The chemistry of
the thiol group
Part 2

THE CHEMISTRY OF FUNCTIONAL GROUPS

A series of advanced treatises under the general editorship of Professor Saul Patai

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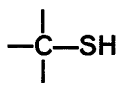

The chemistry of **the thiol group**

Part 2

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Foreword

This volume, 'The Chemistry of the Thiol Group', is again organized and presented according to the general lines described in the 'Preface to the series' printed in the following pages.

Since the last volume in the series 'The Chemistry of the Functional Groups' appeared, there has been one new development in this project: a volume is now in preparation which is planned to contain chapters on subjects which were not included in the previously published volumes either because promised manuscripts have not been delivered or because they represent new developments in rapidly and significantly progressing fields during the last several years. The first such supplementary volume will include material on double-bonded groups (C=C, C=O, C=N). If this venture should prove successful, it is intended to publish further similar supplementary volumes.

The original plan of the present volume also included the following chapters which did not materialize: 'Free radical reactions involving thiols', 'Electrochemistry of the thiol group', 'Enethiols' and 'The thiol-disulphide interchange'.

Jerusalem, May 1974 SAUL PATAI

The Chemistry of Functional Groups
Preface to the series

The series 'The Chemistry of Functional Groups' is planned to cover in each volume all aspects of the chemistry of one of the important functional groups in organic chemistry. The emphasis is laid on the functional group treated and on the effects which it exerts on the chemical and physical properties, primarily in the immediate vicinity of the group in question, and secondarily on the behaviour of the whole molecule. For instance, the volume *The Chemistry of the Ether Linkage* deals with reactions in which the C—O—C group is involved, as well as with the effects of the C—O—C group on the reactions of alkyl or aryl groups connected to the ether oxygen. It is the purpose of the volume to give a complete coverage of all properties and reactions of ethers in as far as these depend on the presence of the ether group, but the primary subject matter is not the whole molecule, but the C—O—C functional group.

A further restriction in the treatment of the various functional groups in these volumes is that material included in easily and generally available secondary or tertiary sources, such as Chemical Reviews, Quarterly Reviews, Organic Reactions, various 'Advances' and 'Progress' series as well as textbooks (i.e. in books which are usually found in the chemical libraries of universities and research institutes) should not, as a rule, be repeated in detail, unless it is necessary for the balanced treatment of the subject. Therefore each of the authors is asked *not* to give an encyclopaedic coverage of his subject, but to concentrate on the most important recent developments and mainly on material that has not been adequately covered by reviews or other secondary sources by the time of writing of the chapter, and to address himself to a reader who is assumed to be at a fairly advanced post-graduate level.

With these restrictions, it is realized that no plan can be devised for a volume that would give a *complete* coverage of the subject with *no* overlap between chapters, while at the same time preserving the readability of the text. The Editor set himself the goal of attaining *reasonable* coverage with *moderate* overlap, with a minimum of cross-references between the chapters of each volume. In this manner, sufficient freedom is given to each author to produce readable quasi-monographic chapters.

The general plan of each volume includes the following main sections:

(a) An introductory chapter dealing with the general and theoretical aspects of the group.

(b) One or more chapters dealing with the formation of the functional group in question, either from groups present in the molecule, or by introducing the new group directly or indirectly.

(c) Chapters describing the characterization and characteristics of the functional groups, i.e. a chapter dealing with qualitative and quantitative methods of determination including chemical and physical methods, ultraviolet, infrared, nuclear magnetic resonance, and mass spectra; a chapter dealing with activating and directive effects exerted by the group and/or a chapter on the basicity, acidity or complex-forming ability of the group (if applicable).

(d) Chapters on the reactions, transformations and rearrangements which the functional group can undergo, either alone or in conjunction with other reagents.

(e) Special topics which do not fit any of the above sections, such as photochemistry, radiation chemistry, biochemical formations and reactions. Depending on the nature of each functional group treated, these special topics may include short monographs on related functional groups on which no separate volume is planned (e.g. a chapter on 'Thioketones' is included in the volume *The Chemistry of the Carbonyl Group*, and a chapter on 'Ketenes' is included in the volume *The Chemistry of Alkenes*). In other cases, certain compounds, though containing only the functional group of the title, may have special features so as to be best treated in a separate chapter, as e.g. 'Polyethers' in *The Chemistry of the Ether Linkage*, or 'Tetraaminoethylenes' in *The Chemistry of the Amino Group*.

This plan entails that the breadth, depth and thought-provoking nature of each chapter will differ with the views and inclinations of the author and the presentation will necessarily be somewhat uneven. Moreover, a serious problem is caused by authors who deliver their manuscript late or not at all. In order to overcome this problem at least to some extent, it was decided to publish certain volumes in several parts, without giving consideration to the originally planned logical order of the chapters. If after the appearance of the originally planned parts of a volume it is found that either owing to non-delivery of chapters, or to new developments in the subject, sufficient material has accumulated for publication of an additional part, this will be done as soon as possible.

The overall plan of the volumes in the series 'The Chemistry of Functional Groups' includes the titles listed below:

The Chemistry of Alkenes (*published in two volumes*)
The Chemistry of the Carbonyl Group (*published in two volumes*)
The Chemistry of the Ether Linkage (*published*)
The Chemistry of the Amino Group (*published*)
The Chemistry of the Nitro and the Nitroso Group (*published in two parts*)
The Chemistry of Carboxylic Acids and Esters (*published*)
The Chemistry of the Carbon–Nitrogen Double Bond (*published*)
The Chemistry of the Cyano Group (*published*)
The Chemistry of Amides (*published*)
The Chemistry of the Hydroxyl Group (*published in two parts*)
The Chemistry of the Azido Group (*published*)
The Chemistry of Acyl Halides (*published*)
The Chemistry of the Carbon–Halogen Bond (*published in two parts*)
The Chemistry of the Quinonoid Compounds (*published in two parts*)
The Chemistry of the Thiol Group (*published in two parts*)
The Chemistry of the Carbon–Carbon Triple Bond
The Chemistry of Amidines and Imidates (*in preparation*)
The Chemistry of the Hydrazo, Azo and Azoxy Groups (*in preparation*)
The Chemistry of the SO, $-SO_2-$, $-SO_2H$ and $-SO_3H$ Groups
The Chemistry of the Cyanates and their Thio-derivatives (*in preparation*)
The Chemistry of the $-PO_3H_2$ and Related Groups

Advice or criticism regarding the plan and execution of this series will be welcomed by the Editor.

The publication of this series would never have started, let alone continued, without the support of many persons. First and foremost among these is Dr. Arnold Weissberger, whose reassurance and trust encouraged me to tackle this task, and who continues to help and advise me. The efficient and patient cooperation of several staff-members of the Publisher also rendered me invaluable aid (but unfortunately their code of ethics does not allow me to thank them by name). Many of my friends and colleagues in Israel and overseas helped me in the solution of various major and minor matters, and my thanks are due to all of them, especially to Professor Z. Rappoport. Carrying out such a long-range project would be quite impossible without the non-professional but none the less essential participation and partnership of my wife.

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CHAPTER 11

The radiation chemistry of thiols

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I. INTRODUCTION

High energy radiation interacts with matter causing ionization and excitation, followed by ion–molecule reactions, charge neutralization and dissociation of molecules giving rise to the formation of free radicals. Thus the radiation chemistry of thiols is essentially free radical chemistry, with the thiyl radical, $RS^{\bullet}$, as the most important intermediate species. The thiols which have been most studied are for two main reasons those of biological interest. Firstly the —SH group is very reactive towards free radicals and consequently molecules containing thiol groups play a dominant role in radiation–biological processes. Secondly, it was found in the 1940's that some aminothiols when added to *in vivo* systems gave considerable protection against the harmful effects of ionizing radiation. As thiols occur in nature, mainly as aminoacid residues of peptide-containing molecules, cysteine, $NH_3^+CH(CO_2^-)CH_2SH$, has been the thiol most closely studied. Cysteamine (2-mercaptoethylamine) was early on found to be highly protective and has also been studied extensively. Studies of the basic radiation chemistry of these and related thiols, in aqueous solutions, alone, or in mixtures with model compounds of biological importance have been most informative, and the gap between radiation chemistry and radiation biology has closed considerably in the last five years. Much current work is now centred on large biologically active molecules.

As the radiolysis of a thiol frequently produces the corresponding disulphide as the major product, and as both thiol and disulphide groups are present together in biological systems, some discussion on the radiation chemistry of the disulphide group is an essential part of this chapter.

Radiation chemistry yields are usually expressed as *G*-values, the number of molecules (or radicals) formed (or destroyed) per 100 eV of

energy absorbed by the system. The equation

$$G(-\mathrm{RSH}) = 2\,G(\mathrm{RSSR}) + G(\mathrm{H_2S})$$

implies that disulphide and H_2S are the only sulphur-containing products formed in a particular experimental study.

II. AQUEOUS SOLUTIONS OF THIOLS–OXYGEN-FREE

A. Radiolysis of Aqueous Solutions

The absorption of high energy radiation by water results in the formation of radical and molecular products[1], and for fast electrons or γ-radiation may be represented by reaction (1) where the stoichiometry is expressed in *G*-values[2]. The exact mechanism of the formation of these products is

$$G_{-\mathrm{H_2O}} \longrightarrow G_{\mathrm{H}}\mathrm{H}^+_{aq} + G_{e_{aq}^-}e^-_{aq} + G_{\mathrm{H}}\mathrm{H} + G_{\mathrm{OH}}\mathrm{OH} + G_{\mathrm{H_2}}\mathrm{H_2} + G_{\mathrm{H_2O_2}}\mathrm{H_2O_2}$$

or

$$4{\cdot}2\,\mathrm{H_2O} \longrightarrow 2{\cdot}7\,\mathrm{H}^+_{aq} + 2{\cdot}7\,e^-_{aq} + 0{\cdot}6\,\mathrm{H} + 2{\cdot}7\,\mathrm{OH} + 0{\cdot}45\,\mathrm{H_2} + 0{\cdot}7\,\mathrm{H_2O_2} \qquad (1)$$

still a matter of research and discussion, but it is clear that at about 100 ns after the absorption of the high energy particle the above products have formed and diffused away sufficiently from the particle track to react with solutes in low concentration ($\leqslant 10^{-3}$M) with effectively homogeneous kinetics. The fraction of the incident energy absorbed by the solute is negligibly small for dilute solutions. The situation is therefore different from that found in photochemistry where all the photon energy is absorbed by direct solute–photon interaction. As its concentration is increased above about 10^{-3}M a reactive solute may progressively interfere with spur reactions, reacting with the primary radical products or their precursors during the stage of 'spur diffusion kinetics', and thus alter the radical and molecular yields.

In dilute solutions of a thiol, RSH, it should be possible to explain the radiation chemistry in terms of the reactions of RSH with OH, e^-_{aq} and H at low conversions, but as the radiation products accumulate, competition between these and RSH for the radicals will occur, leading to secondary products. Thus 'initial yields' of products are normally measured experimentally in mechanistic investigations. When a second solute is also present, e.g. O_2, competition for the primary products will occur, and the intermediates formed from RSH may also react with this added solute. The pH of the solution is also important because H^+_{aq} may compete with RSH for e^-_{aq}, and in addition the actual form, and hence the reactivity, of the thiol may change with pH in a manner depending on its acid dissociation constants.

B. Reactions of Thiols with Primary Radicals

1. Hydroxyl radical

The hydroxyl radical reacts rapidly with thiols, product analysis indicating the thiyl radical to be the main product as in reaction (2). This

$$RSH + OH \longrightarrow RS^{\bullet} + H_2O \quad (2)$$

is supported by the work of Armstrong and Humphries[3], who generated OH radicals from Ti^{3+}–H_2O_2 solutions and reacted them with thiols in a flow system. The e.s.r. spectrum corresponded to that of a thiyl radical. Rate constants for various thiols are listed in Table 1. Jayson, Stirling and

TABLE 1. Rate constants for the reaction of OH with RSH[a]

Thiol	pH	Method[b]	10^{-9} k, l mol^{-1} s^{-1}	Reference
Cysteamine	1·4	CNS^-	15	4
Cysteamine	6·5	CNS^-	13	4
Cysteamine	9	CNS^-	13	4
Cysteine	6·5	CNS^-	13	5
Mercaptoethanol	7	CNS^-	25	6
Mercaptoethanol	11	CNS^-	6·2	6
Mercaptoethanol	6·5	CNS^-	17	7
Mercaptoethanol	6·5	$Fe(CN)_6^{4-}$	6·1	7
Mercaptoethanol	6·5	$PhNO_2$[c]	5	7
Methyl mercaptan	7	CNS^-	31	6
Methyl mercaptan	11	CNS^-	9·4	6
t-Butyl mercaptan	7	CNS^-	17	6
Glutathione	6·5	CNS^-	12	5
Homocysteine	7	PNDA[d]	1·7	8

[a] Normalized to rate constants given in reference 9.
[b] Pulse radiolysis except for homocysteine.
[c] Using $k_{OH+PhNO_2} = 4{\cdot}7 \times 10^9$ l mol^{-1} s^{-1}.
[d] *p*-Nitrosodimethylaniline—steady-state radiolysis.

Swallow obtained a higher figure for mercaptoethanol with thiocyanate ion as competition scavenger than with ferrocyanide ion or nitrobenzene[7], and other figures in the table using CNS^- could also possibly be too high. At pH 9 or 11 the thiols listed would be mainly in their thiolate ion form. In the case of mercaptoethanol and methyl mercaptan at pH 11 new transients seen by pulse radiolysis, and not observed at lower pH, were tentatively attributed to radicals obtained by hydrogen atom abstraction from the α-carbon atom with respect to sulphur[6]. Recent e.s.r.-radiolysis studies also give evidence for some H-abstraction from carbon in mercaptocarboxylic acids[10].

2. Aquated electron

The aquated electron reacts rapidly with thiols in near-neutral solutions to give H_2S and the parent hydrocarbon as the major detectable products, according to reactions (3) and (4). Values of $G(H_2S)$ and $G(RH)$ of

$$RSH + e_{aq}^- \longrightarrow R^{\bullet} + HS^- \quad (3)$$

$$R^{\bullet} + RSH \longrightarrow RH + RS^{\bullet} \quad (4)$$

between 2·5 and 3·0 have been reported for cysteine[11,12,13], cysteamine[14], methyl mercaptan[15], and homocysteine[8] for thiol concentrations in the range 10^{-3}–5×10^{-2}M. Lower values of 2·3 have been reported for glutathione (10^{-2}M)[16], and 4-aminobutane-1-thiol (10^{-3}M)[17], while very much lower values of 1·4 and 1·7 for 10^{-2}M and 10^{-1}M mercaptoethanol[7] have been found. The authors in the latter case suggest that nearly half the e_{aq}^- are reacting by reaction (5):

$$e_{aq}^- + HOCH_2CH_2SH \xrightarrow{H^+} HOCH_2CH_2S^{\bullet} + H_2 \quad (5)$$

but the reason for this difference is not understood.

Reported rate constants for the reaction of e_{aq}^- with thiols are listed in Table 2. In pulse radiolysis studies the rate of disappearance of e_{aq}^- is measured directly, whereas in product-yield–scavenger studies, the RSH–e_{aq}^- adduct could in principle transfer the electron to a scavenger, or not yield H_2S quantitatively, thus leading to low values. The figures for cysteine at low and high pH call for comment. Trumbore and coworkers suggest that the fully protonated form of cysteine, carrying an overall positive charge, reacts faster than does the zwitter-ion form[18], while the 100-fold decrease at pH 11·6 found by Braams[20] would be due to the cysteine being present as the thiolate ion, RS^-. It was found in a much earlier study[21] that $G(H_2S)$ drops as the pH is increased above 8, and the thiolate ion is probably unreactive towards e_{aq}^-.

3. Hydrogen atom

In acidic solutions aquated electrons with protons yield hydrogen atoms by reaction (6), and these, together with those formed directly ($G_H = 0{\cdot}6$), may react with the thiol. Armstrong and coworkers have

$$e_{aq}^- + H_{aq}^+ \longrightarrow H \quad (6)$$

shown that lowering pH increases $G(H_2)$ and decreases $G(H_2S)$[15], but even under conditions where all e_{aq}^- are scavenged by H_{aq}^+ some H_2S is still produced. Thus it appears that H may react by reaction (7) or reaction

TABLE 2. Rate constants for the reaction of e^-_{aq} with RSH

Thiol	pH	[RSH], M	Measured quantity	Technique[a]	$10^{-9}k$, l mol^{-1} s^{-1}	Reference
Cysteine	0·7–0·8	10^{-3}	$G(H_2)$	$[H^+]$	11	15
Cysteine	7	—	$G(H_2S)$	$[NO_3^-]$	4·4	12
Cysteine	7	—	$G(H_2S)$	[acetone]	5·4	12
Cysteine	0·5, 1	10^{-3}–10^{-1}	$G(H_2)$	[RSH]	30	18
Cysteine	5–6	10^{-2}	$G(H_2S)$	$[NO_3^-]$	11	11
Cysteine	5–6	10^{-3}	$G(H_2S)$	[acetone]	11	11
Cysteine	7	3×10^{-3}	$G(H_2S)$	$[O_2]$	4	19
Cysteine	6·3	—	$[e^-_{aq}]$	p.r.	8·7	20
Cysteine	11·6	—	$[e^-_{aq}]$	p.r.	0·075	20
Cysteine	6·9	—	$[e^-_{aq}]$	p.r.	20	20
Cysteamine	8·25	—	$[e^-_{aq}]$	p.r.	3·2	20
Glutathione	6·5	—	$[e^-_{aq}]$	p.r.	5·1	20
Penicillamine	5·7–9	—	$[e^-_{aq}]$	p.r.	12	7
Mercaptoethanol	10	—	$[e^-_{aq}]$	p.r.	10	6
Mercaptoethanol	7	—	$[e^-_{aq}]$	p.r.	7·5	6
Methyl mercaptan	0·7–8	5×10^{-3}	$G(H_2)$	$[H^+]$	18	15
Methyl mercaptan	7	—	$[e^-_{aq}]$	p.r.	3	6
t-Butyl mercaptan	7	3×10^{-3}	$G(H_2S)$	$[O_2]$	4·3	8
Homocysteine	7	10^{-3}	$G(H_2S)$	$[O_2]$	4	17

[a] p.r. stands for pulse radiolysis. In the other cases the competitive electron scavenging technique was used, the compound whose concentration was varied being indicated.

(8) with the R$^\bullet$ abstracting H from a second thiol molecule, reaction (4).

$$H+RSH \longrightarrow H_2+RS^\bullet \quad (7)$$

$$H+RSH \longrightarrow H_2S+R^\bullet \quad (8)$$

Trumbore has pointed out[18] that reactions (9) and (10) provide a possible alternative route for the formation of H_2S. No evidence that clearly separates the possibilities has been reported.

$$H+RSH \longrightarrow HS^\bullet+RH \quad (9)$$

$$HS^\bullet+RSH \longrightarrow H_2S+RS^\bullet \quad (10)$$

The rate constant ratio k_7/k_8 (or k_7/k_9) has been determined from $G(H_2)$ and/or $G(H_2S)$ measurements. The following figures have been obtained at room temperature: cysteine, 3·5[22] and 3·7[23]; cysteamine, 2·7[14]; mercaptoethanol, ~ 5[7]. By bubbling H atoms formed by an electric discharge into a solution of cysteine Navon and Stein obtained a value of about 5[24].

C. Mechanism

The products of the radiolysis of a thiol in the absence of O_2 are the disulphide, H_2 and H_2S. The generally accepted mechanism established for cysteine by Armstrong[11,15,22] and by Trumbore[12,19] is:

$$e^-_{aq}+H^+_{aq} \longrightarrow H \quad (6)$$

$$e^-_{aq}+RSH \longrightarrow R^\bullet+HS^- \quad (3)$$

$$H+RSH \longrightarrow RS^\bullet+H_2 \quad (7)$$

$$H+RSH \longrightarrow R^\bullet+H_2S \quad (8)$$

$$OH+RSH \longrightarrow RS^\bullet+H_2O \quad (2)$$

$$R^\bullet+RSH \longrightarrow RH+RS^\bullet \quad (4)$$

$$RS^\bullet+RS^\bullet \longrightarrow RSSR \quad (11)$$

The evidence for this mechanism comes from the effect of pH on $G(-RSH)$, $G(H_2S)$ and $G(H_2)$, the equality of $G(H_2S)$ and $G(RH)$ at all pH, the sulphur mass-balance $G(-RSH) = 2G(RSSR)+G(H_2S)$, and reasonable agreement of $G(-RSH)$ with the values calculated on the above mechanisms in the extreme where all e^-_{aq} react with RSH according to reaction (3). At any pH the relationship

$$G(-RSH) = G_{OH}+G_{e^-_{aq}}+G_H+G(H_2S)$$

holds if the mechanism is correct, and from simple competition kinetics,

$$G(H_2S) = G_{e_{aq}^-}\frac{k_3[RSH]}{k_3[RSH]+k_6[H^+]}+G_{e_{aq}^-}\frac{k_6[H^+]}{k_3[RSH]+k_6[H^+]}\frac{k_8}{k_7+k_8}+G_H\frac{k_8}{k_7+k_8}$$

At low pH we therefore have

$$G(-RSH) = G_{OH}+(G_{e_{aq}^-}+G_H)\left(1+\frac{k_8}{k_7+k_8}\right)$$

and in neutral solution

$$G(-RSH) = G_{OH}+2\,G_{e_{aq}^-}+G_H\left(1+\frac{k_8}{k_7+k_8}\right)$$

The rate constant ratio k_7/k_8 reported in the previous section was obtained by assuming the above mechanism. Taking the figure of 3·5 for this ratio for cysteine, and the radical yields given in reaction (1), $G(-RSH) = 6{\cdot}7$ in acidic and 8·8 in neutral solution respectively.

In all thiols studied, $G(-RSH)$ figures decrease with decreasing thiol concentrations, the decrease being greater than may be expected from rate constant data. The same general mechanism appears to apply to the thiols cysteamine[14], glutathione[16], homocysteine[8] and 4-aminobutane-1-thiol[17], although the values of $G(-RSH)$ were a little low for complete scavenging in some cases.

As mentioned in section II.B.2 mercaptoethanol behaves differently in that only about half the aquated electrons give rise to H_2S[7]. Bronsted acids can react with e_{aq}^- and convert them to H, but the pK_a of the thiol group in mercaptoethanol is not lower than for other thiols, and the explanation must lie elsewhere.

A further reaction which should be considered when deducing mechanism from product yields is that of H_2O_2 with thiols (12):

$$2\,RSH+H_2O_2 \longrightarrow RSSR+2\,H_2O \qquad (12)$$

This reaction is slow in acidic solution, and $G(H_2O_2) = G_{H_2O_2}$ is found. However, in neutral and alkaline solution the rate can be appreciable and the reaction must be allowed for[11]. It has been shown that the reaction involves a nucleophilic attack of the thiolate ion on hydrogen peroxide, the rate being found proportional to $[RS^-]$ in studies on cysteamine[14] and cysteine[25] in which pH was varied.

D. Transients

I. Pulse radiolysis studies

Pulse radiolysis studies have shown the presence of a transient species when thiols are irradiated at a pH where some ionization of the thiol group has occurred. These species have an absorption band from

approximately 350 to 500 nm with a maximum at 400–450 nm and an extinction coefficient of the order of $10^4\,l\,mol^{-1}\,cm^{-1}$.

The first detailed study was on cysteamine by Adams and coworkers[4], who showed that the transient was not RS• as they had first suspected but RS$\bar{S}$R formed by reaction of the thiyl radical with the thiolate ion in an equilibrium reaction (13). Evidence for this came from studying both

$$RS^\bullet+RS^- \rightleftharpoons RS\bar{S}R \qquad (13)$$

cystamine and cysteamine solutions. In pure solutions of the disulphide the rate of formation of the transient matched the rate of decay of the aquated electron, and the addition of nitrous oxide drastically reduced the amount formed. N_2O scavenges e_{aq}^- to produce OH radicals, reaction (14).

$$e_{aq}^-+N_2O \xrightarrow{H^+} OH+N_2 \qquad (14)$$

The decay of absorption was always exponential, suggesting electron attachment to the disulphide followed by dissociation, reactions (15) and (−13). In cysteamine solution N_2O increased the amount of transient

$$e_{aq}^-+RSSR \longrightarrow RS\bar{S}R \qquad (15)$$

$$RS\bar{S}R \longrightarrow RS^\bullet+RS^- \qquad (-13)$$

formed immediately after the pulse, showing OH radicals to be the precursor in this case. The rate of growth of transient was slower than the rate of reaction of thiol with OH radicals (as measured by CNS^- competition scavenging) but increased with thiol concentration. The maximum absorbance obtained after the electron pulse increased with increasing thiol concentration and pH, i.e. with increasing RS^- concentration, implying the equilibrium (13). This was confirmed by the decay kinetics, which were second-order and much slower than the first-order decay (−13). The second-order rate constant decreased with increasing thiol concentration and pH, implying that the rate of disappearance was controlled by dimerization of free thiyl radicals (reaction 11):

$$RS^\bullet+RS^\bullet \longrightarrow RSSR \qquad (11)$$

Similar results have been found for cysteine[25,26], mercaptoethanol[6,7], various alkyl mercaptans[6], H_2S[27], and penicillamine[28].

Further study of the second-order decay of RS$\bar{S}$R as a function of pH and thiol concentration showed that reaction (16) was also important[29], and this was confirmed during further work on cysteine[30].

$$RS^\bullet+RS\bar{S}R \longrightarrow \text{Products} \qquad (16)$$

The products are presumably RSSR and RS^-. Rate constants reported for reactions (11) and (16) are $\geqslant 10^9\,l\,mol^{-1}\,s^{-1}$.

The equilibrium constants for reaction (13) have been determined from either the rate constants of the forward and back reactions or from dependence of maximum absorbance after the pulse upon concentration and pH, and are shown in Table 3 together with reported extinction coefficients and absorption maximum for $RS\bar{S}R$.

Weaker absorptions at shorter wavelengths have been reported and assigned to the thiyl radical for penicillamine[28] at pH 5 ($\lambda_{max} = 330$ nm, $\varepsilon = 1{\cdot}2 \times 10^3\,l\,mol^{-1}\,cm^{-1}$) and for mercaptoethanol[7] at pH 6 ($\lambda_{max} = 360$ nm, $\varepsilon = 1{\cdot}3 \times 10^2\,l\,mol^{-1}\,cm^{-1}$), and tentatively to the radicals $HOCH_2\dot{C}HS^-$ and $^{\cdot}CH_2S^-$ for mercaptoethanol and methyl mercaptan[6] respectively at pH 12 ($\lambda_{max} = 300$ nm).

2. E.s.r. studies

Transient intermediates in radiolysis can also be detected by e.s.r., and this technique has been developed by Fessenden and his coworkers[32]. A radical must build up to some minimum concentration to be detected, and must not have too great a linewidth.

The radical formed by dissociative electron capture, postulated from stable product analysis, has been detected directly for the mercaptoacetate ion[10]. A spectrum consisting of a 21·2-G triplet with $g = 2{\cdot}0032$ has been attributed to the radical $^{\cdot}CH_2CO_2^-$ at pH 12·4 and 8·6 and shown to have e_{aq}^- as a precursor because N_2O prevented its formation. Increasing $-SCH_2CO_2^-$ concentration decreased the signal, this being taken as evidence for reaction (4). The OH radical was shown to abstract hydrogen from carbon as well as sulphur since the mercaptoacetate ion at pH 12·4 gave a 13·4-G doublet with $g = 2{\cdot}0086$, attributed to $\bar{S}\dot{C}HCO_2^-$. This could not be detected at pH 8·6, and it was thought the doubly-charged anion lowered the recombination rate sufficiently for its concentration to build up to detectable amounts. The radicals $-S\dot{C}HCH_2CO_2^-$ and $-SCH_2\dot{C}(NH_2)COO^-$ were detected in alkaline solutions of 3-mercaptopropionate and cysteine respectively, abstraction from the β-carbon atom with respect to sulphur in the latter case being attributed to the extra stability of a tertiary radical.

No thiyl radicals were detected in these studies, possibly because such radicals could react with thiolate anions, reaction (13), and that the G-factor of $RS\bar{S}R$ might cause such line broadening to make it undetectable.

TABLE 3. Data on $RS\bar{S}R$

Thiol	K_{13}, $l\,mol^{-1}$	k_{13}, $l\,mol^{-1}\,s^{-1}$	k_{-13}, s^{-1}	ε_{max}, $l\,mol^{-1}\,cm^{-1}$	λ_{max}, nm	Reference
Cysteamine	6×10^3	$4{\cdot}9 \times 10^9$	8×10^5	$8{\cdot}9 \times 10^3$	410	4, 29
Cysteamine	—	—	$3{\cdot}5 \times 10^5$	$9{\cdot}0 \times 10^3$	415	105
Cysteine	$9{\cdot}5 \times 10^3$	$3{\cdot}1 \times 10^9$	$3{\cdot}2 \times 10^5$	—	420	30
Cysteine	—	—	$2{\cdot}9 \times 10^5$	$\geqslant 8{\cdot}8 \times 10^3$	420	105
Penicillamine	$2{\cdot}5 \times 10^3$	—	$1{\cdot}5 \times 10^6$	$7{\cdot}5 \times 10^3$	450	28
Penicillamine	—	—	$1{\cdot}3 \times 10^6$	$7{\cdot}3 \times 10^3$	450	105
Glutathione	—	—	3×10^5	5×10^3	420	31
Glutathione	—	—	$1{\cdot}5 \times 10^5$	8×10^3	420	105
Mercaptoethanol	$1{\cdot}7 \times 10^3$	—	—	$8{\cdot}3 \times 10^3$	420	6
Hydrogen sulphide	$2{\cdot}5 \times 10^4$	—	—	$\sim 10^4$	380	27
Cysteine methyl ester	—	—	$1{\cdot}9 \times 10^5$	$8{\cdot}3 \times 10^3$	420	105
2-Mercaptoacetic acid	—	—	$2{\cdot}4 \times 10^6$	$9{\cdot}5 \times 10^3$	400	105
3-Mercaptopropanoic acid	—	—	$2{\cdot}7 \times 10^6$	$1{\cdot}5 \times 10^4$	420	105

E. Derivatives of Thiols

1. Disulphides

The OH radical reacts rapidly with disulphides, rate constants greater than $10^9\,l\,mol^{-1}\,s^{-1}$ being reported for cystine[33] and cystamine[34]. There is little direct evidence for the immediate products, the reaction being written as (17) by Purdie for cystine and penicillamine disulphide[35,36], and as (18)

$$RSSR+OH \longrightarrow RSOH+RS^{\bullet} \quad (17)$$

$$RSSR+OH \longrightarrow RS\overset{+}{S}R+OH^{-} \quad (18)$$

by Jayson and Owen and coworkers for cystamine[37,38], with the cation undergoing bond cleavage in subsequent reactions. The formation of an adduct $RS\dot{S}(OH)R$ with a significant lifetime has also been proposed[39]. Purdie has shown that the OH radical also leads to the formation of trisulphides[35,36] and has postulated a second set of products from OH attack, reaction (19). The sulphenic acid, from reaction (17), can react

$$RSSR+OH \longrightarrow RSSOH+R^{\bullet} \quad (19)$$

with RSH produced from e^-_{aq}, reaction (20), or disproportionate, reaction (21), while the trisulphide is also a product of radiation-produced thiol, reaction (22)[36].

$$RSOH+RSH \longrightarrow RSSR+H_2O \quad (20)$$

$$RSOH+RSOH \longrightarrow RSH+RSO_2H \quad (21)$$

$$RSSOH+RSH \longrightarrow RSSSR+H_2O \quad (22)$$

It has been shown that the presence of chloride ion (hydrochloride salts of aminothiols are often used) in acidic solution decreases S—S cleavage and increases ammonia yields with cystine[40]. (Ammonia is a major product of both e^-_{aq} and OH attack on amino acids and peptides not having thiol or disulphide groups[41].)

The aquated electron reacts rapidly with disulphides, rate constants of $1{\cdot}3\times10^{10}$, 2×10^{10}, 9×10^9 and $6{\cdot}4\times10^9\,l\,mol^{-1}\,s^{-1}$ for cystine, cystamine, homocystine and glutathione disulphide, respectively, at pH 6–7 being reported[20]. As discussed in section II.D.1 the adduct $RS\bar{S}R$ is first formed, and in the absence of other solutes breaks down to $RS^{\bullet}$ and RS^-.

H atoms have been reported to react with cystine with a rate constant of $5\times10^9\,l\,mol^{-1}\,s^{-1}$ and to produce cysteine[24], reaction (23). A transient

$$RSSR+H \longrightarrow RSH+RS^{\bullet} \quad (23)$$

intermediate, believed to be $RS\dot{S}HR$, has been reported by Simic and Hoffmann[31] on pulse radiolysis of glutathione disulphide at pH 1 with λ_{max} of 330 nm and extinction coefficient of $600\,l\,mol^{-1}\,cm^{-1}$. The same transient was seen at pH 3·7 where e^-_{aq} would react directly with the disulphide, and thus it was postulated that H atom addition, and protonation of the electron adduct gave the same product[31] *.

$$RSSR+H \longrightarrow RS\dot{S}HR \quad (24)$$

$$RS\bar{S}R+H^+ \longrightarrow RS\dot{S}HR \quad (25)$$

Product yields in oxygen-free solutions of disulphides are low because of concurrent oxidation and reduction. RSOH (or RS^+) and RSH effectively give back the starting material, reactions (17), (15) and (20).

2. Large molecules of biological interest

In the previous sections reactions associated with the thiol or disulphide groups themselves have been mainly discussed, although some of the molecules mentioned do have other functional groups which show varying degrees of reactivity towards the primary radiolysis products of water. However, product analysis shows that in these cases reactions at other sites in the molecule are at the most only minor, the high reactivity of the —SH and —S—S— groups being the dominating factor.

Recently work has been done on enzymes which contain both thiol and/or disulphide groups[42], including lysozyme[43,44], trypsin[45] and papain[46]. In each case pulse radiolysis shows an absorption at 400–430 nm associated with $RS\bar{S}R$, and shown to have e^-_{aq} as precursor. Sixty per cent of e^-_{aq} are estimated to react with the cystine residue in trypsin[45] and perhaps only 25% in the case of papain[46] where 20% of the adducts decayed with a half-life of about 30 μs, the remainder having a lifetime longer than 0·05 s showing it to be very stable. It was noted that there are three disulphide bridges in papain, and it is possible that electron transfer from one of these to other groups could occur in a time too short to allow detection.

OH attack is shown to occur mainly not at free thiol groups, but at tyrosine residues for papain[46] and tryptophan residues for lysozyme[43] and

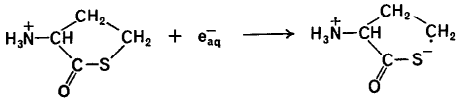
* Shafferman has since shown that this assignment of the transient to $RS\dot{S}HR$ is incorrect, and that the species seen was the thiyl radical of glutathione, $RS^{\bullet}$ [106]. For glutathione disulphide he measured k_{15}, k_{23} and k_{25} as $2{\cdot}7\times10^9$, $1{\cdot}1\times10^{10}$ and $2{\cdot}6\times10^{10}\,l\,mol^{-1}\,s^{-1}$ respectively. Further work by Hoffman and Hayon is in agreement with Shafferman's conclusions[105] and these authors also give λ_{max} and extinction coefficients for other thiyl radicals together with more extensive figures for k_{15} including their pH dependence.

trypsin[45], and that loss of enzyme acitivity may be associated with this for lysozyme, although this loss of activity has recently also been attributed to polymerization through intermolecular —S—S— bond formation[47].

A comparison of pulse radiolysis transient yields and final values of $G(-\text{tryptophan})$ and $G(\text{RSH})$ for trypsin shows that a reconstitution or back reaction occurs, as final yields are low[45]. Oxygen prevented this back reaction. Recent work on ribonuclease shows the same general features as the enzymes mentioned above[107].

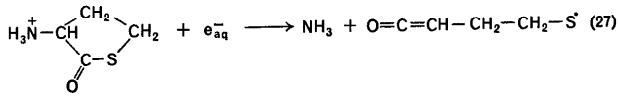
3. Thiolactone

Homocysteine lactonizes readily in acidic solutions, and a study of the aqueous radiation chemistry of this thiolactone was undertaken to see how bonding of the sulphur to a carbonyl carbon modifies its reactivity to the aquated electron[48]. (The normal H atom abstraction from sulphur by OH cannot occur.) The dissociative electron capture reaction which gives H_2S in the case of free thiols can be formulated:

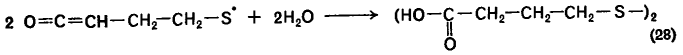


(26)

Resonance stabilization of the thiocarboxylate group might have been expected to favour this reaction. The aquated electron reacted fast with the thiolactone, $k = 3{\cdot}6 \times 10^{10}\,\text{l mol}^{-1}\,\text{s}^{-1}$, but it was found that reductive deamination occurred, this being the typical reaction for amino acid derivatives[41]. 4,4′-Dithiodibutyric acid was found to be a product, and the following steps involving ring opening were postulated:



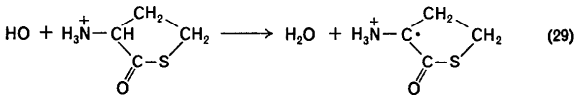
(27)



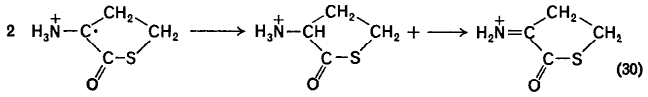
(28)

The OH radical leads to oxidative deamination and ketoacid formation in a manner similar to that for amino acids[41]. This shows that H atom

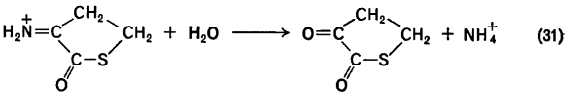
abstraction occurs from the tertiary carbon atom, rather than from that α to sulphur, as found in e.s.r. studies with cysteine[10].



(29)



(30)



(31)

F. Reactions with Secondary Radiation-produced Radicals

Many organic radicals will abstract hydrogen from the thiyl group, reaction (4) being one example. Where the organic radical has been produced by H atom abstraction by OH or H, this hydrogen transfer from thiol restores the molecule to its original form, and effectively protects it from radiolysis damage. This topic is dealt with more fully in section VI on radiation protection.

Inorganic radicals, formed from the reaction between anions and OH can also oxidize thiols to free thiyl radicals. The species $(CNS)_2^-$, Br_2^-, Cl_2^- and I_2^- (formed by radiolysis of N_2O-saturated solutions of CNS^-, Br^-, Cl^-, I^-) are reduced by cysteine with rate constants of $0{\cdot}5\text{–}8{\cdot}5 \times 10^8\,\text{l mol}^{-1}\,\text{s}^{-1}$, and the rate constants for (CNS_2^-), Br_2^- and I_2^- increase by approximately a factor of 10 when the thiol is converted to the thiolate anion[49]. (Cl_2^- exists only in acidic solution.) CO_3^- is also reduced rapidly by the thiolate anion of cysteine[49].

Whereas thiols may be oxidized, disulphides may be reduced by electron transfer from radicals. Willson has shown by pulse radiolysis[50] that the electron adduct of the lipoate anion $[\overline{S\text{—}SCH_2CH_2}CH(CH_2)_4CO_2^-]$ is formed in the reaction of lipoate with $(CH_3)_2\dot{C}OH$, $CH_3\dot{C}HOH$, CO_2^- and the electron-thymine adduct with rate constants of $1\text{–}6 \times 10^8\,\text{l mol}^{-1}\,\text{s}^{-1}$.

Thiyl radicals themselves react with disulphides leading to new products where two different alkyl groups are present[51,39].

$$RS^{\bullet} + RSSR' \longrightarrow RSSR + R'S^{\bullet} \qquad (32)$$

III. AQUEOUS SOLUTIONS OF THIOLS—CONTAINING OXYGEN

A. Products and Yields

Cysteine has been the most extensively studied thiol in oxygenated aqueous solutions. Although reported yields vary from group to group the following general features have been found:

1. Oxygen lowers $G(H_2)$ and $G(H_2S)$ and increases $G(H_2O_2)$ with respect to oxygen-free yields.

2. At low doses, and provided [RSH] $\geqslant \sim 10^{-3}$M the disulphide cystine is still the only major sulphur-containing product, but large doses do result in higher oxidation products being formed.

3. Increasing cysteine concentration increases yields, this effect being greater when the free base is used instead of the hydrochloride (Table 4 shows figures from different research groups) and at pH $\leqslant 5$, there is an approximately equimolar increase in hydrogen peroxide and disulphide. Oxygen concentration has little effect on the yields.

TABLE 4. Variation in $G(-RSH)$ or $2G(RSSR)$ with [RSH] for oxygenated cysteine solutions

[RSH], M	$2G$(RSSR)		$G(-RSH)$						
10^{-4}	5·5	5·6				7		13	
3×10^{-4}	10	9·5	7·6	7·6					
5×10^{-5}					5		15		
10^{-3}		15	15	8·2	7	9	20	20	14
3×10^{-3}	18	18		9·2	10	11	36	24	
10^{-2}			24						
O_2	air[a]	air	air	air	air	1 atm[b]	1 atm	1 atm	air
pH	1	3	0–1	1·35	4	3–4	7	7	7
Reference	23	52	53	25	54	25	19	25	53

Note: Dose rate $0{\cdot}8$–$1{\cdot}4\times10^{18}$ eV l^{-1} s^{-1}.

[a] Equilibrated with air at 1 atm.

[b] Equilibrated with oxygen at 1 atm.

4. As the pH is raised above 5 a marked increase in $G(-RSH)$ and G(RSSR) occurs, but $G(H_2O_2)$ does not increase until the pH is greater than 7, and then the value of $G(H_2O_2)$ is less than that of G(RSSR)[25]. Figure 1 illustrates this.

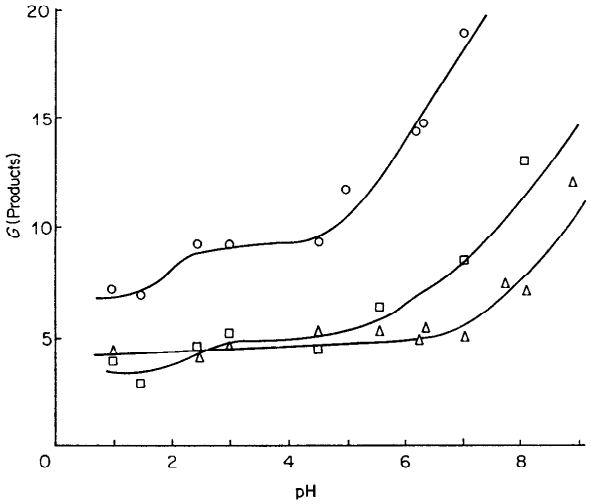


FIGURE 1. G(Products) as a function of pH for 10^{-3} M cysteine saturated with oxygen[25].

○ = $G(-RSH)$ □ = G(RSSR) △ = $G(H_2O_2)$

Mercaptoethanol has been studied[7] in the pH range 0–5·8, and oxygen increases both G(RSSR) and $G(H_2O_2)$, while increasing the thiol concentration from 10^{-2}M to 10^{-1}M causes a major increase as shown in Table 5.

TABLE 5. Product yields from aerated aqueous mercaptoethanol solution[a]

[RSH], M	pH	Aeration	$G(H_2O_2)$	G(RSSR)
10^{-2}	3·1	None	0·56	3·45
10^{-2}	3·1	Air	6·5	6·5
10^{-1}	3·3	None	0·45	4·4
10^{-1}	3·0	Air	36·1	36·1

Note: Dose rate $7{\cdot}8\times10^{16}$ eV l^{-1} s^{-1}.

[a] Taken from Table 1, reference 7.

Cysteamine[14] was found to differ from the above two thiols in that little disulphide and hydrogen peroxide were formed at low pH, whereas at higher pH the yields were at least qualitatively similar to those of cysteine. Figure 2 illustrates these points. Presumably products with sulphur in a higher oxidation state than in cystamine are formed in acidic solution.

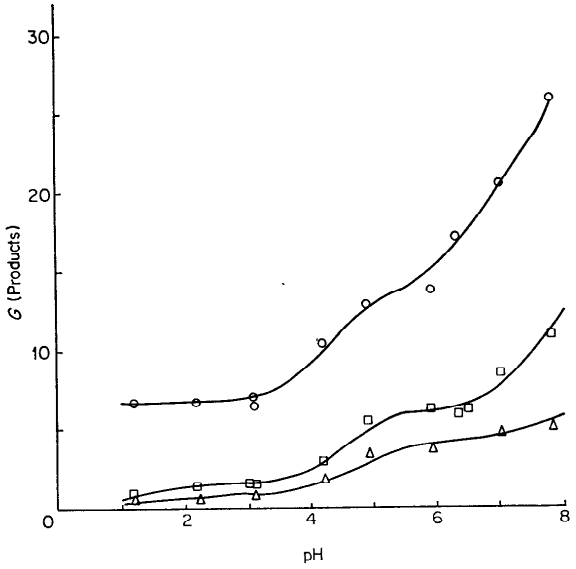


FIGURE 2. G(Products) as a function of pH for aerated 10^{-3} M cysteamine[14].
○ = $G(-\text{RSH})$ □ = $G(\text{RSSR})$ △ = $G(H_2O_2)$

Very high yields of disulphide from some *n*-alkyl mercaptides with G(RSSR) up to 650 have been reported[55]. Quantitative product yields are however difficult to obtain at pH > 8 because of autoxidation, and because the thermal reaction between hydrogen peroxide and thiol proceeds at an appreciable rate.

Disulphides have been studied in the presence of oxygen by Purdie[35, 36, 39, 51, 56] and Owen and coworkers[37, 38, 57, 59]. Sulphonic acids become a major product, Owen consistently reporting higher yields than Purdie, who finds significant amounts of sulphinic acids are still formed.

B. Effect of Oxygen on Radical Reactions

1. Competition for primary radicals

Oxygen does not react with OH except at high pH[59] where the latter exists as O^-. As thiols are readily autoxidized in alkaline solution no detailed studies of oxygenated solutions at high pH have been reported. However, oxygen reacts rapidly with both H and e^-_{aq} to give HOO• and O_2^- with rate constants k_{33} and k_{34} of 2×10^{10} and $1{\cdot}88\times10^{10}$ l mol^{-1} s^{-1} respectively[59], and would be expected to lower $G(H_2)$ and $G(H_2S)$ with

$$H + O_2 \longrightarrow HOO^{\bullet} \qquad (33)$$

$$e^-_{aq} + O_2 \longrightarrow O_2^- \qquad (34)$$

respect to oxygen-free solutions. This has generally been found. Al-Thannon found for example that air lowered $G(H_2)$ from 3·10 to 0·65 in 10^{-4}M cysteine solution and from 3·4 to 3·06 for 10^{-2}M cysteine[33], competition being less effective at the higher thiol concentration as expected. Competition between O_2 and RSH for e^-_{aq} has been used by the Auckland group[8, 17, 19] to determine k_3 for various thiols at pH 7 (Table 2). Neglecting H_2S from reaction (8) and by plotting $1/G(H_2S)$ against $[O_2]/[RSH]$,

$$G(H_2S) = G_{e_{aq}^-}\frac{k_3[RSH]}{k_3[RSH]+k_{34}[O_2]}$$

or

$$\frac{1}{G(H_2S)} = \frac{1}{G_{e_{aq}^-}}\left(1+\frac{k_{34}[O_2]}{k_3[RSH]}\right)$$

k_3 can be found. This technique of determining rate constant ratios by competitive scavenging is common in radiation chemistry.

It has been found for mercaptoethanol that oxygen lowers $G(H_2S)$ much more than would be expected from the known values of k_3 for this thiol determined by measuring the rate of disappearance of e^-_{aq} by pulse-radiolysis and k_{34}, and it has been suggested that the electron adduct of the thiol might be sufficiently long-lived to transfer partially the electron to oxygen before dissociating, reactions (35) and (36)[7]. Barton considered

$$e^-_{aq} + HOCH_2CH_2SH \longrightarrow HOCH_2CH_2SH^- \qquad (35)$$

$$HOCH_2CH_2SH^- + O_2 \longrightarrow HOCH_2CH_2SH + O_2^- \qquad (36)$$

that the reason Winchester's figure for k_3 for cysteine[19] was only about half that measured directly by pulse radiolysis[20] might be due to similar reactions, but by re-analysing Winchester's results where both [RSH] and

[O_2] were varied, he showed this not to be the case[25]. Again it appears as though the reaction between e^-_{aq} and mercaptoethanol is somewhat anomalous.

2. Reaction of HOO• with RSH

The hydroperoxy radical has a pK_a of 4·88 and thus it exists as HOO• in acidic solutions and as its conjugate base, O_2^-, in neutral and alkaline solution[60]. By studying the formate ion–oxygen–cysteine system as a function of pH Barton has found that HOO• does not react with cysteine, but that O_2^- does[25,30]. In the absence of thiol, reactions (37), (38) and (39) in addition to (33) or (34) occur, giving a yield of hydrogen peroxide,

$$OH + HCOOH\ (HCOO^-) \longrightarrow H_2O + {}^{\bullet}COOH\ (CO_2^-) \quad (37)$$

$$^{\bullet}COOH\ (CO_2^-) + O_2 \longrightarrow CO_2 + HOO^{\bullet}\ (O_2^-) \quad (38)$$

$$2\,HOO^{\bullet}\ (O_2^-) \longrightarrow H_2O_2 + O_2 \quad (39)$$

$G(H_2O_2) = G_{H_2O_2} + \frac{1}{2}(G_{OH} + G_{e_{aq}^-} + G_H) = 3{\cdot}7$. If the peroxy radical abstracts H from the thiol, the yield of H_2O_2 should increase as each OH, e^-_{aq} or H now gives rise to one molecule of H_2O_2

$$HOO^{\bullet} + RSH \longrightarrow H_2O_2 + RS^{\bullet} \quad (40)$$

$$O_2^- + RSH \xrightarrow{H^+} H_2O_2 + RS^{\bullet} \quad (41)$$

and $G(H_2O_2) = G_{H_3O_2} + G_{OH} + G_{e_{aq}^-} + G_H = 6{\cdot}7$. In solutions where [HCOOH] ≫ [cysteine], Barton found $G(H_2O_2) = 3{\cdot}7$ and $G(-RSH) = 0$ in acidic solution, these increasing to 6·2 and 5·8 respectively as the pH is raised to 5·1. From this work he estimated k_{41} as $1{\cdot}8 \times 10^4\ l\,mol^{-1}\,s^{-1}$ within a factor of five. Using the same method cysteamine was also found[19] to be unreactive towards HOO•.

The reason for the enhanced reactivity of O_2^- probably lies in the free energy of protonation of the peroxide anion (pK_a of $H_2O_2 = 11{\cdot}8$). On bond strength figures, reaction (40) would be nearly thermoneutral[61,62].

3. Reaction of RS$\bar{S}$R with oxygen

The transient RS$\bar{S}$R has been found to react with oxygen in pulse radiolysis studies of cystine[30] and lipoate[50] with rate constants of $4{\cdot}3 \times 10^8$ and $9 \times 10^8\ l\,mol^{-1}\,s^{-1}$ respectively. Oxygen enhanced the rate of first-order decay, the increase in rate being proportional to oxygen concentration. The reaction is thought to involve electron transfer from disulphide to oxygen, reaction (42).

$$RS\bar{S}R + O_2 \longrightarrow RSSR + O_2^- \quad (42)$$

4. Reaction of thiyl radicals with oxygen

It has been assumed that oxygen reacts with the thiyl radical according to reaction (43) when possible mechanisms for thiol radiolysis in the

$$RS^{\bullet} + O_2 \longrightarrow RSOO^{\bullet} \quad (43)$$

presence of oxygen have been postulated[13,52,62], but direct evidence for this reaction has only been found recently and is limited. Purdie found oxygen inhibited reaction (32) and concluded that oxygen reacts with the thiyl radical[39,51]. When neutral and slightly alkaline solutions of cysteine saturated with N_2O were irradiated it was found that oxygen markedly decreased the amount of RS$\bar{S}$R formed immediately after the pulse as well as greatly increasing its rate of decay by reaction (42). This decrease was considered to be caused by competition between RS^- and O_2 for the thiyl radicals, reactions (13) and (43), and assuming such competition, and plotting A^0_{max}/A_{max} against $[O_2]/[RS^-]$, a value of $k_{43} = 8 \times 10^9\ l\,mol^{-1}\,s^{-1}$ was found, A^0_{max} and A_{max} being the maximum absorbances after the pulse in the absence and presence of oxygen respectively[30].

Swallow and coworkers found a weak absorption with λ_{max} at 560 nm when mercaptoethanol was irradiated in acidic oxygen-saturated solutions[7]. From the variation in the amount formed on changing mercaptoethanol concentration and pH it was concluded that the transient was $HOCH_2CH_2SOO^{\bullet}$ and that it had an extinction coefficient of $180 \pm 35\ l\,mol^{-1}\,cm^{-1}$. A weak transient, λ_{max} at 530 nm, was also detected by Packer on irradiating acidic cysteine solutions in the presence of oxygen, the amount formed increasing slightly with cysteine concentration and more definitely with oxygen concentration. The decay kinetics were complex, but decays were rapid with half-lives of a few microseconds decreasing as the pulse length (i.e. dose) increased, suggesting radical–radical reactions[64]. The data were not inconsistent with the transient being $NH_3^+CH(CO_2^-)CH_2SOO^{\bullet}$.

5. Reaction of alkyl radicals with oxygen

Dissociative electron capture by thiol leads to an alkyl radical, reaction (3). Oxygen, by competing for e^-_{aq} lowers the yield of alkyl radicals, and as it adds to them rapidly, reaction (44), should further lower the yield of

$$R + O_2 \longrightarrow ROO^{\bullet} \quad (44)$$

alkane by preventing H atom transfer from an unreacted thiol molecule, reaction (4). No data that show the fate of such alkylperoxy radicals have

been reported. If they abstract H from the thiol group an alkyl hydroperoxide would form, but none has been identified, and anyway may be reduced in the presence of thiol. Serine, the expected reduction product from cysteine, is formed in low yield[53].

C. Mechanisms

1. Cysteine

For cysteine there appear to be three regions of pH involving distinctly different mechanistic features[25], namely 0–5, 5–7 and >7.

The pH region 0–5 has been studied by several research groups. Recently Barton[25] has collected all the available data and carried out calculations on the 'extra' product yields due to oxygen. From the known rate constants for reactions (2), (3), (6), (7), (33) and (34) he calculated the initial values of $G(RS^{\bullet})$, $G(HOO^{\bullet})$ and $G(O_2^{-})$. Considering the equilibrium between $HOO^{\bullet}$ and O_2^{-}, and assuming that reactions (39) and (41) but not (40) occurred, and that each $RS^{\bullet}$ radical gave rise to half a molecule of cystine, RSSR, he determined $G(-RSH)$, $G(RSSR)$ and $G(H_2O_2)$ arising from these reactions. There is a small possible error as the fate of $R^{\bullet}$ in the presence of oxygen is not known. Subtracting these values from the experimental yields, he obtained the 'extra' product yields which he labelled $G(-RSH)_c$, $G(RSSR)_c$ and $G(H_2O_2)_c$ as the results seemed best explained by a short chain-type mechanism. The essential facts to emerge were that $G(RSSR)_c \sim G(H_2O_2)_c$ for all sets of data; that $G(RSSR)_c$ was proportional to (dose-rate)$^{-\frac{1}{2}}$ from the results of Al-Thannon[23]; and that these 'extra' yields increased slowly with increasing cysteine concentration. He postulated the following scheme:

$$RS^{\bullet} + O_2 \longrightarrow RSOO^{\bullet} \quad (43)$$

$$RSOO^{\bullet} + RSH \longrightarrow RSOOH + RS^{\bullet} \quad (45)$$

as the propagating steps, and

$$RS^{\bullet} + RS^{\bullet} \longrightarrow RSSR \quad (11)$$

$$RS^{\bullet} + RSOO^{\bullet} \longrightarrow RSSR + O_2 \quad (46)$$

$$RSOO^{\bullet} + RSOO^{\bullet} \longrightarrow RSSR + 2\,O_2 \quad (47)$$

as possible termination steps. Reaction (45) must be relatively slow as the 'chain' yields are very small[63], and in view of the fact that reaction (40) does not occur[25,30] this seems reasonable. Reactions (48), or (49) and (20), account for the equality of $G(RSSR)_c$ and $G(H_2O_2)_c$. Owen and Brown

$$RSOOH + RSH \longrightarrow RSSR + H_2O_2 \quad (48)$$

$$RSOOH + H_2O \longrightarrow RSOH + H_2O_2 \quad (49)$$

$$RSOH + RSH \longrightarrow RSSR + H_2O \quad (20)$$

found a slow post-irradiation increase in cystine at pH ~ 4·5 and suggest that reaction (48) was slow[52], but Barton was unable to reproduce their results[25].

The relatively low 'chain' yields imply that oxygen which reacts fast with the thiyl radical does not get reduced, and reaction (47) is proposed to account for this. As a result of his disulphide studies Purdie[39] has suggested that $RSOO^{\bullet}$ radicals react together according to reaction (50), the

$$RSOO^{\bullet} + RSOO^{\bullet} \longrightarrow RSO_2SR + O_2 \quad (50)$$

product being a dioxide, not a peroxide. Assuming the dioxide would be reduced to disulphide by thiol, reaction (50) would lead to a considerable increase in $G(RSSR)$ without a corresponding increase in $G(H_2O_2)$, contrary to what is observed. The possibility of reaction (43) being reversible and giving rise to an equilibrium between $RS^{\bullet}$ and $RSOO^{\bullet}$ comes from the observation that the maximum absorbance at 530 nm following a pulse of electrons in acidified cysteine solution increased with increasing oxygen concentration at concentrations where reaction (43) would be complete were it a fast irreversible reaction[64]. The decay, assuming the transient to be $RSOO^{\bullet}$, was too fast for it to occur by reactions (11) and (−43) alone. Purdie[57] has measured G(cystine) in oxygenated solutions of the mixed disulphide of cysteine and cysteamine as a function of this disulphide concentration. Oxygen and the disulphide compete for cysteinyl radicals from reaction (17), cystine arising from the latter reaction (32). He proposes reaction (46) to account for the fact that G(cystine) has a value of 1·5 when extrapolated to zero mixed disulphide concentration, implying that reaction (43) does not go to completion.

The mechanism requires the 'chain' yield to be proportional to cysteine concentration, but the dependence is much less than first-order. A first-order decay of $RSOO^{\bullet}$ in competition with reactions (45) and (47) would account for this, but a possible reaction is difficult to visualize, and it is concluded that the mechanism is not yet fully understood. A reaction such as (51) is also possible in acidic solution.

$$RSOO^{\bullet} + HOO^{\bullet} \longrightarrow RSOOH + O_2 \quad (51)$$

In the pH region 5–7 $G(RSSR)_c$ increases with pH while $G(H_2O_2)_c$ remains almost constant. The increase in $G(RSSR)_c$ has tentatively[35] been attributed to reaction (52) being much faster than (45), and the divergence

$$RSOO^{\bullet} + RS^{-} \xrightarrow{H^+} RSOOH + RS^{\bullet} \quad (52)$$

of $G(RSSR)_c$ and $G(H_2O_2)_c$ to the fact that the intermediate sulphenyl hydroperoxide is reduced to water by cysteine as the pH increases, reactions (53) and (20).

$$RSOOH + RSH \longrightarrow 2\,RSOH \quad (53)$$

A different chain reaction at pH > 7, involving $RS\bar{S}R$ and producing equimolar amounts of cystine and H_2O_2 was postulated by Packer and Winchester[13], and direct evidence for reactions (42) and (41) was subsequently found[30]. Barton suggests that the two competing chain reactions,

$$RS^{\bullet} + RS^{-} \longrightarrow RS\bar{S}R \quad (13)$$

$$RS\bar{S}R + O_2 \longrightarrow RSSR + O_2^{-} \quad (42)$$

$$O_2^{-} + RSH \xrightarrow{H^+} H_2O_2 + RS^{\bullet} \quad (41)$$

(13), (42), (41) and (43), (52) with (53) and (20) best explain the experimental yields in this higher pH region.

2. Other thiols

As mentioned in section III.A, mercaptoethanol and cysteamine are the only other thiols that have been studied in any detail. As Table 5 shows, increasing mercaptoethanol concentration in acidic solution substantially increases $G(RSSR)$ and $G(H_2O_2)$, and a mechanism similar to that proposed above for cysteine has been postulated[7].

As no detailed product analysis has been done no mechanism for the radiolysis of cysteamine in strongly acidic solution can be postulated. However, it is of interest to note that both Owen[38] and Purdie[51] obtain higher yields of taurine ($NH_3^+CH_2CH_2SO_3H$) from oxygenated cystamine radiolysis than they do the corresponding sulphonic acid from other disulphide solutions. Possibly $NH_3^+CH_2CH_2SOO^{\bullet}$ is readily oxidized by $HOO^{\bullet}$ or H_2O_2 at low pH. Sims [14] has calculated 'chain' or 'extra' yields over the pH range in a similar manner to Barton, and finds that $G(RSSR)_c \sim G(H_2O_2)_c$ at pH of about 4, with both increasing as the pH is further increased, $G(RSSR)_c$ rising faster than $G(H_2O_2)_c$. Thus at higher pH the mechanisms for cysteamine and cysteine would appear to be essentially the same.

3. Disulphides

Owen considers sulphonic acids are formed by reactions (18), (54) and (55), his values of $G(RSO_3H)$ being close to G_{OH}. His values of $G(H_2O_2)$ are consistent with reactions (42) and (39) being important[38]. In his

$$RSSR + OH \longrightarrow \overset{+}{RS}\bar{S}R + OH^{-} \quad (18)$$

$$\overset{+}{RS}\bar{S}R + O_2 \longrightarrow [RSSR^{\bullet}O_2]^{+} \longrightarrow RSO_2^{+} + RS^{\bullet} \quad (54)$$

$$RSO_2^{+} + OH^{-} \longrightarrow RSO_3H \quad (55)$$

earlier papers[35] Purdie considered the sulphonic acid to come from reaction (56), but after further work[51] has suggested that it may be formed

$$RSOH + O_2^{-} \longrightarrow RSO_2^{-} + H \quad (56)$$

by reaction (57). Both authors also consider a number of other reactions to explain the various products and yields.

$$RSO_2^{\bullet} + RSOH \longrightarrow RSO_2H + RS^{\bullet} \quad (57)$$

4. Conclusions

It is clear from the above discussion that more work is needed before a definitive understanding of the reactions involved in the radiolysis of oxygenated solutions of thiols and disulphides is achieved. The variations in yields with experimental conditions, the distinctly different yields from different thiols or disulphides under similar conditions, and the analytical problems in determining yields of sulphur compounds in various oxidation states makes this a complex and difficult field to work in.

IV. THIOLS IN THE LIQUID STATE

Only two studies of thiols irradiated in the pure liquid state have been reported. It was of interest to compare ethanethiol[65] with ethanol where the main products, in addition to H_2, are ethylene glycol and acetaldehyde, which come partly from $CH_3\dot{C}HOH$ as precursor[66]. In ethanol the α-C—H bond is weaker than the O—H bond, whereas in the thiol the relative strengths are reversed. $G(H_2)$ was 7·1, greater than for ethanol (possibly because of the lower ionization energy of ethanethiol), and of the main products $C_2H_5S—SC_2H_5$ contributed 80%, and $C_2H_5SC_2H_5$, 15%. No butane-2,3-dithiol or thioacetaldehyde were found[65]. No mention was made of H_2S, a product that might be expected in view of the sulphide yield.

For thiophenol[67] G values found were: H_2, 4·2; PhS—SPh, 4·6; C_6H_6, 1·4; H_2S, 0·44; PhSPh, 0·049. Hentz considered that reaction (58), involving the parent positive ion and equivalent to that occurring in liquids exhibiting hydrogen-bonding, would be unimportant, and thought reactions (59) and (60) unlikely on thermodynamic grounds although electron capture to give $PhSH^-$ as an intermediate could well occur.

$$\dot{PhSH}^+ + PhSH \longrightarrow PhS^\cdot + PhS^+H_2 \quad (58)$$

$$PhSH + e^- \longrightarrow Ph^\cdot + SH^- \quad (59)$$

$$PhSH + e^- \longrightarrow PhS^- + H \quad (60)$$

As dissociation of the lowest triplet excited state was not possible, he concluded that breakdown occurred from the lowest excited singlet state following charge neutration of the parent ion, with S—H cleavage and to a lesser extent Ph—S cleavage the only important processes. (Johnsen[65] also considered the equivalent of reaction (58) to be less important with ethanethiol, and that the difference from ethanol may well be attributed to the much weaker hydrogen bonding as well as to the relative bond strengths mentioned above.) In benzene–thiophenol mixtures, energy transfer from benzene to thiophenol was shown to occur, leading to products similar to those from pure thiophenol. By using deuterated benzene it was shown that very few H atoms arose from benzene radiolysis. Prior to this work it was not entirely clear whether the very low values of $G(H_2)$ found in aromatic systems implied a low yield of H atoms or were due to the fact that they add to the aromatic ring. Thiophenol was the first aromatic compound studied which gave an appreciable yield of H_2, and this work clearly shows the dominating role that the —SH group exerts when it is present in a molecule, H-abstraction from sulphur preventing the usual ring addition almost entirely.

V. THIOLS IN THE SOLID STATE

A. Pure Compounds

1. Product analysis

Most solid state studies have involved only e.s.r. measurements of the radicals produced on irradiation. However, Garrison and coworkers[68] have irradiated dry degassed cysteine at room temperature, dissolved the irradiated solid in water and analysed the products, finding the following yields: $G(H_2) = 3{\cdot}1$; $G(H_2S) = 1{\cdot}5$; $G(NH_3) = 1{\cdot}8$; G(cystine) $= 5{\cdot}0$; $G(NH_2$-free compounds$) = 1{\cdot}0$; G(total carbonyl compounds$) \leqslant 0{\cdot}1$. In aqueous solution the —SH group appears to be the locus of all significant

reactions as the predominant reactions for amino acid derivatives[41], reductive and oxidative deamination (the latter leading to carbonyl compounds), are negligible. It was of interest to see if this was the case in the solid state also. As the results show oxidative deamination was absent, but reductive deamination competes with loss of HS^-. The following steps were postulated:

$$RSH \rightsquigarrow RS^\cdot + H^+ + e \quad (61)$$

$$RSH \rightsquigarrow RS^\cdot + H \quad (62)$$

reaction (61) involving proton transfer from the radical ion to a neighbouring group, and (62) dissociation of an excited molecule, followed by

$$H^\cdot + RSH \longrightarrow H_2 + RS^\cdot \quad (7)$$

$$H^\cdot + RSH \longrightarrow H_2S + NH_3^+CH(\dot{C}H_2)CO_2^- \quad (63)$$

$$e^- + RSH \longrightarrow NH_3 + {}^\cdot CH(CH_2SH)CO_2^- \quad (64)$$

$$e^- + RSH \longrightarrow H_2 + NH_2CH(CH_2S^\cdot)CO_2^- \quad (65)$$

$$e^- + RSH \longrightarrow H_2S + NH_2CH(\dot{C}H_2)CO_2^- \quad (66)$$

$$RSH + \dot{C}H(CH_2SH)CO_2^- \longrightarrow RS^\cdot + CH_2(CH_2SH)CO_2^- \quad (67)$$

$$RSH + NH_2CH(\dot{C}H_2)CO_2^- \longrightarrow RS^\cdot + NH_2CH(CH_3)CO_2^- \quad (68)$$

$$2\,RS^\cdot \longrightarrow RSSR \quad (11)$$

It was suggested that dimerization of thiyl radicals to give disulphide occurred mainly on dissolution of the irradiated solid. In the case of cysteamine hydrochloride, where there is no carbonyl group to trap the electron prior to deamination, it was found that $G(NH_3) < 0{\cdot}1$. In spite of this $G(H_2S)$ was only 1·2, lower than for cysteine, but the value of $G(H_2)$ of 5·1 was much higher. This is the same situation as was found for mercaptoethanol[7] but not for cysteamine itself[14] in aqueous solution.

2. E.s.r. studies

Several e.s.r. studies have been made on single crystals of cysteine hydrochloride monohydrate. On irradiation with 1·5 MeV electrons at 77 K Akasaka[69] observed an isotropic doublet as the main radical species with a high anisotropic but axial symmetric g factor, and attributed this to the ${}^\cdot SCH_2CH(COOH)NH_3^+Cl^-$ radical, as found by Kurita and Gordy for cystine[70]. Remarkable broadening was observed on warming and at

225 K the spectrum had almost disappeared, although this was not due to radical decay as the spectrum reappeared on cooling[69]. Wheaton and Omerod[71] used ^{60}Co γ-irradiation at 77 K and then warmed or illuminated their crystal with u.v. light. They observed six radicals, four of which were RS$^{\bullet}$. Their initial spectrum was a triplet, which Akasaka did not see, probably because his electron beam warmed the crystals above 77 K. Conformational changes on warming lead to interaction between spin on the sulphur atom and neighbouring —SH groups to give large anisotropies in the spectroscopic splitting factor g. Warming also gave higher radical concentrations suggesting to them that the original damage was not paramagnetic. Further warming beyond 200 K caused nearly complete disappearance of radicals suggesting that the thiyl radicals in fact dimerized in the solid state.

Recent work by Budzinski and Box[72] has shown that 77 K is not a sufficiently low temperature to stabilize the primary radicals initially formed and have found evidence for electron capture by the carboxyl group, providing direct evidence for Garrison's mechanism[69]. They were able to get better defined spectra with penicillamine hydrochloride than with cysteine, and reported detailed work on this compound. At 4 K they observed three radical species, two due to oxidation which they assigned to a chlorine atom and to $^{\bullet}SC(CH_3)_2CH(NH_3^+Cl^-)COOH$ and one due to reduction, $HSC(CH_3)_2CH(NH_3^+Cl^-)\dot{C}(OH)O^-$ formed by electron capture. On warming to 200 K, hole transfer from the chlorine atom occurred to give a different thiyl radical, and the initial thiyl radical underwent a change in conformation to give the same radical. The electron adduct also underwent a conformational change and then on further warming deamination occurred to give the radical $HSC(CH_3)_2\dot{C}HCOOH$. On further warming to 275 K this radical abstracted hydrogen from sulphur to give another thiyl radical, and those already formed underwent a further conformational change, so that at room temperature thiyl radicals were the only type present. Presumably dissociative electron capture by the —SH group would give a radical which would also abstract H from thiol, so although this radical was not observed, this work was not in disagreement with Garrison's mechanism. Box also had a higher yield of radicals from oxidation than from reduction, supporting Garrison's dissociation reaction, as H atoms would abstract hydrogen from the thiyl group. The anisotropy of the thiyl radical was again observed. In agreement with the previous work, the final thiyl species observed at ambient temperature has undergone bending of the carbon–carbon bond α to the sulphur atom.

Ramsbottom, Pintar and Forbes[73] have studied the radical recombination in irradiated polycrystalline cysteine HCl monohydrate at temperatures 340–390 K. Above 333 K a second phase, glassy in nature, was found to form and it was presumed that this was caused by free water molecules around lattice imperfections because irradiated samples had a greater percentage of this new phase. This phase which was absent in anhydrous samples would contain no radicals, and the observed decay was of radicals in the crystalline phase. The decay exhibited second-order kinetics over 4–5 half-lives and an activation energy of 50 kJ mol^{-1} (12 kcal mol^{-1}). They concluded that the decay mechanism involved dimerization of thiyl radicals and that H atom transfer between the thiol group and thiyl radicals could occur above 378 K. The half-life at 375 K was approximately 200 min, and these results would seem to throw doubt on Omerod's observation[71] that radicals decayed at 200 K.

Clear ethanethiol glasses[74,75] have been irradiated at 77 K. A deep orange colour formed, and an absorption maximum at 430 nm was observed. Bleaching with visible light caused the colour to fade to clear yellow, with λ_{max} at 405 nm. E.s.r. measurements before bleaching showed two species, one attributed to $C_2H_5S^{\bullet}$ and the other to an ionic radical. It was the latter that disappeared on bleaching and the peak at 405 nm was attributed to $C_2H_5S^{\bullet}$. Initial yields[75] were given as $G(C_2H_5S^{\bullet}) = 0{\cdot}5$ and $G(\text{ion}) = 2{\cdot}9$.

A series of alkyl mercaptans[76–78] have been irradiated at 77 K. The thiyl radical, RS$^{\bullet}$, is the predominant species for molecules with three or less carbon atoms, but the relative concentration of alkyl radicals increases with the size of the alkyl group.

Disulphides have also been studied. The final stable radical from a single crystal of L-cystine hydrochloride is an RS$^{\bullet}$ radical[70], but irradiation as 4 K gives $RS\overset{+}{S}R$ and $RS\overset{-}{S}R$, the former having an optical absorption maximum at 550 nm and the latter at 420 nm[79]. Some RS$^{\bullet}$ is also produced at 4 K. Both $RS\overset{+}{S}R$ and $RS\overset{-}{S}R$ are present in the dark at 77 K, but light causes all of $RS\overset{+}{S}R$ and about half $RS\overset{-}{S}R$ to decay without a concurrent increase in RS$^{\bullet}$ radicals[80]. In the dark $RS\overset{+}{S}R$ disappears in about 30 min at 125 K but $RS\overset{-}{S}R$ is stable at this temperature.

In studies of peptides and proteins containing sulphydryl and disulphide groups irradiation at 77 K leads to non-S radicals, but on warming migration of spins to sulphur occurs and the stable radicals at higher temperature are thiyl radicals[81,82]. Other forms of energy transfer must also occur as the concentration of thiyl radicals eventually formed is higher than the total radical concentration at 77 K.

B. Frozen Solutions and Glasses

The radicals formed on irradiating frozen aqueous solutions of cysteamine at 77 K and their behaviour on annealing have been studied by e.s.r.[83,84] Solutions of pH less than 2 before freezing formed thiyl radicals on annealing to 178 K, but these thiyl radicals could not be detected when the pH of the initial solutions was greater than 3. At higher pH increases in radical yield and in radical stability to annealing occur and these increases follow the ionization curve of the —SH group. The radicals here were tentatively attributed to $RS\bar{S}R$.

The absorption spectra of species formed on γ-irradiation of methyltetrahydrofuran and hydrocarbon glasses containing thiols and disulphides[85] at 77 K show that $RS\bar{S}R$ radicals are formed by electron capture and that RS$^{\bullet}$ radicals are formed on warming by H atom abstraction by solvent radicals. The electron scavenger CCl_4 inhibited formation of the former but not the latter. In contrast Skelton and Adam[86] were unable to detect thiyl radicals when simple mercaptans in glassy 3-methylpentane were γ-irradiated, although thiyl radicals were formed and were stable at room temperature when the same glasses were photolysed.

VI. RADIATION PROTECTION BY THIOLS

A. Mechanisms

The phenomenon of chemical protection of mammals against the harmful effects of ionizing radiation was discovered in 1949, and aminothiols or compounds that could give rise to free thiol groups were found to be the most active. Much work on synthesizing and testing new compounds of this class has been undertaken. The phenomenon of protection has been the subject of a book[87]. Whereas most compounds containing thiol or disulphide groups act as protecting agents in laboratory studies, only some of them are effective in the body, problems of solubility, transport, toxicity and other factors outside the province of physical chemistry being involved. Only simple chemical theories and work related to them are discussed here.

That the mechanism of protection is partly chemical (i.e. involving fast free radical reactions) rather than biochemical (i.e. involving slow reactions of protecting agents with the biologically important molecules prior to or after irradiation as suggested, for example, in the mixed disulphide theory[88,89]) has been shown by mixing cysteine with bacteria[90] or lysozyme[91] in a rapid flow system. Protection was found with a pre-irradiation mixing time as short as 4 ms but no protection was found if mixing occurred 5 ms after irradiation.

There are two simple mechanisms for this chemical protection, 'competition scavenging' and 'repair'. In both of these the thiol is thought to prevent or reduce damage caused by attack of free radical precursors on the biological solute, the so-called 'indirect action'. As cells are 60–80% water there is little doubt that these precursors are OH, H, or e^-_{aq}, and the number of them reacting with the biological substrate is reduced by competitive scavenging of the thiol, yielding thiyl radicals. These are relatively unreactive towards the biological molecules and consequently damage is reduced. Where the primary radicals do react directly with the substrate a free radical formed by H atom abstraction is a likely product and further reactions of this may lead to permanent biological damage. In the repair mechanism the thiol is thought to transfer a hydrogen atom from sulphur to the radical, restoring the biological molecule to its original form and replacing it by the innocuous thiyl radical.

The repair mechanism can also operate where a radical has been formed by H atom loss after a direct ionization of a biological molecule, and energy transfer, especially to a disulphide group, is also a possibility.

Evidence for these mechanisms has come from radiolysis experiments, including e.s.r. measurements on model systems.

B. Solution Studies

Adams and coworkers have made quantitative measurements on both possible mechanisms, using monomers[92] and polymers[29] as model substrates and cysteamine as the protecting agent. On pulse irradiating mixtures of alcohols and cysteamine they found $RS\bar{S}R$ to be formed in two reactions, one of these being complete 3 μs after the pulse with the other slower reaction occurring during the next 10–100 μs. Increasing the alcohol concentration at fixed cysteamine concentration decreased the amount of $RS\bar{S}R$ formed 3 μs after the pulse, showing normal competitive kinetic behaviour, reactions (2) and for methanol (69), but the total

$$OH + RSH \longrightarrow H_2O + RS^{\bullet} \quad (2)$$

$$OH + CH_3OH \longrightarrow H_2O + {}^{\bullet}CH_2OH \quad (69)$$

amount finally formed remained the same. The rate of formation of $RS\bar{S}R$ in the slower reaction was independent of alcohol concentration

but was proportional to cysteamine concentration, suggesting the repair reaction (70) was being observed. (Recent work on the γ-radiolysis of

$$\cdot CH_2OH + RSH \longrightarrow CH_3OH + RS\cdot \quad (70)$$

isopropanol in D_2O substantiates this since it was found that addition of thiol induces deuteration of the alcohol and lowers the yield of acetone[93].) Analysis of the oscillograms of growth of RSS̄R yielded 'repair' rate constants for a series of alcohols, the values ranging from $1{\cdot}8 \times 10^7$ l mol^{-1} s^{-1} for *t*-butanol to 42×10^7 l mol^{-1} s^{-1} for isopropanol[92]. Using polyethylene oxide (PEO) polymers of varying molecular weights, the same two kinetic pathways of RSS̄R formation were again observed, and repair rate constants of $5\text{–}10 \times 10^6$ l mol^{-1} s^{-1} were found. With high molecular weight PEO reactions of PEO radicals with RS· and RSS̄R were also detected. pH studies on both monomer and polymer systems showed the thiolate anion barely repaired the radicals, if at all[29].

The repair mechanism does not appear to function where attack occurs on pyrimidine bases. Here primary radicals add to the 5:6 double bond and hydrogen transfer to the intermediate radical would complete an addition across this bond. This reaction has been shown to occur between cysteine and the protonated electron-adduct of cytosine[94], and in this case cysteine increases $G(-\text{cytosine})$ by blocking the reconstitution reaction which occurs between OH-adduct and electron-adduct. Adams[92] found the rate constants for reaction between cysteamine and the OH-adducts of allyl alcohol, thymidine and uracil to be less than 10^7 l mol^{-1} s^{-1}, his findings being confirmed very recently in a pulse radiolysis study using e.s.r. to detect transient intermediates[95]. Both cysteamine and cysteine were used and the corresponding rate constants for uracil and thymine being shown to be less than 10^6 l mol^{-1} s^{-1}. For these compounds all protection was due to thiol scavenging of OH. This technique gave figures comparable to Adams's for repair of alcohol radicals[92], and also showed the repair mechanism functioned for dihydrothymine.

There is evidence that radicals other than primary ones can add to pyrimidine bases and hence damage DNA function, and it has been shown that thiols can prevent this by repairing the intermediate radicals prior to their attack on the base[96]. The repair mechanism has also been shown to operate in e.s.r.-flow studies of biochemical molecules where OH radicals are generated chemically[97].

Studies of protection of two enzymes, lysozyme[44] and papain[98], have been made in aqueous solution, and in addition to scavenging protection,

a reaction between cysteine and the OH-adduct of lysozyme has been observed[44]. A slow post-irradiation repair reaction was found for papain[98], probably involving cysteine as a reducing agent.

In some systems it is found that the presence of oxygen lowers the protection given by added thiol[99], an explanation being that oxygen reacts with the substrate radical in an irrepairable step in competition with the hydrogen transfer reaction with thiol. Pulse radiolysis studies with cysteamine were not inconsistent with this[92].

C. *Solid State Studies*

E.s.r. studies in the solid state also give considerable evidence for the repair mechanism of thiols. Mention of migration of spins to sulphur in proteins and from solvent to thiol in glasses has been made in section V. In a simple model system a single crystal of 2-aminobutyric acid HCl containing 2% of cysteine HCl was irradiated. The main radical detected at 220°K was $CH_3\dot{C}HCH_2COOH$, but on warming to room temperature the free thiyl radical appeared, implying transfer of H from the thiol[100]. Work prior to 1965 has been reviewed[101] and many systems involving mixtures of thiols and model compounds or biological material in the dry or glassy state have been studied since and have provided clear examples of the repair mechanism. However the factors controlling transfer of spin to the added thiols are complex, as recent work by Milvy has shown[102, 103].

VII. ADDITION OF THIOLS TO OLEFINS

Radiolysis of mixtures of thiols and olefins in the absence of oxygen leads to anti-Markovnikov addition across the double bond in a long chain reaction involving free radicals. The propagation steps for a terminal olefin are:

$$RS\cdot + R'CH{=}CH_2 \longrightarrow R'\dot{C}H{-}CH_2SR \quad (71)$$

$$RSH + R'\dot{C}H{=}CH_2SR \longrightarrow R'CH_2{-}CH_2SR + RS\cdot \quad (72)$$

This reaction is not specific to radiolysis, and the initiating free radicals may also be generated thermally or photochemically. The general field of free radical addition of thiols to unsaturated compounds has recently been reviewed[104].

Thiols may be formed by radiolysis of H_2S with olefins, the mechanism being similar to that above, but as the thiol formed undergoes loss of hydrogen by radical abstraction more readily than H_2S, a mixture of thiol and sulphides is likely to be formed.

VIII. REFERENCES

1. I. G. Draganic and Z. D. Draganic, *The Radiation Chemistry of Water*, Academic Press, New York and London, 1971.
2. G. V. Buxton, *Proc. Roy. Soc.* (*London*), **A328**, 9 (1972).
3. W. A. Armstrong and W. G. Humphries, *Can. J. Chem.*, **45**, 2589 (1967).
4. G. E. Adams, G. S. McNaughton and D. B. Michael in *The Chemistry of Ionisation and Excitation* (Ed. G. Scholes and G. R. A. Johnson), Taylor and Francis, London, 1967, p. 281.
5. G. S. McNaughton, private communication, 1967.
6. W. Karmann, A. Granzow, G. Meissner and A. Henglein, *Int. J. Radiat. Phys. Chem.*, **1**, 395 (1969).
7. G. G. Jayson, D. A. Stirling and A. J. Swallow, *Int. J. Radiat. Biol.*, **19**, 143 (1971).
8. J. P. Barton, M. Sc. Thesis, University of Auckland, 1968.
9. R. L. Willson, G. L. Greenstock, G. E. Adams, R. Wageman and L. M. Dorfman, *Int. J. Radiat. Phys. Chem.*, **3**, 211 (1971).
10. P. Neta and R. W. Fessenden, *J. Phys. Chem.*, **75**, 2277 (1971).
11. V. G. Wilkening, M. Lal, M. Arends and D. A. Armstrong, *J. Phys. Chem.*, **72**, 185 (1968).
12. A. El. Samahy, H. L. White and C. N. Trumbore, *J. Amer. Chem. Soc.*, **86**, 3177 (1964).
13. J. E. Packer and R. V. Winchester, *Can. J. Chem.*, **48**, 417 (1970).
14. R. J. Sims, Ph.D. Thesis, Univeristy of Auckland, 1972.
15. D. A. Armstrong and V. G. Wilkening, *Can. J. Chem.*, **42**, 2631 (1964).
16. M. Lal, D. A. Armstrong and M. Wieser, *Radiation Res.*, **37**, 246 (1969).
17. J. R. Clement, M.Sc. Thesis, University of Auckland, 1969.
18. A. Al-Thannon, R. M. Peterson and C. N. Trumbore, *J. Amer. Chem. Soc.*, **72**, 2395 (1968).
19. R. V. Winchester, Ph.D. Thesis, University of Auckland, 1968.
20. R. Braams, *Radiation Res.*, **27**, 319 (1966).
21. W. M. Dale and J. V. Davies, *Biochem. J.*, **48**, 129 (1951).
22. V. G. Wilkening, M. Lal, M. Arends and D. A. Armstrong, *Can. J. Chem.*, **45**, 1209 (1967).
23. A. Al-Thannon, Ph.D. Thesis, Univeristy of Delaware, 1967.
24. G. Navon and G. Stein, *Israel J. Chem.*, **2**, 151 (1964).
25. J. P. Barton, Ph.D. Thesis, Univeristy of Auckland, 1972.
26. G. E. Adams in *Current Topics in Radiation Research*, Vol. 3 (Ed. M. Ebert and A. Howard), North-Holland, Amsterdam, 1967, p. 35.
27. W. Karmann, G. Meissner and A. Henglein, *Z. Naturforsch.*, **22B**, 273 (1967).
28. J. W. Purdie, H. A. Gillis and N. V. Klassen, *J. Chem. Soc.* (*D*), 1163 (1971).
29. G. E. Adams, R. C. Armstrong, A. Charlesby, D. E. Michael and R. L. Willson, *Trans. Faraday Soc.*, **65**, 732 (1969).
30. J. P. Barton and J. E. Packer, *Int. J. Radiat. Phys. Chem.*, **2**, 159 (1970).
31. M. Simic and M. Z. Hoffman, *J. Amer. Chem. Soc.*, **92**, 6096 (1970).
32. K. Eiben and R. W. Fessenden, *J. Phys. Chem.*, **75**, 1186 (1971).
33. G. Scholes, P. Shaw, R. L. Willson and M. Ebert in *Pulse Radiolysis* (Ed. M. Ebert, J. P. Keene, A. J. Swallow and J. H. Baxendale), Academic Press, New York, 1965, p. 151.

34. G. G. Jayson and A. C. Wilbraham, *J. Chem. Soc.* (*D*), 461 (1968).
35. J. W. Purdie, *J. Amer. Chem. Soc.*, **89**, 226 (1967).
36. J. W. Purdie, *Can. J. Chem.*, **47**, 1029 (1969).
37. G. G. Jayson, T. C. Owen and A. C. Wilbraham, *J. Chem. Soc.* (*B*), 944 (1967).
38. T. C. Owen, A. C. Wilbraham J. A. G. Roach and D. R. Ellis, *Radiation Res.*, **50**, 234 (1972).
39. J. W. Purdie, *Radiation Res.*, **48**, 474 (1971).
40. D. Giles and D. W. Grant, *Chem and Ind.* (London), 1437 (1970).
41. W. M. Garrison in *Current Topics in Radiation Research*, Vol. 4 (Ed. M. Ebert and A. Howard), North-Holland, Amsterdam, 1968, p. 83.
42. G. E. Adams, R. B. Cundall and R. L. Willson in *Chemical Reactivity and Biological Role of Functional Groups in Enzymes* (Ed. R. M. S. Smellie), Academic Press, London, 1970, p. 171.
43. G. E. Adams, R. L. Willson, J. E. Aldrich and R. B. Cundall, *Int. J. Radiat. Biol.*, **16**, 333 (1969).
44. J. V. Davies, M. Ebert and R. J. Shalek, *Int. Radiat. Biol.*, **14**, 19 (1969).
45. T. Masuda, J. Ovadia and L. I. Grossweiner, *Int. J. Radiat. Biol.*, **20**, 447 (1971).
46. J. R. Clement, D. A. Armstrong, N. V. Klassen and H. A. Gillis, *Can. J. Chem.*, **50**, 2833 (1972).
47. Yamamoto and Kazuhiko, *J. Radiat. Res.*, **12**, 133 (1971); *Chem. Abstr.*, **77**, 44876 (1972).
48. R. F. Anderson and J. E. Packer, unpublished results.
49. G. E. Adams, J. E. Aldrich, R. H. Bisby, R. B. Cundall, J. L. Redpath and R. L. Willson, *Radiation Res.*, **49**, 278 (1972).
50. R. L. Willson, *J. Chem. Soc.* (*D*), 1425 (1970).
51. J. W. Purdie, *Can. J. Chem.*, **49**, 725 (1971).
52. T. C. Owen and M. T. Brown, *J. Org. Chem.*, **34**, 1161 (1969).
53. D. A. Armstrong, private communication, 1969.
54. S. L. Witcher, M. Rotheram and N. Todd, *Nucleonics*, **11** (8), 30 (1953).
55. E. M. Nanobashvili and G. G. Chirakadze, *Issled. v Obl. Electrokhim. i Radiats. Khim., Acad. Nauk Gruz. SSR, Inst. Neorgan. Khim. i Electrokhim.*, 40 (1965).
56. J. W. Purdie, *Can. J. Chem.*, **47**, 1037 (1969).
57. T. C. Owen, M. Rodriguez, B. G. Johnson and J. A. G. Roach, *J. Amer. Chem. Soc.*, **90**, 196 (1968).
58. T. C. Owen and A. C. Wilbraham, *Radiation Res.*, **50**, 253 (1972).
59. B. H. J. Bielski and J. M. Gebicki in *Advances in Radiation Chemistry*, Vol. 2 (Ed. M. Burton and J. L. Magee), Wiley-Interscience, New York, 1970, p. 269.
60. D. Behar, G. Czapski, J. Rabani, L. M. Dorfman and H. A. Schwarz, *J. Phys. Chem.*, **74**, 3209 (1970).
61. E. C. Kooyman, *Pure & Applied Chem.*, **15**, 81 (1967).
62. V. I. Vedeneev, L. V. Gurvich, Y. N. Kontrat'yev, V. A. Medvedev and Ye. L. Frankevic in *Bond Energies, Ionisation Potentials and Electron Affinities*, Arnold, London, 1966, p. 76.
63. J. E. Packer, *J. Chem. Soc.*, 2320 (1963).
64. J. E. Packer, unpublished results.

65. J. J. J. Myron and R. H. Johnsen, *J. Phys. Chem.*, **70**, 2951 (1966).
66. R. A. Basson in *The Chemistry of the Hydroxyl Group* (Ed. S. Patai), Interscience, London, 1971, Chap. 17.
67. G. Lunde and R. R. Hentz, *J. Phys. Chem.*, **71**, 863 (1967).
68. D. B. Peterson, J. Holian and W. M. Garrison, *J. Phys. Chem.*, **73**, 1568 (1969).
69. K. Akasaka, *J. Chem. Phys.*, **43**, 1182 (1965).
70. Y. Kurita and W. Gordy, *J. Chem. Phys.*, **34**, 282 (1961).
71. R. F. Wheaton and M. G. Omerod, *Trans. Faraday Soc.*, **65**, 1638 (1969).
72. E. E. Budzinski and H. C. Box, *J. Phys. Chem.*, **75**, 2564 (1971).
73. J. V. Ramsbottom, M. M. Pintar and W. F. Forbes, *Radiation Res.*, **48**, 454 (1971).
74. S. B. Milliken, K. Morgan and R. H. Johnsen, *J. Phys. Chem.*, **71**, 3238 (1967).
75. A. Tarikai, S. Sawada, K. Fueki and Z. Kuri, *Bull. Chem. Soc. Jap.*, **43**, 1617 (1970).
76. K. Akasaka, S. Ohnishi and H. Hatano, *Kogyo Kagaku Zasshi*, **68**, 1548 (1965); *Chem. Abstr.*, **63**, 15758 (1965).
77. A. D. Bichiashvili, R. G. Barsegov and E. M. Nanobashvili, *Khim. Vys. Energ.*, **3**, 182 (1969).
78. A. D. Bichiashvili, E. M. Nanobashvili and R. G. Barsegov, *Soobshch. Akad. Nauk. Gruz. SSR.*, **53**, 337 (1969).
79. H. C. Box and H. G. Freund, *J. Chem. Phys.*, **41**, 2571 (1964).
80. K. Akasaka, S. Kominami and H. Hatano, *J. Phys. Chem.*, **75**, 3747 (1971).
81. F. Patten and W. Gordy, *Radiation Res.*, **14**, 573 (1961).
82. T. Henriksen, *J. Chem. Phys.*, **36**, 1258; **37**, 2189 (1962).
83. E. S. Copeland and H. M. Swartz, *Int. J. Radiat. Biol.*, **16**, 293 (1969).
84. E. S. Copeland and W. L. Earl, *Int. J. Radiat. Biol.*, **19**, 401 (1971).
85. J. Wendenburg, H. Möckell, A. Granzow and A. Henglein, *Z. Naturforsch.* **21B**, 632 (1966).
86. J. Skelton and F. C. Adam, *Can. J. Chem.*, **49**, 3536 (1971).
87. Z. M. Bacq, *Chemical Protection Against Ionising Radiation*, Charles C. Thomas, Illinois, 1965.
88. L. Eldjarn and A. Pihl, *J. Biol. Chem.*, **225**, 499 (1957).
89. A. Pihl and L. Eldjarn, *Pharmacol. Rev.*, **10**, 437 (1958).
90. G. E. Adams in *Proceedings 2nd International Symposium Radiation Protection and Sensitization* (Ed. N. Moroson and M. Quintiliani), Taylor and Francis, London, 1970, p. 12.
91. T. Brustad and W. B. G. Jones, in *Proceedings 2nd International Symposium Radiation Protection and Sensitization* (Ed. N. Moroson and M. Quintiliani), Taylor and Francis, London, 1970, pp. 95–101.
92. G. E. Adams, G. S. McNaughton and D. B. Michael, *Trans. Faraday Soc.*, **64**, 902 (1968).
93. S. G. Cohen and F-L. Lam, *Radiation Res.*, **45**, 462 (1971).
94. J. Holian and W. M. Garrison, *Nature*, **221**, 57 (1969).
95. G. Nuclifora, B. Smaller, R. Remko and E. C. Avery, *Radiation Res.*, **49**, 96 (1972).
96. H. Loman, S. Voogd and J. Blok, *Radiation Res.*, **42**, 437 (1970).
97. B. B. Singh and C. Nicolau, *Progr. Biophys. Mol. Biol.*, **23**, 21 (1971).

98. G. M. Gaucher, B. L. Mainman, G. P. Thompson and D. A. Armstrong, *Radiation Res.*, **46**, 457 (1971).
99. P. Howard-Flanders, *Nature (Lond.)*, **186**, 485 (1960).
100. H. C. Box, H. G. Freund and E. E. Budzinski, *J. Chem. Phys.*, **45**, 2324 (1966).
101. K. G. Zimmer and A. Müller in *Current Topics in Radiation Research* (Ed. M. Ebert and A. Howard), North Holland, Amsterdam, 1965, p. 1.
102. P. Milvy and I. Pullman, *Radiation Res.*, **34**, 265 (1968).
103. P. Milvy, *Radiation Res.*, **47**, 83; **48**, 206 (1971).
104. K. Griesbaum, *Angew. Chem. (Int. Ed. in English)*, **9**, 273 (1970).
105. M. Z. Hoffman and E. Hayon, *J. Amer. Chem. Soc.*, 94, 7950 (1972).
106. A. Shafferman, *Israel J. Chem.*, **10**, 725 (1972)
107. N. N. Lichtin, J. Ogdan and G. Stein, *Biochim. Biophys. Acta*, **263**, 14 (1972); **276**, 124 (1972).

CHAPTER 12

Synthetic uses of thiols

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I. INTRODUCTION

The use of thiols in the synthesis of bivalent organosulphur compounds is well known[1]. Thiols can be converted to sulphides, disulphides, sulphonium salts, sulphoxides, sulphones, sulphonic acids, thioacetals and thioacids; these transformations being effected generally by nucleophilic displacement, addition, oxidation or condensation reactions involving the sulphur function. In the above cases a thiol is used in the preparation of a new compound containing sulphur and this is often the main purpose for effecting the reaction. In this chapter we have chosen not to cover *per se* these types of reactions; certain of these reactions are covered in various detail in other chapters in this volume. We have chosen instead to treat reactions in which a thiol is an important and necessary reagent, being incorporated into the molecule to promote the desired transformation, following which the sulphur function is removed to yield the final reaction product. The thiol, therefore, functions in an accessory role in the synthetic transformation. An example is the conversion of a carbonyl group to a

methylene group by Raney nickel desulphurization of an intermediate thioacetal; the thioacetal being prepared by reaction of a thiol with the ketone or aldehyde.

Examples of a thiol functioning in a synthetic transformation involving only one step are minimal. Most cases covered in this chapter require more than one step with several steps being involved in the conversion of the reactant, *via* reaction with a thiol, into the final product. This necessitates that the thiol be transformed into a bivalent organosulphur derivative, i.e. a sulphide, thioacetal or a higher oxidized sulphur function such as a sulphone or sulphonium salt, followed by subsequent conversion to final product. Thus, many of the reactions covered could be considered as examples of the synthetic use of sulphides, sulphones, etc., equally as well as synthetic uses of thiols[2]. The general criteria used in selection of reactions for the chapter have been: (a) a thiol has been or readily could be used in preparation of the intermediate organosulphur derivative, (b) the purpose of the transformation is not the preparation of an organosulphur derivative, thus (c) the sulphur function is normally and conveniently removed to give the final product.

The following reactions have been excluded as being beyond the scope of this chapter: the variety of synthetically useful reactions of dimethyl sulphoxide (DMSO) and dimethyl sulphide, which include reactions involving dimsyl anion, oxidation reactions involving DMSO, methylene transfer reactions of corresponding sulphonium methylides, and reaction of stabilized sulphonium ylids normally prepared from dimethyl sulphide[3].

II. DITHIOACETALS

The formation of a dithioacetal as an intermediate in organic synthesis is not new to most chemists. However, in recent years there has been a continuing improvement in the methods of preparation as well as the subsequent reactions. The early use of the dithioacetal group as a means to reduce carbonyl functions with Raney nickel has been expanded to extensive use as a protecting group, methylene blocking group and as an intermediate in the preparation of complex hydrocarbons, olefins, aldehydes and ketones.

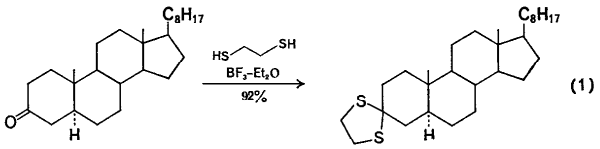
A. Carbonyl Protection

The protecting ability of dithioacetals has become well established[4]. These groups are stable towards both mild acid and mild base and show reasonable stability towards such varied reagents as lithium aluminium hydride, chromium trioxide and Grignard reagents[5]. However, the method

has rarely been utilized because of the difficulty in regenerating the carbonyl. Recent developments in this area should change the situation and give dithioacetals a prominent place in synthetic organic chemistry.

I. Preparation

Early workers reacted the ketone with an excess of the thiol in the presence of an acid catalyst such as zinc chloride[6], hydrogen chloride[7,8] or *p*-toluenesulphonic acid[9] to prepare dithioacetals. The results were erratic and the yields often disappointing. The use of boron trifluoride etherate has led to consistently better results[8,10]. This method is particularly effective when the thiol is used for the solvent of the ketone as the boron trifluoride etherate is added. Ethanedithiol and propanedithiol are usually the thiols of choice forming 1,3-dithiolanes and 1,3-dithianes respectively. For example, the 1,3-dithiolane of cholestane-3-one (equation 1) can be prepared in high yield by this method[10]. Occasionally the choice



(1)

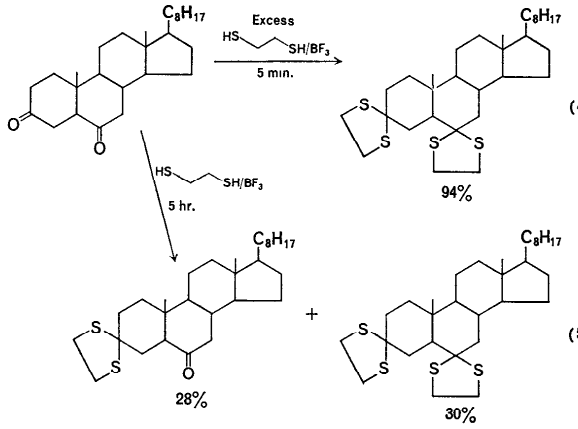
of solvent is very important and it has been noted that a more acidic medium such as acetic acid may be useful in accelerating product formation and reducing side reactions[11]. A newer method involving the use of alkyl orthothioborates gives nearly quantitative yields of the dithioacetals of simple aldehydes and ketones (equation 3) under neutral conditions[12]. The orthothioborates are easily prepared from sulphurated sodium borohydride (equation 2) but the use of dithiols would seem to be excluded.

$$NaBH_2S_3 + EtSH \longrightarrow (EtS)_3B + H_2 + (EtS)_2 + NaS_3H \quad (2)$$

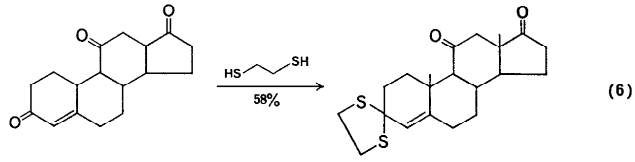
$$CH_3CH_2\overset{O}{\overset{\|}{C}}CH_3 + (EtS)_3B \longrightarrow CH_3CH_2\overset{Et\ Et}{\overset{S\ \ S}{C}}CH_3 + B_2O_3 \quad (3)$$

In the formation of 1,3-dithiolanes of di- and tricarbonyl compounds, there is considerable selectivity. Normally one does obtain a mixture of the monodithiolane contaminated by varying amounts of the bisdithiolane

but separation is generally not difficult. Apparently the formation of two isomeric and hard-to-separate monothiolanes is seldom a problem. Cholestane-3,6-dione with excess ethanedithiol gives a high yield of the bis-1,3-dithiolane (equation 4) in just 5 min[10]. Restricting the quantity of thiol and extending the reaction time led to a mixture containing a reasonable yield of the cholestane-3,6-dione-3-(1,3-dithiolane) (equation 5)[10].

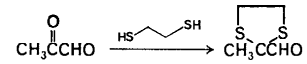


Where the nature of the carbonyls of a dicarbonyl compound differ greatly, one isomer of the monodithiolane may become the only product. In the conversion of 4-androstene-3,11,17-trione to 4-androstene-3,11,17-trione-3-(1,3-dithiolane) no bis- or trisdithiolane was observed (equation 6)[9]. The condensation of an equimolar amount of 1,2-ethanedithiol with



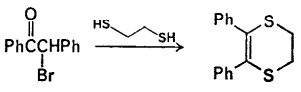
(6)

an α-keto aldehyde such as pyruvaldehyde leads to the formation of 1,3-dithiolane-2-carboxaldehydes[13] with little or none of the isomeric 1,3-dithiolan-2-yl ketones being observed (equation 7).



(7)

Although the formation of dithioacetals generally is a simple reaction, side reactions become prevalent when a reasonable leaving group is in the α-position to the carbonyl or to a conjugated double bond. In the reaction of 2-bromo-2-phenylacetophenone with ethanedithiol, 2,3-diphenyl-5,6-dihydro-1,4-dithiin (equation 8) was obtained[14,15]. Similarly, the dihydrodithiin (**1**) was obtained from 6-β-acetoxy-4-cholesten-3-one (equation 9)[3]. Additional examples exist for the formation of dihydro-1,4-dithiins *via* halides[16], epoxides[17] and even amides[18].



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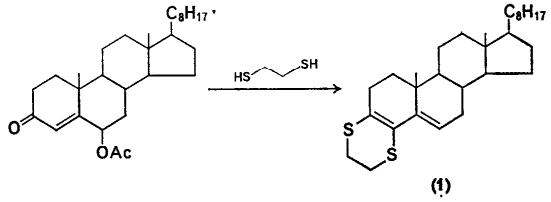
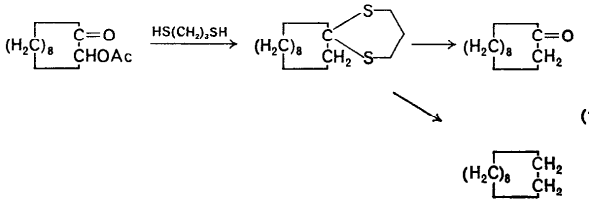


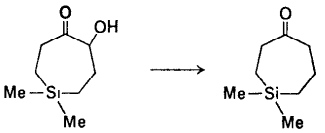
(9)

(1)

Under slightly different conditions, using 1,3-propanedithiol, acyloins and acyloin acetates lead to the formation of 1,3-dithianes where hydrogen has replaced the hydroxyl or acetoxyl group[19]. Hydrolysis to the ketone provides a method of converting acyloins to ketones and desulphurization allows conversion of acyloins to hydrocarbons (equation 10). Reduction of 1,1-dimethyl-5-hydroxysila-4-cycloheptanone gave 1,1-dimethylsila-4-cycloheptanone by this method (equation 11)[20]. A similar reaction is believed to be involved in the action of D-proline reductase[21].



(10)



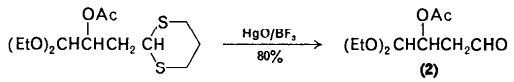
(11)

Numerous other examples of dithioacetal formation, including selective formations, have been well documented[4,22-24], but the above suggest the scope.

2. Removal

Early workers relied completely on the use of heavy metal salts in the hydrolysis of dithioacetals. The initial use of mercuric chloride with cadmium carbonate in hydroxylic medium[25,19] was modified and generally improved by the substitution of mercuric oxide for the cadmium carbonate[26,27]. However, in some cases the results of this method of hydrolysis have been disappointing. This is especially true in the recovery of aldehydes and steroidal ketones from their dithioacetals. Recently, numerous new methods of hydrolysis have emerged significantly changing the stature of dithioacetals as blocking groups.

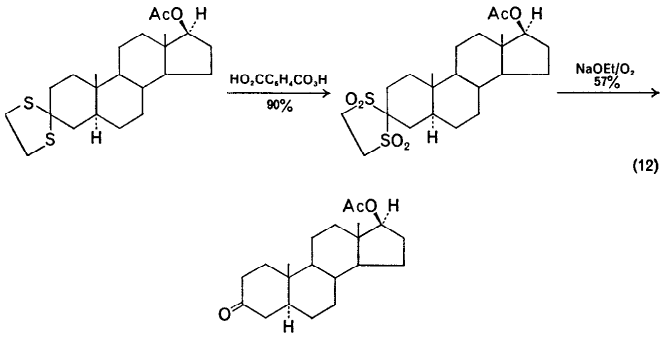
The use of mercuric oxide and boron trifluoride etherate in aqueous tetrahydrofuran gave good yields of aldehydes[28], even those such as **2** where mercuric chloride only destroyed the starting material. This method has also proved useful in carbohydrate chemistry[29,30,31].



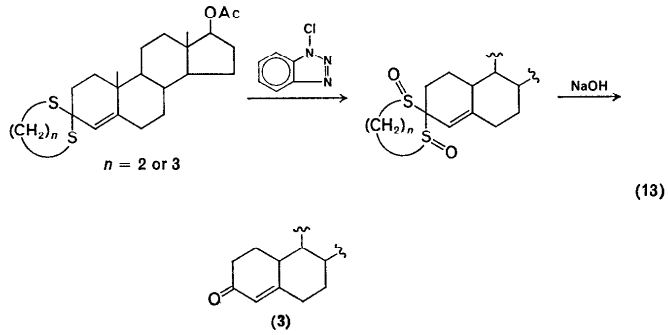
(2)

Oxidation of 1,3-dithiolanes with monoperphthalic acid[32] or hydrogen peroxide[33] gives ethylenedisulphones in high yields. These compounds are stable in acid, but are easily decomposed with base in the presence of oxygen to give the original carbonyl group. Thus, 17β-hydroxy-5α-androstan-3-one-3-(1,3-dithiolane) acetate was converted to the disulphone, which in the presence of sodium ethoxide and oxygen gave the original ketone (equation 12)[32]. Besides the fact that this method is effective in the steroid series, there are the advantages of being able to hydrolyse acid-sensitive compounds or work in acid media without fear of decomposing the blocking group.

The use of a mild oxidizing agent such as 1-chlorobenzotriazole[34] with 1,3-dithiolanes and 1,3-dithianes leads to the formation of disulphoxides.

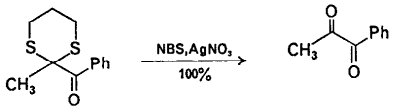


The disulphoxides generally are not isolated but are decomposed with sodium hydroxide to the ketone[35]. The reaction works well in the steroids, with 17β-acetoxytestosterone (3) easily being regenerated from its dithioacetal (equation 13).



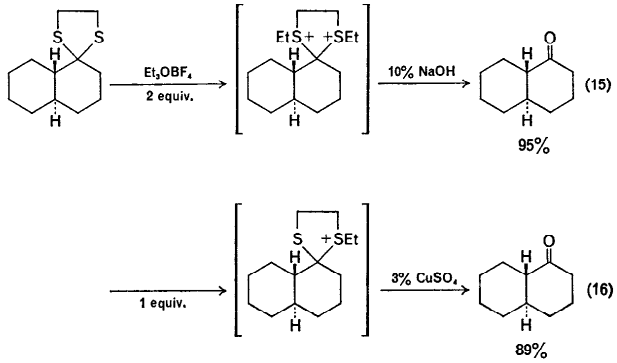
Oxidative hydrolysis of 1,3-dithianes using N-halosuccinimides has been extensively investigated[36]. The yields were consistently high when using N-bromosuccinimide (NBS), usually in acetonitrile. Unlike earlier methods 2-acyl-1,3-dithianes were efficiently hydrolysed to 1,2-dicarbonyl compounds. For example, 1-phenyl-1,2-propanedione was prepared in quantitative yield from the 2-benzoyl-2-methyl-1,3-dithiane (equation 14). Silver salts often aid the reaction, but it has been noted[3] that NBS in the

presence of silver ion reacts with double bonds. However, N-chlorosuccinimide (NCS) even with silver nitrate is compatible with double bonds and still gives comparable yields.



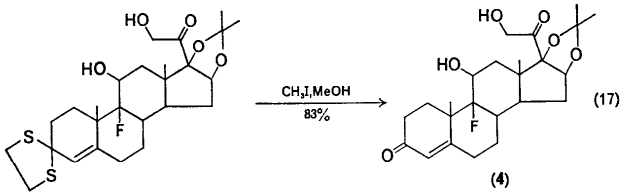
A few other methods for hydrolysis of 1,3-dithianes have recently been discovered but have not been thoroughly investigated. Use of sodium N-chloro-*p*-toluenesulphonamide (chloramine-T) leads to the corresponding ketones in consistently high yields[37]. The procedure requires only short reaction times in aqueous alcohol and should prove to be a very powerful method.

Alkylation of 1,3-dithiolanes with two equivalents of triethyloxonium tetrafluoroborate leads to bissulphonium salts. Treatment with 10% sodium hydroxide gives excellent yields of the corresponding ketones[38]. If only one equivalent of the oxonium salt is used, the resulting monosulphonium salt gives the ketone in high yield if a mild oxidizing agent such as copper sulphate or hydrogen peroxide is present. Equations (15) and (16) demonstrate the effectiveness of this method in the recovery of *trans*-1-decalone.



The sulphonium salt also seems to be involved in a procedure using methyl iodide in aqueous alcohol[39]. Mild conditions and high yields are typical. That the reaction is quite selective is apparent from the hydrolysis

of the 1,3-dithiolane of 9-fluoro-11β,16α,17,21-tetrahydroxypregn-4-ene-3,20-dione-16,17-acetonide (**4**) in high yield (equation 17).

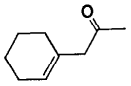
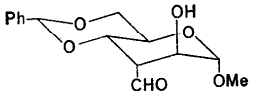


(17)

(4)

Some further representative examples of the hydrolysis of dithioacetals are given in Table 1.

TABLE 1. Hydrolysis of dithioacetals to carbonyl compounds

Dithioacetal of	Reagent	Yield, %	Reference
Cholestan-3-one	Chloramine-T	75	37
	1-Chlorobenzotriazole	50	35
	Monoperphthalic acid	52	32
	(1) $Et_3O^+BF_3^-$; (2) NaOH	81	38
Cyclohexanone	Chloramine-T	95	37
	(1) $Et_3O^+BF_3^-$; (2) $CuSO_4$	81	38
	$HgO-BF_3$	25	28
Fluorenone	Chloramine-T	86	37
PhCH=CHCHO	$HgO-BF_3$	86	28
Ph_3SiCCH_3 (O)	$HgCl_2$, aq. acetone–benzene	82	40
$CH_3CH_2CHCH_2CPh$ (CH_3, O)	$HgCl_2$, HgO, aq. MeOH	70	41
	NCS, $AgNO_3$	94	36
	$HgO-BF_3$	80	29, 30
$PhCH_2CCO_2Et$ (O)	NBS, acetone	78	36

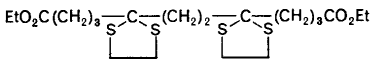
B. Carbonyl Reduction

Since the Raney nickel desulphurization of dithioacetals to the corresponding methylene was first observed by Wolfrom[42], the reaction has become one of the most reliable and mild ways of reducing the carbonyl group. Outstanding reviews can be found concerning the application of nickel desulphurizations to all types of organosulphur compounds[22,23] as well as a detailed discussion of the mechanism[42]. However, a brief mention of the scope of the reaction as well as some of the more recent modifications seems in order.

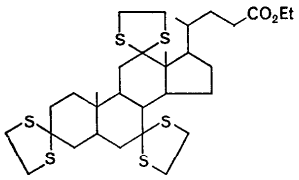
1. Reduction to saturated hydrocarbons

Typically desulphurization reactions are carried out with a large excess of Raney nickel. The reaction is not truly catalytic in nature since the hydrogen used to replace the sulphur usually comes from hydrogen retained by the metal during its preparation. In addition the nickel is consumed by the combination with the sulphur to form nickel sulphide. In practice a minimum ratio of 2·6 : 1 for nickel atoms to sulphur atoms is necessary[44].

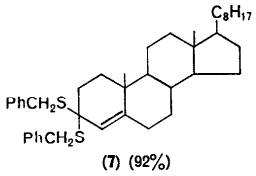
The Raney nickel catalyst is prepared through the action of aqueous alkali on a nickel–aluminium alloy. The conditions employed allow the preparation of the catalyst with a specific activity. Furthermore, the catalyst may be deactivated by refluxing with hydrogen acceptors, by degassing or by ageing. For details the reader is referred to the reviews mentioned above.



(5) (87%)



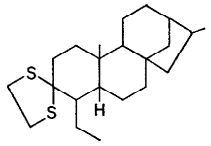
(6) (86%)



(7) (92%)

Although desulphurizations are very successful on most dithioacetals, a few have been somewhat unsatisfactory. Compounds **5**[45], **6**[6] and **7**[6] are typical of the high yields which often accompany desulphurizations. On

the other hand, *n*-heptanal diethylthioacetal gave only 40% yield of heptane[42] and compound **8** gave only 33% of desulphurized product[23]. Other functional groups generally do not affect the results. Desoxytetrahydrohelenaline (**9**) gave the desired product quantitatively[23] and isatin 1,3-dithiolane (**10**) gave oxindol without complication[23].



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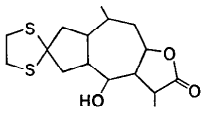

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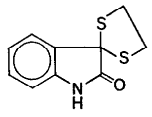
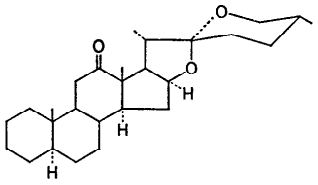


(10)

A recent modification in the use of Raney nickel may greatly enhance its utility. Industrial use of the standard procedure has been limited by the necessity to use such large quantities of the very expensive Raney nickel. It now appears that the use of the nickel–aluminium alloy itself in formic acid leads to very efficient desulphurizations with Ni/S ratios of only 0·2[44]. High proportions of the aluminium seem to give the best results, apparently because of the ability of the aluminium to regenerate the active nickel catalyst. Similar results were obtained using nickel or cobalt salts in the presence of auxiliary metals such as aluminium or iron.

The use of deuterium oxide and sodium deuterium oxide in the preparation of Raney nickel leads to the formation of deutero Raney nickel suitable for replacing dithioacetals with deuterium[46, 47]. The method suffers from some scrambling of the isotope often leading to products of low isotopic purity. Deuteration of (25R)-5α-spirostan-12-one (**11**) by this



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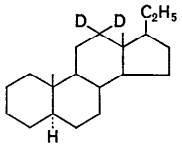
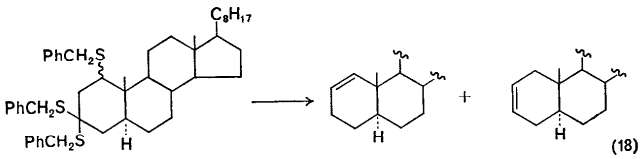


(12)

method led to an isotopic mixture consisting of 4% d_0, 44% d_1, 49% d_2 and 3% d_3 products[48]. At times fairly pure products are obtained, such as the preparation of 12,12-d_2-pregnane (**12**) with 76% d_2[49].

2. Reduction to olefins

The formation of an olefin during desulphurization was first noted when 1,3,3-tribenzylthiocholestane gave a mixture of cholest-1-ene and cholest-2-ene (equation 18) with Raney nickel deactivated by boiling in acetone[50]. Similar conditions gave predominantly olefin with the 1,3-dithiolane from 14β-$\Delta^{5,7,9}$-anthraergostatriene-15-one (equation 19)[51].



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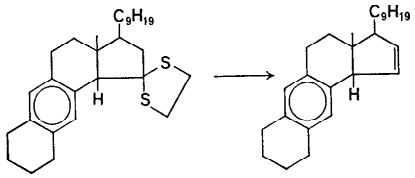
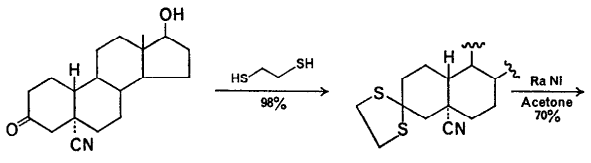


(19)

More extensive investigations[46, 52] have led to the use of W-2 Raney nickel in refluxing acetone to obtain olefins in 55–75% yields based on starting ketone. Even the synthesis of dienes from α,β-unsaturated ketones was successful[52]. Using this method 5α-cyano-17β-hydroxyestran-3-one was converted to the corresponding olefin (equation 20)[53]. Surprisingly, the 5β-cyano isomer gave low yields in the first step and no olefin in the second step. Both the *cis*- and *trans*-isomers in the 2-keto-10-cyano series have been converted to olefins[54, 55]. Other examples of this reaction include the conversion of dihydrogedunin (**13**) to the olefin[56] and the partial formation of olefin from 17-norphyllocladan-16-one (**14**)[57]. Groups in the α-position to the ketone may be lost during the reaction as seen by the formation of 5α-cholest-2-ene as the sole product from 2α-chloro-5α-cholestan-3-one[58].



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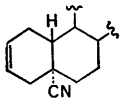

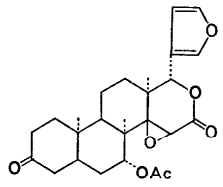

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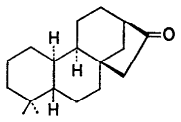
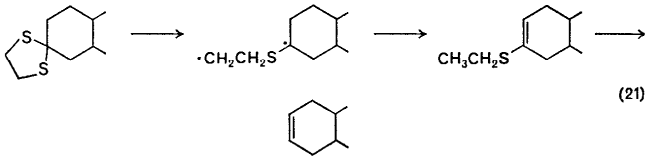


(14)

The mechanism of this reaction seems to involve formation of a diradical intermediate which, if the concentration of hydrogen radicals is low, gives the thioenol ether[46]. Further desulphurization gives the olefin (equation 21). If the alkyl radical is responsible for the $C_{(2)}$ hydrogen



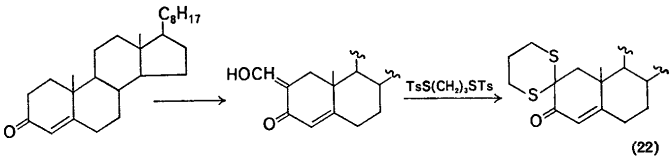
(21)

abstraction, it would seem necessary that it remain near the reaction site so that homolysis takes place before addition of hydrogen from the catalyst. Analogy with studies of the mechanism of desulphurization in monothioacetals[59] and thiazolidines[60] suggests that the abstraction may very well come from an external radical.

C. Methylene Blocking Group

In the presence of ethyl formate and sodium methoxide, the most reactive methylene group of a ketone is converted to its hydroxymethylene

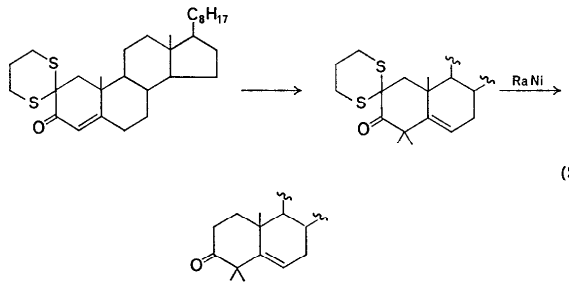
derivative[61]. Further reaction with the ditosylate of propane-1,3-dithiol[62] leads to the formation of the 1,3-dithiane[63] (equation 22). Thus the active position of the ketone is effectively blocked with a group easily removed by Raney nickel.



(22)

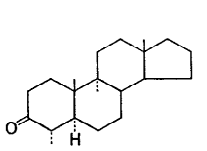
I. Alkylations

The presence of the dithioacetal does reduce the reactivity of the ketone toward alkylations at its other available positions[64], but nevertheless the sequence has been effectively utilized. This is clearly demonstrated by the formation of 4,4-dimethylcholestenone by this procedure (equation 23)[63]. Other examples of the successful use of this method include the



(23)

preparation of 4α,9α-dimethyl-5α-androstan-3-one (**15**)[65] and 4α-methyl-B-nor-5α-cholestan-3-one (**16**)[66].



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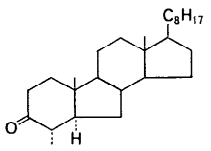
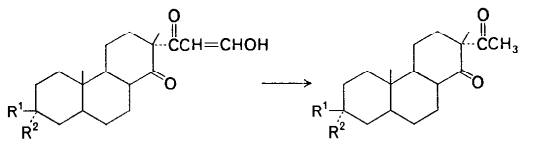


(16)

2. Decarbonylations

The formation of 1,3-dithianes from hydroxymethylene compounds, which are enol tautomers of β-keto aldehydes, has been shown to be useful in itself. When followed by desulphurization the net reaction is the decarbonylation to the ketone. This has been used to advantage in the formation of the methyl ketone (**17**)[67] in equation (24). Similarly the methyl ketone (**18**) was formed from its hydroxymethylene derivative[68].

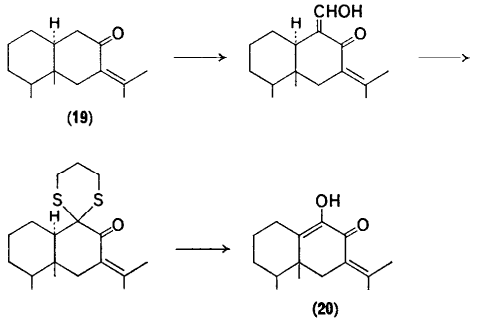


(24)

(**17**) R^1 = OAc, R^2 = H
(**18**) R^1 = CO_2Me, R^2 = Me

3. Formation of dicarbonyl compounds

The treatment of the intermediate 1,3-dithiane, either before or after alkylation with reagents such as mercuric chloride–cadmium carbonate (see section II.A.2) gives hydrolysis to the carbonyl. Thus *trans*-fukinone (**19**) was converted to (+)-hydroxyeremophilone (**20**) (equation 25)[69]. In *cis*-fukinone, the 1,3-dithiane could not be formed from the 1-hydroxymethylene fukinone, presumably for steric reasons.



(**19**)

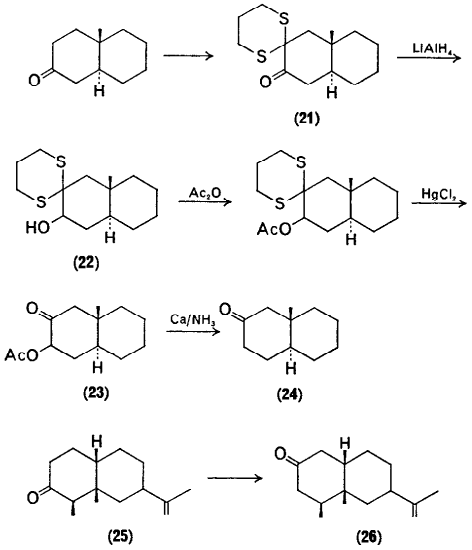
(25)

(**20**)

4. Ketone transposition

Modification of the above sequence to include reduction of the original ketone before hydrolysis is the basis for a new method of ketone transposition[70]. For example, the keto 1,3-dithiane (**21**) was prepared in the

usual manner followed by reduction of the carbonyl with lithium aluminium hydride to the alcohol (**22**). Conversion to the acetate and hydrolysis of the dithiane with mercuric chloride led to the keto acetate (**23**). Reduction with calcium in ammonia resulted in the formation of the new methyl decalone (**24**) in 58% overall yield (equation 26). The same



(**21**)

(**22**)

(**23**)

(**24**)

(26)

(**25**)

(**26**)

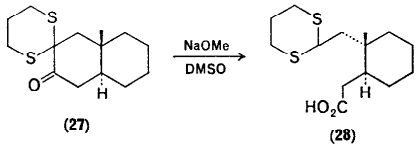
(27)

sequence was used to convert decalone (**25**) into the isomeric decalone (**26**) in 46% overall yield (equation 27). These conversions have been shown to take place with complete stereochemical integrity. Alternative methods of removing the carbonyl from the keto 1,3-dithiane so far have not been satisfactory.

5. Selective carbon—carbon bond cleavage

Keto 1,3-dithianes are susceptible to nucleophilic attack at the carbonyl with subsequent cleavage occurring preferentially between the carbonyl and the dithiane functions[71]. In the one instance reported, the keto dithiane (**27**) was cleaved with sodium methoxide in dimethyl sulphoxide to acid (**28**). The explanation as to why the acid is formed instead of the

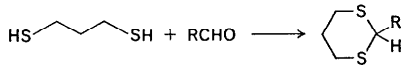
methyl ester is not apparent. The reaction conditions are mild and do not seem to put serious limitations on the nature of the rest of the molecule. Most importantly, after cleavage, the 1,3-dithiane grouping is suitable for many conversions such as reduction, alkylation, acylation or hydrolysis.



(27) (28) (28)

D. Synthetic Applications of 2-Lithio-1,3-dithianes

Corey and Seebach have reported[72] the use of 2-lithio-1,3-dithianes as useful reagents in organic synthesis. The method involves the use of 1,3-propanedithiol, which is caused to react with an aldehyde to yield the 1,3-dithiane (equation 29). Lithiation of the dithiane, normally with *n*-butyllithium in tetrahydrofuran at lowered temperatures, gives the 2-lithio-1,3-dithiane (equation 30). The R group can be alkyl, aryl or hydrogen.



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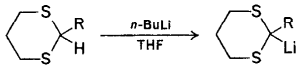
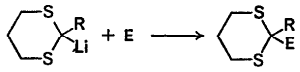


(30)

2-Lithio-1,3-dithianes have been shown[73] to undergo reaction with a variety of electrophiles, E, to give substituted dithianes (equation 31). Removal of the dithioketal function generates the newly synthesized carbonyl compound (equation 32) having the group E substituted for the aldehydic hydrogen of the original aldehyde. The dithioketal is most often hydrolysed using the mercuric chloride : mercuric oxide method[26] or by oxidative hydrolysis with N-halosuccinimides[27]. It is possible also to remove the dithioacetal function by desulphurization (Raney Ni) to yield the corresponding methylene derivative (equation 33). For a general treatment of removal of the dithioacetal function, see section II.A.2.

2-Lithio-1,3-dithiane reagents are in effect masked nucleophilic acylating agents and can be considered equivalent to the presently unknown



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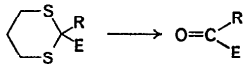

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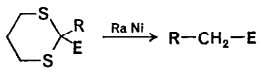
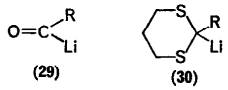


(33)

acyllithium reagent (**29**). Thus, by use of a thiol, the carbonyl carbon of an aldehyde can be transformed from an electrophilic site to the nucleophilic centre in the lithiated dithiane derivative (**30**). The ability of sulphur to



(29) (30) (34)

stabilize carbanions α to the sulphur atom is significant in the readily accomplished lithiation of 1,3-dithianes. The preparation and reactions of 2-lithio-1,3-dithianes have been reviewed[73].

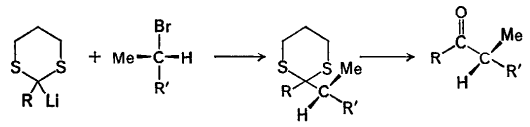
The following is a general outline of the various types of reactions that these reagents are known to undergo, including a comprehensive treatment of reactions reported since the review article by Seebach[73].

1. Reaction with alkyl halides

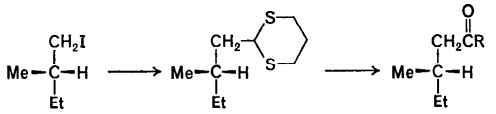
2-Lithio-1,3-dithianes undergo alkylation at the 2-position upon reaction with alkyl halides. This reaction appears to be S_N2 in nature as it is applicable to primary and secondary alkyl halides[72], occurs most readily with alkyl iodides[72], and with optically active secondary halides gives inverted products[74]. It has been shown[41,72] that reaction with optically active alkyl halides provides a useful route for the preparation of optically active aldehydes or ketones (equations 35 and 36).

Cycloalkylation has been effected by reaction with α,ω-dihaloalkanes to give, upon hydrolysis, cyclic ketones[72,75] (equation 37). Likewise, the

dithiane derivatives of α,β-unsaturated aldehydes undergo hydrochlorination followed by cycloalkylation to yield substituted cyclopropanes (equation 38)[76c]. Cyclic 1,3-diones are available[72] by the

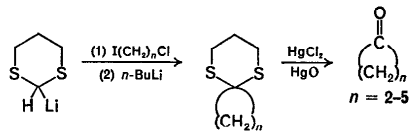


(35)



(36)

alkylation of the bis-dithiane **31** (equation 39). It also has been observed that the use of α,ω-dibromoalkanes in cycloalkylation reactions is complicated by formation of sulphonium salts (equation 40), a reaction not observed with use of α-chloro-ω-iodo or α,ω-dichloroalkanes.



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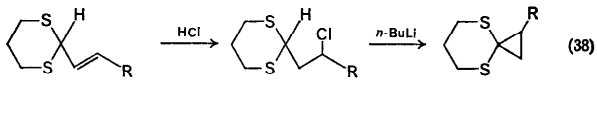

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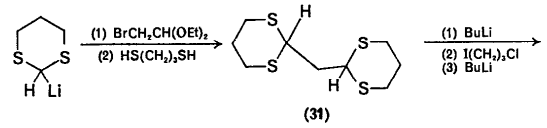
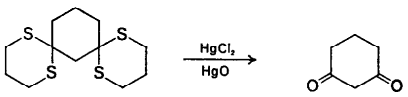
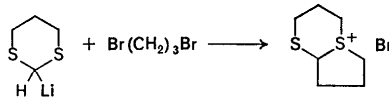


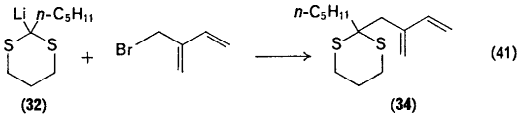
(39)



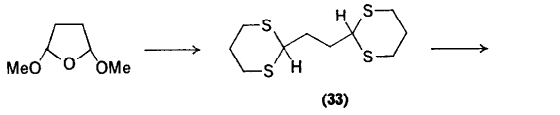


(40)

Corey and coworkers[76a], in a synthesis of prostaglandins, prepared diene **34** by alkylation of the lithiodithiane **32** with 2-bromomethyl-1,3-butadiene (equation 41). A synthesis of jasmone (**35**), in an overall yield of 50%, has been reported by Ellison and Woessner[76b] in which the bisdithianylethane **33** was sequentially alkylated, followed by hydrolysis and cyclization (equation 42). A similar route for preparation of 4-hydroxy-2-cyclopenten-1-ones has been reported[77]. This method appears to provide a general route to 1,4-diketones *via* 1,3-dithianes.



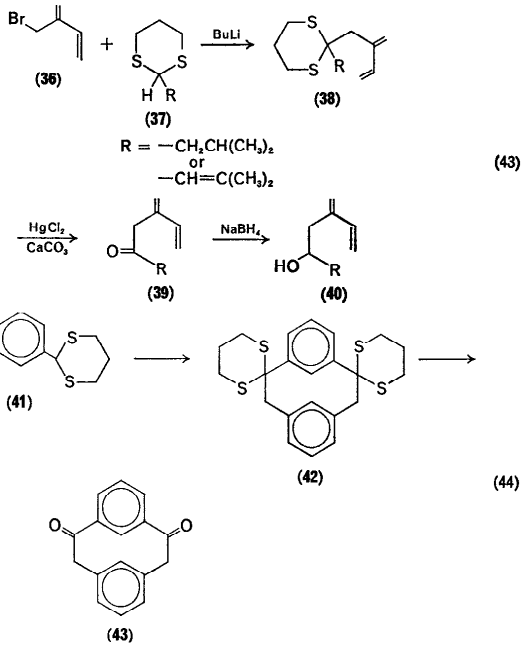
(41)



(42)

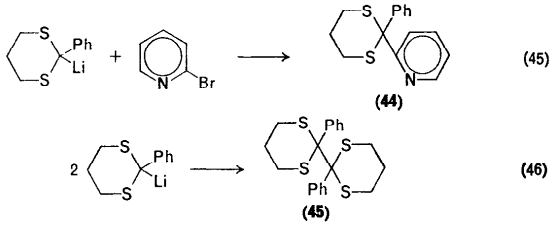
The synthesis of the monoterpene components **40** of the sex attractant of the bark beetle has been accomplished[78] as outlined in equation (43). The alkylation of the dithiane **37** was a key step in the synthesis since efforts to prepare **40** by addition of the magnesium or lithium derivatives of the bromoalkene **36** to the appropriate aldehyde failed.

Hylton and Boekelheide[79] prepared the cyclophanedione **43** by alkylation of the bisdithiane **41** followed by hydrolysis. An improved procedure for the preparation of **41** has been reported[80].



2. Reaction with aryl halides

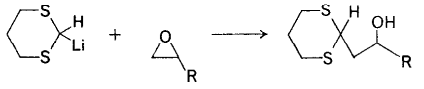
Treatment of 2-lithio-2-phenyl-1,3-dithiane with 2-bromopyridine gave the substituted pyridine **44** in 50% yield[81]. However, reaction with 2,4-dinitrobromobenzene gave none of the substitution product, but rather compound **45** resulting from oxidative dimerization of the dithiane



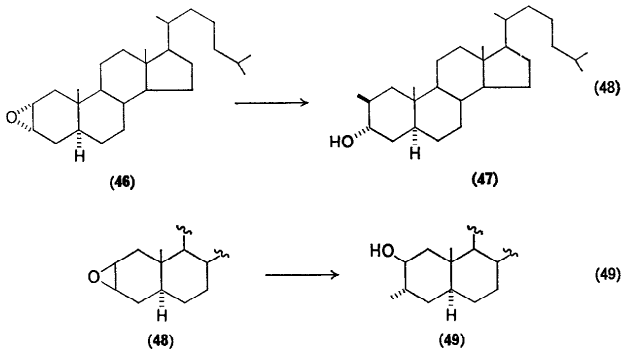
moiety[81]. Such oxidative dimerizations (see section II.D.7) of 2-lithio-1,3-dithianes are known and have been reported[72] to occur with nitro compounds.

3. Reaction with epoxides

Epoxides effect alkylation of 2-lithio-1,3-dithianes[72,75] (equation 47); opening of the epoxide ring occurs in the fashion typical of reactions with nucleophiles. The reported yields are in the range of 70–95% and appear to be free of side reactions common with other organometallic reagents[73].

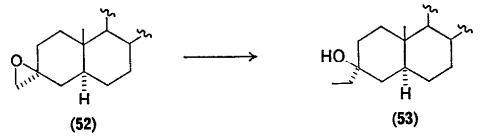


Recently, Jones and Grayshan[82–84] have reported the reaction of lithiodithiane derivatives with steroidal epoxides to effect preparation of modified steroids. Treatment of 2α,3α-oxiranyl-5α-cholestane (**46**) with 2-lithio-1,3-dithiane, followed by desulphurization, yielded the 2β-methyl-3α-cholestanol **47** (equation 48). Conversely, reaction with the epimeric epoxide **48** furnished 3α-methyl-5α-cholestan-2β-ol (**49**) (equation 49)[82].



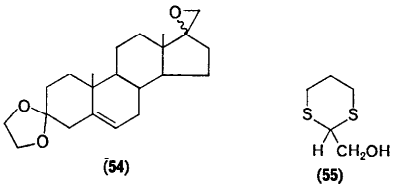
The spiroepoxide **50**, prepared from 5α-cholestan-3-one, was cleanly converted to the 3β-ethyl derivative **51**; the 3α-ethyl derivative **53** was obtained in an analogous manner from the epimeric spiroepoxide **52**[83] (equation 51). Similar results were obtained[84] when this method was applied

(50) (51)



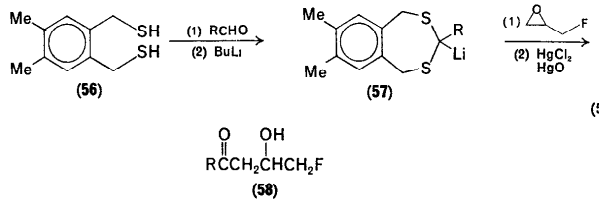
(52) (53)

to the epimeric spiroepoxide **54**. This method appears to be the most suitable synthetic route to these modified steroids. However, attempts to utilize the lithium derivative of 2-hydroxymethyl-1,3-dithiane (**55**), or the corresponding tetrahydropyran derivative, to prepare corticoid steroids were unfruitful[84].



(54) (55)

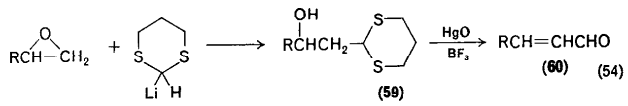
The preparation of some γ-fluoro-β-hydroxyketones (**58**) by reaction of epifluorohydrin with the lithio derivative **57** has been reported[85]. The dithioacetals prepared from dithiol **56** are reported to be crystalline, odourless compounds[86], therefore some advantage may be purported for their use.



(56) (57) (53)

(58)

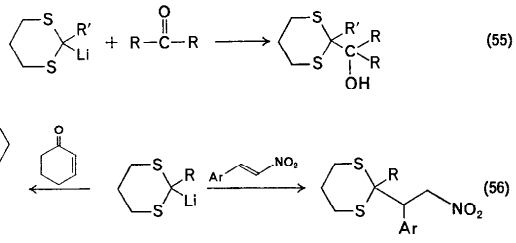
A synthesis[28] of α,β-unsaturated aldehydes has been effected by reaction of 2-lithio-1,3-dithiane with epoxides (equation 54). It was found that treatment of the dithianyl alcohol **59** with mercuric oxide–boron trifluoride caused dehydration and hydrolysis to give the α,β-unsaturated aldehyde **60** in good yield. Standard methods for removal of the thioacetal function were not successful in these cases.



(59) (60) (54)

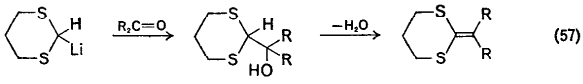
4. Reaction with aldehydes and ketones

2-Lithio-1,3-dithianes add to the carbonyl group of aldehydes and ketones to provide mercaptal derivatives of α-hydroxy aldehydes or ketones[72] (equation 55). The yields are normally quite high. Reaction with α,β-unsaturated ketones has been observed[72,87] to give only 1,2-addition; however, Seebach and Lietz have reported[88] 1,4-addition to occur in reactions with α,β-unsaturated nitro derivatives (equation 56). In the case



(55) (56)

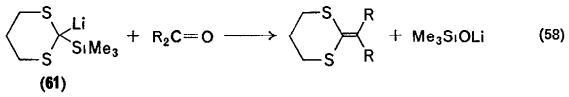
where R′ = H, the addition product obtained from reaction with a ketone can be converted by dehydration to a ketene thioacetal (equation 57).



(57)

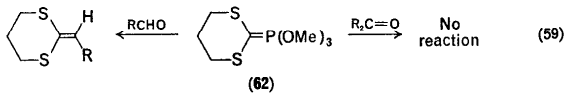
Ketene thioacetals also are readily available[89,90] by a Wittig-type reaction of 2-lithio-2-trimethylsilyl-1,3-dithiane (**61**) with aldehydes or

ketones. The dithiane **61** is prepared[40,91] by reaction of 2-lithio-1,3-dithiane with trimethylchlorosilane followed by lithiation (see section II.D.6). A method employing the phosphite ylid **62** to prepare ketene thioacetals by reaction with aldehydes, but not ketones, has been reported[92] (equation 59).



(58)

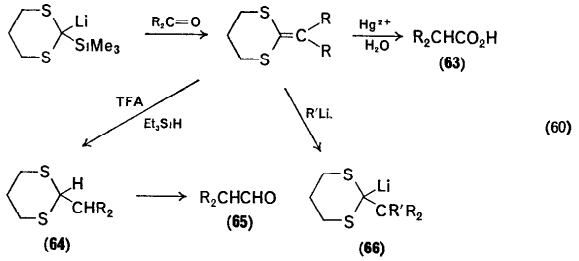
(61)



(59)

(62)

Ketene thioacetals should prove to be useful synthetic intermediates. Hydrolysis[92] of ketene thioacetals yields carboxylic acids (**63**), while protonation–hydride transfer using trifluoroacetic acid-triethylsilane, as reported by Carey and Neergaard[93], provides the thioacetal (**64**) of the homologous aldehyde (**65**).

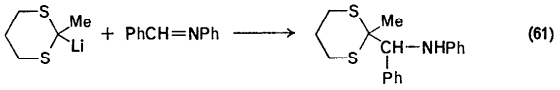


(60)

(64) (65) (66)

Alkyllithium reagents are known[94] to add to ketene thioacetals to give 2-lithio-1,3-dithianes **66** in which R′ has become attached to the ethylidene carbon. Both **64** and **66** are capable of undergoing further reactions available to 2-lithio-1,3-dithianes. Therefore, it should be possible in principle to convert an aldehyde, RCHO, to any of the following *via* the corresponding ketene dithioacetal: RCH_2CO_2H, RCH_2CHO, RR′CHCHO, RR′CHCOR″, and RR′R″CCOR″.

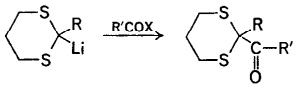
Imines, being nitrogen analogues of carbonyl compounds, are reported[72] to undergo addition with 2-lithio-1,3-dithianes to yield amines (equation 61).



(61)

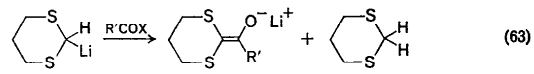
5. Reaction with acylating agents

Acylation of 2-lithio-1,3-dithiane derivatives occurs in satisfactory yields only when a dilute solution of the dithiane derivative is added at −78°C to a solution containing a 20–100-fold excess of the acylating agent[72,73] (equation 62). The above conditions are necessary to circumvent reaction of a molecule of the reactive lithiodithiane with a molecule of previously formed 2-acyldithiane. This method offers, by subsequent removal of the dithioketal function, a route for the preparation of 1,2-dicarbonyl compounds.



(62)

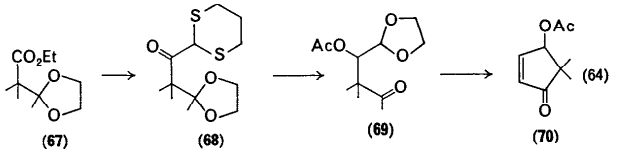
Acylating agents that have been employed[72] are carbon dioxide, alkyl chloroformates, alkyl formates, acid chlorides, esters, benzonitrile and dimethylformamide; the expected acylation products from reaction with the above reagents were formed in each case. However, the N,N-dimethylamide derivatives of higher carboxylic acids did not yield acylated product as in the case of dimethylformamide[73]. When R = H (equation 63), it was necessary to employ two equivalents of the lithiodithiane due to product enolate formation.



(63)

In the total synthesis of illudin M, Matsumoto and coworkers[95] prepared the cyclopentenone **70** by reaction of 2-lithio-1,3-dithiane with

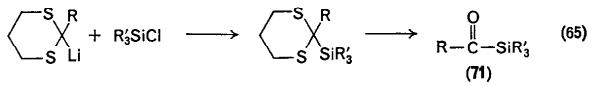
the ester **67** to give **68**. Reduction, acetylation and removal of the dithioacetal function gave **69**, apparently formed by an intramolecular transketalization reaction.



(67) (68) (69) (70)

6. Silylation and related reactions

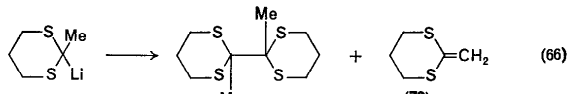
2-Lithio-1,3-dithianes react with trialkyl- and triaryl-chlorosilanes to give the 2-silylated derivatives (equation 65). This method was used in the preparation[40,91] of the previously unknown α-silylketones **71**. Germanylation and stannylation also can be accomplished with the corresponding trialkylhalo derivatives[40].



(71)

7. Oxidative dimerization

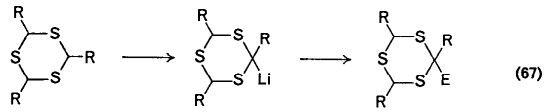
Treatment of 2-lithio-1,3-dithianes with iodine, cupric salts, 1,2-dibromoethane, or nitro compounds effects oxidative dimerization[73] to give the dimer **72** plus a small amount of the 2-methylene derivative **73**.



(72) (73)

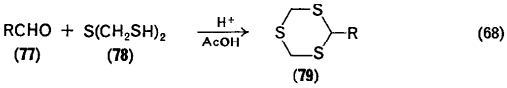
8. Reactions using 1,3,5-trithianes

1,3,5-Trithianes (**74**) undergo lithiation[41,73] with an equivalent of *n*-butyllithium to yield the 2-lithio derivatives, which substances undergo the usual reactions (equation 67) as with 2-lithio-1,3-dithianes. Since additional active hydrogens are present in 1,3,5-trithianes, dimethylation has been observed in some cases[73].



(74) R = H or alkyl (75) (76)

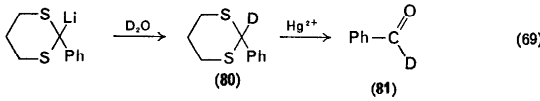
An alternate route not involving 2-lithio-1,3,5-trithianes for the preparation of 2-substituted-1,3,5-trithianes recently has been reported[96]. This method involves reaction of an aldehyde **77** with the dithiol **78** to yield the 2-substituted trithiane **79**.



(77) (78) (79)

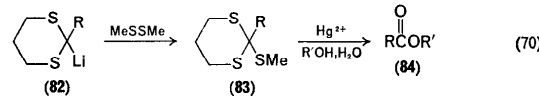
9. Miscellaneous applications

A convenient preparation of 1-deuterioaldehydes (**81**) *via* 2-lithio-1,3-dithianes has been reported by Seebach and coworkers[27] (equation 69). This method appears to be superior to previously reported methods for the preparation of 1-deuterioaldehydes.



(80) (81)

Treatment of 2-lithio-1,3-dithiane derivatives with methyl disulphide yields the orthothioformate **83**, which upon hydrolysis in alcoholic solvents furnishes an ester[97]. This method may provide a useful route for the conversion of sensitive aldehydes to esters and carboxylic acids.



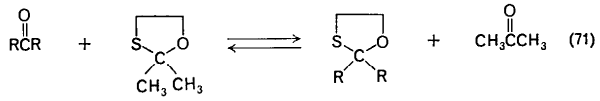
(82) (83) (84)

III. MONOTHIOACETALS

The use of monothioacetals in organic synthesis has not been nearly so extensive as the use of dithioacetals. Generally prepared as 1,3-oxathiolanes and 1,3-oxathianes, the group is resistant toward dilute base and lithium aluminium hydride[14]. Regeneration of the carbonyl is easily accomplished.

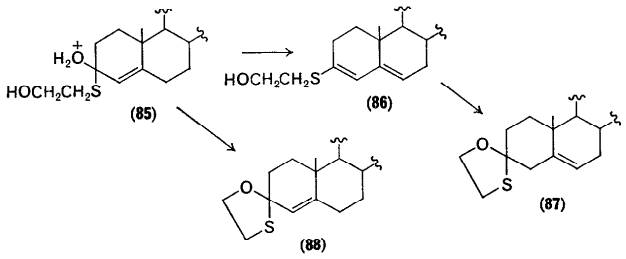
A. Preparation

Condensation of 2-mercaptoethanol or 3-mercaptopropanol with ketones is usually achieved with the aid of an acid catalyst. Hydrogen chloride has been used[98] but more common agents are boron trifluoride[10], freshly fused zinc chloride[79] or *p*-toluenesulphonic acid[100]. An exchange method between 2,2-dimethyl-1,3-oxathiolane or 2,2-dimethyl-1,3-oxathiane and a non-volatile ketone leads to formation of the new monothioacetal and acetone[100]. The equilibrium is displaced by continuous distillation of the acetone formed (equation 71). With saturated ketones, mostly steroids, the yields of the above methods are comparable and are usually in the 60–90% range. With α,β-unsaturated ketones, the yields were significantly lower[100].



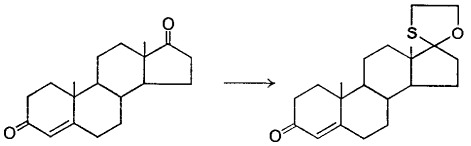
(71)

Unlike the case of 1,3-dithiolane formation, 1,3-oxathiolanes from α,β-unsaturated ketones show a shift of the double bond. It has been proposed[100] that intermediate **85** may undergo nucleophilic attack by the hydroxyl leading to unrearranged product **88**. Alternatively, dehydration would give the conjugated diene **86**, to which the hydroxyl could add giving the rearranged product **87**. Obviously, with ethanedithiol nucleophilic attack of the sulphur must predominate, while with the less nucleophilic hydroxyl, prior dehydration occurs. This is in agreement with the fact that with ethanediol the resulting ketal shows a shifted double bond.



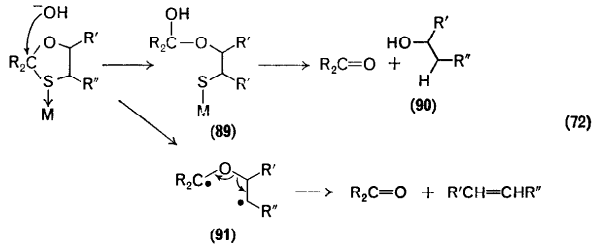
The reduced reactivity of α,β-unsaturated ketones towards 3-mercaptoethanol allows preferential formation of the hemithioacetal of an unconjugated carbonyl present in the molecule. One example of this general

phenomenon is given below in which 4-androstene-3,17-dione was converted to the 17-(1,3-oxathiolane) with zinc chloride catalysis[99]. With *p*-toluenesulphonic acid catalysis, the 3,17-bis(1,3-oxathiolane) could be formed in low yield.



B. Removal

Unlike 1,3-dithiolanes, treatment of 1,3-oxathiolanes with Raney nickel gives regeneration of the carbonyl group[99]. Thus, protection of a carbonyl by condensation with 2-mercaptoethanol allows regeneration in high yields under neutral conditions. Surprisingly, in the usual alcohol or acetone solvent, the ketonic oxygen is not from the oxathiolane. Apparently, association of the sulphur with the electron-deficient metal (equation 72) causes activation of the ring followed by attack of a hydroxide, either from the media or combined with the metal, to give the hemiketal **89**. Normal work-up cleaves the hemiketal which, with further desulphurization, leads to formation of the ketone and the alcohol **90**[59,101]. Solvents such as benzene may also be used and under the right conditions lead to high yields of the ketone[101]. In nonpolar solvents,

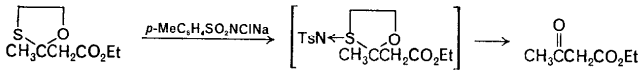


(72)

ionic intermediates are presumably not involved and the diradical (**91**) is the accepted intermediate[59,101]. The desulphurization of 1,3-oxathianes behaves similarly with the ketone being the major product[101]. Additional information may be found in the previously mentioned reviews[22,23,43].

The hydrolysis of 1,3-oxathiolanes with acid[102,103] or mercuric ion[101] also provides a suitable procedure for regenerating the ketone. The mechanism involved appears similar to that with Raney nickel, but with a proton or mercuric ion taking the place of the nickel.

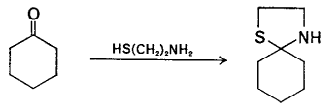
The most recent method of removal of the 1,3-oxathiolane group is by the use of N-chloro-*p*-toluenesulphonamide(chloramine-T)[104] in water, methanol or ethanol (equation 73). Again basically the same mechanism appears involved with prior association of the sulphur to form an unstable sulphilimine. The reaction times are short (2 min), conditions are mild and yields are high (85–100%).



(73)

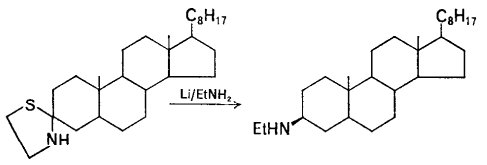
IV. THIAZOLIDINES

Just as 2-mercaptoethanol will condense with ketones to produce 1,3-oxathiolanes, so will 2-mercaptoethylamine react to produce thiazolidines[60]. Usually *p*-toluenesulphonic acid is used as a catalyst in benzene with yields being quite good, 94% in the case of cyclohexanone (equation 74). The uses of the thiazolidines have not been throughly



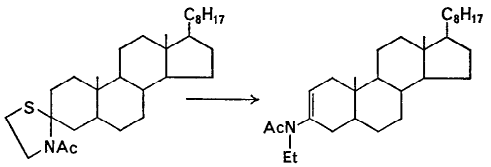
(74)

investigated, but it appears that they offer no advantages over previously mentioned protecting groups. Although Raney nickel desulphurization gives unsatisfactory yields of the starting ketone, lithium in ethylamine offers promise in the preparation of amines. 3β-Ethylamino-5α-cholestane was prepared in 87% yield[60] when desulphurized in this manner (equation 75).



(75)

More thoroughly investigated has been the desulphurization of N-acetylated thiazolidines to form acetylated enamines. Thus 31-day-old Raney nickel in benzene gives a 90% yield of 3-(N-ethylacetamido)-5α-cholest-2-ene (equation 76) from the corresponding N-acetylthiazolidine[60,105]. The conditions for this reaction are rather sensitive to solvent



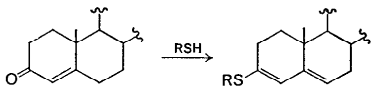
(76)

and catalyst age. The unsaturated amide is the favoured product in benzene with aged catalyst, but with fresh catalyst the major product in acetone is the ketone and in ethanol the saturated amide. The mechanism of desulphurization is believed[60] to be similar to the first step in the formation of olefins from 1,3-dithiolanes (see section II.B.2).

V. THIOENOL ETHERS

A. Carbonyl Protecting Group

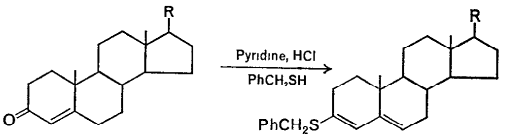
It has been noted (see section III.A) that protecting reagents such as 2-mercaptoethanol react preferentially with the saturated carbonyl when it is in the presence of an α,β-unsaturated carbonyl. Thioenol ethers are equally useful because they are formed almost exclusively from α,β-unsaturated carbonyls (equation 77).



(77)

Normally the reaction of thiols with carbonyls, saturated or unsaturated, leads to the formation of dithioacetals when acid catalysts such as zinc chloride or *p*-toluene-sulphonic acid are present (see section II.A.1). Occasionally, under special reaction conditions thioenol ethers have been formed using these same catalysts[106,107], but never in the presence of acid-sensitive substituents. Pyridine hydrochloride as the catalyst has been successfully used to give excellent yields of the thioenol ethers of Δ^4-3-ketosteroids even in the presence of sensitive groups[108]. Thus, desoxycorticosterone acetate (**92**) was converted to its 3-benzylthioenol ether

(93) in 60% yield (equation 78). The selectivity of the reaction using these conditions is very high. Unlike the case with zinc chloride, progesterone (94) with pyridine hydrochloride and benzyl mercaptan gives no observable reaction at $C_{(20)}$ with the only product being progesterone 3-benzyl thioenol ether (95)[108].

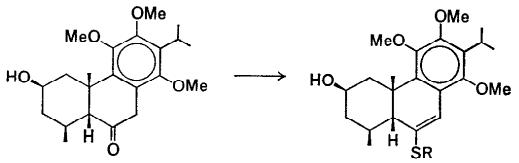


(78)

(92) R = $COCH_2OAc$
(94) R = $COCH_3$

(93) R = $COCH_2OAc$
(95) R = $COCH_3$

Other condensing agents which have proved useful under certain conditions are boron trifluoride[8], formic acid with *p*-toluenesulphonic acid[8] and hydrochloric acid in acetic acid[8,107]. One unusual example of a thioenol ether formed from a saturated ketone has been reported using hydrogen chloride as the catalyst[109]. In this case, compound **96** was converted to either its benzylthioenol ether **97** or its ethylthioenol ether **98** (equation 79). Benzyl mercaptan normally seems to be the reagent of choice in most conversions because of its easily crystallized products.

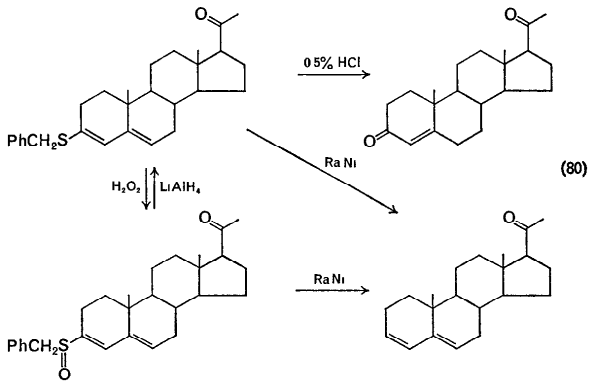


(79)

(96)

(97) R = $PhCH_2$—
(98) R = Et

The thioenol ethers are stable towards base[8] and lithium aluminium hydride[106,108], but are reconverted to the parent compound on dilute acid hydrolysis. Raney nickel desulphurization can be used to form the diene[108]. Hydrogen peroxide oxidation will convert the acid-labile thioenol ether to an acid-stable sulphoxidoenol ether[8,108]. The sulphoxidoenol ether may be desulphurized with Raney nickel to the diene, or with lithium aluminium hydride reconverted to the thioenol ether for hydrolysis to the α,β-unsaturated ketone[108]. These reactions are depicted in equation (80).

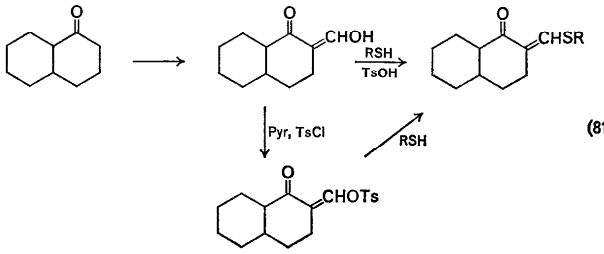


(80)

B. Methylene Blocking Group

In the continuing search for the ideal methylene blocking group, considerable effort has been expended in looking at derivatives of hydroxymethylenes. These are readily prepared from a ketone, ethylformate and sodium methoxide[61].

Ireland and Marshall[64] found that alkanethiols form very versatile derivatives with hydroxymethylenes. The reaction with a thiol, accompanied by a *p*-toluensulphonic acid catalysed water separation, leads to formation of the corresponding thioenol ether (equation 81). If acid-labile substituents are present, a procedure involving displacement from an intermediate tosylate (**99**) by the thiol is used. Although other thiols

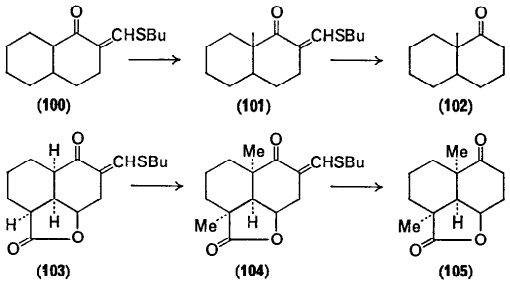


(81)

(99)

have been used, *n*-butanethiol appears to be the most convenient in this reaction. The yields of the thioenol ethers from hydroxymethylenes are generally greater than 80% using the acid catalysed method and only slightly lower with the basic pyridine procedure.

Alkylations of the protected ketones are very facile. The thioenol ether generally need only be left in contact with the base a few minutes before addition of the alkyl halide. Such short contact with the base allows easy isolation of the alkylated, blocked ketones[64]. Thus, 2-*n*-butylthiomethylene-1-decalone (**100**) was converted to 9-methyl-2-butylthiomethylene-1-decalone (**101**) in 85% yield. This procedure was used in the difficult dimethylation of **103** to give the lactone **104**.



Although the *n*-butylthiomethylene group is subject to acid hydrolysis, basic conditions for hydrolysis have been developed[64] and these seem to be preferred in actual practice. A typical procedure uses a mixture of a 25% aqueous potassium hydroxide solution with ethylene glycol heated at reflux. In this manner thioenol ether **101** was converted to 9-methyl-1-decalone (**102**) in 78% yield[64] (equation 82). The rare use of acid hydrolysis is exemplified by the use of concentrated hydrochloric acid to hydrolyse the blocked lactone (**104**) to **105** (equation 83)[110]. Additional examples of conversions using a thioenol ether intermediate are shown in Table 2.

C. *Monomethylation via Reduction*

Just as the blocking of active sites to permit alkylations on less reactive sites has been a recurring problem, so has the problem of preventing polyalkylations on reactive sites. The use of the alkylthiomethylene group offers a convenient intermediate from which monomethylated products are prepared by desulphurization with Raney nickel. In this way, 2,3,5,5-tetramethylcyclohexanone was prepared[119] in 58% overall yield from

3,3,5-trimethylcyclohexanone (equation 84). The same procedure was used[120] in the conversion of 7-oxobicyclo[3.2.1]octane to the 6-methyl derivative (equation 85). Similarly, 10-carbethoxy-2,7,7-trimethyl-*cis*-

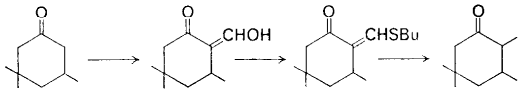


TABLE 2. Alkylation of ketones using thioenol ethers as a methylene blocking group

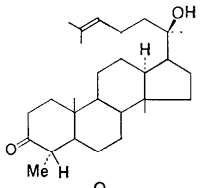
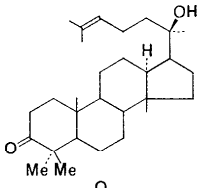
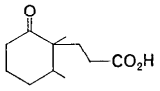
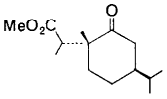
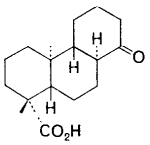
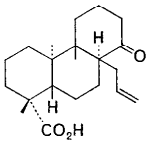
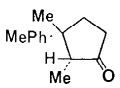
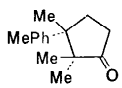
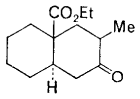
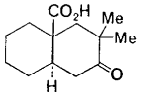
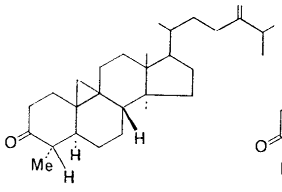
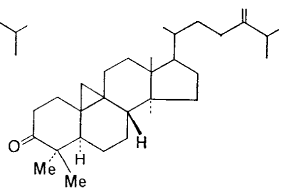
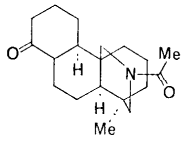
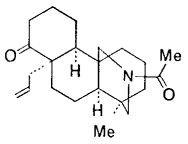
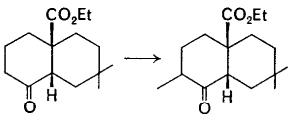
Reactant	Product	Overall yield, %	Reference
		73	111
		60	112
		62	113
		82	114
		31	115

TABLE 2 (*cont.*)

Reactant	Product	Overall yield %	Reference
		62	116
		35	117
		36	118

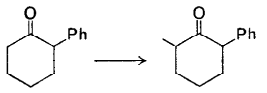
decal-1-one (equation 86) was prepared in 73% overall yield using this method[121]. In those cases where partial reduction of the carbonyl accompanies desulphurization, the crude mixture is oxidized before purification[119].

(85)



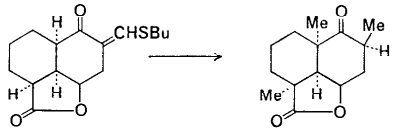
(86)

The methylation of a very active but substituted position is easily avoided by the alkylthiomethylene approach. A high yield of 6-phenyl-2-methylcyclohexanone was obtained from 6-phenylcyclohexanone (equation 87)[64,122].



(87)

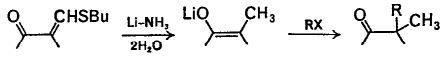
Of course, the use of the alkylthiomethylene group first for blocking and later as a route to monomethylation further expands its utility. Thus, compound **103** was methylated and desulphurized to give the trimethyl derivative **106**[110].



(103) (106)

D. Geminal Alkylation

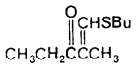
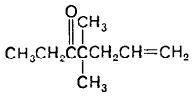
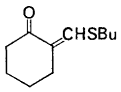
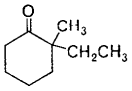
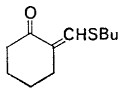
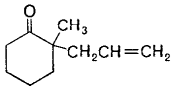
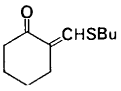
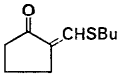
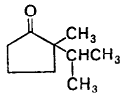
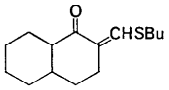
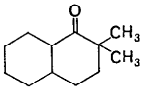
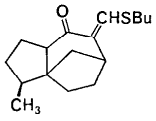
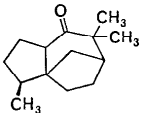
In attempting alkylations leading to highly substituted ketones, careful choice of methods is required to avoid difficulties. Selective geminal alkylations can be achieved by blocking all other available sites, but this is not always possible as with α,α,α′-trisubstituted acetones. An interesting new method has evolved incorporating the lithium–ammonia reduction of *n*-butylthiomethylene derivatives of ketones to their methyl-substituted enolate anions with subsequent alkylation[123]. This reduction–alkylation leads to the introduction of one methyl group and a second variable geminal substituent at any position which will condense with ethyl formate (equation 88). Reaction times as brief as 30 s plus the use of water



(88)

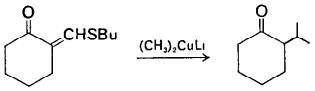
as a proton donor minimize any over-alkylation. Table 3 lists some typical conversions using this procedure.

TABLE 3. Geminal alkylation of ketones *via* thioenol ether derivatives

Ketone derivative	Product	Yield from *n*-butylthiomethylene derivative, %	Reference
		82	123
		75	123
		85	123
		56	123
		40	123
		70	123
		69	128

E. Symmetrical α-Branched Alkylation

The reaction of dialkylcopper lithium reagents with α,β-unsaturated ketones leads to selective conjugate addition[124]. It has been observed that *n*-butylthiomethylene derivatives undergo a double conjugate addition, with loss of the alkylthio group, upon reaction with dimethylcopper lithium. Thus, dimethylcopper lithium reacts with 2-*n*-butylthiomethylenecyclohexanone to give almost quantitatively 2-isopropylcyclohexanone (equation 89)[123]. This reaction should prove useful for the preparation of ketones having a symmetrically branched alkyl substituent in the α-position.



(89)

F. α, β-Unsaturated Aldehydes

Ketones with blocking groups of the isopropoxymethylene type are readily converted to α,β-unsaturated aldehydes by reduction followed by acid-catalysed rearrangement[125,126]. However, the use of this blocking group has the drawback of being moisture-sensitive and of having a deactivating effect on the other α-position. Fortunately, the *n*-butylthiomethylene grouping does not suffer from these drawbacks and is still readily converted to the α,β-unsaturated aldehyde[64,127]. Thus 2-*n*-butylthiomethylene-6,6-dimethylcyclohexanone (**107**) is reduced with lithium aluminium hydride and the resulting alcohol hydrolysed in acid to the α,β-unsaturated aldehyde **110**[129]. The alcohol **111** typically makes up

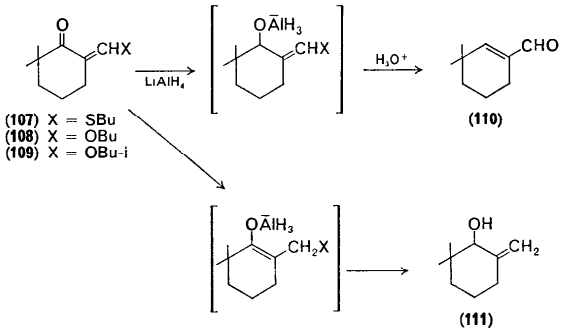
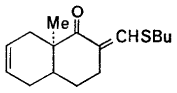
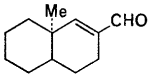
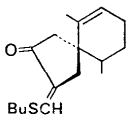
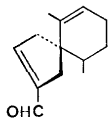
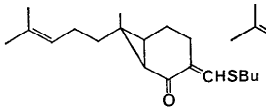
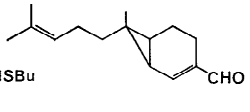
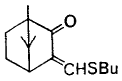
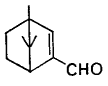
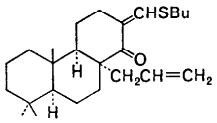
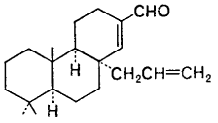
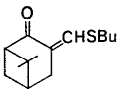
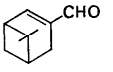


TABLE 4. Preparation of α,β-unsaturated aldehydes by $LiAlH_4$ reduction of α-*n*-butylthiomethylene ketone derivatives

Ketone derivative	Product	Yield from *n*-butylthiomethylene, %	Reference
		63	127
		81	131, 132
		—	133
		—	130
		38	134
		52	135

about 5% of the product. A comparison of the *n*-butylthiomethylenes with butoxy- and isobutoxymethylenes (**108** and **109**) shows[119,129] that the latter two are significantly more prone to 1,4-addition leading to alcohols such as **111**. The use of lithium aluminium hydride instead of the originally suggested sodium borohydride[127] also seems to minimize the 1,4-addition[130]. Table 4 provides some further examples of this reaction.

VI. SULPHUR EXTRUSION REACTIONS

Reactions in which a sulphur atom that bridges or interconnects two carbon groups is extruded with formation of a carbon—carbon bond between the two carbon groups is termed a sulphur extrusion reaction (equation 90). These types of reactions have proven to be of synthetic utility and are treated in this section.

$$R{-}S{-}R \longrightarrow R{-}R + S \qquad (90)$$

Thiols can serve as reagents in the extrusion reaction by being converted to a sulphide or a corresponding higher oxidized derivative upon which the extrusion process is effected. While for many of the cases covered in this section the organosulphur compound used in the extrusion reaction was not prepared directly from a thiol, the potential exists for thiols to be utilized in these types of reactions.

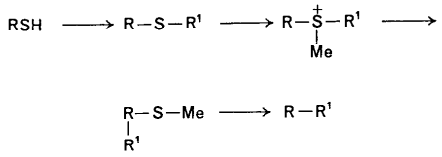
A. Stevens Rearrangement of Sulphonium Salts

The Stevens rearrangement of a sulphonium salt[136] involves treatment of the salt with base and leads to migration of a group from sulphur to an adjacent carbon atom (equation 91). Analogous Stevens rearrangement of ammonium salts[137] and the related Wittig rearrangement[137] of ethers are well known.

$$R{-}\overset{+}{S}(R){-}CH_2R^1 \xrightarrow{\text{base}} R{-}S{-}CHR^1 \qquad (91)$$

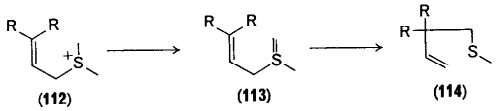
The sulphonium salts used in the Stevens rearrangement need not be prepared initially from a thiol; however, this is feasible and is often the case. This method, therefore, allows the conversion of a thiol to a sulphonium salt, followed by rearrangement with concomitant carbon—carbon bond formation. Removal of the sulphur moiety following rearrangement permits, in effect, a thiol to function in a reaction that leads to bond formation between two R groups that originally were attached to sulphur (equation 92).

The Stevens rearrangement of sulphonium salts is known to proceed through the intermediacy of the corresponding sulphonium ylid[138]. There appears to be two distinct mechanistic pathways, depending upon the structure of the ylid, leading to rearranged product. Rearrangement of



(92)

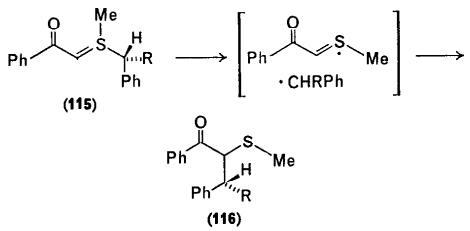
allyl sulphonium salts[139] (**112**), proceeding via the ylid **113**, has been shown[140] to occur by a [2,3] sigmatropic reaction (equation 93); a minor amount of product also arises by what is equivalent to a [1,2] shift[140]. These rearrangements are examples of what appear to be a general class of electrocyclic reactions of sulphonium ylids[141].



(93)

(112) (113) (114)

A second type of rearrangement involves ylids derived from non-allyl sulphonium salts. Baldwin and coworkers[142] have reported that rearrangement of the sulphonium ylid **115** in toluene at reflux temperatures occurs by a radical pair mechanism (equation 94), in which the benzyl group migrates with predominant retention of configuration to yield **116**.



(94)

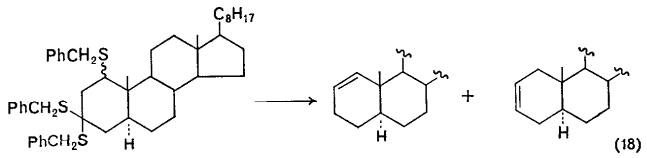
(115) (116)

Thompson and Stevens[143], in their first paper on the rearrangement of sulphonium salts, reported obtaining the sulphide **116** upon treatment of **117** with sodium methoxide. However, more recent work has shown[138,144]

method led to an isotopic mixture consisting of 4% d_0, 44% d_1, 49% d_2 and 3% d_3 products[48]. At times fairly pure products are obtained, such as the preparation of 12,12-d_2-pregnane (**12**) with 76% d_2[49].

2. Reduction to olefins

The formation of an olefin during desulphurization was first noted when 1,3,3-tribenzylthiocholestane gave a mixture of cholest-1-ene and cholest-2-ene (equation 18) with Raney nickel deactivated by boiling in acetone[50]. Similar conditions gave predominantly olefin with the 1,3-dithiolane from 14β-$\Delta^{5,7,9}$-anthraergostatriene-15-one (equation 19)[51].



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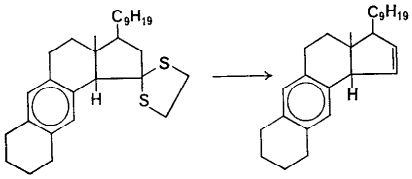
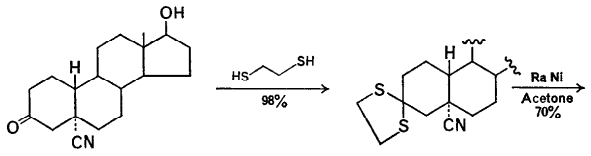


(19)

More extensive investigations[46,52] have led to the use of W-2 Raney nickel in refluxing acetone to obtain olefins in 55–75% yields based on starting ketone. Even the synthesis of dienes from α,β-unsaturated ketones was successful[52]. Using this method 5α-cyano-17β-hydroxyestran-3-one was converted to the corresponding olefin (equation 20)[53]. Surprisingly, the 5β-cyano isomer gave low yields in the first step and no olefin in the second step. Both the *cis*- and *trans*-isomers in the 2-keto-10-cyano series have been converted to olefins[54,55]. Other examples of this reaction include the conversion of dihydrogedunin (**13**) to the olefin[56] and the partial formation of olefin from 17-norphyllocladan-16-one (**14**)[57]. Groups in the α-position to the ketone may be lost during the reaction as seen by the formation of 5α-cholest-2-ene as the sole product from 2α-chloro-5α-cholestan-3-one[58].



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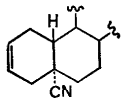

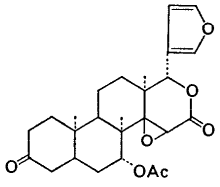

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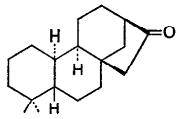
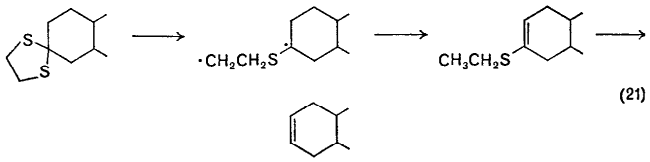


(14)

The mechanism of this reaction seems to involve formation of a diradical intermediate which, if the concentration of hydrogen radicals is low, gives the thioenol ether[46]. Further desulphurization gives the olefin (equation 21). If the alkyl radical is responsible for the $C_{(2)}$ hydrogen



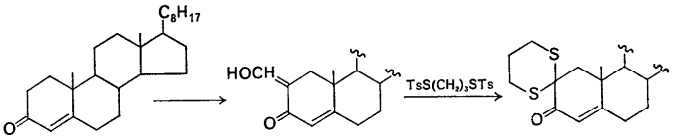
(21)

abstraction, it would seem necessary that it remain near the reaction site so that homolysis takes place before addition of hydrogen from the catalyst. Analogy with studies of the mechanism of desulphurization in monothioacetals[59] and thiazolidines[60] suggests that the abstraction may very well come from an external radical.

C. Methylene Blocking Group

In the presence of ethyl formate and sodium methoxide, the most reactive methylene group of a ketone is converted to its hydroxymethylene

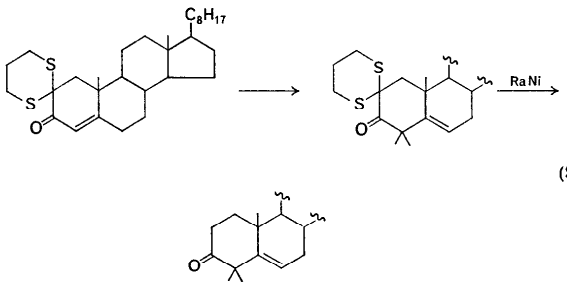
derivative[61]. Further reaction with the ditosylate of propane-1,3-dithiol[62] leads to the formation of the 1,3-dithiane[63] (equation 22). Thus the active position of the ketone is effectively blocked with a group easily removed by Raney nickel.



(22)

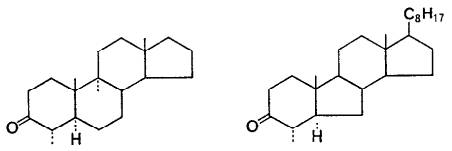
I. Alkylations

The presence of the dithioacetal does reduce the reactivity of the ketone toward alkylations at its other available positions[64], but nevertheless the sequence has been effectively utilized. This is clearly demonstrated by the formation of 4,4-dimethylcholestenone by this procedure (equation 23)[63]. Other examples of the successful use of this method include the



(23)

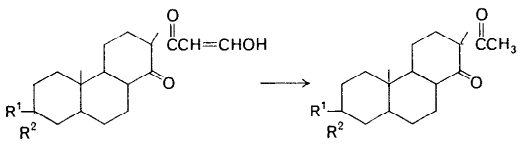
preparation of 4α,9α-dimethyl-5α-androstan-3-one (**15**)[65] and 4α-methyl-B-nor-5α-cholestan-3-one (**16**)[66].



(15) (16)

2. Decarbonylations

The formation of 1,3-dithianes from hydroxymethylene compounds, which are enol tautomers of β-keto aldehydes, has been shown to be useful in itself. When followed by desulphurization the net reaction is the decarbonylation to the ketone. This has been used to advantage in the formation of the methyl ketone (**17**)[67] in equation (24). Similarly the methyl ketone (**18**) was formed from its hydroxymethylene derivative[68].

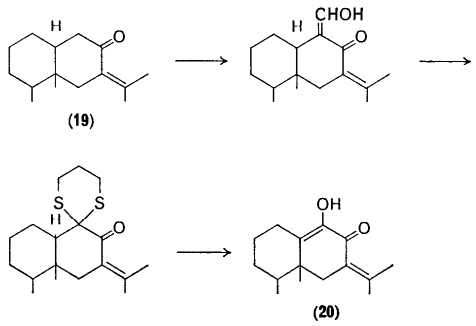


(24)

(**17**) R¹ = OAc, R² = H
(**18**) R¹ = CO_2Me, R² = Me

3. Formation of dicarbonyl compounds

The treatment of the intermediate 1,3-dithiane, either before or after alkylation with reagents such as mercuric chloride–cadmium carbonate (see section II.A.2) gives hydrolysis to the carbonyl. Thus *trans*-fukinone (**19**) was converted to (+)-hydroxyeremophilone (**20**) (equation 25)[69]. In *cis*-fukinone, the 1,3-dithiane could not be formed from the 1-hydroxymethylene fukinone, presumably for steric reasons.



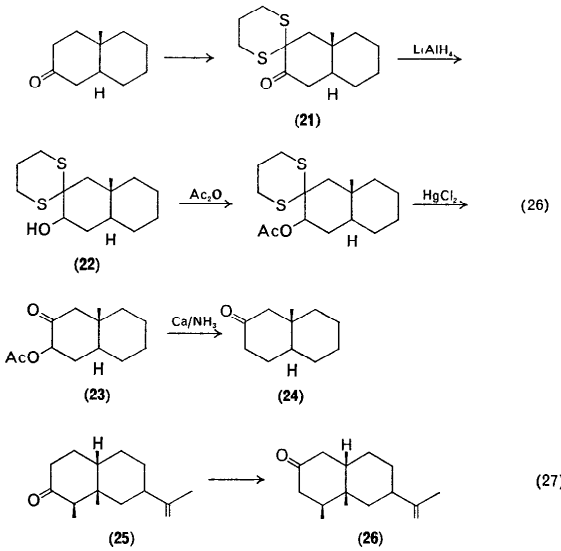
(**19**)

(25)

(**20**)

4. Ketone transposition

Modification of the above sequence to include reduction of the original ketone before hydrolysis is the basis for a new method of ketone transposition[70]. For example, the keto 1,3-dithiane (**21**) was prepared in the usual manner followed by reduction of the carbonyl with lithium aluminium hydride to the alcohol (**22**). Conversion to the acetate and hydrolysis of the dithiane with mercuric chloride led to the keto acetate (**23**). Reduction with calcium in ammonia resulted in the formation of the new methyl decalone (**24**) in 58% overall yield (equation 26). The same



(**21**)

(**22**)

(**23**) (**24**)

(26)

(**25**) (**26**)

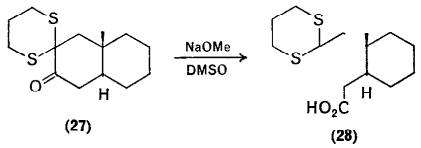
(27)

sequence was used to convert decalone (**25**) into the isomeric decalone (**26**) in 46% overall yield (equation 27). These conversions have been shown to take place with complete stereochemical integrity. Alternative methods of removing the carbonyl from the keto 1,3-dithiane so far have not been satisfactory.

5. Selective carbon—carbon bond cleavage

Keto 1,3-dithianes are susceptible to nucleophilic attack at the carbonyl with subsequent cleavage occurring preferentially between the carbonyl and the dithiane functions[71]. In the one instance reported, the keto dithiane (**27**) was cleaved with sodium methoxide in dimethyl sulphoxide to acid (**28**). The explanation as to why the acid is formed instead of the

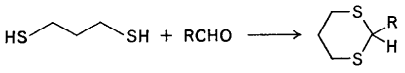
methyl ester is not apparent. The reaction conditions are mild and do not seem to put serious limitations on the nature of the rest of the molecule. Most importantly, after cleavage, the 1,3-dithiane grouping is suitable for many conversions such as reduction, alkylation, acylation or hydrolysis.



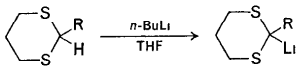
(27) (28) (28)

D. Synthetic Applications of 2-Lithio-1,3-dithianes

Corey and Seebach have reported[72] the use of 2-lithio-1,3-dithianes as useful reagents in organic synthesis. The method involves the use of 1,3-propanedithiol, which is caused to react with an aldehyde to yield the 1,3-dithiane (equation 29). Lithiation of the dithiane, normally with *n*-butyllithium in tetrahydrofuran at lowered temperatures, gives the 2-lithio-1,3-dithiane (equation 30). The R group can be alkyl, aryl or hydrogen.



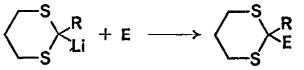
(29)



(30)

2-Lithio-1,3-dithianes have been shown[73] to undergo reaction with a variety of electrophiles, E, to give substituted dithianes (equation 31). Removal of the dithioketal function generates the newly synthesized carbonyl compound (equation 32) having the group E substituted for the aldehydic hydrogen of the original aldehyde. The dithioketal is most often hydrolysed using the mercuric chloride : mercuric oxide method[26] or by oxidative hydrolysis with N-halosuccinimides[27]. It is possible also to remove the dithioacetal function by desulphurization (Raney Ni) to yield the corresponding methylene derivative (equation 33). For a general treatment of removal of the dithioacetal function, see section II.A.2.

2-Lithio-1,3-dithiane reagents are in effect masked nucleophilic acylating agents and can be considered equivalent to the presently unknown



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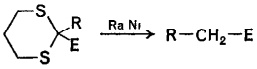
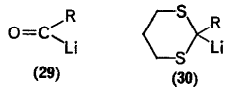
(32)



(33)

acyllithium reagent (**29**). Thus, by use of a thiol, the carbonyl carbon of an aldehyde can be transformed from an electrophilic site to the nucleophilic centre in the lithiated dithiane derivative (**30**). The ability of sulphur to



(29) (30) (34)

stabilize carbanions α to the sulphur atom is significant in the readily accomplished lithiation of 1,3-dithianes. The preparation and reactions of 2-lithio-1,3-dithianes have been reviewed[73].

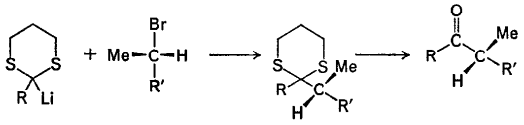
The following is a general outline of the various types of reactions that these reagents are known to undergo, including a comprehensive treatment of reactions reported since the review article by Seebach[73].

1. Reaction with alkyl halides

2-Lithio-1,3-dithianes undergo alkylation at the 2-position upon reaction with alkyl halides. This reaction appears to be S_N2 in nature as it is applicable to primary and secondary alkyl halides[72], occurs most readily with alkyl iodides[72], and with optically active secondary halides gives inverted products[74]. It has been shown[41,72] that reaction with optically active alkyl halides provides a useful route for the preparation of optically active aldehydes or ketones (equations 35 and 36).

Cycloalkylation has been effected by reaction with α,ω-dihaloalkanes to give, upon hydrolysis, cyclic ketones[72,75] (equation 37). Likewise, the

dithiane derivatives of α,β-unsaturated aldehydes undergo hydrochlorination followed by cycloalkylation to yield substituted cyclopropanes (equation 38)[76c]. Cyclic 1,3-diones are available[72] by the



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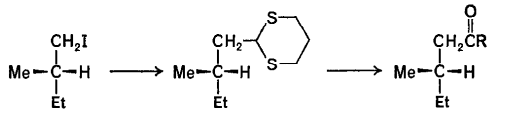
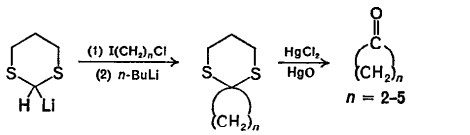


(36)

alkylation of the bis-dithiane **31** (equation 39). It also has been observed that the use of α,ω-dibromoalkanes in cycloalkylation reactions is complicated by formation of sulphonium salts (equation 40), a reaction not observed with use of α-chloro-ω-iodo or α,ω-dichloroalkanes.



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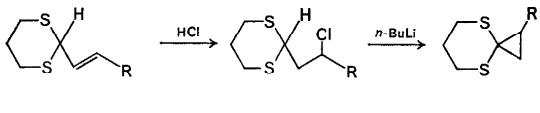

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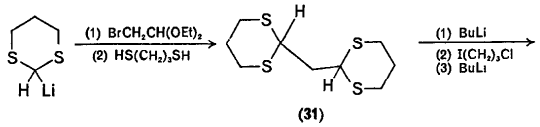

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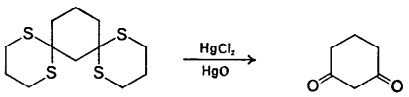
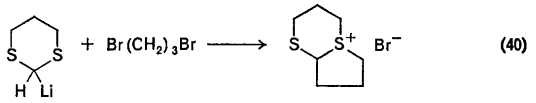
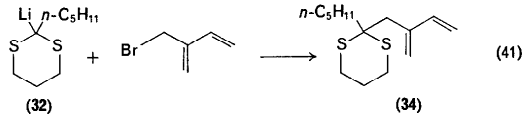




(40)

Corey and coworkers[76a], in a synthesis of prostaglandins, prepared diene **34** by alkylation of the lithiodithiane **32** with 2-bromomethyl-1,3-butadiene (equation 41). A synthesis of jasmone (**35**), in an overall yield of 50%, has been reported by Ellison and Woessner[76b] in which the bisdithianylethane **33** was sequentially alkylated, followed by hydrolysis and cyclization (equation 42). A similar route for preparation of 4-hydroxy-2-cyclopenten-1-ones has been reported[77]. This method appears to provide a general route to 1,4-diketones *via* 1,3-dithianes.



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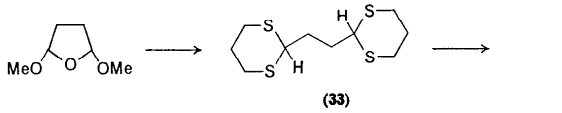
(32) (34)



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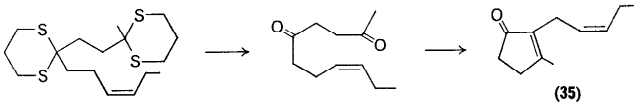
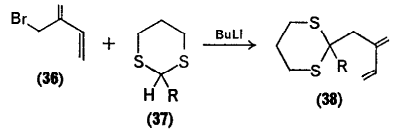


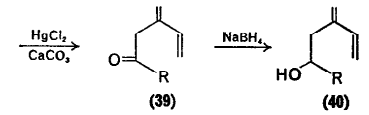
(35)

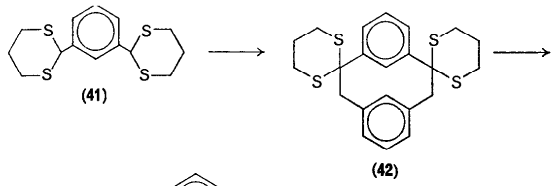
The synthesis of the monoterpene components **40** of the sex attractant of the bark beetle has been accomplished[78] as outlined in equation (43). The alkylation of the dithiane **37** was a key step in the synthesis since efforts to prepare **40** by addition of the magnesium or lithium derivatives of the bromoalkene **36** to the appropriate aldehyde failed.

Hylton and Boekelheide[79] prepared the cyclophanedione **43** by alkylation of the bisdithiane **41** followed by hydrolysis. An improved procedure for the preparation of **41** has been reported[80].

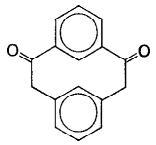


$R = -CH_2CH(CH_3)_2$ or $-CH{=}C(CH_3)_2$ (43)



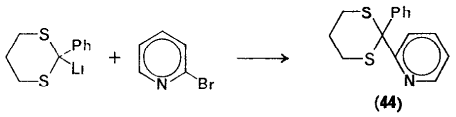


(44)



2. Reaction with aryl halides

Treatment of 2-lithio-2-phenyl-1,3-dithiane with 2-bromopyridine gave the substituted pyridine **44** in 50% yield[81]. However, reaction with 2,4-dinitrobromobenzene gave none of the substitution product, but rather compound **45** resulting from oxidative dimerization of the dithiane



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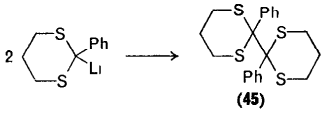
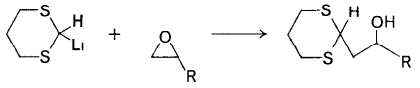


(46)

moiety[91]. Such oxidative dimerizations (see section II.D.7) of 2-lithio-1,3-dithianes are known and have been reported[72] to occur with nitro compounds.

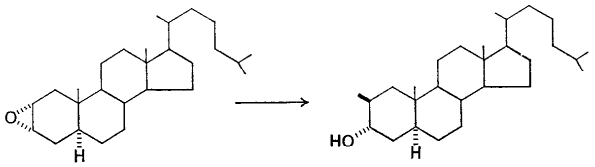
3. Reaction with epoxides

Epoxides effect alkylation of 2-lithio-1,3-dithianes[72,75] (equation 47); opening of the epoxide ring occurs in the fashion typical of reactions with nucleophiles. The reported yields are in the range of 70–95% and appear to be free of side reactions common with other organometallic reagents[73].



(47)

Recently, Jones and Grayshan[82-84] have reported the reaction of lithiodithiane derivatives with steroidal epoxides to effect preparation of modified steroids. Treatment of 2α,3α-oxiranyl-5α-cholestane (**46**) with 2-lithio-1,3-dithiane, followed by desulphurization, yielded the 2β-methyl-3α-cholestanol **47** (equation 48). Conversely, reaction with the epimeric epoxide **48** furnished 3α-methyl-5α-cholestan-2β-ol (**49**) (equation 49)[82].



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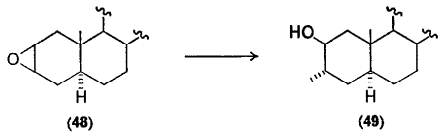
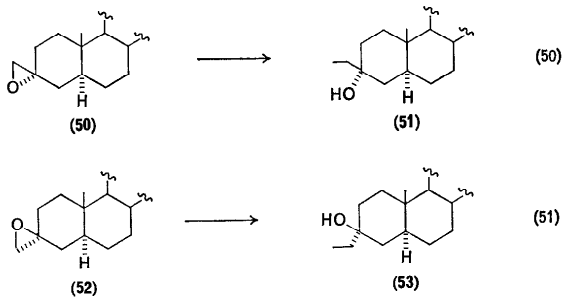


(49)

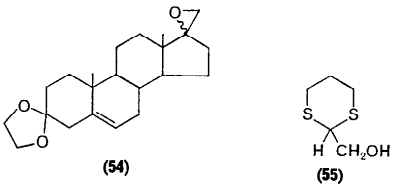
The spiroepoxide **50**, prepared from 5α-cholestan-3-one, was cleanly converted to the 3β-ethyl derivative **51**; the 3α-ethyl derivative **53** was obtained in an analogous manner from the epimeric spiroepoxide **52**[83] (equation 51). Similar results were obtained[34] when this method was applied



(50)

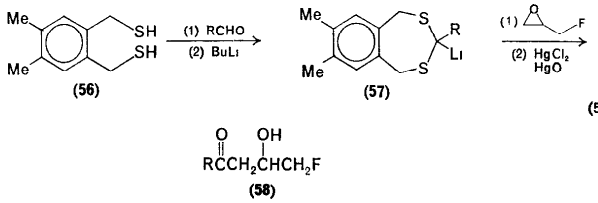
(51)

to the epimeric spiroepoxide **54**. This method appears to be the most suitable synthetic route to these modified steroids. However, attempts to utilize the lithium derivative of 2-hydroxymethyl-1,3-dithiane (**55**), or the corresponding tetrahydropyran derivative, to prepare corticoid steroids were unfruitful[84].



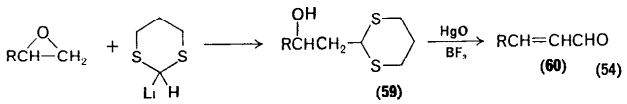
(52)

The preparation of some γ-fluoro-β-hydroxyketones (**58**) by reaction of epifluorohydrin with the lithio derivative **57** has been reported[85]. The dithioacetals prepared from dithiol **56** are reported to be crystalline, odourless compounds[86], therefore some advantage may be purported for their use.



(53)

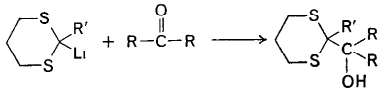
A synthesis[28] of α,β-unsaturated aldehydes has been effected by reaction of 2-lithio-1,3-dithiane with epoxides (equation 54). It was found that treatment of the dithianyl alcohol **59** with mercuric oxide–boron trifluoride caused dehydration and hydrolysis to give the α,β-unsaturated aldehyde **60** in good yield. Standard methods for removal of the thioacetal function were not successful in these cases.



(54)

4. Reaction with aldehydes and ketones

2-Lithio-1,3-dithianes add to the carbonyl group of aldehydes and ketones to provide mercaptal derivatives of α-hydroxy aldehydes or ketones[72] (equation 55). The yields are normally quite high. Reaction with α,β-unsaturated ketones has been observed[72,87] to give only 1,2-addition; however, Seebach and Lietz have reported[88] 1,4-addition to occur in reactions with α,β-unsaturated nitro derivatives (equation 56). In the case



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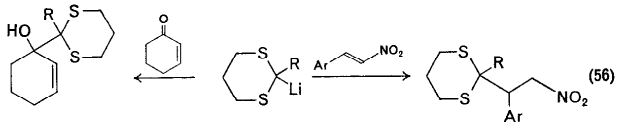
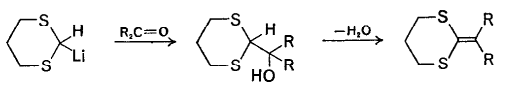


(56)

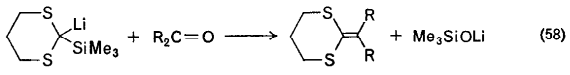
where R′ = H, the addition product obtained from reaction with a ketone can be converted by dehydration to a ketene thioacetal (equation 57).



(57)

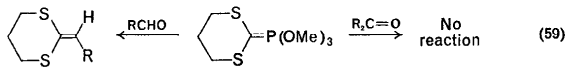
Ketene thioacetals also are readily available[89,90] by a Wittig-type reaction of 2-lithio-2-trimethylsilyl-1,3-dithiane (**61**) with aldehydes or

ketones. The dithiane **61** is prepared[40,91] by reaction of 2-lithio-1,3-dithiane with trimethylchlorosilane followed by lithiation (see section II.D.6). A method employing the phosphite ylid **62** to prepare ketene thioacetals by reaction with aldehydes, but not ketones, has been reported[92] (equation 59).



(58)

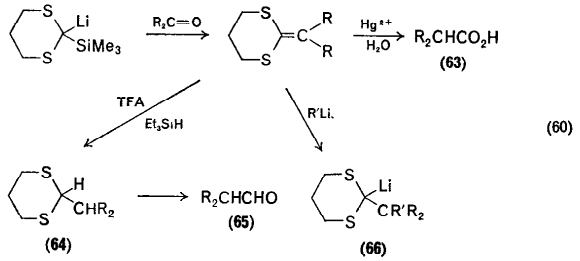
(61)



(59)

(62)

Ketene thioacetals should prove to be useful synthetic intermediates. Hydrolysis[92] of ketene thioacetals yields carboxylic acids (**63**), while protonation–hydride transfer using trifluoroacetic acid-triethylsilane, as reported by Carey and Neergaard[93], provides the thioacetal (**64**) of the homologous aldehyde (**65**).

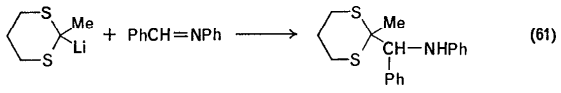


(60)

(64) (65) (66)

Alkyllithium reagents are known[94] to add to ketene thioacetals to give 2-lithio-1,3-dithianes **66** in which R′ has become attached to the ethylidene carbon. Both **64** and **66** are capable of undergoing further reactions available to 2-lithio-1,3-dithianes. Therefore, it should be possible in principle to convert an aldehyde, RCHO, to any of the following *via* the corresponding ketene dithioacetal: RCH_2CO_2H, RCH_2CHO, RR′CHCHO, RR′CHCOR″, and RR′R″CCOR″.

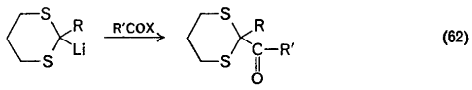
Imines, being nitrogen analogues of carbonyl compounds, are reported[72] to undergo addition with 2-lithio-1,3-dithianes to yield amines (equation 61).



(61)

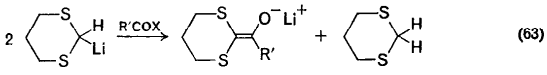
5. Reaction with acylating agents

Acylation of 2-lithio-1,3-dithiane derivatives occurs in satisfactory yields only when a dilute solution of the dithiane derivative is added at −78°C to a solution containing a 20–100-fold excess of the acylating agent[72,73] (equation 62). The above conditions are necessary to circumvent reaction of a molecule of the reactive lithiodithiane with a molecule of previously formed 2-acyldithiane. This method offers, by subsequent removal of the dithioketal function, a route for the preparation of 1,2-dicarbonyl compounds.



(62)

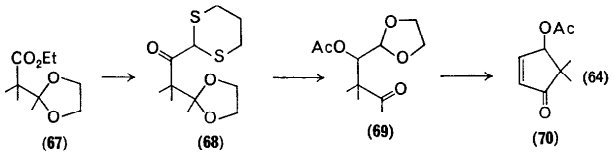
Acylating agents that have been employed[72] are carbon dioxide, alkyl chloroformates, alkyl formates, acid chlorides, esters, benzonitrile and dimethylformamide; the expected acylation products from reaction with the above reagents were formed in each case. However, the N,N-dimethylamide derivatives of higher carboxylic acids did not yield acylated product as in the case of dimethylformamide[73]. When R = H (equation 63), it was necessary to employ two equivalents of the lithiodithiane due to product enolate formation.



(63)

In the total synthesis of illudin M, Matsumoto and coworkers[95] prepared the cyclopentenone **70** by reaction of 2-lithio-1,3-dithiane with

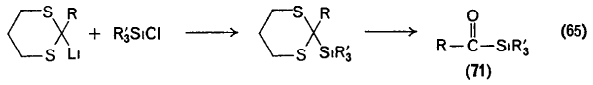
the ester **67** to give **68**. Reduction, acetylation and removal of the dithioacetal function gave **69**, apparently formed by an intramolecular transketalization reaction.



(67) (68) (69) (70)

6. Silylation and related reactions

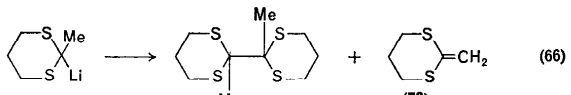
2-Lithio-1,3-dithianes react with trialkyl- and triaryl-chlorosilanes to give the 2-silylated derivatives (equation 65). This method was used in the preparation[40,91] of the previously unknown α-silylketones **71**. Germanylation and stannylation also can be accomplished with the corresponding trialkylhalo derivatives[40].



(71)

7. Oxidative dimerization

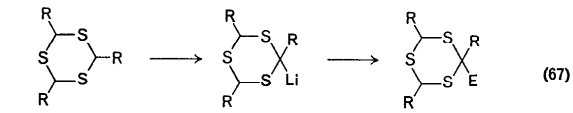
Treatment of 2-lithio-1,3-dithianes with iodine, cupric salts, 1,2-dibromoethane, or nitro compounds effects oxidative dimerization[73] to give the dimer **72** plus a small amount of the 2-methylene derivative **73**.



(72) (73)

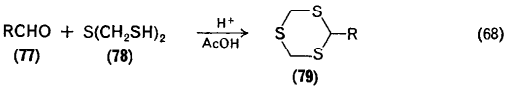
8. Reactions using 1,3,5-trithianes

1,3,5-Trithianes (**74**) undergo lithiation[41,73] with an equivalent of *n*-butyllithium to yield the 2-lithio derivatives, which substances undergo the usual reactions (equation 67) as with 2-lithio-1,3-dithianes. Since additional active hydrogens are present in 1,3,5-trithianes, dimethylation has been observed in some cases[73].



(74) R = H or alkyl (75) (76)

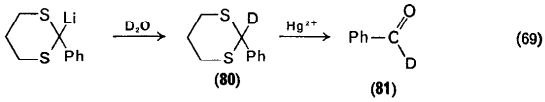
An alternate route not involving 2-lithio-1,3,5-trithianes for the preparation of 2-substituted-1,3,5-trithianes recently has been reported[96]. This method involves reaction of an aldehyde **77** with the dithiol **78** to yield the 2-substituted trithiane **79**.



(77) (78) (79)

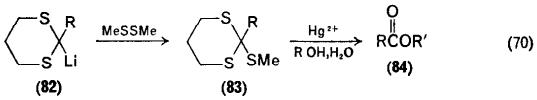
9. Miscellaneous applications

A convenient preparation of 1-deuterioaldehydes (**81**) *via* 2-lithio-1,3-dithianes has been reported by Seebach and coworkers[27] (equation 69). This method appears to be superior to previously reported methods for the preparation of 1-deuterioaldehydes.



(80) (81)

Treatment of 2-lithio-1,3-dithiane derivatives with methyl disulphide yields the orthothioformate **83**, which upon hydrolysis in alcoholic solvents furnishes an ester[97]. This method may provide a useful route for the conversion of sensitive aldehydes to esters and carboxylic acids.



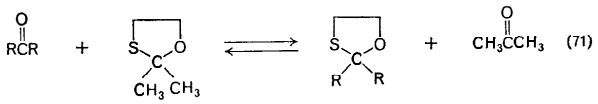
(82) (83) (84)

III. MONOTHIOACETALS

The use of monothioacetals in organic synthesis has not been nearly so extensive as the use of dithioacetals. Generally prepared as 1,3-oxathiolanes and 1,3-oxathianes, the group is resistant toward dilute base and lithium aluminium hydride[14]. Regeneration of the carbonyl is easily accomplished.

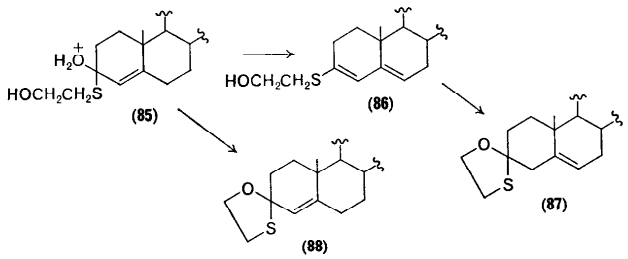
A. Preparation

Condensation of 2-mercaptoethanol or 3-mercaptopropanol with ketones is usually achieved with the aid of an acid catalyst. Hydrogen chloride has been used[98] but more common agents are boron trifluoride[10], freshly fused zinc chloride[99] or *p*-toluenesulphonic acid[100]. An exchange method between 2,2-dimethyl-1,3-oxathiolane or 2,2-dimethyl-1,3-oxathiane and a non-volatile ketone leads to formation of the new monothioacetal and acetone[100]. The equilibrium is displaced by continuous distillation of the acetone formed (equation 71). With saturated ketones, mostly steroids, the yields of the above methods are comparable and are usually in the 60–90% range. With α,β-unsaturated ketones, the yields were significantly lower[100].



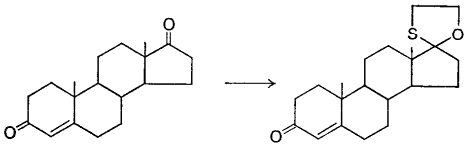
(71)

Unlike the case of 1,3-dithiolane formation, 1,3-oxathiolanes from α,β-unsaturated ketones show a shift of the double bond. It has been proposed[100] that intermediate **85** may undergo nucleophilic attack by the hydroxyl leading to unrearranged product **88**. Alternatively, dehydration would give the conjugated diene **86**, to which the hydroxyl could add giving the rearranged product **87**. Obviously, with ethanedithiol nucleophilic attack of the sulphur must predominate, while with the less nucleophilic hydroxyl, prior dehydration occurs. This is in agreement with the fact that with ethanediol the resulting ketal shows a shifted double bond.



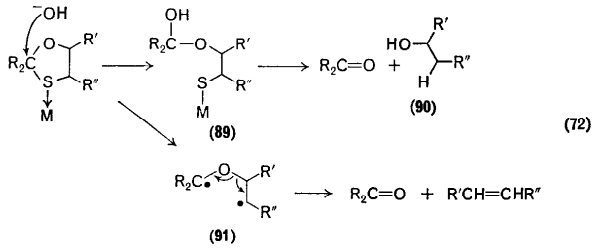
The reduced reactivity of α,β-unsaturated ketones towards 3-mercaptoethanol allows preferential formation of the hemithioacetal of an unconjugated carbonyl present in the molecule. One example of this general

phenomenon is given below in which 4-androstene-3,17-dione was converted to the 17-(1,3-oxathiolane) with zinc chloride catalysis[99]. With *p*-toluenesulphonic acid catalysis, the 3,17-bis(1,3-oxathiolane) could be formed in low yield.



B. Removal

Unlikc 1,3-dithiolanes, treatment of 1,3-oxathiolanes with Raney nickel gives regeneration of the carbonyl group[99]. Thus, protection of a carbonyl by condensation with 2-mercaptoethanol allows regeneration in high yields under neutral conditions. Surprisingly, in the usual alcohol or acetone solvent, the ketonic oxygen is not from the oxathiolane. Apparently, association of the sulphur with the electron-deficient metal (equation 72) causes activation of the ring followed by attack of a hydroxide, either from the media or combined with the metal, to give the hemiketal **89**. Normal work-up cleaves the hemiketal which, with further desulphurization, leads to formation of the ketone and the alcohol **90**[59,101]. Solvents such as benzene may also be used and under the right conditions lead to high yields of the ketone[101]. In nonpolar solvents,

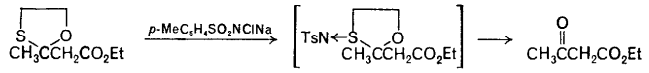


(72)

ionic intermediates are presumably not involved and the diradical (**91**) is the accepted intermediate[59,101]. The desulphurization of 1,3-oxathianes behaves similarly with the ketone being the major product[101]. Additional information may be found in the previously mentioned reviews[22,23,43].

The hydrolysis of 1,3-oxathiolanes with acid[102, 103] or mercuric ion[101] also provides a suitable procedure for regenerating the ketone. The mechanism involved appears similar to that with Raney nickel, but with a proton or mercuric ion taking the place of the nickel.

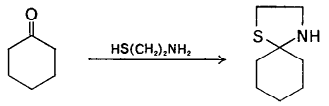
The most recent method of removal of the 1,3-oxathiolane group is by the use of N-chloro-*p*-toluenesulphonamide(chloramine-T)[104] in water, methanol or ethanol (equation 73). Again basically the same mechanism appears involved with prior association of the sulphur to form an unstable sulphilimine. The reaction times are short (2 min), conditions are mild and yields are high (85–100%).



(73)

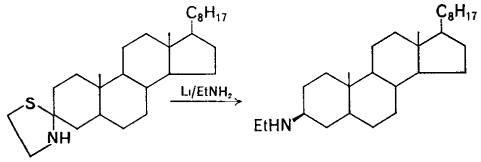
IV. THIAZOLIDINES

Just as 2-mercaptoethanol will condense with ketones to produce 1,3-oxathiolanes, so will 2-mercaptoethylamine react to produce thiazolidines[60]. Usually *p*-toluenesulphonic acid is used as a catalyst in benzene with yields being quite good, 94% in the case of cyclohexanone (equation 74). The uses of the thiazolidines have not been throughly



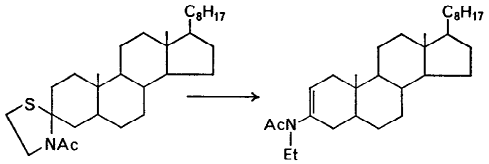
(74)

investigated, but it appears that they offer no advantages over previously mentioned protecting groups. Although Raney nickel desulphurization gives unsatisfactory yields of the starting ketone, lithium in ethylamine offers promise in the preparation of amines. 3β-Ethylamino-5α-cholestane was prepared in 87% yield[60] when desulphurized in this manner (equation 75).



(75)

More thoroughly investigated has been the desulphurization of N-acetylated thiazolidines to form acetylated enamines. Thus 31-day-old Raney nickel in benzene gives a 90% yield of 3-(N-ethylacetamido)-5α-cholest-2-ene (equation 76) from the corresponding N-acetylthiazolidine[60, 105]. The conditions for this reaction are rather sensitive to solvent



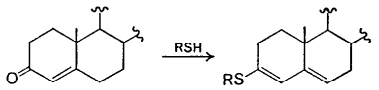
(76)

and catalyst age. The unsaturated amide is the favoured product in benzene with aged catalyst, but with fresh catalyst the major product in acetone is the ketone and in ethanol the saturated amide. The mechanism of desulphurization is believed[60] to be similar to the first step in the formation of olefins from 1,3-dithiolanes (see section II.B.2).

V. THIOENOL ETHERS

A. Carbonyl Protecting Group

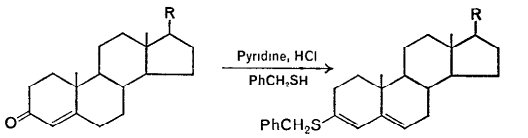
It has been noted (see section III.A) that protecting reagents such as 2-mercaptoethanol react preferentially with the saturated carbonyl when it is in the presence of an α,β-unsaturated carbonyl. Thioenol ethers are equally useful because they are formed almost exclusively from α,β-unsaturated carbonyls (equation 77).



(77)

Normally the reaction of thiols with carbonyls, saturated or unsaturated, leads to the formation of dithioacetals when acid catalysts such as zinc chloride or *p*-toluene-sulphonic acid are present (see section II.A.1). Occasionally, under special reaction conditions thioenol ethers have been formed using these same catalysts[106, 107], but never in the presence of acid-sensitive substituents. Pyridine hydrochloride as the catalyst has been successfully used to give excellent yields of the thioenol ethers of Δ^4-3-ketosteroids even in the presence of sensitive groups[108]. Thus, desoxycorticosterone acetate (**92**) was converted to its 3-benzylthioenol ether

(93) in 60% yield (equation 78). The selectivity of the reaction using these conditions is very high. Unlike the case with zinc chloride, progesterone (94) with pyridine hydrochloride and benzyl mercaptan gives no observable reaction at $C_{(20)}$ with the only product being progesterone 3-benzyl thioenol ether (95)[108].

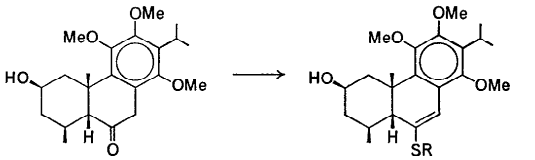


(78)

(92) R = $COCH_2OAc$
(94) R = $COCH_3$

(93) R = $COCH_2OAc$
(95) R = $COCH_3$

Other condensing agents which have proved useful under certain conditions are boron trifluoride[8], formic acid with *p*-toluenesulphonic acid[8] and hydrochloric acid in acetic acid[8,107]. One unusual example of a thioenol ether formed from a saturated ketone has been reported using hydrogen chloride as the catalyst[109]. In this case, compound **96** was converted to either its benzylthioenol ether **97** or its ethylthioenol ether **98** (equation 79). Benzyl mercaptan normally seems to be the reagent of choice in most conversions because of its easily crystallized products.

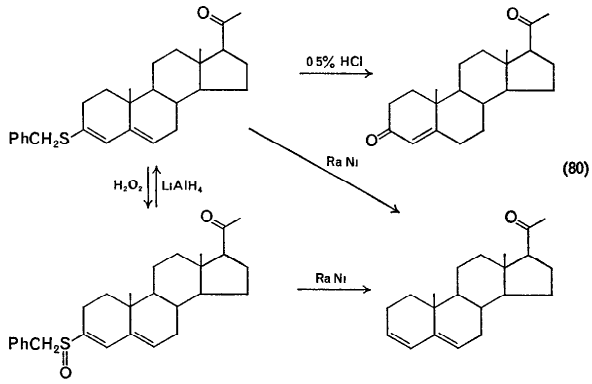


(79)

(96)

(97) R = $PhCH_2$—
(98) R = Et

The thioenol ethers are stable towards base[8] and lithium aluminium hydride[106,108], but are reconverted to the parent compound on dilute acid hydrolysis. Raney nickel desulphurization can be used to form the diene[108]. Hydrogen peroxide oxidation will convert the acid-labile thioenol ether to an acid-stable sulphoxidoenol ether[8,108]. The sulphoxidoenol ether may be desulphurized with Raney nickel to the diene, or with lithium aluminium hydride reconverted to the thioenol ether for hydrolysis to the α,β-unsaturated ketone[108]. These reactions are depicted in equation (80).

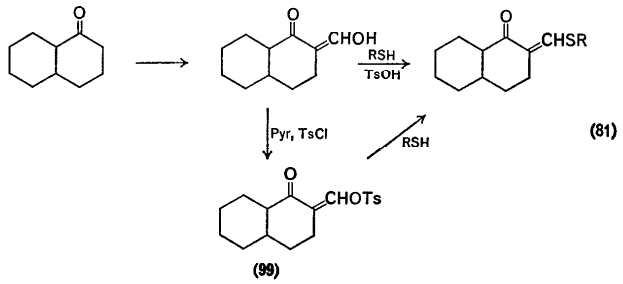


(80)

B. Methylene Blocking Group

In the continuing search for the ideal methylene blocking group, considerable effort has been expended in looking at derivatives of hydroxymethylenes. These are readily prepared from a ketone, ethylformate and sodium methoxide[61].

Ireland and Marshall[64] found that alkanethiols form very versatile derivatives with hydroxymethylenes. The reaction with a thiol, accompanied by a *p*-toluensulphonic acid catalysed water separation, leads to formation of the corresponding thioenol ether (equation 81). If acid-labile substituents are present, a procedure involving displacement from an intermediate tosylate (**99**) by the thiol is used. Although other thiols

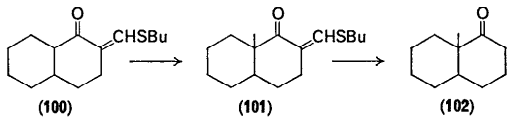


(81)

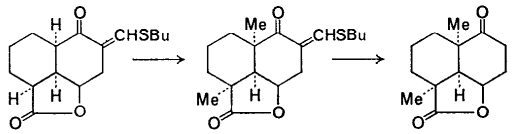
(99)

have been used, *n*-butanethiol appears to be the most convenient in this reaction. The yields of the thioenol ethers from hydroxymethylenes are generally greater than 80% using the acid catalysed method and only slightly lower with the basic pyridine procedure.

Alkylations of the protected ketones are very facile. The thioenol ether generally need only be left in contact with the base a few minutes before addition of the alkyl halide. Such short contact with the base allows easy isolation of the alkylated, blocked ketones[64]. Thus, 2-*n*-butylthiomethylene-1-decalone (**100**) was converted to 9-methyl-2-butylthiomethylene-1-decalone (**101**) in 85% yield. This procedure was used in the difficult dimethylation of **103** to give the lactone **104**.



(100) (101) (102) (82)



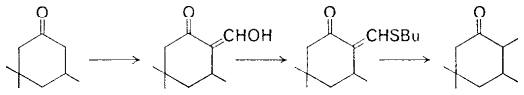
(103) (104) (105) (83)

Although the *n*-butylthiomethylene group is subject to acid hydrolysis, basic conditions for hydrolysis have been developed[64] and these seem to be preferred in actual practice. A typical procedure uses a mixture of a 25% aqueous potassium hydroxide solution with ethylene glycol heated at reflux. In this manner thioenol ether **101** was converted to 9-methyl-1-decalone (**102**) in 78% yield[64] (equation 82). The rare use of acid hydrolysis is exemplified by the use of concentrated hydrochloric acid to hydrolyse the blocked lactone (**104**) to **105** (equation 83)[110]. Additional examples of conversions using a thioenol ether intermediate are shown in Table 2.

C. Monomethylation via Reduction

Just as the blocking of active sites to permit alkylations on less reactive sites has been a recurring problem, so has the problem of preventing polyalkylations on reactive sites. The use of the alkylthiomethylene group offers a convenient intermediate from which monomethylated products are prepared by desulphurization with Raney nickel. In this way, 2,3,5,5-tetramethylcyclohexanone was prepared[119] in 58% overall yield from

3,3,5-trimethylcyclohexanone (equation 84). The same procedure was used[120] in the conversion of 7-oxobicyclo[3.2.1]octane to the 6-methyl derivative (equation 85). Similarly, 10-carbethoxy-2,7,7-trimethyl-*cis*-



(84)

TABLE 2. Alkylation of ketones using thioenol ethers as a methylene blocking group

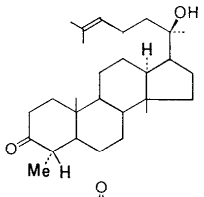
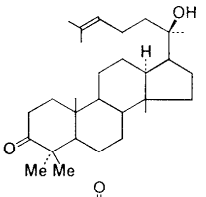
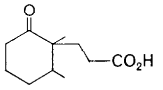
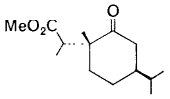
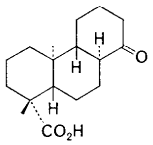
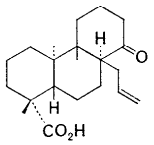
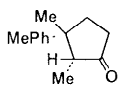
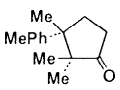
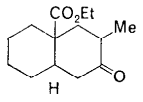
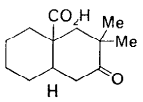
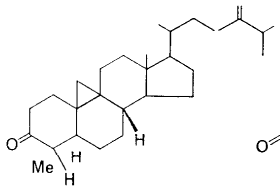
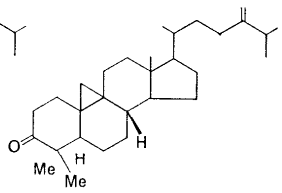
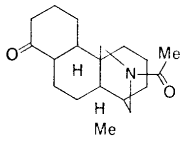
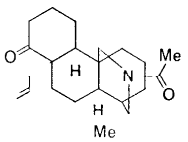
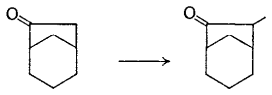
Reactant	Product	Overall yield, %	Reference
		73	111
		60	112
		62	113
		82	114
		31	115

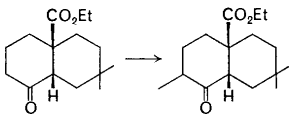
TABLE 2 (*cont.*)

Reactant	Product	Overall yield %	Reference
		62	116
		35	117
		36	118

decal-1-one (equation 86) was prepared in 73% overall yield using this method[121]. In those cases where partial reduction of the carbonyl accompanies desulphurization, the crude mixture is oxidized before purification[119].

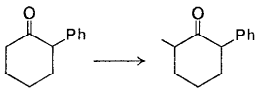


(85)



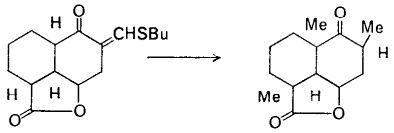
(86)

The methylation of a very active but substituted position is easily avoided by the alkylthiomethylene approach. A high yield of 6-phenyl-2-methylcyclohexanone was obtained from 6-phenylcyclohexanone (equation 87)[64,122].



(87)

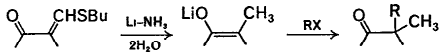
Of course, the use of the alkylthiomethylene group first for blocking and later as a route to monomethylation further expands its utility. Thus, compound **103** was methylated and desulphurized to give the trimethyl derivative **106**[110].



(103) (106)

D. Geminal Alkylation

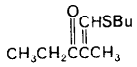
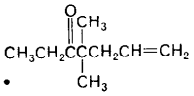
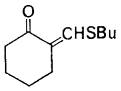
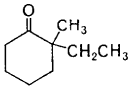
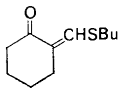
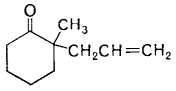
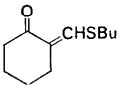
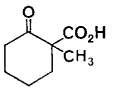
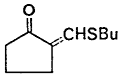
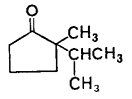
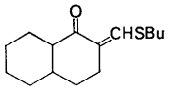
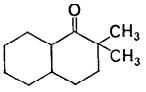
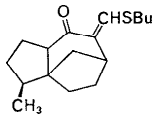
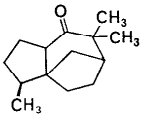
In attempting alkylations leading to highly substituted ketones, careful choice of methods is required to avoid difficulties. Selective geminal alkylations can be achieved by blocking all other available sites, but this is not always possible as with α,α,α'-trisubstituted acetones. An interesting new method has evolved incorporating the lithium–ammonia reduction of *n*-butylthiomethylene derivatives of ketones to their methyl-substituted enolate anions with subsequent alkylation[123]. This reduction–alkylation leads to the introduction of one methyl group and a second variable geminal substituent at any position which will condense with ethyl formate (equation 88). Reaction times as brief as 30 s plus the use of water



(88)

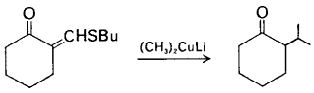
as a proton donor minimize any over-alkylation. Table 3 lists some typical conversions using this procedure.

TABLE 3. Geminal alkylation of ketones *via* thioenol ether derivatives

Ketone derivative	Product	Yield from *n*-butylthiomethylene derivative, %	Reference
		82	123
		75	123
		85	123
		56	123
		40	123
		70	123
		69	128

E. Symmetrical α-Branched Alkylation

The reaction of dialkylcopper lithium reagents with α,β-unsaturated ketones leads to selective conjugate addition[124]. It has been observed that *n*-butylthiomethylene derivatives undergo a double conjugate addition, with loss of the alkylthio group, upon reaction with dimethylcopper lithium. Thus, dimethylcopper lithium reacts with 2-*n*-butylthiomethylenecyclohexanone to give almost quantitatively 2-isopropylcyclohexanone (equation 89)[123]. This reaction should prove useful for the preparation of ketones having a symmetrically branched alkyl substituent in the α-position.



(89)

F. α, β-Unsaturated Aldehydes

Ketones with blocking groups of the isopropoxymethylene type are readily converted to α,β-unsaturated aldehydes by reduction followed by acid-catalysed rearrangement[125,126]. However, the use of this blocking group has the drawback of being moisture-sensitive and of having a deactivating effect on the other α-position. Fortunately, the *n*-butylthiomethylene grouping does not suffer from these drawbacks and is still readily converted to the α,β-unsaturated aldehyde[64,127]. Thus 2-*n*-butylthiomethylene-6,6-dimethylcyclohexanone (**107**) is reduced with lithium aluminium hydride and the resulting alcohol hydrolysed in acid to the α,β-unsaturated aldehyde **110**[129]. The alcohol **111** typically makes up

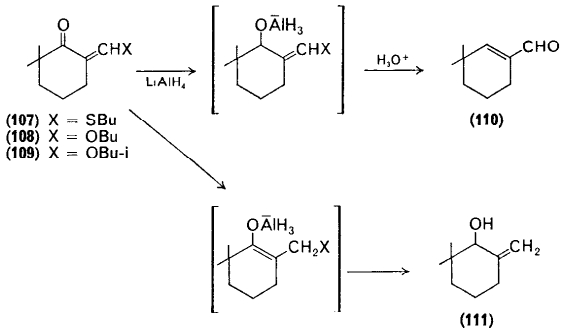
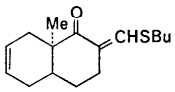
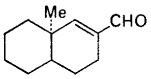
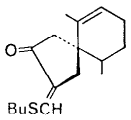
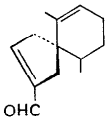
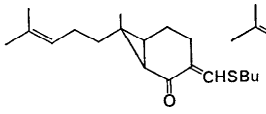
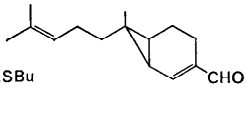
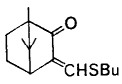
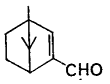
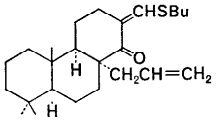
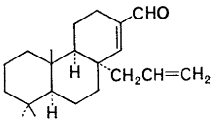
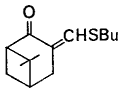
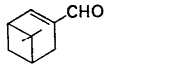


TABLE 4. Preparation of α,β-unsaturated aldehydes by $LiAlH_4$ reduction of α-*n*-butylthiomethylene ketone derivatives

Ketone derivative	Product	Yield from *n*-butylthiomethylene, %	Reference
		63	127
		81	131, 132
		—	133
		—	130
		38	134
		52	135

about 5% of the product. A comparison of the *n*-butylthiomethylenes with butoxy- and isobutoxymethylenes (**108** and **109**) shows[119,129] that the latter two are significantly more prone to 1,4-addition leading to alcohols such as **111**. The use of lithium aluminium hydride instead of the originally suggested sodium borohydride[127] also seems to minimize the 1,4-addition[130]. Table 4 provides some further examples of this reaction.

VI. SULPHUR EXTRUSION REACTIONS

Reactions in which a sulphur atom that bridges or interconnects two carbon groups is extruded with formation of a carbon—carbon bond between the two carbon groups is termed a sulphur extrusion reaction (equation 90). These types of reactions have proven to be of synthetic utility and are treated in this section.

$$R{-}S{-}R \longrightarrow R{-}R + S \qquad (90)$$

Thiols can serve as reagents in the extrusion reaction by being converted to a sulphide or a corresponding higher oxidized derivative upon which the extrusion process is effected. While for many of the cases covered in this section the organosulphur compound used in the extrusion reaction was not prepared directly from a thiol, the potential exists for thiols to be utilized in these types of reactions.

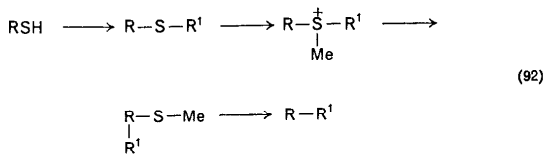
A. Stevens Rearrangement of Sulphonium Salts

The Stevens rearrangement of a sulphonium salt[136] involves treatment of the salt with base and leads to migration of a group from sulphur to an adjacent carbon atom (equation 91). Analogous Stevens rearrangement of ammonium salts[137] and the related Wittig rearrangement[137] of ethers are well known.

$$R{-}\overset{+}{S}(R){-}CH_2R^1 \xrightarrow{\text{base}} R{-}S{-}CHR^1{}_{(R)} \qquad (91)$$

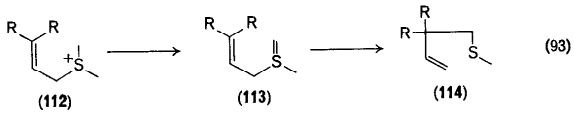
The sulphonium salts used in the Stevens rearrangement need not be prepared initially from a thiol; however, this is feasible and is often the case. This method, therefore, allows the conversion of a thiol to a sulphonium salt, followed by rearrangement with concomitant carbon—carbon bond formation. Removal of the sulphur moiety following rearrangement permits, in effect, a thiol to function in a reaction that leads to bond formation between two R groups that originally were attached to sulphur (equation 92).

The Stevens rearrangement of sulphonium salts is known to proceed through the intermediacy of the corresponding sulphonium ylid[138]. There appears to be two distinct mechanistic pathways, depending upon the structure of the ylid, leading to rearranged product. Rearrangement of



(92)

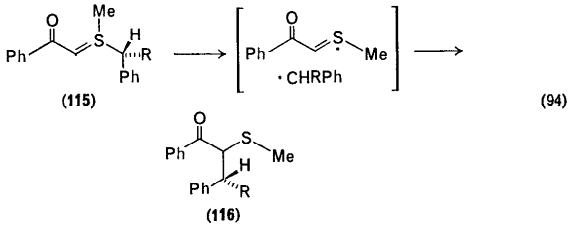
allyl sulphonium salts[139] (**112**), proceeding via the ylid **113**, has been shown[140] to occur by a [2,3] sigmatropic reaction (equation 93); a minor amount of product also arises by what is equivalent to a [1,2] shift[140]. These rearrangements are examples of what appear to be a general class of electrocyclic reactions of sulphonium ylids[141].



(93)

(112) (113) (114)

A second type of rearrangement involves ylids derived from non-allyl sulphonium salts. Baldwin and coworkers[142] have reported that rearrangement of the sulphonium ylid **115** in toluene at reflux temperatures occurs by a radical pair mechanism (equation 94), in which the benzyl group migrates with predominant retention of configuration to yield **116**.



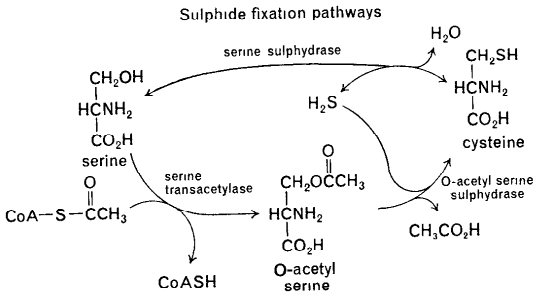
(115) (94)

(116)

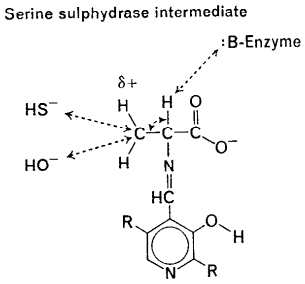
Thompson and Stevens[143], in their first paper on the rearrangement of sulphonium salts, reported obtaining the sulphide **116** upon treatment of **117** with sodium methoxide. However, more recent work has shown[138, 144]

condensed with serine. It presently appears that two different enzyme sequences are possible and both may operate in some organisms.

These are contrasted in the scheme below.



With the direct H_2S–H_2O interchange enzyme, serine sulphydrase, the reaction is completely reversible. Pyridoxal phosphate participates as an essential cofactor which suggests a mechanism involving a pyridoxamine–Schiff base intermediate. By stabilizing an electrophilic centre at the side chain carbon a nucleophilic substitution could be facilitated.



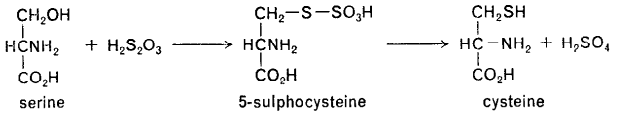
The reversibility of this reaction would allow this enzyme to participate in either sulphuration or desulphuration of cysteine and its real role *in vivo* remains somewhat doubtful.

The other system for sulphide assimilation involves a coupled hydrolysis of acetyl coenzyme A. This enzyme system can only operate in the direction of cysteine synthesis and would ensure the effective trapping of

most available sulphide for this purpose. The acetylation of the serine hydroxyl also provides an effective leaving group so that one might envisage an enzyme-mediated direct nucleophilic displacement mechanism. Pyridoxal cofactors are not thought to participate in the O-acetyl serine sulphydrase reaction, although this remains an unsettled question. The enzymes of the O-acetyl serine pathway are responsive to the metabolic needs of the cell being repressed by cysteine in *Escherichia coli*. There is a biochemical generalization that critical biosynthetic pathways such as this are normally coupled to high energy bond expenditure which guarantees effective utilization of nutrients. Such considerations make it likely that this is the normal biosynthetic route. Similar systems have not been found in all organisms capable of sulphide incorporation however, so an important role of the direct sulphide–hydroxyl exchange system in cysteine synthesis cannot be excluded. There is evidence for a similar system in chick embryo involving a serine phosphate rather than the acetate[7].

Cysteine formation through the addition of thiosulphate to serine or O-acetyl serine may play a role in the sulphur metabolism of some organisms. The reactions involved are similar in form to those described above, with S-sulphocysteine serving an intermediary role. Since thiosulphate is not generally considered to be on the main line of inorganic sulphur metabolism this probably represents an adaptation to certain special environments.

Cysteine formation from thiosulphate



2. Cysteine oxidation[1,2]

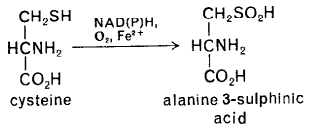
The balance of the 'sulphur cycle' requires that reduced sulphur derivatives eventually be reoxidized to sulphate. A number of photosynthetic and chemosynthetic organisms have the ability to utilize reduced sulphur, particularly H_2S, as critical electron donors for ATP production, but these pathways are not of enough general importance to consider here. Pathways are known for the production of sulphide from cysteine, and it is also clear that the oxidation of sulphide can occur in animals with production of sulphate and thiosulphate. What is not certain is to just what extent specific enzyme reactions are involved. The nonenzymatic

oxidation of sulphite is promoted by a variety of normal cellular constituents, but it is felt that direct sulphide oxidation is of little consequence for animals. Sulphide is an exceedingly toxic material, precluding its normal accumulation in significant amounts and the principal 'detoxification' route seems to be fixation into organic thiol compounds rather than oxidation.

All organic thiols and thiol derivatives are quite susceptible to aerobic oxidation yielding a variety of oxy-derivatives. Actually the biological significance of most of these sulphoxide derivatives is unknown. In certain instances, there are mechanisms to reduce sulphoxides back to divalent sulphur compounds. β-Lipoic acid, an active factor in bioassays, proved to be a sulphoxide derivative of this disulphide cofactor which was generated during purification[8]. Its biological activity implies that it can be reduced to the normal form of the cofactor. A methionine sulphoxide reductase system from yeast has been studied extensively and found to resemble the PAPS reductase and ribonucleotide reductase systems in that electron transport was mediated by a heat-stable protein disulphide factor[9]. Thus, there does seem to be some ability to salvage partially oxidized thiol derivatives, but it is uncertain how widespread this capacity might be.

The only thiol oxidation reaction to oxy-derivatives of general biochemical significance is that of cysteine to alanine 3-sulphinic acid (cysteine sulphinic acid)[1,10]. This is thought to be the initial reaction in the

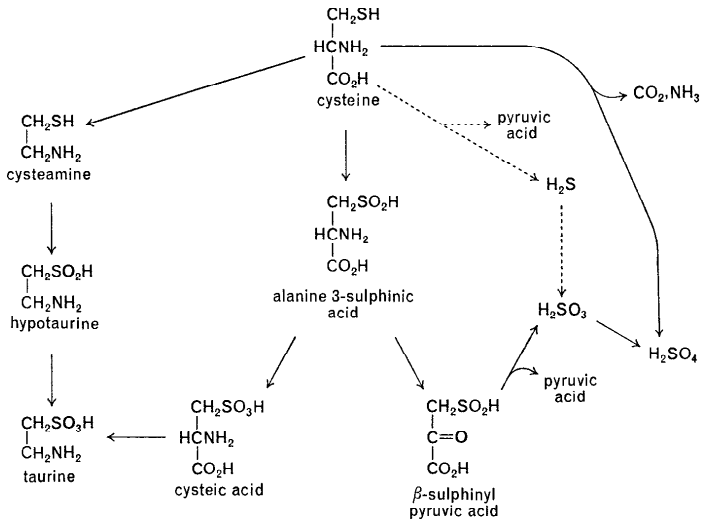
Cysteine oxidase reaction



main pathway for the utilization of cysteine sulphur for sulphate production. Relatively little is known about the details of this oxidation process. Some form of reduced nicotinamide coenzyme, ferrous iron, and possibly other cofactors are required by an enzyme from the soluble fraction of rat liver. There is little information on mechanistic details or possible intermediate states. Cysteine sulphinic acid is further converted to what has been presumed to be β-sulphinyl pyruvic acid and ultimately to inorganic sulphite. This is then oxidized to sulphate. Cysteic acid and taurine may also arise from cysteine sulphinic acid.

There is also a mitochondrial system for cysteine oxidation to sulphate in which there are no known intermediates. It has been suggested that this system is important in cysteine metabolisms and the production of sulphate from sulphur amino acids. A sulphite oxidase deficiency has been reported in the human[11]. Since virtually no sulphate ions occurred in the urine, this would imply an obligatory role for this enzyme in the cysteine to sulphate conversion, and cast doubt on the role of the mitochondrial system. However, the sulphite oxidase is also a mitochondrial enzyme and might function in both pathways. A possible defect in cysteine oxidation has also been considered in another genetic disease, cystinosis. The cysteine to sulphate oxidation has, however, been shown to be normal in the liver of such patients[12]. Greater understanding of these processes should be forthcoming in conjunction with such studies on human genetic disease. The possible routes for the enzymatic oxidation of cysteine sulphur to oxy-acid derivatives are summarized below.

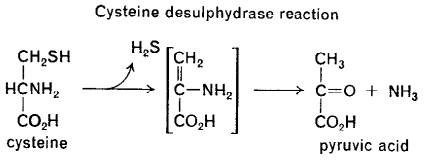
Possible cysteine oxidation pathways



3. Cysteine desulphuration[1, 10]

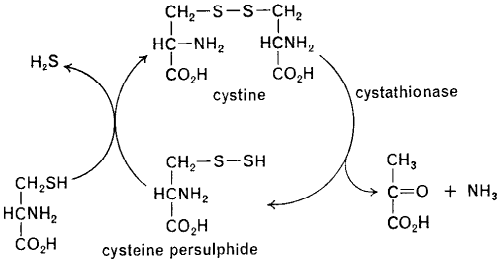
Desulphuration (desulphydration) of cysteine may play a role in thiol catabolism, but there is considerable confusion concerning the existence of a distinct cysteine desulphydrase.

Cysteine desulphydrase reaction



There is no doubt that such a reaction, catalysed by a pyridoxal phosphate-dependent enzyme, can occur in biological systems. It is quite possible, however, that this only represents a side reaction of other enzymes. Cystathionase, for example, will act on cystine with the elimination of a cysteine persulphide and pyruvate. The persulphide then reacts with cysteine to eliminate sulphide and regenerate cystine. The complete cycle would constitute a cysteine desulphydrase activity.

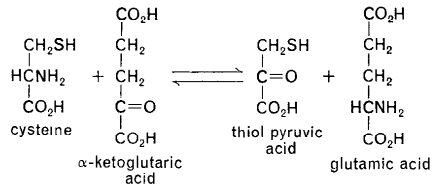
Cysteine desulphydrase activity via cystathionase and cystine



Cystathionase also has a low level of direct cysteine desulphydrase activity. Tryptophanase and tryptophan synthetase are other enzymes capable of carrying out the cysteine desulphydrase reaction. Such considerations have cast doubt on this biological significance of this reaction, although strong arguments have been presented for a true cysteine desulphydrase in *Salmonella*[13].

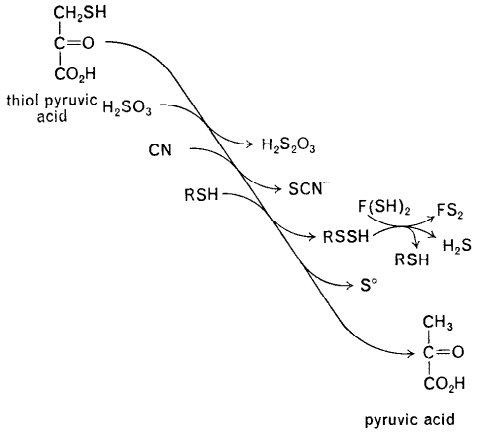
Another route for the removal of the thiol group from cysteine is through the intermediate formation of thiol pyruvic acid, which is the α-keto acid derived from cysteine by transamination:

Cysteine transaminase reaction



The product can be acted on by an enzyme which transfers sulphur to a variety of acceptors *in vitro* to generate thiosulphate (and thiosulphonates), thiocyanate and organic persulphides[1,10,14]. A direct desulphuration to sulphide does not appear to occur but a further reaction of persulphide with dithiol carriers would provide this product. The generation of elemental sulphur can also occur under certain circumstances. The thiol pyruvate sulphurtransferase is though to act through an enzyme persulphide

Reactions of thiol pyruvate sulphurtransferase

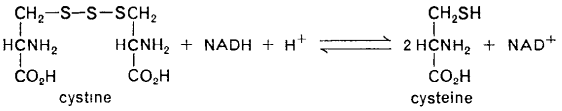


intermediate and will be considered further in a subsequent section. Thiol pyruvate can also be reduced to thiol lactate and decarboxylated to mercaptoethanol.

4. Cysteine–cystine interconversion[13]

While it is doubtful if cystine, the disulphide of cysteine, has any critical biological role as such, it is an ubiquitous constituent of aerobic systems resulting from the facile oxidation of cysteine. It also can arise from the digestion of protein disulphides. Cystine is relatively insoluble and if allowed to build up tends to form crystalline precipitates within the cell. There is normally little of the disulphide in cells, while in the blood the oxidized form dominates. One method for the reduction of cystine to cysteine is *via* a nicotinamide coenzyme-linked dehydrogenase. Glutathione

Cysteine dehydrogenase reaction



also has a critical role in cystine reduction. While this reduction occurs readily without enzymes, it is stimulated by the enzyme cystine–glutathione transhydrogenase.

$$2\ \text{GSH} + \text{cystine} \longrightarrow \text{GSSG} + 2\ \text{cysteine}$$

This latter system appears to be the one dominant in cystine reduction by mammalian cells.

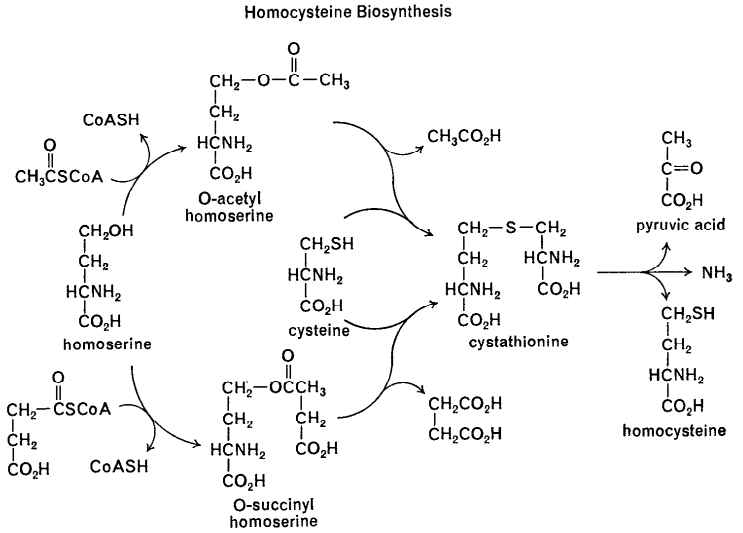
Two human genetic diseases are known which involve this disulphide amino acid. In one, cystinuria[15], there is a transport defect in the intestine and kidney. This results in abnormally high levels of cystine in the urine and can result in the precipitation of cystine crystals and kidney stone formation. In cystinosis[12], cystine crystals form within cells and eventually cause severe kidney damage. The nature of the primary biochemical lesion is unknown; all known cystine reduction systems of the cell appear to be normal.

5. Transsulphuration *via* cystathionine[1,2,16]

Cysteine also donates its sulphur to form homocysteine and eventually the second critical sulphur amino acid, methionine. Methionine is the S-methyl ether of homocysteine, a cysteine analogue with one additional

carbon in the chain. Transsulphuration from cysteine occurs in bacteria, plants, yeast and fungi, but not in animals. The latter rely on dietary sources of methionine. It is actually the homocysteine portion which is required but this does not normally occur in significant quantities.

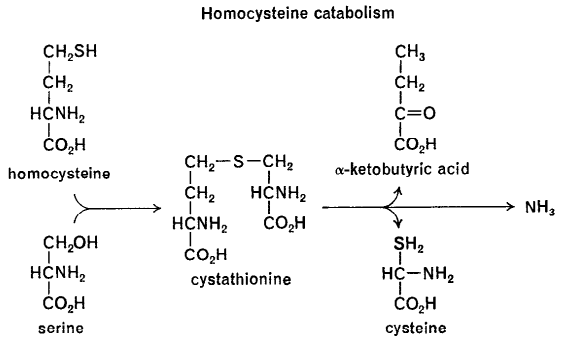
The carbon skeleton of homocysteine is derived from the corresponding hydroxy amino acid, homoserine. The hydroxyl of homoserine is acylated with either a succinyl (bacteria) or acetyl (yeast, fungi, plants) group derived from the corresponding coenzyme A derivative. The O-acyl substituent is then displaced by the thiol group of cysteine producing a mixed thioether, cystathionine. This in turn undergoes a pyridoxal phosphate dependent β-elimination to homocysteine, pyruvate and



ammonia. Homocysteine is then methylated to methionine by pathways to be discussed later. Cystathionine is generally only a trace metabolite, but occurs in reasonably high concentrations (25–50 mg/100 gm) in brain[17]. A direct formation of homocysteine from homoserine (or O-acyl homoserine) and hydrogen sulphide has also been observed in extracts of some organisms. Whether this is only a side reaction of the cystathionine

synthesis system or is a physiologically important route for assimilation of sulphide is uncertain.

In animals homocysteine arises from methionine through its role as a methyl donor, as will be discussed in a subsequent section. It may either be reutilized for methionine production or degraded. In animal tissues the degradative pathway plays a major role in sulphur nutrition[18]. Much of the cysteine sulphur, and through it sulphate, can be derived from dietary methionine. The transsulphuration from homocysteine to produce cysteine is very much like that in the other direction. It also involves cystathionine but is not a reversal of the synthetic pathway. Quite different reactions are involved. Homocysteine reacts with serine to produce the



thioether intermediate. Unlike the route from cysteine and homoserine, no O-acylation step has been implicated. Instead, the homocysteine–serine-condensing enzyme probably requires pyridoxal phosphate as a coenzyme, although this is not unequivocally established.

Cystathionine cleavage in the mammalian transsulphuration system produces cysteine, α-ketobutyrate and ammonia by what is believed to be a pyridoxal-catalysed γ-elimination reaction. Again this reaction is quite similar to the cystathionine cleavage by the other pathway; only the direction of cleavage is different. Actually the bacterial cystathionase is capable of cleaving the thioether in either direction, although that producing homocysteine is dominant. This implies that even the enzyme-bound intermediates are similar and the binding specificity of the particular enzyme site is crucial in ensuring the proper reaction.

Particular interest in this pathway arises from the findings of human genetic diseases associated with each step[19]. Lack of the first enzyme,

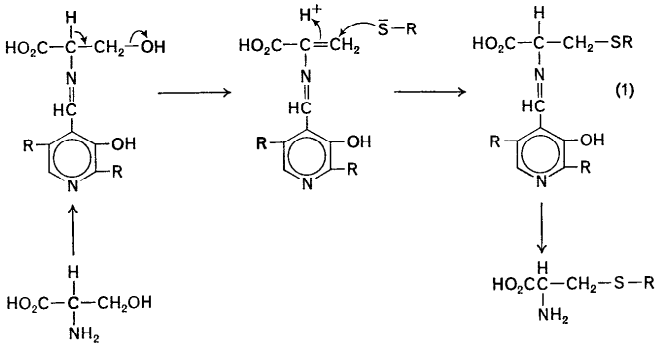
cystathionine synthase, results in homocystinuria[20]. This is one of the most common genetic disorders of amino acid metabolism and is exceeded in frequency only by phenylketonuria. In this disease, homocysteine cannot be metabolized and its disulphide, homocystine, builds up and is excreted. The disorder is often associated with severe symptoms including mental retardation. Actually two distinct autosomal recessive forms of homocystinuria can be differentiated: one type is susceptible to treatment with vitamin B_6 (pyridoxine). Since pyridoxine is the precursor of pyridoxal phosphate, such therapeutic results strongly support a critical role for this coenzyme in the cystathionine synthase. It also implies that, in at least some homocystinurics, the biochemical defect is in coenzyme formation or binding. In the vitamin B_6-unresponsive patients the mutation must affect some other aspect of the enzyme. Actually only a small proportion of the daily methionine intake by homocystinuria patients can be accounted for by the excreted homocysteine and the study of this disease may greatly enhance our knowledge of thiol metabolism. For example, it appears that homocystinurics can make cystathionine to some extent from cysteine and homoserine, a reaction generally believed impossible in animals.

Cystathioninuria, a deficiency of cystathionase, is a much rarer and less clearly defined disorder[17]. While the disease has frequently been associated with mental retardation, this may only reflect the type of individual with which testing most frequently occurs. Patients with normal mental function are also known. Nonetheless, the high levels of cystathionine in brain and the mental defects associated with its faulty metabolism, have led to speculation that this thioether has some special role in nervous function. In tissues from at least one patient, there was evidence that the defect was in pyridoxal phosphate binding by cystathionase and that normal enzyme activity could be achieved at abnormally high levels of coenzyme. This is often quoted as the classical example of a binding or 'K_m' mutant, but not all patients with the disorder give the same effect.

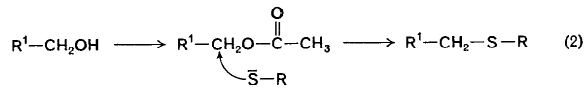
These reactions which lead to homocysteine formation in some creatures and its utilization in others are undoubtedly representative of a general thiol group transfer mechanism. The initial condensation of the donor thiol, most commonly cysteine, with some suitably reactive receptor generates a thioether. The differences in the requirement for O-acylation when starting from serine and homoserine may reflect two completely different mechanisms for this thiol substitution reaction. In the case of serine, the removal of the hydroxyl as hydroxide and the stabilization of an electrophilic centre on the side-chain carbon can be achieved through the pyridoxal phosphate–amino acid adduct. A similar example is in the carbon–carbon condensation between serine and imidazole in tryptophan

synthesis. When homoserine is the receptor a different activation system appears to be necessary. While pyridoxal coenzymes can facilitate γ-elimination of hydroxide from the homoserine structure, stabilization of an electrophilic centre at the appropriate position cannot occur. By first acylating the hydroxyl of homoserine a suitable leaving group for an enzyme-facilitated nucleophilic displacement reaction is created. The two possible mechanisms for formation of a thioether intermediate for transsulphuration are shown below. The thioether then breaks down by the

Possible mechanisms for thioether formation



(1)



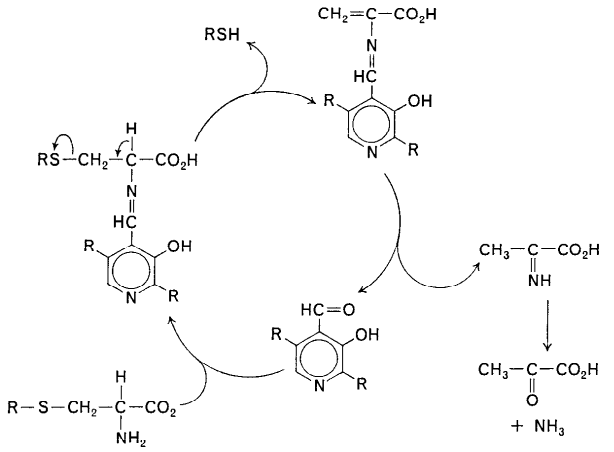
(2)

elimination of a thiolate to complete the transsulphuration sequence. Typically this would be a β-elimination from the cysteine structure potentiated by a pyridoxal phosphate stabilized intermediate as depicted below.

It also appears that thiol pyruvate can serve as sulphur donor for some biological transsulphurations. The thiol nucleotides which occur in small quantities in certain nucleic acids appear to derive their sulphur, at least in part, from thiol pyruvate rather than directly from cysteine[21]. While these reactions have not been extensively studied as yet, ATP is required possibly to activate a group for intermediate thioether formation. Pyruvate elimination could then proceed through an enolate or an intermediate enzyme-bound Schiff base.

A diverse variety of divalent sulphur compounds is found throughout nature. These are often found in small quantities or in restricted species and little is known about their metabolism. It is generally presumed that they all ultimately derive their sulphur from cysteine. Thus more examples of transsulphuration reactions will be described, and it is likely that

β-Elimination from a cysteine thioether



mechanisms involving mixed thioether intermediates will frequently be implicated. Another general route for transsulphuration may be through enzyme-bound persulphides. The existence of such intermediates in the rhodanese and thiol pyruvate sulphur transferase reactions seems reasonably established, although there are no examples of their being involved in the formation of organic thiols.

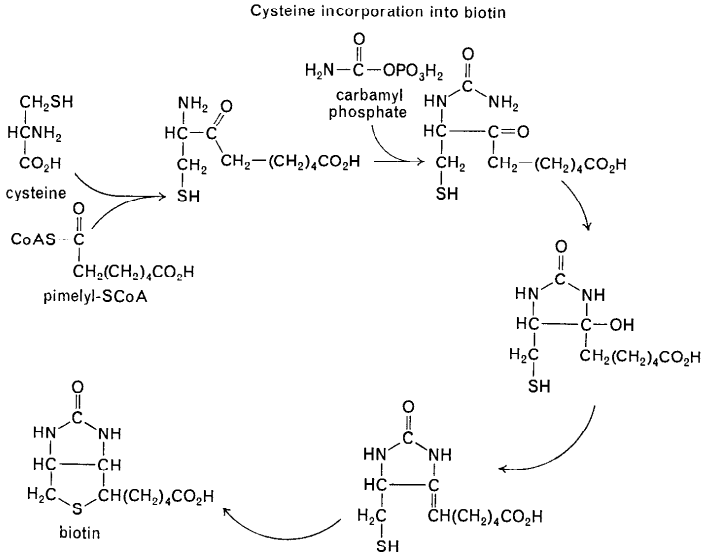
6. Thiol formation by cysteine incorporation

Thiol groups enter some biologically important thiol compounds by the direct incorporation of cysteine itself. Most frequently this involves peptide bond formation. The incorporation of cysteine into proteins does not differ from any other amino acid involving activation as an amino acid adenylate, transfer to a specific transfer ribonucleic acid (t-RNA), and assembly by ribosomal enzymes as coded by messenger ribonucleic acid (m-RNA). It should be pointed out that cystine, the 'two-headed'

disulphide amino acid, is not directly incorporated, but arises in proteins by oxidation of two cysteine residues after assembly of the chain. The formation of glutathione and pantetheine also involves peptide bond formation to cysteine, but the mechanism of formation is quite different from the nucleic acid-coded protein synthesis. These pathways will be included in the discussions of the thiol coenzymes.

An example in which a portion of the cysteine carbon chain is incorporated directly is one of the proposed routes for biotin synthesis by microorganisms[22]. An acyl coenzyme A derivative of pimelic acid condenses with cysteine, eliminating CO_2. Reaction with carbamyl phosphate leads to the formation of an ureido ring system. The thiol then forms a cyclic thioether by addition to a double bond resulting from dehydration.

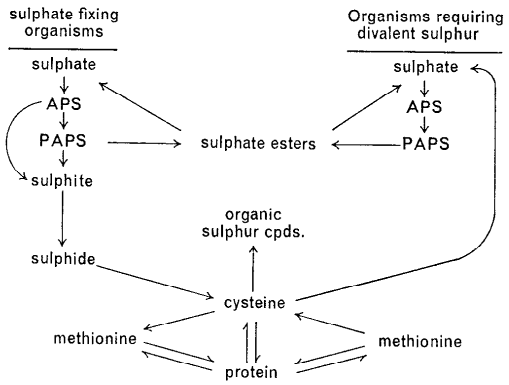
Cysteine incorporation into biotin



Cysteine is the pivotal compound in thiol metabolism. Sulphate and other oxidized forms of sulphur are reduced to the level of sulphide, which enters organic linkage as cysteine. There is no other direct sulphuration pathway of any significance. All biological thiols and subsequently

derivatives such as disulphides, thioesters, thioethers and sulphonium salts derive sulphur through cysteine. This is accomplished either by transsulphuration or by incorporation of the cysteine structure directly. The sulphur metabolism in organisms capable of sulphate fixation and those requiring preformed sulphur amino acids is summarized below.

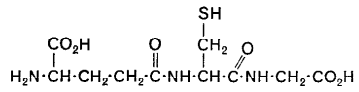
Outline of sulphur metabolism



III. BIOLOGICAL THIOLS AND THEIR FUNCTION

A. Glutathione[23, 24, 25]

While cysteine is the central compound of organic thiol metabolism, a tripeptide derivative, glutathione, is probably the most ubiquitous single thiol compound. Much fascinating biochemical history surrounds this molecule and it has served as the subject of two published volumes[23, 25]. Still, remarkably little is really known concerning its biological importance.



Glutathione (γ-glutamyl cysteinyl glycine)

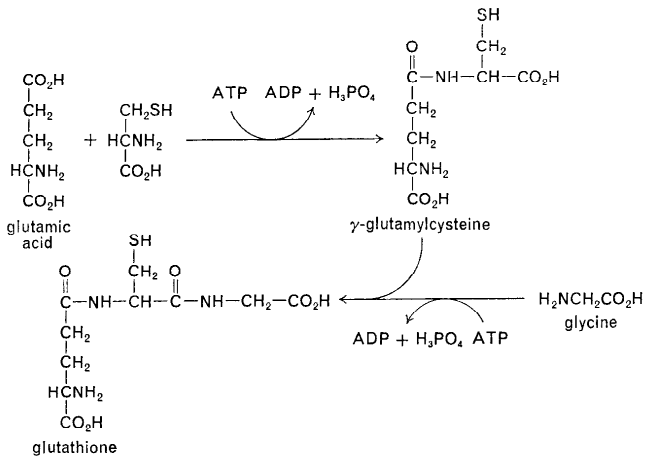
Since glutathione occurs throughout the biological world, it is felt that it must satisfy some critical cellular need. The most likely general function is maintaining a reduced cellular environment. Glutathione can also serve

a variety of additional roles. This peptide functions as cofactor for certain enzymes and it may serve as a γ-glutamyl donor in the synthesis of other γ-glutamyl derivatives. Glutathione is involved in the detoxification of certain organic toxins by some species. There have also been suggestions of special roles for glutathione or its derivatives in brain function and in cell division.

1. Biosynthesis and degradation[24, 26]

Glutathione is assembled from glutamic acid, cysteine and glycine in a protein-directed synthesis. Glutamic acid reacts with cysteine in the presence of ATP to yield ADP, inorganic phosphate and γ-glutamyl cysteine. In a second step the γ-glutamyl cysteine is condensed with glycine to give glutathione. A considerable amount of glutathione synthesis

Biosynthesis of glutathione



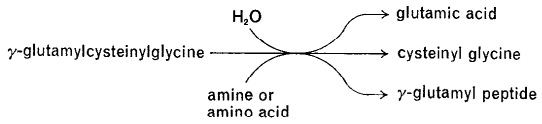
occurs in some cells. Liver may contain 10 mM glutathione which turns over every 2 to 10 h. Such high rates of synthesis and breakdown only add to the mystery of glutathione's importance.

A human disease associated with impaired glutathione synthesis has been reported[27]. Red blood cells from this patient lacked the second enzyme of the synthetic sequence, but the activities of enzymes involved

in glutathione utilization were all normal. Red blood cell glutathione was only reduced to 10–20% of normal. This implies either that the enzyme defect is tissue specific and other tissues can supply some glutathione to the red cell or that the cell produces a less stable enzyme which had become inactivated by the time of analysis. Aside from their intrinsic medical interest, such natural mutants can be expected to provide considerable information about the biochemistry of glutathione. For example, this person was reasonably normal with problems only appearing under stress. This is surprising if the defect was really general and the roles of glutathione are as critical as suggested. On the other hand, an increased sensitivity of this individual's red cells to oxidative stress favours an anti-oxidant role for glutathione.

One special enzyme, that cleaving the γ-glutamyl bond, is involved in glutathione degradation. This enzyme, usually referred to as glutathionase, also has γ-glutamyl transpeptidase activity under certain assay conditions.

Action of glutathionase



It is unclear if the transpeptidation activity represents a way for glutathione to serve as a synthetic γ-glutamyl donor or is simply an insignificant transferase activity typical of many hydrolases. This enzyme probably also participates in mercapturic acid formation[28], and this can be viewed as a variation of the direct hydrolysis reaction in which a substituted glutathione is substrate.

2. Maintenance of the reduced cell[24]

Glutathione can be oxidized to its disulphide by oxygen, oxidized electron transport carriers, free radicals and a variety of disulphides. While most of these reactions are facilitated by enzymes, they also can occur spontaneously. It must be assumed that the ease of nonenzymatic oxidation is an important attribute in the protection of other cellular constituents. The idea that glutathione serves to keep thiols in a reduced state is a direct extension of its usefulness in maintaining extracted enzyme systems in a functional form. The nonenzymatic disulphide interchange reaction of glutathione is facile and a number of enzymatic activities promoting such reactions have also been described.

Disulphide interchange reaction of glutathione (GSH)

$$2\,GSH + RSSR \rightleftharpoons GSSG + 2\,RSH$$

Glutathione reductase is an ubiquitous enzyme to be discussed mechanistically in the section on dithiol enzymes. Through its action, metabolic reducing power generated as reduced pyridine nucleotides can be coupled to the maintenance of the reduced environment.

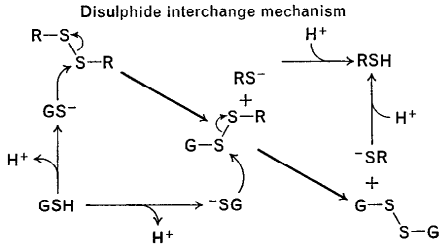
Glutathione reductase reaction

$$GSSG + NADPH + H^+ \rightleftharpoons 2\,GSH + NADP^+$$

Crucial thiols such as cysteine and coenzyme A and the numerous cellular enzymes requiring thiol groups for proper function are kept reduced by the high glutathione levels within cells. Oxidized glutathione in turn is reduced by glutathione reductase and NADPH-generating systems.

Glutathione-mediated disulphide reductions whether enzyme mediated or spontaneous probably proceed through an intermediate mixed disulphide *via* a thiolate displacement mechanism.

Disulphide interchange mechanism



Relatively high glutathione concentration would be required to ensure complete reduction. Some glutathione bound as a mixed disulphide is found in cellular proteins as would be expected from this scheme, but it is uncertain if this actually existed within the cell or was produced on extraction.

A few systems are known in which glutathione serves as a reductant for molecules other than disulphides[10,24]. Probably the most critical of these in animals is the glutathione peroxidase of the red cell. Along with catylase this enzyme is responsible for destroying peroxides and thereby preventing lipid peroxidation and haemoglobin inactivation. The

Glutathione peroxidase reaction

$$2\,GSH + H_2O_2 \longrightarrow GSSG + 2\,H_2O$$

functional importance of this reaction can be deduced from the effects of genetic disorders such as erythrocyte glucose-6-phosphate dehydrogenase deficiency. Where there is a lack of NADPH production the inability to maintain glutathione in the reduced form results in decreased red cell stability and haemolytic anaemias[29].

Related to this is the action of glutathione as a free radical scavenger in protection against radiation damage[25]. Thiols readily react with free radicals producing thiol radicals which eventually combine to disulphides. It is felt that the ease of this reaction and the ready availability of glutathione minimizes damage to critical biological structures by the free

Glutathione reaction with free radicals

$$GSH + HO^{\bullet} \longrightarrow GS^{\bullet} + H_2O$$

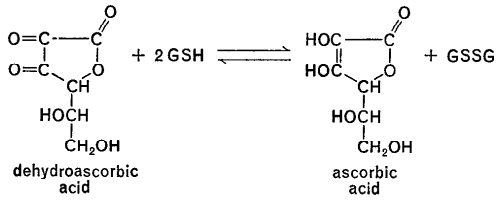
$$2\,GS^{\bullet} \longrightarrow GSSG$$

radicals produced by ionizing radiation. Some consider one important mechanism of cellular ageing to be a slow accumulation of radiation-induced damage. Glutathione might therefore be considered also to have an anti-ageing role.

3. Other electron transport roles[24, 25]

Plants have an enzyme system linking the oxidation of glutathione to the reduction of dehydroascorbic acid. A similar enzyme may occur in animal tissues, although in this case a facile non-enzymatic reaction could possibly account for the observed activity. The plant enzyme provides a

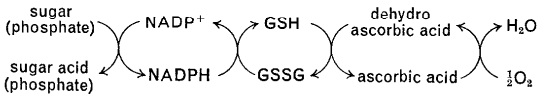
Dehydroascorbate reductase reaction



pathway whereby oxidized ascorbate can be reduced thereby enhancing its potential as an antioxidant. The full appreciation of the biological significance of this reaction suffers from the almost complete ignorance of the role of ascorbic acid. Coupled with a NADPH-linked glutathione reductase, the NADP reduction activity of the pentose shunt enzymes, and

a dehydroascorbic acid oxidase, a complete respiratory chain for the oxidation of glucose is possible. Its actual operation if it occurs at all appears restricted to the earliest phases of plant development.

Glutathione-ascorbic acid respiratory chain

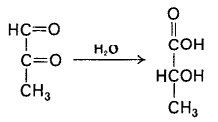


Glutathione is also the reductant for an organo-nitrate ester-reducing enzyme from liver. This so-called nitroglycerin reductase reacts with glycerol, erythritol or mannitol nitrates to yield free alcoholic hydroxyls and nitrite ions. The normal physiological substrate for this system is unclear. While its study has provided interesting enzymology it has not yielded any insight into the biological significance of glutathione.

4. Use as an enzyme cofactor

The best established functional role for glutathione is as a cofactor in certain enzymatic processes. The most extensively studied example is the glyoxylase system[24, 30]. This catalyses an internal oxidation–reduction, or dismutation, of certain α-keto aldehydes to α-hydroxy acids.

Glyoxylase reaction

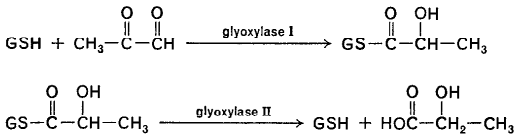


The idea that this system played a crucial role in carbohydrate metabolism forms an important, but now largely forgotten, aspect of the history of biochemistry[24]. The discovery of the importance of glutathione in the glyoxylase reaction was, in fact, the critical finding which has relegated this reaction to its present metabolic obscurity. In muscle preparations the glyoxylase system was found to be inoperative without added glutathione. However, glycolytic activity continued precluding any direct role for glyoxylase in this important metabolic process. While the bulk of intermediary metabolism has been traced out in the intervening forty years, glyoxylase function remains undefined. At present it is assigned a detoxification role in protecting against α-keto aldehydes, although

Szent-Györgyi has proposed that the glyoxylase system may be important in the control of cell division[31].

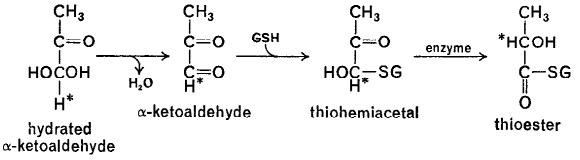
The glyoxylase reaction is promoted by two enzymes found in almost all living creatures. The first protein catalyses the condensation of the α-keto aldehyde with glutathione followed by an internal disproportionation producing a thioester of an α-hydroxy acid. A second enzyme cleaves the thioester regenerating glutathione.

Glyoxylase system



The present conception of the glyoxylase I mechanism involves a non-enzymatic condensation of the thiol of glutathione with the α-keto aldehyde to produce a thiohemiacetal. The enzyme then promotes an intramolecular hydride migration generating an α-hydroxy acid-thioester. The original aldehydic hydrogen is retained in the final product. It has been compared to the Cannizzaro and benzilic acid rearrangements of organic chemistry.

Glyoxylase I mechanism

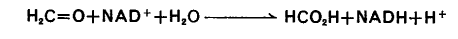


The second enzyme of the glyoxylase system, the thioesterase, is specific for thioesters of glutathione and its analogues. Thioester hydrolysis and transacylation will be discussed in subsequent sections.

At one time glutathione was also thought to constitute part of the active centre of glyceraldehyde 3-phosphate dehydrogenase[32] with similar thiohemiacetal and thioester intermediates. While the analogous involvement of an enzyme thiol in the enzyme reaction has been well established, an analysis of the amino acid sequence at the active site of the enzyme has shown that glutathione is not a part of the enzyme[33]. Glutathione does

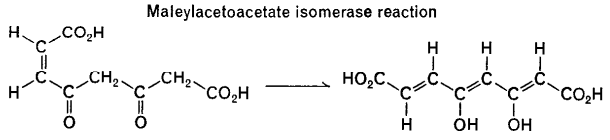
appear to have a valid role in a similar reaction, that of formaldehyde dehydrogenase[24].

Formaldehyde dehydrogenase reaction



Glutathione also acts as coenzyme for a completely different type of reaction, the isomerization of maleylacetoacetate to fumarylacetoacetate[25].

Maleylacetoacetate isomerase reaction



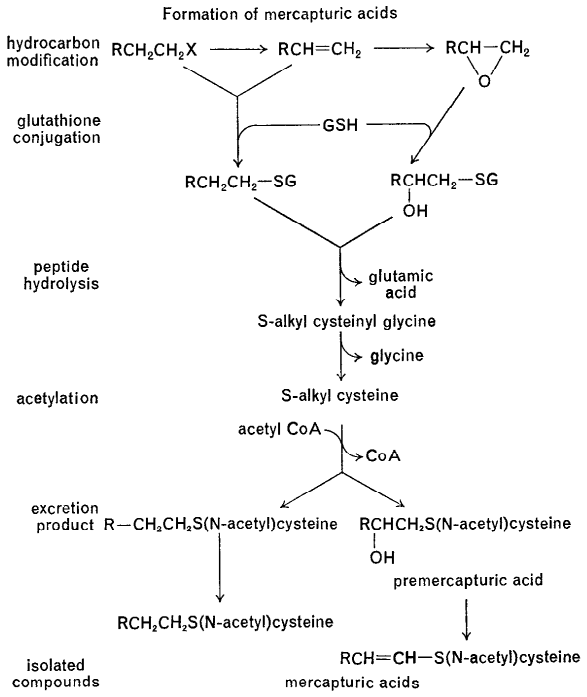
The reaction is thought to proceed through a reversible addition of the thiol to the double bond. Glutathione can catalyse the isomerization of this and other α-β-unsaturated acids even in the absence of enzyme. A glutathione addition product can be isolated with such substrates but does not appear to be a true intermediate in the enzymatic process, as it is not acted on by the enzyme. An enzyme-bound adduct is thus implicated.

5. Mercapturic acid formation and detoxification[10,28]

Glutathione is involved in the conjugation of certain toxic hydrocarbons by the liver. These are eventually excreted as mercapturic acids, S-substituted N-acetyl-cysteines. Such compounds have been isolated from the urine of many animals including man.

Benzene, halobenzenes, naphthalenes and a variety of other aromatic or unsaturated hydrocarbons are conjugated by reaction with glutathione. Many of these compounds readily react with thiols nonenzymatically, and their rapid sequestration would be critical in protecting the functional thiols of the cell. A group of enzymes concentrated in the liver and kidneys, the glutathione-S transferases, catalyse the condensation with glutathione. Cysteine or other biological thiols do not serve as acceptors. After formation of the hydrocarbon adduct the glutathione peptide bonds are hydrolysed and the cysteine residue is N-acetylated before excretion. In many cases the product actually excreted is a so-called premercapturic acid which contains a hydroxyl adjacent to the thioether substituent. Water is eliminated during isolation to produce the mercapturic acid. The

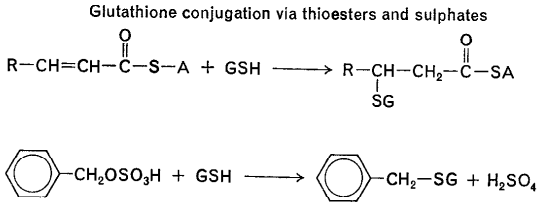
frequent occurrence of an α-hydroxy substituent suggests that the hydrocarbon has undergone epoxidation of a double bond prior to reaction with glutathione. The condensation reaction would then involve an attack on the epoxide ring by a thiolate. Direct addition of the thiol to a double bond or even halogen displacement may also occur in certain cases giving rise to metabolic products without α-hydroxy substituents.



In humans mercapturic acid formation appears less significant than detoxification pathways involving glucuronide or sulphate ester formation, but is of considerable importance in other species. Halobenzenes which can cause liver damage lead to mercapturic acid formation in the rat, while non-toxic compounds such as *p*-dibromobenzene do not. Such facts strongly support the idea that this pathway has a detoxification role.

Mercapturic acid production seems to have first call on the sulphur amino acid reserves and serious deficiency states can be induced in rats by hepatotoxic hydrocarbons. Diets high in cysteine and methionine will protect against the liver damage. Some mercapturic acid production may also result from reaction of protein thiol groups with the hydrocarbons, hydrolysis of the protein to the S-substituted cysteine and its N-acylation. However, the vast majority is formed via the glutathione adducts if the hydrocarbon dose is not so great as to deplete the glutathione reserves of the liver.

The intermediate production of aralkyl sulphate esters or thioacyl derivatives prior to conjugation with glutathione seems likely for certain types of compounds since enzymes of the following types have been characterized[34,35].



Mercapturic acid formation has been shown to occur in a variety of mammals, birds, reptiles, amphibians and fish. Insects also form glutathione conjugates but do not N-acylate the eventual S-substituted cysteine derivatives to any great extent. It is also possible that the S-carboxyalkylcysteines of plants have a similar genesis. Mercapturic acid formation is certainly one of the best studied and documented protective functions for glutathione.

Thus, in spite of many years of investigation and speculation, no universal functional role has been established for glutathione which would explain its broad distribution and high concentration in biological systems. The most satisfying concept is that the glutathione system establishes the reduced state of the cell, at least in so far as preventing the oxidation of cellular thiols. In fact the thiol protective effect is multifaceted. Glutathione preferentially reacts with agents of all types which otherwise would inactivate thiol metabolites, coenzymes and proteins. If inappropriate disulphide formation should occur, activity can be restored by the disulphide interchange. Whether such a general protective action is the universal glutathione role has been difficult to prove, and the

concept has been gently derided by labelling it the euphoristic theory of glutathione action (see reference 24).

While the overriding function of glutathione may be protective, a number of more specific roles have evolved. It serves as a coenzyme for certain enzymatic processes, and it may moderate critical rearrangements of cellular architecture. If for no other reason glutathione could be regarded as the most important cellular thiol on a purely quantitative basis, and it is likely that it has functions of correspondingly critical significance.

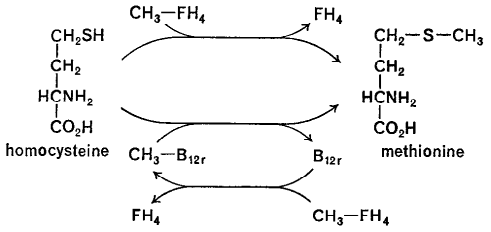
B. Methionine and S-Adenosyl Methionine[36, 37]

The biological importance of the second thiol amino acid, homocysteine, is as the thioether and sulphonium ion derivatives. The free thiol occurs only as a metabolic intermediate. Methionine, the methyl thioether, is one of the twenty amino acids utilized for protein synthesis. Our concepts of the special significance of methionine in protein structure and function are only beginning to be developed, and will not be considered here. N-Formyl methionine also has the distinctive role of being a chain initiator in protein synthesis[38]. The most extensively studied form of this thiol is S-adenosyl methionine or SAM, the sulphonium ion cofactor. This is the principal methylating reagent of biological systems and other alkyl transfers from the sulphonium ion are also known.

1. Methylation of homocysteine

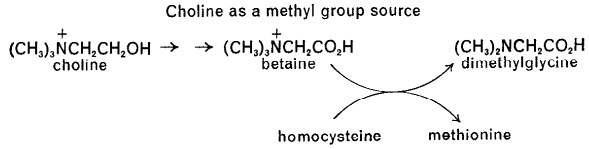
Methylation of homocysteine to methionine can be accomplished by one of several sequences. A major route is from a N^5-methyl-tetrahydrofolic acid ($CH_3—FH_4$) derivative. In some organisms a coenzyme derivative of vitamin B_{12} is also required, where it functions in its reduced form (B_{12r} in the following scheme) as an intermediate methyl carrier:

Methylation of homocysteine by folic acid derivatives



Animals also derive methyl groups from dietary choline, which can partially substitute for the methionine nutritional requirement. An oxidation product of choline, betaine, is the actual methyl donor to homocysteine. This probably represents a salvage pathway for methyl groups in the catabolism of choline, but it can be of considerable importance if the capacity for *de novo* methyl synthesis is limited.

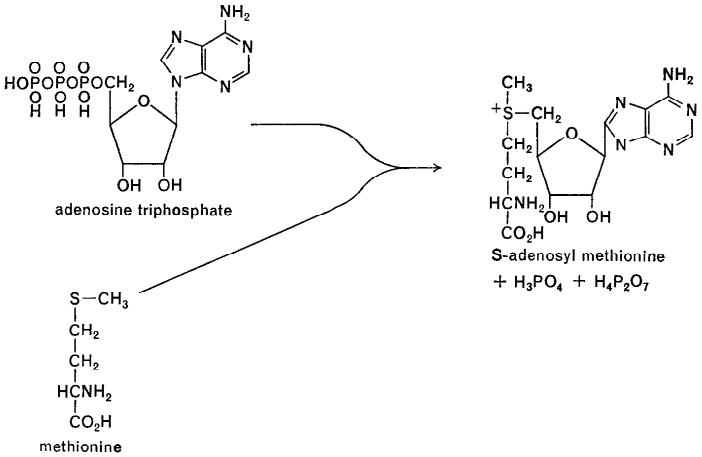
Choline as a methyl group source



2. S-Adenosyl methionine and transmethylation

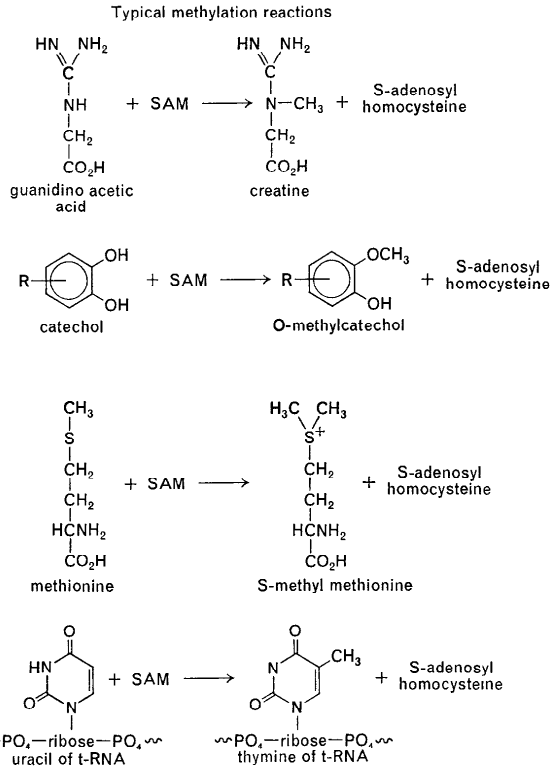
Methionine reacts with ATP to produce S-adenosyl methionine (SAM) with the release of both an orthophosphate and pyrophosphate residue.

Biosynthesis of S-adenosyl methionine



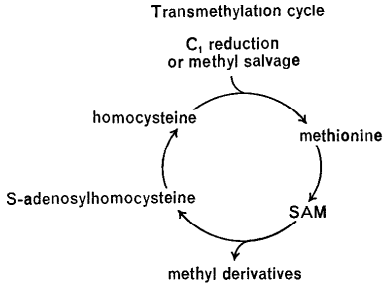
This sulphonium compound, often referred to as 'active methyl', serves as a methyl donor for biological synthesis. The list of compounds which derive methyl groups by transmethylation from SAM is extensive and

includes many types. Oxygen, nitrogen, sulphur and carbon atoms can act as acceptor. A few representative reactions are indicated below.



The S-adenosyl homocysteine produced in the transmethylation reactions is generally cleaved to adenosine and homocysteine. The latter can be degraded as previously discussed or be remethylated to methionine and eventually regenerate S-adenosyl methionine. Thus the operation of a methionine cycle provides a route whereby one-carbon metabolites reduced through the tetrahydrofolic acid sequence provide methyl groups for biosynthetic pathways. Certain other sulphonium compounds such as

S-methyl methionine and dimethyl β-propiothetin are apparently capable of serving as methylating agents in some organisms but do not have the general biological significance of S-adenosyl methionine.

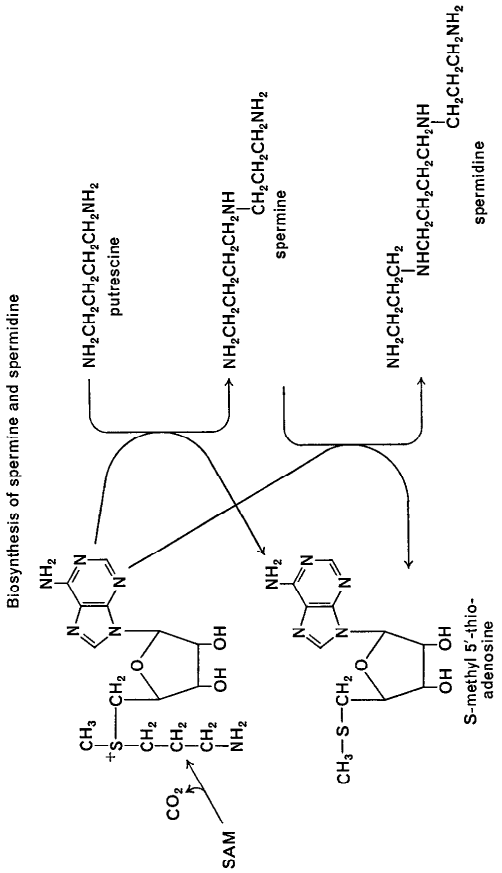


3. Other sulphonium ion alkylations

Methyl transfer is not the only kind of alkylation that can be effected by the sulphonium centre. The best studied example is the synthesis of the polyamines spermine and spermidine, important counter ions for nucleic acids. S-Adenosyl methionine undergoes a decarboxylation of the homocysteine side chain producing a thiopropyl amine derivative. The propyl amine residue then is transferred, first to one and then to the other amino group of putrescine yielding in turn spermine and spermidine[39].

S-Adenosyl methionine provides an interesting example of how thiol derivatives can promote what are normally considered to be difficult organic reactions. Few alkylating reagents employed by the chemist are compatible with the conditions of biochemical systems. Sulphonium ions can however be readily formed under biological conditions and are sufficiently stable in an aqueous environment to have their reaction controlled by enzyme specificity. The wide biological distribution of S-adenosyl methionine-mediated transmethylation attests to the fact that alkylation through sulphonium ion intermediates is among the most ancient biological group transfer reactions.

The chemical rationalization for the alkyl-transferring capacity of the sulphonium (and other 'onium') compounds is that the positively charged sulphur induces a partial positive charge on the immediately adjacent carbon atom. Such a positive carbon centre then becomes susceptible to nucleophilic attack. The thioether serves as an excellent leaving group particularly if a relatively nonpolar reactive centre is envisaged. Reactions involving S-adenosyl methionine as a methyl donor at neutral pH, generally



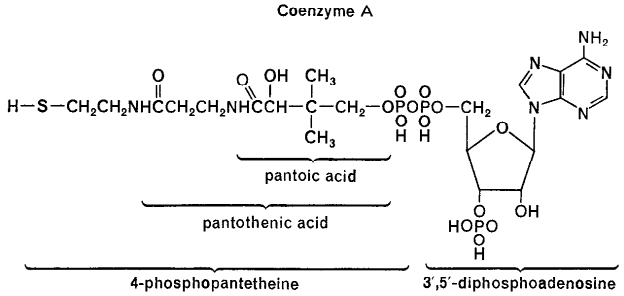
have favourable free energies of −7 (or more) kilocalories per mole. Thus, the intermediary role of SAM in biological transmethylations and occasionally in other transalkylation reactions reflects both thermodynamic and mechanistic attributes of sulphonium ions. Sulphonium ion reactions in turn constitute one of the fundamental functional roles of a thiol in biological systems.

C. Pantetheine Cofactors

The most clearly defined functional role of cellular thiols is that of coenzyme A and related cofactors[40,41,42]. Coenzyme A was first recognized as a carrier for activated acyl groups. The general sequence for acylation in biological systems is acyl activation to a thioester followed by acyl transfer to form amides, esters and acid anhydrides. In addition the thioester linkage enhances the carbonyl nature of the carboxylate group leading to a variety of reactions within the acyl carbon chain. Recently it has been recognized that the phosphopantetheine portion of the coenzyme A molecule also occurs in proteins, where it serves a similar role. A great deal of mechanistic information has been accumulated on enzyme reactions mediated by the thioesters of coenzyme A and related structures[43]. This is one of the areas in which the physical organic chemists' approach to biochemistry has proved most fruitful.

1. Biosynthesis of coenzyme A[44]

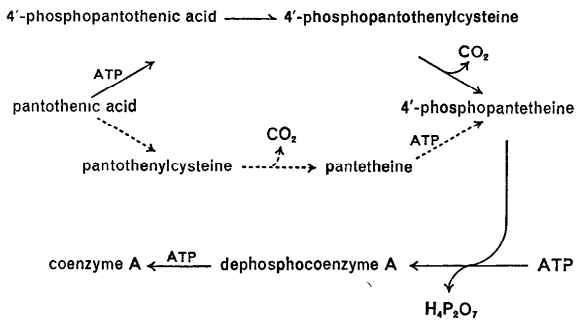
Coenzyme A is a complex organic molecule with a nucleotide portion of adenine, ribose and phosphoryl groups linked through a pyrophosphoryl bridge to an unusual peptide, pantetheine. This structure has a branched-chain dihydroxy acid, pantoic acid, linked to β-alanine which in turn is bonded to thioethylamine. In spite of the complexity of the coenzyme A



molecule our understanding of its function relates only to the fact that it is a thiol. The remainder of the molecule is presently relegated to imparting water solubility to acyl derivatives and providing highly specific structures for enzyme binding. In fact its catalytic function in several enzyme systems can be met by various simple N-acyl cysteamine models, although enzyme affinity is considerably lowered. While viewing coenzyme A simply as a thiol is generally recognized as being a gross oversimplification, evidence of any functional significance for other structural elements is sparse.

In microorganisms the pantoic acid carbon chain is derived from valine and 'active formaldehyde' and the β-alanine from aspartic acid. Higher organisms are unable to synthesize the pantothenic acid portion of the molecule and it is a required vitamin. Pantothenic acid is first phosphorylated to 4-phosphopantothenic acid and then condensed with cysteine to produce 4'-phosphopantothenyl cysteine. The cysteine residue then undergoes decarboxylation to 4'-phosphopantetheine. An adenylate is transferred from ATP to generate dephospho coenzyme A and a final phosphorylation of the 3'-hydroxyl of ribose provides the biologically active molecule. A slightly different sequence was thought to operate at one time, and still may be possible in some organisms. It differs only in that condensation with cysteine and the decarboxylation precedes the phosphorylation of the pantothenic acid hydroxyl group.

Biosynthesis of coenzyme A



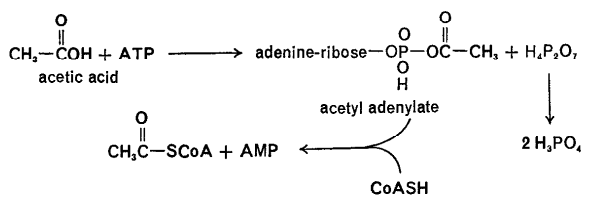
Coenzyme A can readily be oxidized to an inactive disulphide in air and mixed disulphides with other thiols such as cysteine and glutathione are also readily formed. In fact any reagent used to probe for enzyme thiols will also react with coenzyme A making studies of protein thiols much more difficult in coenzyme A-requiring systems.

2. Formation of coenzyme A thioesters[40,41]

In its biological function the sulphydryl group of coenzyme A is converted to a thioester. The acid is almost always a carboxylic acid although there have been some indications that coenzyme A thiophosphate esters might play a role in certain reactions. Thioesters have a sufficiently large negative free energy of hydrolysis to place them among the so-called 'high energy' compounds of biochemical energetics. Their synthesis must be driven by exergonic metabolic processes. Actually coenzyme A thioesters participate in the metabolic energy exchange system serving as an intermediate repository for the biochemical energy quanta represented by the squiggle (~) bond. Thioesters are formed by nucleoside triphosphate-dependent reactions, by oxidative processes or by thiolytic cleavage of β-keto thioesters. The coenzyme A derivative can donate the acyl to amino, thiol, hydroxyl and carbanion centres in energetically favourable reactions. It can also drive the formation of pyrophosphate linkages of nucleoside triphosphates. Coupled with this high reactive potential of the thioester is an amazing kinetic stability. Spontaneous decomposition mechanisms are not available in an aqueous environment at neutral pH and physiological temperatures. Such a situation is biochemically ideal, a high reactivity which can be completely controlled by enzymatic catalysis.

The direct route of acyl coenzyme A synthesis from a free carboxylic acid is catalysed by a group of nucleoside triphosphate-requiring enzymes, collectively known as thiokinases. The general mechanism, as exemplified for acetate activation by acetyl thiokinase, proceeds as follows. The carboxylic acid is first activated by acetyl adenylate formation with the displacement of pyrophosphate from ATP. While the initial reaction is fully reversible, subsequent action of pyrophosphatase drives the reaction

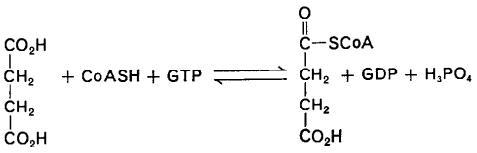
Acyl thiokinase reaction via an acyladenylate



process. The thiol of coenzyme A then displaces adenylic acid in a second step to produce the acetyl thioester.

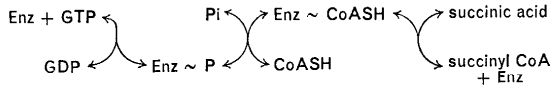
Acyl adenylate intermediates seem the general rule for acyl activation, but alternate mechanisms are known. An example is the succinyl thiokinase reaction[45]. The mammalian enzyme system utilizes guanosine triphosphate (GTP) or inosine triphosphate (ITP), although similar ATP-requiring enzymes are known from plants and bacteria. In addition to the coenzyme A derivative, a nucleoside disphosphate and inorganic phosphate are produced.

Succinyl thiokinase reaction



The products suggest activation as a phosphoryl rather than as a nucleotidyl derivative. Both succinyl phosphate and thiophosphoryl coenzyme A have been suggested as intermediates. However, neither is included, at least as a freely dissociable intermediate, in current formulations of this reaction. An enzyme-bound phosphoryl histidine intermediate is thought to be involved, as is some sort of activated enzyme–CoA complex. Many aspects of the enzyme mechanism are still in doubt, but the sequence below is consistent with most available data.

Proposed succinylthiokinase mechanism



This mode of thioester formation is not as energetically favourable as that involving pyrophosphate release and its eventual cleavage. This probably reflects different biological roles for the two types of thiokinases. Succinyl thiokinase and probably other nucleoside diphosphate–inorganic phosphate type enzymes normally operate in the other direction, with thioacyl coenzyme A driving the synthesis of nucleoside triphosphate. One type of enzyme system produces coenzyme A thioesters efficiently at the expense of nucleoside triphosphate, while the other helps to couple metabolic processes to the synthesis of high energy phosphates.

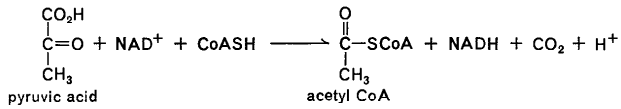
Another way to generate particular acyl coenzyme A derivatives is at the expense of others. The succinyl–acetoacetyl coenzyme A transferase reaction is an important example.

Acyl interchange reaction

An intermediate enzyme–coenzyme A complex in which the energy of the thioester bond is preserved has been demonstrated. Here the coenzyme A thioester is involved in a transfer reaction quite different from its usual acyl donor role. Functionally this enzyme allows metabolically generated coenzyme A derivatives to be utilized directly for carboxylic acid activation, without intermediary formation of nucleoside triphosphates.

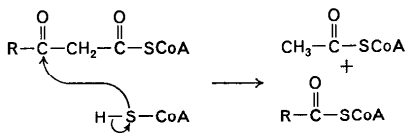
A metabolically important route for the generation of acyl coenzyme A derivatives is through the oxidation of α-keto acids. The α-keto acid dehydrogenase complexes, of which pyruvate dehydrogenase complex is typical, are large multienzyme aggregates. They carry out a complex reaction sequence to be discussed in section III.D on lipoic acid. The overall reaction given below is an oxidative decarboxylation coupled to thioester formation.

Pyruvate decarboxylase-dehydrogenase reaction



The final process for coenzyme A thioester synthesis is by the thiolytic cleavage of β-keto acyl coenzyme A derivatives. The thiolase reaction is the principal metabolic process for degrading the hydrocarbon chain of fatty acids.

β-ketofatty acyl CoA thiolase reaction

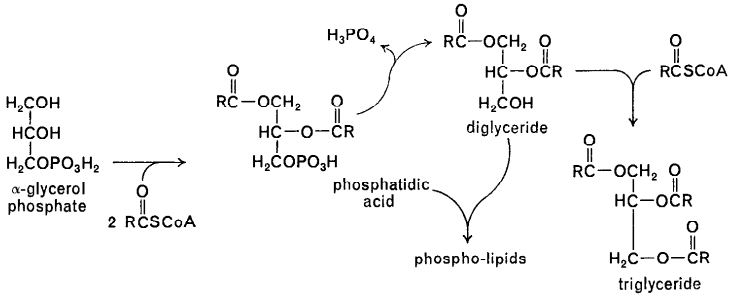


3. Reactions of coenzyme A thioesters[42,43]

Examples of acylation by acyl coenzyme A derivatives are numerous[41]. The quantitatively most important example is the transfer of fatty acyl

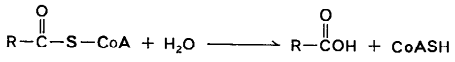
residues from coenzyme A in the synthesis of glycerides. In this case the acyl acceptors are the hydroxyl groups of glycerol derivatives and the products are oxygen esters. Acyl coenzyme A hydrolases can also be

Pathways for glycerol lipid synthesis



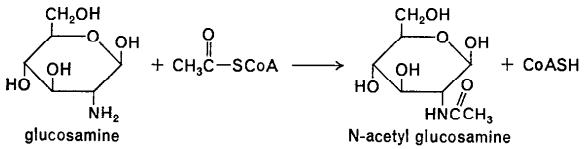
looked upon as acyl O-transferases of a special type with water acting as acceptor.

Acyl-coenzyme A hydrolase reaction



Transfer from an acyl coenzyme A derivative to a nitrogen nucleophile is also quite common. Typical is the N-acetylation of the amino sugars such as glucosamine. The conversion of palmityl coenzyme A to palmit-

Glucosamine Acetylation



aldehyde by reduced pyridine nucleotide can be considered, at least formally, as an acyl transfer reaction. Here the acyl acceptor can be envisaged as a hydride ion derived from NADH.

Reactions where phosphate, thiol and even cyanide accept the substituent from acyl coenzyme A derivatives have been described in biological

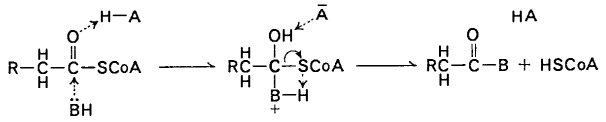
Acyl coenzyme A reductase reaction

systems. Carbon is also an important acyl acceptor, generally reacting as a resonance-stabilized carbanion. Examples are the Claisen type ester condensation reactions to be discussed in section III.E.1.

The increased acyl transfer potential of thioesters as compared to corresponding oxygen esters is explained as being due to less double bond character in the bridging bond. The unpaired sulphur electrons do not have as high a tendency towards double bond formation as those of oxygen, and less electron delocalization or resonance stabilization of the bonding system is possible. This results in a longer and more easily displaced linkage. The lack of resonance with the ester sulphur also results in an enhanced electrophilic character of the carbonyl carbon. Thus, attack by nucleophiles at this position is facilitated.

The general mechanism for acyl transfer reactions from thioesters is envisaged as a nucleophilic attack at the positively polarized carbonyl carbon, accompanied by or followed by thiol elimination.

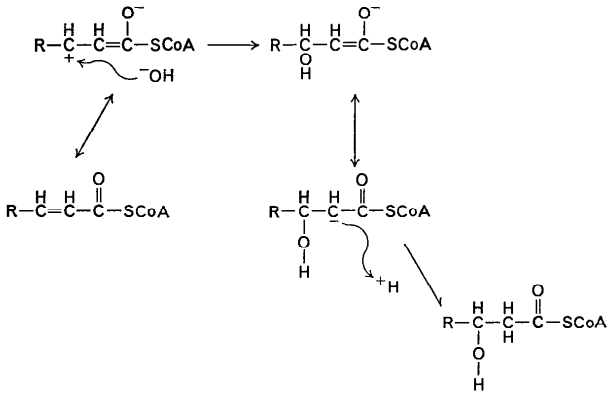
General transacylation mechanism from acyl coenzyme A



It is supposed that the enzyme participates by providing general acid and general base groups which facilitate the attack of the entering nucleophile, the departure of the thiolate and the polarization of the carbonyl. An intermediate acylated enzyme may occur in some reactions but this can simply be envisioned as a case where binding centre, catalytic groups and the initial attacking nucleophile are all provided by the enzyme.

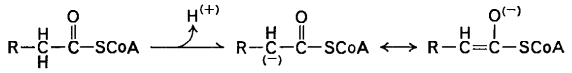
Coenzyme A thioesters can also promote nucleophilic attack at the β-carbon in α,β-enoyl derivatives. In these cases an electrophilic centre is stabilized at the β-carbon by resonance with the carbonyl system. This could be particularly favoured by hydrogen bonding or protonation of the carbonyl oxygen by an enzyme. An example is the enoyl coenzyme A hydratase reaction of fatty acid degradation.

β-substitution in α,β-enoyl coenzyme A thioesters



α-Activation is the other crucial aspect of thioester and acyl coenzyme A biochemistry. The formation of the thioester considerably increases the ketone-like character of the carbonyl group of the carboxylic acid. In addition to increasing the electrophilic behaviour of the carbonyl carbon, it enhances the acidity of the hydrogens at the α-position. This is normally attributed to the possibility for resonance stabilization involving the enolate anion.

Enolate stabilization in coenzyme A thioesters

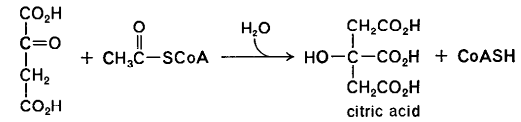


Enolate ion formation allows coenzyme A-bound acyl groups to serve as nucleophiles and to react at electrophilic centres. This permits thioesters to participate in the formation or degradation of carbon—carbon linkages by mechanisms analogous to the aldol condensation or more specifically the Claisen type ester condensation. There are few available mechanisms for carbon—carbon bond formation or cleavage which can be employed under biological reaction conditions, and pathways which depend on coenzyme A thioesters for this purpose are widespread.

The classic example is the reaction by which acetate carbon enters the tricarboxylic acid cycle, the citrate synthase reaction. Extensive mechanistic studies have established the involvement of the enolate of the acetyl

thioester in the enzyme reaction[42]. Exchange of the acetate hydrogens of acetyl coenzyme A with deuterium or tritium in the solvent is catalysed by the enzyme under conditions in which the condensation cannot occur.

Citrate synthase reaction

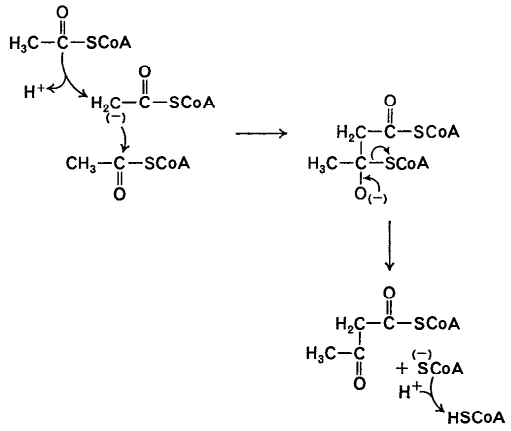


oxaloacetic acid citric acid

Initially this was not observed, exchange only being measurable when oxaloacetate was also present. This absence of exchange is now believed to result from a need to have oxaloacetate bound to the enzyme before the proper catalytic configuration can be achieved. This function can be served by certain other dicarboxylic acids which are not capable of undergoing the condensation reaction and the exchange activity has been demonstrated. A coenzyme A-facilitated enolization mechanism seems firmly established.

An example in which reactivity of both the attacking nucleophile and the electrophilic acceptor is dependent on the special character of acyl thioesters is in the condensation of two acetyl coenzyme A units to form acetoacetyl coenzyme A. This is the reverse of the thiolase reaction

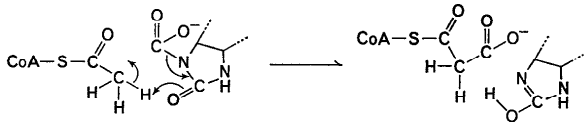
Condensation of two acetyl coenzyme A units



discussed previously. The actual mechanism of this reaction may involve an initial transfer of one acetyl grouping to an enzyme thiol prior to condensation, but the general reaction scheme is unchanged as thioacyl activation would still be involved.

α-Carbon activation is also involved in the biotin-mediated carboxylation of acetyl coenzyme A to malonyl coenzyme A, a critical and distinctive step in fatty acid biosynthesis[46]. Carbon dioxide is initially attached to a ureido carbon of biotin and then transferred to the methyl carbon of acetyl coenzyme A. A concerted mechanism for this transfer has been suggested rather than a pre-equilibrium enolization of the acetyl coenzyme A on the basis of the stereochemistry of the condensation[42]. The proposed reaction sequence is an example of how concerted substitution on the α-carbon of thioesters could be facilitated.

Mechanism of acetyl coenzyme A carboxylation

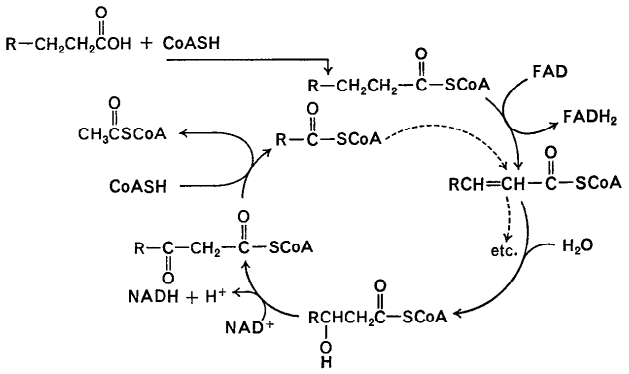


The thioester promotes the acidity of the α-hydrogens favouring hydrogen-bonded interaction with the ureido oxygen. In this case the promoting base and the electrophilic centre being attacked are part of the same structure, permitting a concerted electronic rearrangement without the necessity of an actual enolate ion. Since similar advantageous arrangements of reacting and catalytic functions are possible on enzymes, it is conceivable that other examples may also circumvent the pre-equilibrium enolate formation which would be predicted from analogy to solution chemistry. This does not alter the concept that thioesters facilitate such reactions by enhancing the acidity of α-hydrogens.

A convenient way to summarize the reactions of coenzyme A thioesters is by reviewing the β-oxidation pathway for fatty acids[46]. Fatty acid activation occurs by acylation of the coenzyme A thiol by way of an acyl adenylate. This is then dehydrogenated to an α,β-enoyl acyl coenzyme A derivative by a flavin-dependent dehydrogenase. The ability of the adjacent carbonyl to provide resonance stabilization of the product appears to be an important aspect of this reaction. Such flavin-dependent dehydrogenations occur in other reaction sequences, but only where carbonyl resonance stabilization is possible. Water adds to the α,β-enoyl thioester to generate a β-hydroxy fatty acid derivative, a reaction facilitated by β-carbonium ion stabilization in enoyl thioesters. The β-hydroxyl is next

reduced to a β-keto group. Such nicotinamide coenzyme-linked reductions to alcohols are common and no special advantage can be ascribed to the thioester. Thiolytic cleavage of the β-keto thioester releases acetyl coenzyme A and leaves a fatty acid derivative two carbons shorter than the original. The desaturation, hydration, dehydrogenation, thiolation sequence is repeated to reduce the chain by two carbons at a time with almost every step dependent on the unique properties of coenzyme A thioesters.

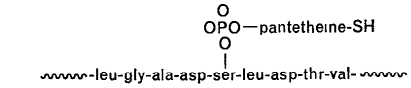
Fatty acid oxidation spiral



4. Phosphopantetheine proteins

Protein-bound phosphopantetheine has been found in recent years to be involved in acyl binding and reaction in much the same manner as coenzyme A [47]. A 77 amino acid protein was isolated from *E. coli* which acted as an acyl carrier in fatty acid synthesis. This protein completely lacked cysteine or other thiol amino acid, yet functioned by binding various acyl intermediates as thioesters. The reactive centre was phosphopantetheine linked to the protein through a phosphodiester bridge to serine. Similar acyl carrier proteins, or ACPs, have now been isolated from a variety of organisms and extensively characterized. An active ACP protein chain has even been prepared synthetically. ACP *per se* has been

Phosphopantetheine linkage in *E. coli* acyl carrier protein



difficult to demonstrate in higher organisms in which the intermediates of fatty acid synthesis are bound to high molecular weight complexes. It is reasonably certain that protein-bound phosphopantetheine is involved however, and an analogous protein cofactor is believed to be present in a tightly bound form. Phosphopantetheine prosthetic groups are now also known to function in other pathways.

Coenzyme A is the precursor of the enzyme-bound phosphopantetheine. The prosthetic group is added to the prosthetic group free protein (apo-ACP), by a phosphoryl transfer reaction employing coenzyme A as donor, yielding the functional complex protein, holo-APC:

Attachment of 4-phosphopantetheine to protein

apo-ACP + coenzyme A ⟶ holo-ACP + 3′,5′-adenosine diphosphate

The phosphopantetheine prosthetic group of ACP, fatty acid synthetase complexes, and presumably other enzyme systems, turn over rapidly, possibly as part of a cellular control mechanism. A specific phosphodiesterase cleaves holo-ACP to 4′-phosphopantetheine and the apoprotein:

Removal of 4-phosphopantetheine from protein

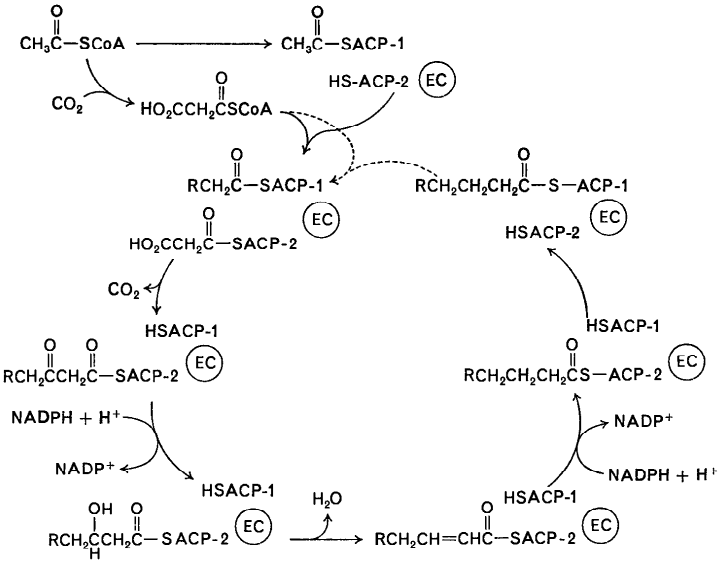
holo-ACP $\xrightarrow{H_2O}$ 4-phosphopantetheine + apo-ACP

The role of phosphopantetheine linked to protein is analogous to that in coenzyme A. Mechanistically fatty acid synthesis is pretty much a reversal of the β-oxidation pathway discussed earlier. There are however a few minor and one major differences. ACP rather than coenzyme A derivatives participate in synthesis and a nicotinamide coenzyme rather than a flavin cofactor is involved in double bond reduction. The major difference is that in the chain-elongating thioester condensation reaction the attacking nucleophilic carbon derives from a malonyl rather than an acetyl thioester. As indicated previously, malonyl coenzyme A is produced from acetyl coenzyme A by a biotin- and ATP-dependent CO_2 fixation reaction. Both acetyl and malonyl groupings are transacylated to ACP for fatty acid synthesis. Enzyme thiols, in addition to those of the phosphopantetheine prosthetic group, are also implicated in the process. In the yeast system, at least, a thioacyl linkage to a cysteinyl residue participates at one stage.

A turn of a generalized fatty acid synthesis spiral is presented below where the intermediate carriers are represented as ACP units tightly bound to a multienzyme complex, (EC).

Specific details vary somewhat from species to species, but this scheme illustrates a typical phosphopantetheine protein involvement.

Generalized fatty acid synthesis spiral



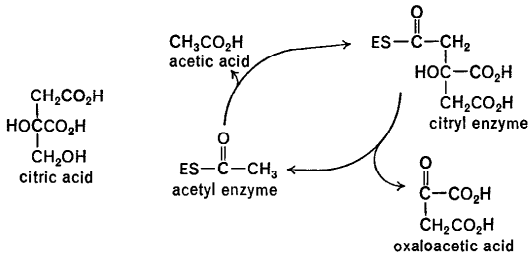
Acetyl coenzyme A transfers its substituent to ACP-1 of the synthetase complex where it serves as the start of the growing chain. A subsequent acetyl coenzyme A unit is carboxylated to malonyl coenzyme A and transferred to ACP-2. The acetyl (or higher homologue) segment then reacts with the malonyl methylene carbon accompanied by the release of CO_2 and freeing the thiol of ACP-1. The β-keto derivative of ACP-2 is then reduced to the β-hydroxy, dehydrated to the α,β-enoyl and reduced to the saturated fatty acid derivative. The acyl group is next transferred to ACP-1. With the entry of a new malonyl unit on ACP-2 the sequence repeats and the chain is built up two carbon units at a time. No intermediates are released from the complex until the long-chain fatty acid is completed. The fatty acyl linkage is then transferred from ACP-1 to coenzyme A for use in the synthesis of complex lipids. A direct utilization of the ACP thioester for acylation of lipids probably occurs in some systems.

The point of note is the special role of the malonyl thioester in the chain elongation process. The presence of the additional carboxylate group

adjacent to the methylene carbon increases the stabilization of a carbanion at this position. This further facilitates proton dissociation and attack at the carbonyl of the other ACP-bound thioester. The concerted loss of CO_2 renders the reaction essentially irreversible and provides a thermodynamic situation favourable for chain elongation.

Multienzyme complexes responsible for the assembly of the cyclic polypeptide antibiotics, gramicidin and tyrocidine, also contain protein-bound phosphopantetheine. This presumably participates in the enzyme-directed peptide bond assembly as an amino acyl carrier. Citrate lyase catalyses the cleavage of citrate to oxaloacetate and acetate without the involvement of coenzyme A. This has posed somewhat of a dilemma since thioester activation is considered mechanistically important in the oxaloacetate–acetate condensation sequence and presumably should also be necessary for decondensation. Recent evidence implies that the enzyme contains a phosphopantetheine unit which is acetylated in the active enzyme[48]. The reaction is envisaged as an acyl exchange with citrate, releasing acetate and generating a citryl thioenzyme. This then undergoes a thioester-promoted decondensation releasing oxaloacetate and re-generating the S-acetyl enzyme.

Proposed citrate lyase mechanism

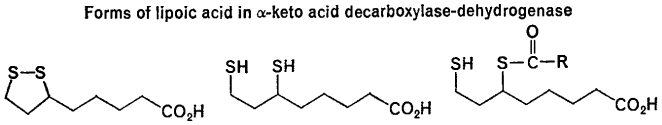


Thus the biological importance of the phosphopantetheine group as a catalytic centre is widespread. Numerous examples of the role of coenzyme A are known and the list of phosphopantetheine enzyme centres is growing. The principal reactive element is the thiol, although other attributes of the unique peptide will undoubtedly prove important. The thiol serves as the site of thioester formation and its particular chemical attributes facilitate acyl transfer, carbon chain modification and condensation reactions. The phosphopantetheine thiol represents the most extensively investigated example of this functional group in biochemical processes.

D. Lipoic Acid[8,49]

Lipoic acid is a five-membered cyclic disulphide ring with a five-carbon carboxylic acid chain. When reduced it provides a constrained dithiol centre. This disulphide–dithiol cofactor is covalently bound to one of the enzymes in a multienzyme complex which catalyses oxidative decarboxylation of α-keto acids. In the course of the reaction three forms of the prosthetic group participate; the cyclic disulphide, the dithiol and a thioester of the dithiol form.

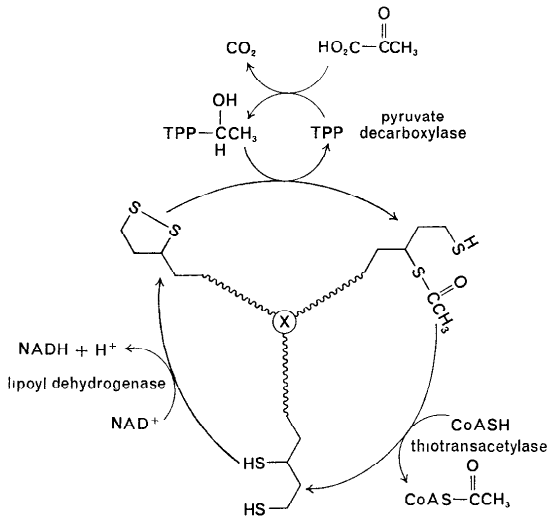
Forms of lipoic acid in α-keto acid decarboxylase-dehydrogenase



The reactions of the α-keto acid decarboxylase system occur in a highly organized complex of enzymes which utilizes a number of cofactors in addition to lipoic acid[50]. It has been proposed that a long flexible arm resulting from the amide linkage of the lipoyl carboxylate to an ε-amino group of a protein lysine permits the disulphide–dithiol centre to swing from one active site to another within the confines of the complex. The lipoic acid centre therefore may serve a physical transport role within its special environment, in addition to its chemical participation in the reaction sequence. In the initial reaction of the α-keto acid system a thiamine pyrophosphate-mediated decarboxylation results in a thiamine–aldehyde adduct. This is oxidized by the lipoic acid disulphide and the resulting acyl transferred from thiamine to the thiol at carbon-6 of the dihydrolipoyl residue. A second enzyme of the complex then transfers the thioacyl from the dithiol to coenzyme A. This system thus provides one of the major routes for acyl coenzyme A production from sugar and amino acid metabolites. At the reactive centre of the third enzyme of the complex the lipoyl disulphide is regenerated by oxidation of the dithiol by a nicotinamide coenzyme. The dihydrolipoyl dehydrogenase is an unusual flavoprotein which will be discussed subsequently as an example of a dithiol–disulphide electron transfer protein.

Lipoic acid links two of the major biochemical roles of thiol groups, being both involved in electron transfer and the generation of high

Action of lipoic acid in pyruvate decarboxylase complex



energy thioester bonds. By positioning the two thiol groups in a close relationship specific oxidation is facilitated. The presence of strain in the five-membered dithiolane ring system also may be an important aspect of lipoic acid biochemistry, but its functional significance has remained moot.

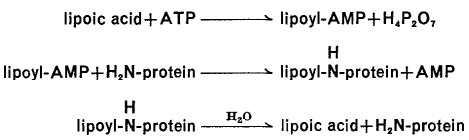
There are relatively large amounts of lipoic acid and dihydrolipoyl dehydrogenase in photosynthetic tissues. Their presence still lacks a satisfactory explanation in terms of a particular functional role. Proposals implicating the lipoate dithiolane ring system in primary energy trapping or in the transfer and utilization of chlorophyll-trapped energy has not gained any real acceptance[51].

Photosynthetic carbon dioxide fixation into α-keto acids has recently been found to be the major pathway in some organisms. The process appears to be essentially a reversal of the mitochondrial oxidative decarboxylation process[52]. The photoreduction is mediated through a ferredoxin system similar to the photosynthetic nicotinamide coenzyme reductase. The involvement of lipoic acid has not yet been shown, but it would be expected and could provide the long-sought role of lipoate in photosynthesis.

The really unique reaction of the lipoate centre in α-keto acid metabolism is the oxidative thioester formation from a thiamine-coordinated 'active aldehyde'. Thiol transacetylase and dithiol–disulphide oxidation reduction roles are well-known attributes of other biological thiols. Unfortunately mechanistic studies on this reductive acylation of a cyclic disulphide have so far received little attention. Proposals that a lipoic acid–thiamine pyrophosphate compound was the functional entity in α-keto acid oxidation have been completely abandoned, but data supporting this concept remain unexplained. Investigations in this area might have some relevance for the reductive acylation process.

Enzyme systems have been found for the formation and hydrolysis of the lipoyl amide linkage at appropriate lysine ε-amino groups of enzymes[49]. The lipoic acid is activated by ATP to form a lipoyl adenylate, possibly as an enzyme-bound form, which then transfers the lipoyl group to the protein amino group.

Attachment and release of enzyme-bound lipoic acid



The specific cofactor attachment and removal system could reflect an effective enzyme control mechanism. At present there is no evidence that such a control is manifest within cells, and these reactions must be viewed as synthetic and degradative processes.

It should be noted that most enzyme studies concerning this disulphide–dithiol coenzyme have actually been carried out with either free lipoic acid or lipoamide and not a protein-bound cofactor. While this has been a pragmatic necessity, certain reserve should be maintained in extrapolating from such studies to the protein-bound prosthetic group.

The only established lipoic acid function is that in the α-keto acid decarboxylase–dehydrogenase complexes, although several examples of this type of enzyme with varying substrate specificities are known. Other examples of lipoic acid enzymes have been sought, but other dithiol–disulphide enzymes have been shown to be free of lipoic acid residues. Sulphoxide derivatives of lipoic acid are easily isolated, and their possible biological function has also been suggested. However, presently accepted dogma dismisses the more oxidized forms of lipoic acid as artifacts of air oxidation during isolation.

E. Thiol Proteins[53-56]

A large number of functional proteins are known in which substitution of some or all of the thiols of cysteine residues interferes with activity. Most frequently this is only a reflection of a requirement for the thiol in maintaining a proper configuration or subunit interaction. In some cases a thiol group is believed to exist in or near the active site and possibly play a role in substrate or cofactor binding. In a few enzymes the cysteine thiol is known to play a critical role in the catalytic process. In all of these cases enzyme activity or other biological function can be influenced by reaction of the protein with thiol-specific reagents. The diverse spectrum of chemicals used to probe for thiol function in biological reaction systems will not be discussed here, nor will the limits of their supposed specificity. Other sources should be consulted for information on these fascinating but overly extensive topics[57-59]. It is probably important to point out, however, that a variety of types of chemicals are commonly employed including metal ions, organometallics, alkylating agents, and disulphide oxidants. Sometimes quite different results are achieved with different agents. Furthermore, their specificity for thiol functions is not complete. Thus evidence for thiol groups based on thiol-specific reagents must always be viewed with caution. Only in those cases where there is strong collaborating evidence can indications for thiol function be considered secure.

Those proteins for which the thiol has no known specific function are not really of interest for the present discussion since no particular aspect of thiol chemistry can be related to the biological activity. Most of the emphasis will be reserved for thoses cases where the thiol group participation in the reaction is clearly established. Examples where thiol involvement is merely postulated will be mentioned only if they represent particularly interesting possibilities of thiol function.

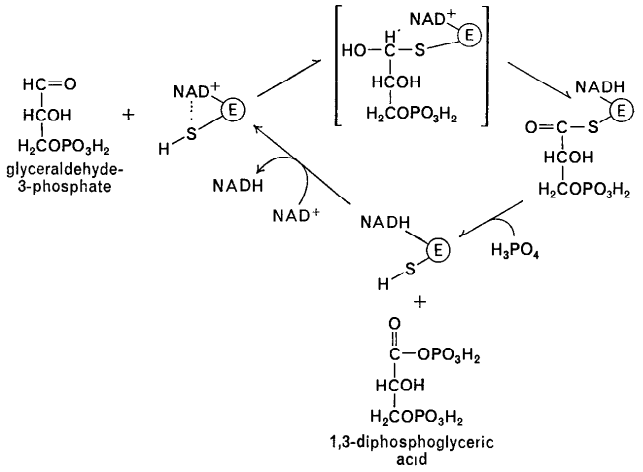
I. Thioester enzyme intermediates

Glyceraldehyde phosphate dehydrogenase probably holds the distinction of being the classic thiol enzyme in the minds of most biochemists[60,61]. The thiol is believed to be involved in the initial attachment of the aldehyde substrate as a thiohemiacetal. The enzyme-bound thiohemiacetal is then oxidized by NAD^+ generating an enzyme-bound thioester. In more sophisticated proposals for this mechanism the nicotinamide cofactor interacts with the active centre thiol as a charge transfer type of complex. This facilitates the reaction of the thiol with the carbonyl of the substrate. The thiol addition and the electron transfer to nicotinamide occur

simultaneously so that the thiohemiacetal actually does not build up as true steady state intermediate.

The thioester of phosphoglyceric acid is generated as an enzyme-bound reaction intermediate. It possesses a highly negative free energy of hydrolysis and is capable of driving ATP synthesis. The freely reversible interaction of a thiol with an aldehyde carbonyl followed by oxidation of the thiohemiacetal has provided the cell with a mechanism for trapping part of the energy released in the conversion of an aldehyde to an acid. The enzyme-bound thioester undergoes phosphorolysis in the normal course of events, freeing the enzyme thiol and producing 1,3-diphosphoglyceric acid. This enzyme system is fully reversible and the thioester intermediate can be generated from the acyl phosphate.

Glyceraldehyde phosphate dehydrogenase reaction

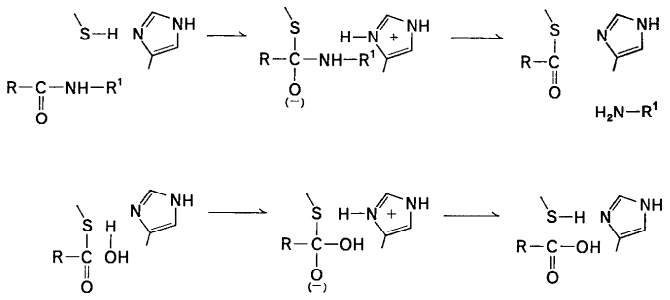


Treatment of the enzyme with acyl phosphate in the complete absence of reduced cofactor has allowed the thiol enzyme derivative to be prepared and separated from its reaction mixture. This in turn has permitted considerable characterization of the enzyme thiol. No special cofactor is involved. The thiol of a cysteine residue from the main peptide chain of the enzyme provides the reactive centre[53]. This enzyme demonstrates that the acyl transfer role of thioesters in biological systems is not restricted to phosphopantetheine and dihydrolipoate derivatives. The reactions of the

enzyme thioester are analogous with the transacylations to phosphate and hydride ion described previously (section III.C.3). Acyl transferase reactions to hydroxylamine, arsenate, methylmercaptan and even a nitrogen within the enzyme itself can be demonstrated with acylated glyceraldehyde phosphate dehydrogenase. These reactions probably have no biological significance but have proven useful in substantiating and characterizing the thioester intermediate.

The thiol enzyme for which the most detailed mechanistic formulations have been proposed is papain[62,63]. In this enzyme a cysteine thiol group appears to function in the same manner as the serine hydroxyl of other proteases and esterases. In the hydrolysis of proteins by this plant protease there is an intermediate formation of an acyl thiol, which is subsequently cleaved by water.

Mechanism of papain proteolysis

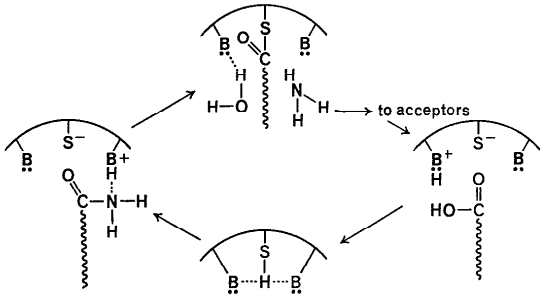


Imidazole from an enzyme histidine and possibly an enzyme carboxylate group are thought to participate in the reaction. X-ray crystal analysis of the protein[64] has established that a cysteine at position 25 and a histidine at position 159 are so positioned that they can participate in a hydrogen-bonded reactive centre. An aspartic acid at position 158 is also close enough to influence the reaction. The papain-active-centre thiol shows exceedingly rapid rates of reaction with certain thiol reagents. This suggests an enhanced nucleophilic character due to interaction with the imidazole and possibly other functional groupings in the reactive centre. The participation of a cysteine thiol in papain and other plant proteases must be considered unusual from the standpoint of thiol chemistry. Acyl transfer from amide nitrogen to sulphur is not considered thermodynamically reasonable, except under unusual circumstances. In this regard it is interesting to note that the active-centre serine hydroxyl of the

bacterial protease, subtilisin, can be chemically converted to a thiol and still retain certain enzymatic activities[65]. This stresses the critical importance of the proper juxtaposition of appropriate reactive groupings as opposed to the precise chemical attributes of any single functional group in enzymatic catalysis.

An intermediate formation of a thioester, facilitated by adjacent acid and base groups, has also been proposed as a general mechanism for glutamine-mediated amination reactions[66]. The apparent function of glutamine in such reactions is to provide a source of unhydrated ammonia at the reactive centre. This is accomplished by hydrolysis of the amide with the following type of mechanism being suggested:

Proposed reactions for ammonia generation from glutamine



Thus thioacyl cysteines appear to participate in the catalytic function of diverse types of enzymes, even when the conservation of a high energy bond is not the prime consideration.

2. Persulphide enzyme intermediates[1,67]

Rhodanese provides an example of a thiol enzyme of a somewhat different type. This enzyme, which is widely distributed throughout nature, catalyses the formation of thiocyanate from thiosulphate and cyanide. This reaction probably does not represent the true biological

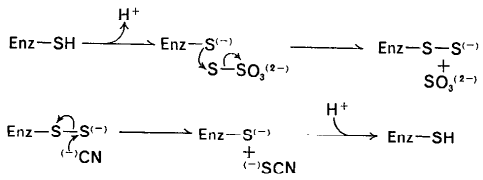
Rhodanese reaction

$$HSSO_3H + {}^{(-)}CN \longrightarrow {}^{(-)}SCN + H_2SO_3$$

action of the enzyme, although it could provide a system for the detoxification of cyanide. The reaction is more likely only a convenient means for the *in vitro* assay of some uncharacterized sulphur-transferring

system. The proposed mechanism involves an initial transfer of sulphur from the donor to an enzyme thiol group producing an enzyme persulphide. The persulphide sulphur is then displaced by the acceptor-regenerating enzyme thiol.

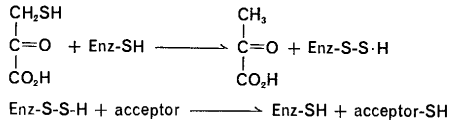
Persulphide enzyme mechanism for rhodanese



Some doubt that enzyme persulphide *per se* exists in the enzyme intermediate has been indicated, but at least an enzyme-stabilized equivalent of persulphide seems generally accepted. A release of the intermediate persulphide sulphur from the enzyme can be effected by heat or trichloroacetic acid treatment.

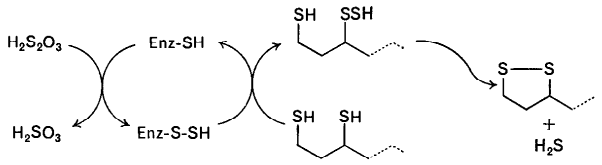
The enzyme transferring sulphur from 3-mercapto pyruvate appears to have a similar mechanism, involving a persulphide-like enzymatic intermediate. The possible role of this enzyme in transsulphuration from cysteine has been discussed earlier.

Thiol pyruvate transsulphurase reaction



In the presence of disulphide-reducing agents there is a production of sulphide from persulphide enzyme intermediates. Dihydrolipoate (or more likely a protein-bound form) may be a natural acceptor substrate for such enzymes. Only one optical isomer reacted in the rhodanese system, suggesting the presence of a specific binding site. It was presumed that one of the dihydrolipoyl thiols acted as the sulphur acceptor with a subsequent release of sulphide through displacement by the adjacent thiol. Therefore these enzymes may normally function in reductive desulphuration. Alternatively, transsulphuration by way of the enzyme persulphide may be the important biological process. It has been proposed that rhodanese, and by inference other enzyme persulphide transferases, may be the

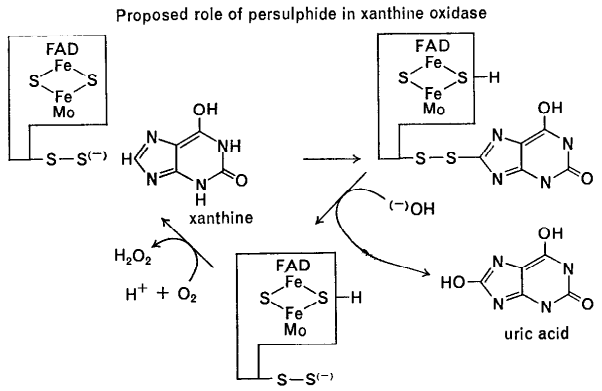
Possible persulphide mediated desulphydration mechanism



immediate donor of the 'labile sulphide' for the biosynthesis of nonhaem iron proteins such as ferredoxin[68].

Evidence for the presence of a persulphide group in the active form of xanthine oxidase has recently been presented, and a direct catalytic role for the group is proposed[69]. Thus protein persulphides may play a significant functional group role in their own right.

Proposed role of persulphide in xanthine oxidase

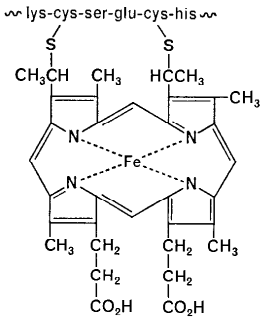


3. Thiol-binding centres

Another way thiols can participate in enzyme reactions is by binding substrates or coenzymes at the active site. A clear differentiation between involvement in catalytic and binding functions is seldom possible, but a binding role is presumed when protection of the critical thiol is afforded by the presence of substrate and no specific catalytic role is suspected. There are only a few proven examples of thiol substrate binding other than those already discussed in which a precise catalytic role is also proposed.

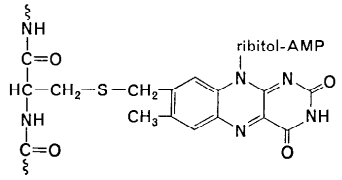
The clearest examples of thiol-binding centres are those in which the linkage is covalent. Attachment of the haem group to the cytochrome c protein occurs through two cysteine thiol residues[54]. The sulphydryls add across the double bond of two vinylic side chains of the iron tetrapyrrole, providing thioether bridges between the protein and the prosthetic group.

Haem linkage to human cytochrome c



The binding of a flavin prosthetic group to hepatic monamine oxidase has recently been reported to involve a thiol[70]. FAD is linked as a thioether formed between a cysteine and a methyl substituent on the dimethyl isoalloxazine. While binding is generally conceived to be a reversible process and these cases must be viewed as an extreme, they do provide clear examples of the general concept.

Flavin binding to monamine oxidase



A frequently postulated binding role for thiols is in the attachment of metals to metalloproteins[71]. The involvement of thiol ligands will influence the strength and specificity of metal-complexing centres and in this way could affect the structure and function of proteins in rather specific ways.

Polythiol metal-binding sites will be discussed in section III.F.4, but single thiols acting in conjunction with oxygen and nitrogen ligands are also quite important.

Loss of titratable thiol in the presence of zinc and the magnitudes of the stability constants for a series of enzyme–metal complexes has implicated a nitrogen–sulphur metal-binding centre in bovine carboxypeptidase. However, no cysteine side chains were found within the zinc coordination sphere on X-ray crystallographic analysis, casting considerable doubt on these conclusions[72]. A thiol has also been implicated in metal binding by human carbonic anhydrase, but the complete lack of cysteine in the bovine enzyme makes this contention somewhat uncertain since zinc binding by both enzymes is very similar. Metallothiol centres may themselves act as binding sites. A metal ion bridge is thought to be involved in nicotinamide coenzyme binding by alcohol dehydrogenase and there is evidence that the protein centre includes a thiol.

Most claims for thiol participation in binding are based on protection of sulphydryl groups by the presence of the ligand or on lack of binding if thiol groups have been blocked. Unfortunately, it has become increasingly obvious that such evidence does not necessarily mean that the thiol is directly involved or even that it is near the binding site. Attachment of substrate can simply mask an otherwise uninvolved thiol, or can induce a conformational shift which alters thiol reactivity. Conversely, the integrity of distant thiol groups may be necessary for the proper binding configuration of the protein. Their derivatization could produce structural rearrangements which would eliminate binding and activity in distant parts of the molecule. In fact, certain enzyme activities can be enhanced by thiol substitution, implying that the thiol effect must be taking place away from the active centre. Early studies showed that substitution of sulphydryl groups on haemoglobin altered the nature of the oxygen binding and eliminated haem–haem interactions[54]. This would now be explained as being due to alterations in subunit interaction since it is known that thiols are not in or near the oxygen-binding site[73].

4. Thiols and disulphides in protein structure[54]

The most common thiol role is participation in the overall structural integrity of proteins. Except for the special case of the disulphide linkage this can be viewed as a rather nonspecific and passive function. This is not to imply that in any given circumstance that another amino acid side chain might serve as effectively as cysteine or methionine, but rather to point out that these amino acids are no more critical in their place than are any other in theirs. From an experimental standpoint there is one

special significance of the sulphydryl group in protein structure. It is the ease and specificity with which it can be modified. The list of enzymes which have their activity influenced by thiol-specific reagents far exceeds the number for which a defined role in binding or catalysis can be established. In most of these cases it must be concluded that the thiol reagent sensitivity represents the loss of some critical structural feature upon thiol modification.

It is also not surprising that quite contradictory effects can sometimes be achieved with various thiol reagents since these introduce different bulk, ionic charge or hydrogen-binding capabilities at the site of substitution.

While offering little information on the active structure of proteins, modification of these 'structural' sulphydryl residues has been helpful to the biochemist in many instances[74]. As examples one can cite the increasing success of thiol reagents in dissociating subunit enzymes and releasing tightly bound cofactors without destroying covalent linkages. When the thiol blocking agent can subsequently be removed, as is the case with organic mercurials, the reassembly of functioning units can sometimes be achieved.

It is as the disulphide that the structural importance of the thiol in proteins can best be appreciated[75]. Covalent disulphide bonds provide bridges that are much stronger than the hydrophobic and hydrogen-bonded interactions believed responsible for initial protein folding. The real uniqueness of the thiol–disulphide structural system lies in the ease with which it may be formed, broken down and reformed under reasonable biological conditions. The principal method for the making and breaking of protein disulphides is by disulphide interchange. This process, as mediated by glutathione, can be coupled to cellular redox systems by a specific reduced nicotinamide coenzyme–disulphide reductase. Thus, protein disulphide structure can be formed, be rearranged and broken up by systems involving low molecular weight thiol–disulphide couples.

However, a major disulphide contribution to structures within the cell is made unlikely by the observation that disulphide bonds are relatively rare in intercellular proteins. In fact we have already discussed the possible role of glutathione in maintaining protein thiols in the reduced state. It is really with proteins that operate outside the cell that one finds the great importance of disulphide-stabilized structures. One can reasonably rationalize this fact in two ways. Since the protein must operate without the protective environment of the cell, random disulphide formation would eventually occur. By initially fixing most thiols as disulphides in an active configuration the chances for deleterious random disulphide formation

would be reduced. Another view would contend that extracellular proteins must survive and function in a much more variable and hostile environment than cellular enzymes. They therefore require greater rigidity and an ability to function even if partially damaged. These attributes are afforded by disulphide cross linking. Both explanations probably have some truth with the inevitability of disulphide formation and the increase in structural stability once formed contributing to the importance of this system. Since many of the most abundant and best studied proteins are extracellular many examples are known in which functional structure is dependent on disulphide bridges. Only a few examples illustrating certain generalizations will be discussed.

It is important to remember that the position of disulphide bonds cannot be directly specified by the genetic code and disulphide formation must occur subsequent to the assembly of the peptide chain. There is now strong evidence for the idea that the initial three-dimensional folding of a protein is totally a consequence of the primary amino acid sequence. The same is true for the association of subunits into functional complexes. It is only after weak interactions have brought about a highly favoured configuration that the disulphide formation occurs to 'lock in' the protein structure. Disulphide cross linking does not create form, but only fixes what was initially dictated by the linear peptide sequence and weak bonding forces.

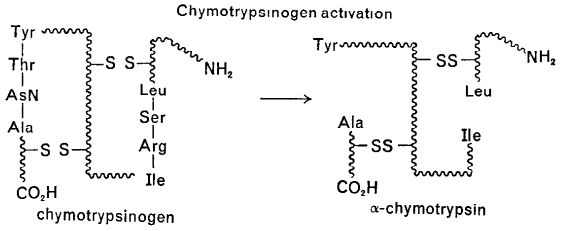
The exact nature of the oxidant for the normal biogenesis of disulphides is uncertain. Low molecular weight protein disulphide–dithiol electron transport carriers are implicated. The cytological localization of the process is more certain. A membrane-bound microsomal enzyme which catalyses a protein disulphide interchange is probably responsible for assembly of disulphide-stabilized structures. This activity is most prevalent in those cells which are producing and excreting disulphide-stabilized proteins. The enzyme occupies a position on the microsomal membranes at or near the site for ribosome binding. It is therefore directly available to act on the newly assembled peptide chains. Assay of this enzyme depends on its ability to reform the active, disulphide-stabilized, structure of ribonuclease from a randomly cross-linked material. Of the 105 possible disulphide combinations, only one is proper and active. Actually this one 'correct' structure can reform in reasonably high yield if oxidation conditions are properly controlled. The microsomal disulphide interchange enzyme facilitates the process by promoting rearrangement of inappropriate disulphide patterns. The interchange capacity of the system is important because it allows the newly formed protein to achieve its best and presumably proper folding pattern even if some premature oxidation

might occur. It also seems reasonable to assume for the present that this same enzyme is responsible for the initial oxidation of the thiols on the newly synthesized protein. Since this probably involves an intramolecular disulphide exchange with a disulphide–dithiol redox carrier no new catalytic capacity need be involved. The exclusive association of this disulphide interchange activity with rough endoplasmic reticulum is consistent with the idea that disulphide proteins only occur extracellularly. These are the cellular structures believed responsible for assembly and vacuolization of excretory proteins.

It is also possible that disulphide bond formation and rearrangement occurs after excretion of the protein from its cell of synthesis in some cases. This would best account for assembly of very large sulphur-rich aggregates such as hair. Exact cross linking fidelity is probably not so critical in these cases and complete assembly of such large cross-linked meshworks within a cell is clearly impossible.

The most dramatic examples of the importance of disulphides for biological function are found among enzymes which are initially produced as inactive precursor proteins. Chain folding and disulphide bonding patterns reflect the primary peptide structure of this inactive zymogen molecule. Activation usually involves the cleavage of peptide bonds and sizeable peptide segments may be removed[76]. The protein arrangement is no longer one that would form spontaneously. The maintenance of the active structure is completely dependent on the disulphide linkages.

Chymotrypsinogen, as synthesized by the pancreatic cells, is a single polypeptide chain which can maintain its native configuration if the disulphide links are reduced. Activation, by a series of peptide bond cleavages, eventually results in three separate polypeptide segments held together by disulphides, as indicated diagrammatically below. Destruction



of the disulphide links now results in separation of subunits. Reassembly cannot occur and activity is completely and irreversibly lost.

An example involving another familiar protein is the biosynthesis of insulin[77]. This hormone is assembled as a continuous chain of 73 amino acids. Subsequent to folding and the establishment of disulphide bonds, a 22-amino-acid segment is removed from the centre of the protein. This provides the two-standard, disulphide cross-linked structure of the active molecule. Thus one general function which can clearly be assigned to disulphides is the maintenance of appropriate structure after secondary protein modifications have occurred.

A closely related role for disulphide bridging is in 'freezing' subunit arrangements[76]. The four peptide chains of the typical antibody molecule are held together in proper position by disulphide bonds. A vast variety of individual antibodies can coexist in the blood without any mixing of subunits. If the disulphide bonds holding the chains together are reduced, the proper type of subunit interaction can be maintained under certain experimental conditions. However an interchange of subunits can now occur. The presence of the disulphide bridges in the native structure ensures that subunits forming the two identical and highly specific binding sites will remain together in the general circulation. While such subunit assemblies must be formed by spontaneous and reversible interactions at their point of synthesis, they can be prevented from undergoing subsequent rearrangement by disulphide bonding.

Large disulphide-linked aggregates are found in hair and related animal keratins[78,79]. In fact the cardinal characteristic of wool, nails, horns, feathers, etc. is their high sulphur content. The basic keratin system is believed by most to be composed of two protein subtypes. One type forms filamentous fibrils which are wound arrays of protein strands. Differing arrangements of fibres and patterns of protein folding distinguish the α- and β-keratins. Fibril proteins are rather low in cysteine content and hydrogen bonding and hydrophobic interactions impart their strong fibre-forming tendencies. The keratin fibres are embedded in a protein matrix having no recognizable order. The matrix proteins are extremely rich in cysteine and also enriched in serine, threonine and proline. The high sulphur proteins are extensively crosslinked to each other, and to the sulphur-poor fibrous constituents through disulphide bonds. The sulphur-rich fraction probably does not represent a single protein but rather a mixture of related proteins. The nature of this mixture and the amounts of the individual constituents vary with the type of structure formed (hair, feather, horn, etc.) and to some extent with the diet of the animal. Newly synthesized hair proteins are actually soluble, but by 4 to 6 hours they can no longer be extracted into water and by 18 to 20 hours much of the material cannot even be solubilized by urea. This suggests that assembly

of the crosslinked disulphide meshwork occurs long after the initial peptide assembly is completed. The high sulphur proteins have been extremely hard to study because of the difficulty in dissolving them without modifying backbone structures. The biochemistry of this complex system is only beginning to be unravelled, principally by chemists interested in modification of the basic structures for textile or cosmetic application. However, there is little doubt that disulphide bonds constitute the principal structural feature of hair and other keratin assemblies.

Another area in which a critical functional role for protein disulphides has been suggested is in the action of the polypeptide hormones[80]. A small cyclic disulphide loop is a common feature in many of these molecules. This has drawn attention as a possible site for hormone binding to the target cell. The greatest amount of evidence supporting this idea concerns the action of antidiuretic hormone or vasopressin. The hormone is bound in the kidney by a thiol-cleavable bond, and no such interaction occurs with other tissues. Thiol reagents prevent binding, and reduction of the hormone's disulphide causes inactivation. Diuretic effects can be achieved by a wide variety of compounds which share an ability to react with thiols. The idea of a disulphide loop being a site for attachment to a target thiol by disulphide interchange is attractive and may prove to be a generally significant disulphide function.

Almost all proteins contain some cysteine, but in only a minority of these can the thiol group be assigned a definite role. Nonetheless the list of thiol functions in proteins is long and clearly exemplifies the importance of this group in biological systems.

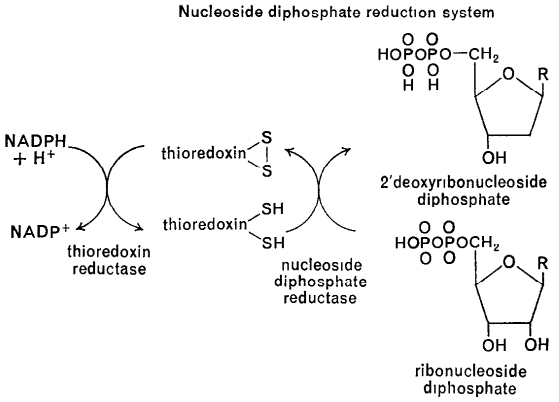
F. Dithiol and Polythiol Proteins[82]

A special type of thiol functional group can be achieved by constraining more than one thiol group into a polythiol centre. An example has already been considered, lipoic acid, where the presence of two thiols on the same carbon chain facilities a dithiol–disulphide redox system. A similar functional centre can be created by the close positioning of two cysteine thiols through appropriate secondary and tertiary folding of a polypeptide chain. Inhibition by arsenite or by cadmium has been considered to be indicative of a dithiol involvement in enzyme action. Unfortunately, a lack of knowledge about the precise chemical specificity of these dithiol reagents has left most suggestions of an enzyme dithiol in doubt. Several examples have now been supported by direct thiol assay or active site isolation, strengthening the dithiol enzyme concept. Recent studies on dithiol criteria should increase confidence in specific reagents when used appropriately[83], but also emphasize the deficiencies in the way such

criteria have often been applied. The division between mono- and dithiol functions is quite arbitrary. However, it does emphasize that something more than just a summation of two independent groups is achieved by making it possible for them to act in concert.

I. Thioredoxins[84]

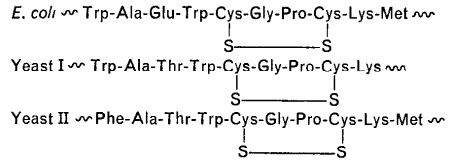
A dithiol protein, thioredoxin, functions in the transport of electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to ribonucleotides in the biosynthesis of 2′-deoxyribonucleotides. A thioredoxin type carrier is involved in both vitamin B_{12} dependent and independent type systems. Thioredoxin is frequently described as a polypeptide cofactor rather than an enzyme because its activity is not destroyed



by heating, and the molecular weight is relatively low (approximately 12,000). As such it is only one example of a class of small proteins carrying reactive centres which have been recognized in recent years.

Thioredoxin from *E. coli* contains only two cysteine residues which are linked as a disulphide in the oxidized form of the molecule. These residues are separated by two intervening amino acids, glycine and proline, providing a small polypeptide as the functional centre of the molecule. When two thioredoxins from yeast and the one from *E. coli* were compared, the amino acid sequences were identical in the immediate vicinity of the disulphide–dithiol centre, and quite similar for a considerable distance beyond.

Sequencies of thioredoxin active centres

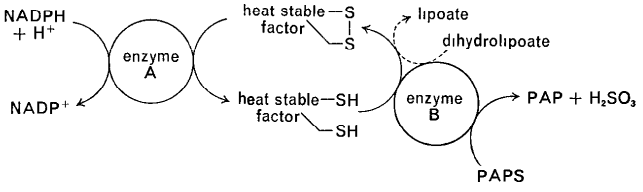


The thioredoxins appear to have a highly specific relationship with the enzyme carrying out their reduction. Yeast thioredoxin for example is not reduced by the thioredoxin reductase from *E. coli*. In contrast reduced thioredoxins may donate electrons to a variety of acceptors. Reduced thioredoxin is a good general disulphide reductant. In combination with its reductase a disulphide reductase system is formed which is capable of reducing lipoic acid, oxidized glutathione and other similar structures. In these cases the thioredoxin–disulphide redox system does not appear to require additional enzymatic components.

Reducing equivalents from a given thioredoxin can be donated to a variety of reductase enzymes. They are not specific for the nucleotide reductase or for enzymes from the same organism. Reduced yeast thioredoxin will serve as reductant for methionine sulphoxide reductase, sulphate reductase and the *E. coli* nucleoside diphosphate reductase. Heat-stable protein cofactors are known to be involved in each of these systems.

The sulphate reductase factor which has already been mentioned was the first of these polypeptide dithiol–disulphide cofactors to be recognized[3]. In this case the reduction of PAPS to PAP and sulphite was shown possible with a dithiol reductant such as dihydrolipoate or with NADPH and two protein components. One of the protein factors was not inactivated by heating. Incubation of the two protein fractions with NADPH generated

Electron transport system for sulphate reduction



approximately two moles of thiol associated with the heat-stable component. The heat-labile reductase enzyme could not of itself reduce lipoamide or other disulphides.

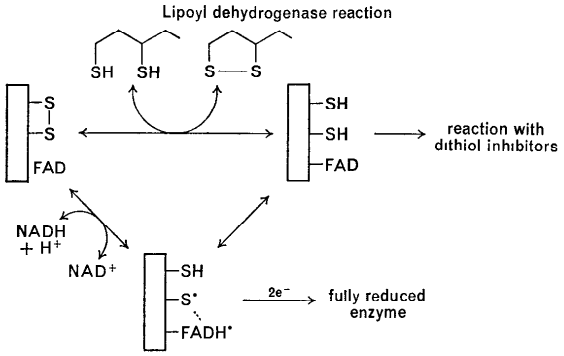
There is a growing literature on similar electron transport systems and it seems likely that such small protein disulphide–dithiol factors will be found to have a general biological role. It seems reasonable to refer to all cofactors of this type as thioredoxins, emphasizing that this is a class of compounds of similar but not identical structures which can show a high degree of specificity for a given organism or reaction.

2. Dithiol–flavin enzymes[86]

Disulphide–dithiol redox centres are also found in a number of high molecular weight electron-transporting enzymes. Most extensively studied of these are the group of flavoproteins which carry electrons between disulphide cofactors and nicotinamide nucleotides. These include lipoyl dehydrogenase, glutathione reductase and the thioredoxin reductases. These enzymes are unique in utilizing a combined flavin–disulphide centre for the oxidation of reduced nicotinamide coenzyme. Each reactive enzyme centre is composed of a disulphide formed from two cysteine sulphydryls and a tightly bound flavin adenine dinucleotide (FAD). Upon reduction by reduced nicotinamide cofactor the two-electron equivalents are shared between the dithiol and the flavin prosthetic groups. A fully reduced four-electron enzyme containing a dithiol and $FADH_2$ does not occur during the normal catalytic cycle. The reduced enzyme site is envisaged as some sort of mixed free radical with one electron on sulphur and the other in the flavin system. This is not a conventional flavin semi-quinone and the possibility of a charge transfer complex between the active elements of the redox centre has been proposed. Complete electron transfer to the dithiol centre and reduction of the disulphide substrate through a disulphide interchange sequence completes the catalytic cycle.

These dithiol–flavoproteins transport electrons over a redox potential range considerably more reducing than is associated with free flavin and most other types of flavin enzymes. By acting in conjunction with the protein dithiol centre, flavin is transformed into a much more powerful reducing agent.

The sequences of the dithiol active centres of two enzymes of this type from *E. coli* have recently been reported[87,88]. The lipoyl dehydrogenase dithiol peptide has four amino acids intervening between the two cysteines and is rich in hydrophobic amino acids. This has been taken as a reflection of a highly hydrophobic pocket at the catalytic centre, as had been implicated by model substrate studies. The thioredoxin reductase dithiol



centre has only two residues between the cysteines. The dithiol peptide of thioredoxin itself is the same size as that of its reductase but the sequences are quite distinct.

Amino acid sequences of dithiol-disulphide centres

Lipoyl dehydrogenase ~ val-cys-leu-asn-val-gly-cys-ilu-pro-ser
S——S

Thioredoxin reductase ~ ala-cys-ala-thr-cys-asp-gly-phe ~
S——S

Amino acid sequences of the known dithiol–disulphide redox centres provide little hope that any specific peptide structure will be found associated with this particular activity. Even the size of the disulphide ring systems vary, so one must conclude that it is the overall folding of the protein which is responsible for the correct juxtaposition of the functional elements.

3. Other dithiol enzymes[82]

The presence of dithiol centres at the active sites of a variety of additional enzymes has been proposed on the basis of inhibition studies. For example, the investigations on aldehyde dehydrogenase represent one of the earliest uses of arsenite as a dithiol diagnostic reagent. The overall data strongly support the presence of a polythiol site as a general feature of aldehyde oxidases, but its functional role has not been established. Because of the ease of thioacetal formation a dithiol would make a chemically attractive

aldehyde binding site. The failure to find lipoic acid as a part of these enzymes makes it likely that the dithiol centre arises from the juxtaposition of cysteine thiol residues.

The light-emitting luciferase system from fireflies has been extensively studied and there is strong support for a functional dithiol[89]. The extensive thiol involvement in fatty acid biosynthesis has already been indicated, and some enzyme components have characteristics expected of dithiol centres. There are many additional systems where there is some evidence for dithiol involvement, but proof for a clear functional role of a dithiol is lacking.

4. Polythiol metal-binding centres[90]

A polythiol centre can serve as a highly specific metal ion binding site. For example, polythiol ligands have come to be thought of as relatively selective for cadmium. Actually a number of important metal ions including mercury, zinc, lead, copper and iron bind quite well at such centres[91]. The order of relative binding affinities for polythiol chelates is different from those involving nitrogen or oxygen ligands. Cadmium, mercury and to a lesser degree zinc form the most avid complexes.

The polythiol metal complexes can provide functional centres with unique properties, an example being the nonhaem iron proteins to be discussed in the next section. They also are capable of serving rather distinctive structural roles, since the binding of metals can influence the overall configuration of the protein. Studies on cadmium and zinc binding to thiol-substituted dextran polymers showed that these metals can actually organize polythiol-binding centres and might appreciably change the folding of a polypeptide chain[90].

Metals with a high affinity for multithiol coordination thus could serve to generate and stabilize particular protein conformations. Several examples of structurally important metal–polythiol interaction have recently appeared. *E. coli* aspartate transcarbamylase, the subject of extensive investigations concerned with mechanisms for enzyme control, has been shown to contain zinc. Zinc binding appears to occur at a dithiol centre. The metal is required to maintain the regulatory subunit in a configuration suitable for binding to the catalytic subunit[92]. Histidine ammonia-lyase is dependent on cadmium when enzyme disulphides have been reduced, and this has been shown to be due to the formation of a metallo-dithiol complex[93]. The reactive thiols appear to be contributed by separate subunits and the complex formation establishes an appropriate interaction of the individual components. In the oxidized enzyme, these thiols are linked as a disulphide and this form of metal ion activation is

not required. In bovine superoxide dismutase the proper conformation for the binding of an active site copper ion is maintained by a distinct zinc-binding centre[94]. Two sulphydryl groups per zinc are uncovered on removal of this metal implicating a dithiol-binding site. Metal binding at specific dithiol or polythiol sites could constitute a general mechanism for stabilizing protein conformation or facilitating interaction between subunits.

A cadmium-rich protein, metallothionein, has been isolated from kidney and other tissues[91]. It is a small protein of about 7000 molecular weight and is exceedingly rich in thiol groups. One out of every four to five amino acids is cysteine, and three thiols are involved in each cadmium-binding site. The biological importance of metallothionein is unknown. The simplest role envisaged is scavenging toxic metal ions which might otherwise interfere with critical enzymatic processes. Metallothionein from kidneys of patients treated with mercurial diuretics contained increased amounts of mercury which could reflect a toxic ion sequestering action of the protein. Another interpretation might be that metallothionein is an undegradable and unexcretable end product. It might have been derived from a thiol-rich centre of a protein(s) which had been inactivated by cadmium. The isolated material would merely be the accumulating debris of toxic insult. A somewhat intermediate viewpoint would ascribe a normal trace metal-binding role to metallothionein. A similar constituent has been isolated from liver and contains primarily zinc and copper. If the protein's normal function was the storage or mobilization of these two critical trace metals, cadmium would interfere because of its avid binding. This would eventually lead to inactive cadmium- (or mercury-) saturated forms such as those isolated from the kidney. Whatever its role, metallothionein is an excellent example of polythiols serving as selective metal-binding sites.

5. Iron–sulphur redox proteins[95,98]

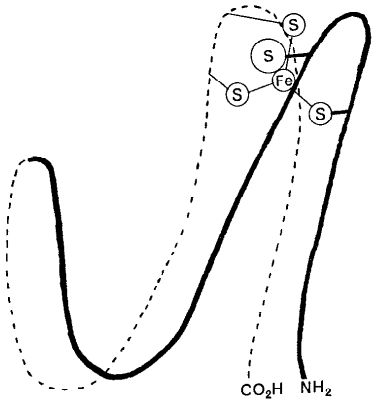
One rapidly advancing area of thiol biochemistry involves a group of iron–sulphur redox proteins, most commonly referred to as the nonhaem iron proteins. This designation derives from the fact that more iron was present in electron transport complexes than could be accounted for by the haem content. In the investigation of bacterial nitrogen fixation a low molecular weight iron containing protein was isolated which functioned as an electron transport carrier. This was named ferredoxin. An unusual characteristic was that when the protein was treated with acid to release iron, hydrogen sulphide was also produced. A component of the photosynthetic nicotinamide coenzyme reductase system was recognized as

having similar properties and has come to be referred to as plant ferredoxin. Adrenodoxin, putidaredoxin, rubredoxin and high potential iron-protein are additional nonhaem iron electron transport proteins of a similar character. A variety of high molecular weight electron transporting enzymes also have been found to have nonhaem iron centres. A triad of characteristics has come to be associated: (1) a tightly bound iron, not accountable for as haem iron; (2) an unusual e.p.r. signal in the vicinity of $G = 1{\cdot}96$, not characteristic of typical iron chelates; and (3) the release of iron on acidification accompanied by the unmasking of protein thiol groups and the generation of hydrogen sulphide. While each of these characteristics is not always demonstrable, they have served to delineate a heretofore unrecognized redox centre of wide distribution.

An intense effort by physicists, physical chemists, biochemists, inorganic chemists and X-ray crystallographers has now defined the common attribute of the nonhaem iron proteins. It is an iron centre tetrahedrally coordinated by four sulphur ligands. A number of variations within this theme are recognized. The simplest case is found to be rubredoxin from *Clostridium pasteurianum*, a 6000 molecular weight protein whose exact electron transport function is unknown.

A single iron atom is bound by four cysteine sulphurs with no acid labile sulphur being involved. A detailed crystallographic analysis of this molecule has been carried out, the general features of which are indicated below[97].

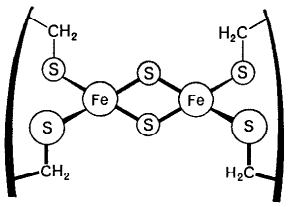
Iron binding site of rubredoxin



The peptide chain can be roughly described as a bent hairpin. The iron-binding centre consists of two small dithiol peptide segments. These dithiol centres are quite distant in the linear peptide sequence occurring in the end halves of the two legs of the hairpin. The peptide folding transforms these into a compact tetrathiol iron-binding centre. While this simplified description does great injustice to the details of the X-ray structure analysis, it serves to illustrate the critical features of the metal-binding centre. It also draws attention to a possible relationship of the iron–sulphur proteins to the disulphide–dithiol redox carriers considered previously.

The more typical iron–sulphur centre contains two iron atoms, two sulphides and four cysteine sulphurs[98]. It is believed that each iron is surrounded by four sulphur ligands in an approximate tetrahedral array. Every iron is coordinated by two sulphurs from cysteine and two from sulphide, with each of the sulphides binding both irons. The general structure of the two-iron redox centre is depicted below.

Proposed structure of a two-iron centre



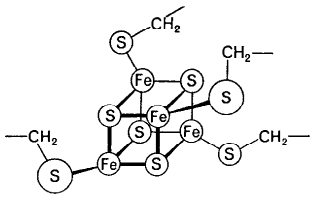
Another arrangement for an iron–sulphur redox centre is found in HiPIP (high potential iron–protein) from *Chromatium* and the bacterial ferredoxins[99,100]. These structures have been elegantly established by X-ray crystallography. The redox centre contains four iron atoms, four sulphide sulphurs and four cysteine sulphurs from the protein. Each iron is again surrounded by four sulphur ligands in an approximate tetrahedral arrangement, but each sulphide sulphur now interacts with three irons. The irons are bonded to three sulphides and one cysteine sulphur. The iron–sulphur array is roughly cubical with the corners being either a sulphide sulphur or an iron linked to the protein shell.

Bacterial ferredoxin actually contains eight irons and eight labile sulphides, but these are arranged as two distinct four-iron clusters.

Iron–sulphur proteins participate as one electron carriers, even those with reactive sites containing four irons. Each centre rather than each

iron must be counted as an electron transport unit. Only the bacterial ferredoxin acts as a two-electron acceptor and even in this case it is really two Fe_4S_4 one-electron centres acting independently.

Arrangement of the Fe_4S_4 nonhaem iron centre



Iron–sulphur prosthetic groups of a similar nature are also implicated in more complex higher molecular weight electron-transferring enzymes. Examples such as xanthine oxidase have been extensively examined and the type of iron–sulphur centre seems analogous to those in the lower molecular weight cofactors. For the present at least the nonhaem iron centres of complex electron transport chains can also be envisaged as enzyme polythiol sites generated by the juxtaposition of two protein dithiol sequences. Coordinated within this tetrathiol cavity is an iron–sulphide core.

What is it about divalent sulphur which results in such unique and biologically useful complexes of iron? The special feature of these prosthetic groups is that the iron is held in what approximates a tetrahedral complex, while all common iron complexes with oxygen and nitrogen ligands are octahedral. Tetrahedral iron–oxygen complexes are known, but only as high molecular weight networks where there seems to be a requirement for a condensed packing. One critical difference in the sulphur ligand could simply be effective size. Since the outer orbitals of sulphur are occupied it might be difficult to pack six ligands around an iron. The less crowded tetrahedral arrangement would therefore be favoured. The capacity of sulphur for expansion of the valence shell also might be considered of importance for moving electrons into and out of the complex and for delocalizing electrons in the reduced complex. The intense research activity currently focused on these iron–sulphur proteins ensures that our understanding of this aspect of thiol biochemistry will improve rapidly.

The variety of oxidation–reduction carriers having a dithiol centre as a part of their structure suggests a possible evolutionary relationship.

Dithiol–disulphide redox roles in primitive systems would have favoured the development of dicysteinyl peptides, restraining two thiols in close proximity. Thioredoxin type molecules would have evolved from these small prototype dithiol peptides. Similar centres would also have developed as parts of more complex enzymes. The binding of a flavin coenzyme near a dithiol centre could eventually have produced the combined disulphide–flavoprotein centre with its special redox properties.

The propensity for dithiols to bind metals would have led to a further evolution of function. Metal–protein bridging may have preceded disulphides as a method of holding proteins in effective organization particularly before the oxidizing environment developed. Iron–sulphur complexes had redox potentials different from other iron carriers and the dithiol. They eventually developed into the powerful reducing system of the ferredoxins by combining two dithiol ligands around one iron. Simple iron sulphide aggregates were also incorporated giving rise to the two iron-two sulphide and four iron-four sulphide variants. As more iron–sulphur atoms condensed into a single site, the redox possibilities increased and the iron–sulphur centre became involved in the variety of different redox roles seen today.

Cytochrome C might also have arisen from a dithiol redox protein as the haem-binding centre is nothing more than a dithiol peptide. While such speculations on the evolution of biomolecules are only mental games, they point out how the dithiol can be modified to carry out a variety of related functions.

IV. CONCLUSION

The thiol and its simple derivatives represent an exceedingly important and versatile functional centre in biological molecules. A number of basic metabolic processes are dependent on the particular chemical characteristics of thiol derivatives. It is difficult to imagine how metabolism might have evolved without the rich supply of thiols which probably were available in the 'primordial soup'. Actually thiols or their derivatives participate in so many biological reactions that one is amazed to find they have no indispensable role in the central dogma of molecular biology. Self-replication, transcription and translation rely only periferally on thiols. It is in the realms of catalysis and structure, the domains of the enzymologist and protein physical chemist, that the thiol is of central importance.

Thiols have been of foremost importance in the development of the functional group concept in biochemistry. Because of its ease of manipulation the thiol, particularly that of glutathione, has fascinated the biochemist. All manner of roles have been suggested but most of these have not been proven, and many are totally forgotten. Still the chemical approach to biochemistry and the attempt to explain how biological reactions occur in terms of model organic systems had much of its initial success in explaining thiol-mediated reactions. The sulphur of the thioester provides activation for acyl transfer, and an intermediate in amide and ester hydrolysis. It facilitates α-hydrogen dissociation and provides a mechanism for carbon–carbon condensation and chain modifications. Reduction of carboxylic acids is preceded by thioester formation. Energy released by oxidative metabolism is trapped as a thioester, a form suitable for driving the synthesis of ATP.

The disulphide and certain thiol–metal ion derivatives serve as carriers of electrons and function in biological redox reactions of diverse types. Thiols and their metal derivatives provide strong binding centres for substrates and cofactors. They often help maintain proper protein configurations and subunit interactions. The disulphides of extracellular proteins are of profound structural importance. They make relatively permanent the arrangements of peptide chains initially established by weaker bonding forces. Often they become totally responsible for holding the active structure together, particularly where covalent modification of the protein chain is involved in activation. Animal keratins are particularly rich in sulphur, deriving their inertness from extensive disulphide cross linking.

The sulphonium ion serves as an alkylating reagent. The bulk of biological methylations proceed through S-adenosyl methionine. Persulphides, thiophosphates, thiocyanates and thiosulphonate derivatives have been postulated to have significant functional roles. The plant kingdom in particular is full of strange thiols and thiol derivatives which impart characteristic tastes and smells. Their functions are unknown, but could range from insect attractant to water repellent. Vitamins such as thiamine and biotin have heterocyclic sulphur which can be viewed as thiol derivatives. Even the simplest thiol of all, sulphide, finds a critical biochemical involvement in the iron–sulphur electron transport centres.

Thiols provide the living systems with a link to their genesis in a reducing environment. Glutathione helps maintain the cellular interior in a state in which enzyme activities evolved in the absence of oxygen can still function. Protection from all sorts of injurious agents, detoxification and anti-radiation roles can be added to complete the listing of thiol functions in biological systems. The intense fascination of the biochemist with the thiol functional group can certainly be appreciated.

REFERENCES*

1. A. B. Roy and P. A. Trudinger, *The Biochemistry of Inorganic Compounds of Sulphur*, Cambridge University Press, London, 1970.
2. P. A. Trudinger in *Advances in Microbial Physiology*, Vol. 3 (Ed. A. H. Rose and J. F. Wilkinson), Academic Press, New York, 1969, pp. 111–158.
3. R. S. Bandurski in *Plant Biochemistry* (Ed. J. Bonner and J. E. Varner), Academic Press, New York, 1965, pp. 467–489.
4. K. Torii and R. S. Bandurski, *Biochim. Biophys. Acta*, **136**, 286 (1967).
5. E. C. Hatchikian, J. LeGall, M. Bruschi and M. Dubourdieu, *Biochim. Biophys. Acta*, **258**, 701 (1972).
6. A. Yoshimoto and R. Sato, *Biochim. Biophys. Acta*, **153**, 576 (1968).
7. A. Sentenac, F. Chapeville and P. Fromageot, *Biochim. Biophys. Acta*, **67**, 672 (1963).
8. L. J. Reed in *The Enzymes*, 2nd ed., Vol. 3 (Ed. P. D. Boyer, H. Lardy and K. Myrbäck), Academic Press, New York, 1960, pp. 195–223.
9. S. Black, E. M. Harte, B. Hudon and L. Wartofsky, *J. Biol. Chem.*, **235**, 2910 (1960).
10. E. Kun in *Metabolic Pathways*, 3rd ed., Vol. 3 (Ed. D. M. Greenberg), Academic Press, New York, 1969, pp. 375–401.
11. S. H. Mudd, F. Irreverre and L. Laster, *Science*, **156**, 1599 (1967).
12. J. A. Schneider and J. E. Seegmiller in *The Metabolic Basis of Inherited Disease*, 3rd ed. (Ed. J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson), McGraw-Hill, New York, 1972, pp. 1581–1064.
13. N. M. Kredich, B. S. Keenan and L. J. Footc, *J. Biol. Chem.*, **247**, 7157 (1972).
14. H. Vachek and J. L. Wood, *Biochim. Biophys, Acta*, **258**, 133 (1972).
15. S. O. Thier and S. Segal in *The Metabolic Basis of Inherited Disease*, 3rd. ed. (Ed. J. B. Stanbury, J. B. Wyngaarden and S. D. Fredrickson), McGraw-Hill, New York, 1972, pp. 1504–1519.
16. J. F. Thompson, *Ann. Rev. Plant Physiol.*, **18**, 59 (1967).

* The aim of this chapter is to present a general coverage of the biochemistry of thiols rather than a review of recent advances. As such this is a departure from the usual coverage of material in this volume. The biochemical literature encompassed by this subject is immense, and many excellent reviews, monographs and symposia have been devoted to certain aspects of the topic. If possible referencing has been restricted to these secondary sources, since it was presumed that an interested reader would prefer to see these before proceeding to original material. It has of course been necessary to cite also primary literature when no suitable secondary source could be found. The article cited, however, is usually one of the most recent in that area and not necessarily the most pertinent to the subject being discussed. No attempt was made to include a comprehensive coverage of even the recent literature, but often typical papers have been cited in order to provide a reasonable point of entry to the literature of rapidly expanding areas. This heavy reliance on secondary sources and the desire to present the material in as generalized a form as possible have done great injustice to original data and to its original interpretations in many cases. It is also realized that many excellent papers and ideas have been ignored or completely missed. While this is regrettable, the scope of the subject probably makes it inevitable, and only a simple apology can be offered.

17. G. W. Frimpter in *The Metabolic Basis of Inherited Disease*, 3rd ed. (Ed. J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson), McGraw-Hill New York, 1972, pp. 413–425.
18. J. D. Finkelstein in *Symposium: Sulfur in Nutrition* (Ed. O. H. Muth and J. E. Oldfield), The Avi Publishing Co., Westport, Connecticut, 1970, pp. 46–60.
19. S. H. Mudd in *Symposium: Sulfur in Nutrition* (Ed. O. H. Muth and J. E. Oldfield), The Avi Publishing Co., Westport, Conn., 1970, pp. 222–243.
20. T. Gerritsen and H. A. Waisman in *The Metabolic Basis of Inherited Disease*, 3rd ed. (Ed. J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson), McGraw-Hill, New York, 1972, pp. 404–412.
21. T. W. Wong, S. B. Weiss, G. L. Eliceiri and J. Bryant, *Biochemistry*, **11**, 2376 (1970).
22. D. B. McCormick and L. D. Wright in *Comprehensive Biochemistry*, Vol. 21 (Ed. M. Florkin and E. H. Stotz), Elsevier Publishing Co., Amsterdam, 1971, pp. 81–110.
23. S. Colowick, A. Lazarow, E. Racker, D. R. Schwarz, E. Stadtman and H. Waelsch, Eds., *Glutathione*, Academic Press, New York, 1954.
24. W. E. Knox in *The Enzymes*, 2nd ed., Vol. 2 (Ed. P. D. Boyer, H. Lardy and K. Myrbäck), Academic Press, New York, 1960, pp. 253–294.
25. E. M. Crook, Ed., *Glutathione* (Biochemical Society Symposium 17), Cambridge University Press, Cambridge, 1959.
26. A. Meister, *Biochemistry of the Amino Acids*, 2nd ed., Vol. 1, Academic Press, New York, 1965, pp. 452–454.
27. D. N. Mohler, P. W. Majerus, V. Minnich, C. E. Hess and M. D. Garrick, *New Eng. J. Med.*, **283**, 1253 (1970).
28. J. L. Wood in *Metabolic Conjugation and Metabolic Hydrolysis*, Vol. 2 (Ed. W. H. Fishman), Academic Press, New York, 1970, pp. 261–299.
29. E. Beutler in *The Metabolic Basis of Inherited Disease*, 3rd ed. (Ed. J. B. Stanbury, J. B. Wyngaarden and D. S. Frederickson), McGraw-Hill, New York, 1972, pp. 1358–1388.
30. D. L. Vander Jagt, L. B. Han and C. H. Lehman, *Biochemistry*, **11**, 3735 (1972).
31. A. Szent-Györgyi, *Science*, **161**, 988 (1968).
32. I. Krimsky and E. Racker, *J. Biol. Chem.*, **198**, 721 (1952).
33. A. H. Gold and H. L. Segal, *Biochemistry*, **3**, 778 (1964).
34. T. W. Speir and E. A. Barnsley, *Biochem. J.*, **125**, 267 (1971).
35. B. Gillham, *Biochem. J.*, **121**, 667 (1971).
36. S. H. Mudd and G. L. Cantoni in *Comprehensive Biochemistry*, Vol. 15 (Ed. M. Florkin and E. H. Stotz), Elsevier Publishing Co., Amsterdam, 1964, pp. 1–47.
37. S. K. Shapiro and F. Schlenk, Eds., *Transmethylation and Methionine Biosynthesis*, University of Chicago Press, Chicago, 1965.
38. J. Lucas-Lenard and F. Lipmann, *Ann Rev. Biochem.*, **40**, 409 (1971).
39. P. Hannonen, A. Raina and J. Jänne, *Biochim. Biophys. Acta*, **273**, 84 (1972).
40. L. Jaenicke and F. Lynen in *The Enzymes*, 2nd ed., Vol. 3 (Ed. P. D. Boyer, H. Lardy and K. Myrbäck), Academic Press, New York, 1960, pp. 3–103.

41. P. Goldman and P. R. Vagelos in *Comprehensive Biochemistry*, Vol. 15 (Ed. M. Florkin and E. H. Stotz), Elsevier Publishing Co., Amsterdam, 1964, pp. 71–92.
42. F. Lynen in *Chemical Reactivity and Biological Role of Functional Groups in Enzymes* (Ed. R. M. S. Smellie), Academic Press, New York, 1970, pp. 1–19.
43. T. C. Bruice and S. Benkovic, *Bioorganic Mechanisms*, Vol. 1, W. A. Benjamin, Inc., New York, 1966, pp. 259–297.
44. G. M. Brown in *Comprehensive Biochemistry*, Vol. 21 (Ed. M. Florkin and E. H. Stotz), Elsevier Publishing Co., Amsterdam, 1971, pp. 73–80.
45. J. M. Lowenstein in *Metabolic Pathways*, 3rd ed. Vol. 1, (Ed. D. M. Greenberg), Academic Press, New York, 1967, pp. 146–270.
46. S. J. Wakil and E. M. Barnes in *Comprehensive Biochemistry*, Vol. 18 (Ed. M. Florkin and E. H. Stotz), Elsevier Publishing Co., Amsterdam, 1971, pp. 57–104.
47. D. J. Prescott and P. R. Vagelos, *Adv. Enzymol.*, **36**, 269 (1972).
48. P. A. Srere, B. Böttger and G. C. Brooks, *Proc. Nat. Acad. Sci. USA*, **69**, 1201 (1972).
49. L. J. Reed in *Comprehensive Biochemistry*, Vol. 14 (Ed. M. Florkin and E. H. Stotz), Elsevier Publishing Co., Amsterdam, 1966, pp. 99–126.
50. L. J. Reed and D. J. Cox in *The Enzymes*, 3rd ed., Vol. 1 (Ed. P. D. Boyer), Academic Press, New York, 1970, pp. 213–240.
51. A. F. Wanger and K. Folkers, *Vitamins and Coenzymes*, Wiley, New York, 1964, pp. 259–262.
52. B. B. Buchanan in *The Enzymes*, 3rd ed., Vol. 6 (Ed. P. D. Boyer), Academic Press, New York, 1972, pp. 193–216.
53. E. S. G. Barron, *Adv. Enzymol.*, **11**, 201 (1951).
54. R. Benesch, R. E. Benesch, P. D. Boyer, I. M. Klotz, W. R. Middlebrook, A. G. Szent-Györgyi, and D. R. Schwarz, Eds., *Sulfur in Proteins*, Academic Press, New York, 1959.
55. R. Cecil and J. R. McPhee, *Adv. in Protein Chem.*, **14**, 255 (1959).
56. R. Cecil in *The Proteins*, 2nd ed., Vol. 1 (Ed. H. Neurath), Academic Press, New York, 1963, pp. 379–476.
57. H. Gutfreund and C. H. McMurray in *Chemical Reactivity and Biological Role of Functional Groups in Enzymes* (Ed. R. M. S. Smellie), Academic Press, New York, 1970, pp. 39–47.
58. J. L. Webb, *Enzyme and Metabolic Inhibitors*, Vols. 2 and 3, Academic Press, New York, 1966.
59. A. N. Glazer, *Ann. Rev. Biochem.*, **39**, 101 (1970).
60. P. D. Boyer in *The Enzymes*, 2nd ed., Vol. 1 (Ed. P. D. Boyer, H. Lardy and K. Myrbäck), Academic Press, New York, 1959, pp. 511–588.
61. S. F. Velick and C. Furfine in *The Enzymes*, 2nd ed., Vol. 7 (Ed. P. D. Boyer, H. Lardy and K. Myrbäck), Academic Press, New York, 1963, pp. 243–273.
62. K. Wallenfels and B. Eisele in *Chemical Reactivity and Biological Role of Functional Groups in Enzymes* (Ed. R. M. S. Smellie), Academic Press, New York, 1970, pp. 21–35.
63. A. N. Glazer and E. L. Smith in *The Enzymes*, 3rd ed., Vol. 3 (Ed. P. D. Boyer), Academic Press, New York, 1971, pp. 501–546.

64. J. Drenth, J. N. Jansonius, R. Koekoek and B. G. Wolthers in *The Enzymes*, 3rd ed., Vol. 3 (Ed. P. D. Boyer), Academic Press, New York, 1971, pp. 485–499.
65. F. S. Markland and E. L. Smith in *The Enzymes*, 3rd ed., Vol. 3 (Ed. P. D. Boyer), Academic Press, New York, 1971, pp. 561–608.
66. A. Levitzki and D. E. Koshland, *Biochemistry*, **10**, 3365 (1971).
67. J. Westley and D. Heyse, *J. Biol., Chem.*, **246**, 1468 (1971).
68. A. Finazzi Agró, C. Cannella, M. T. Graziani and D. Cavallini, *FEBS Letters*, **16**, 172 (1971).
69. D. Edmondson, V. Massey, G. Palmer, L. M. Beacham and G. B. Elion, *J. Biol. Chem.*, **247**, 1597 (1972).
70. W. H. Walker, E. B. Kearney, R. L. Seng and T. P. Singer, *Eur. J. Biochem.*, **24**, 328 (1971).
71. B. L. Vallee and J. E. Coleman in *Comprehensive Biochemistry*, Vol. 12 (Ed. M. Florkin and E. H. Stotz), Elsevier Publishing Co., Amsterdam, 1964, pp. 165–235.
72. D. Eisenberg in *The Enzymes*, 3rd ed., Vol. 1 (Ed. P. D. Boyer), Academic Press, New York, 1970, pp. 1–89.
73. E. Antonini and M. Brunori, *Ann. Rev. Biochem.*, **39**, 977 (1970).
74. I. M. Klotz, N. R. Langerman and D. W. Darnall, *Ann. Rev. Biochem.*, **39**, 25 (1970).
75. C. B. Anfinsen, *Biochem. J.*, **128**, 737 (1972).
76. A. R. Williamson in *Essays in Biochemistry*, Vol. 5 (Ed. P. N. Campbell and G. D. Greville), Academic Press, New York, 1969, pp. 139–175.
77. P. T. Grant and T. L. Coombs in *Essays in Biochemistry*, Vol. 6 (Ed. P. N. Campbell and F. Dickens), Academic Press, New York, 1970, pp. 69–92.
78. A. G. Lyne and B. F. Short, Eds., *Biology of Skin and Hair Growth*, Elsevier Publishing Co., New York, 1965.
79. W. G. Crewther, Ed., *Symposium on Fibrous Proteins*, Plenum Press, New York, 1968.
80. O. K. Behrens and E. L. Grinnan, *Ann. Rev. Biochem.*, **38**, 83 (1969).
81. J. P. Vincent and M. Lazdunski, *Biochemistry*, **11**, 2967 (1972).
82. B. P. Gaber and A. L. Fluharty, *Quart. Rep. Sulfur Chem.*, **3**, 318 (1968).
83. B. P. Gaber and A. L. Fluharty, *Bioinorganic Chem.*, **2**, 135 (1972).
84. P. G. Porqué, A. Baldesten and P. Reichard, *J. Biol. Chem.*, **245**, 2363 (1970).
85. D. E. Hall, A. Baldesten and P. Reichard, *Eur. J. Biochem.*, **23**, 328 (1971).
86. A. H. Neims and L. Hellerman, *Ann. Rev. Biochem.*, **39**, 867 (1970).
87. B. D. Burleigh and C. H. Williams, *J. Biol. Chem.*, **247**, 2077 (1972).
88. S. Ronchi and C. H. Williams, *J. Biol. Chem.*, **247**, 2083 (1972).
89. R. Lee and W. D. McElroy, *Biochemistry*, **8**, 130 (1969).
90. B. P. Gaber and A. L. Fluharty, *Bioinorganic Chem.*, **1**, 65 (1971).
91. B. L. Vallee and D. D. Ulmer, *Ann. Rev. Biochem.*, **41**, 91 (1972).
92. M. E. Nelbach, V. P. Pigiet, J. C. Gerhart and H. K. Schachmann, *Biochemistry*, **11**, 315 (1972).
93. C. B. Klee, *J. Biol. Chem.*, **247**, 1398 (1972).
94. G. Rotillo, L. Calabrese, F. Bossa, D. Barra, A. Finzazi Agró and B. Mondovì, *Biochemistry*, **11**, 2182 (1972).

95. A. San Pietro, Ed., *Non-heme Iron Proteins: Role in Energy Conversion*, Antioch Press, Yellow Springs, Ohio, 1965.
96. R. Malkin and J. C. Rabinowitz, *Ann. Rev. Biochem.*, **36**, 113 (1967).
97. K. D. Watenpaugh, L. C. Sieker, J. R. Herriott and L. H. Jensen, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 359 (1971).
98. W. R. Dunham, G. Palmer, R. H. Sands and A. J. Bearden, *Biochim. Biophys. Acta*, **253**, 373 (1971).
99. C. W. Carter, S. T. Freer, N. H. Xuong, R. A. Alden and J. Kraut, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 381 (1971).
100. L. C. Sieker, E. Adman and L. H. Jensen, *Nature*, **235**, 40 (1972).

Since completion of this chapter, two volumes on Thiol biochemistry have been published (References 101 and 102).

101. P. C. Jocelyn, *Biochemistry of the SH Group*, Academic Press, New York, 1972.
102. M. Friedman, *Chemistry and Biochemistry of the Sulphydryl Group in Amino Acids, Peptides and Proteins*, Pergamon Press, Oxford, 1973.

CHAPTER 14

Protection of the thiol group

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I. INTRODUCTION

The thiol group readily undergoes a variety of chemical reactions (e.g. oxidation, alkylation, acylation), so there is a need to protect it while other sites in the molecule are undergoing chemical changes. This can be done by converting the thiol group to a derivative which is stable under the reaction conditions to be employed, and from which the thiol group can be regenerated without affecting the rest of the molecule.

The great interest in thiol-protecting groups is due mainly to the significant development in the chemical synthesis of peptides, polypeptides and proteins. It is important to note that only one kind of a protecting group is needed to protect the various cysteine thiols during a protein synthesis. This results from the spontaneous refolding of proteins which takes place upon reoxidation of their sulphydryl groups produced by reduction of the protein with mercaptoethanol. This phenomenon was observed first with ribonuclease where the reoxidized molecule retained its full biological activity[1] and extended to a large number of proteins (e.g. insulin, lysozyme).

We shall try in this chapter to concentrate on the processes involved in the formation and deblocking of various types of protecting groups.

II. DISULPHIDES AS A PROTECTING GROUP

Disulphides are much less prone to participate in organic reactions (e.g. oxidation, alkylation, acylation) then the corresponding free thiol[2], and as such could serve as a protection for the thiol group. Furthermore, in some cases the removal of some protecting groups results in the formation of the disulphide first (e.g. sections III. B,C; V. A,C) which, later on, is reduced to the free thiol.

Disulphides are obtained by oxidation of the corresponding thiols, by a variety of reagents, e.g. oxygen, hydrogen peroxide, iodine, bromine, hypohalites, ferric chloride, nitrous oxide, sulphonyl chloride[3], diethyl azocarboxylate (**1**)[4], N-bromosuccinimide[5], tetranitromethane[6], peroxy-

$$EtOOC{-}N{=}N{-}COOEt$$

(1)

acetyl nitrate[7]. The free thiols are obtained from the disulphides by reduction, which again may be carried out by a large variety of reagents, e.g. tin and acid, sodium in xylene, ether or liquid ammonia, lithium aluminium hydride, sodium borohydride, sodium dithionate and various organic thiols[8]. The most widely used thiols are thioglycolic acid and mercaptoethanol. Of special interest among the thiols used is dithioerythritol (**2**)[9], which is a powerful reducing agent and reduces disulphides in much lower concentration than other mercaptans (e.g. mercaptoethanol) presumably due to the formation of a stable six-member ring containing a disulphide bond. Disulphides could also be reduced to free thiols by means of electrolytic reduction[8] as well as by water soluble phosphines (e.g. trihydroxymethylphosphine, tricarboxymethylphosphine) which were recently used for disulphide cleavage in proteins[10].

$$R{-}S{-}S{-}R + HSCH_2{-}CHOH{-}CHOH{-}CH_2SH \longrightarrow RSH + \underset{(2)}{R{-}S{-}S{-}CH_2{-}CHOH{-}CHOH{-}CH_2SH}$$

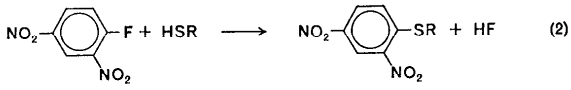
$$\longrightarrow RSH + \text{(cyclic dithiane-diol)} \qquad (1)$$

A much more detailed discussion of the reduction of disulphides to mercaptans is given in Chapter 4 on the preparation of thiols.

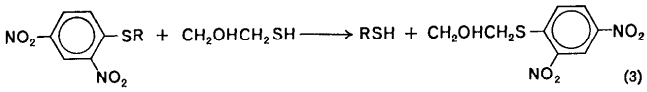
III. THIOETHERS

Simple saturated aliphatic thioethers are generally not easily cleaved to yield the free thiols[11]. However, there are some exceptions in which the alkyl radical of an alkyl-phenyl-thioether is cleaved by means of sodium in liquid ammonia, lithium in dimethylamine[12] or lithium in monomethylamine[13] to give the corresponding thiophenols. (See Chapter 4.)

2,4-Dinitrophenyl-thioethers are cleaved under very mild conditions ('thiolysis' of the thioether), with mercaptoethanol at pH 8[14]. These derivatives are obtained by reacting the thiol with 2,4-dinitrofluorobenzene in presence of base.



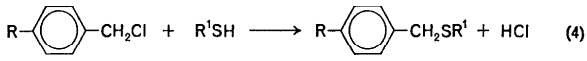
(2)



(3)

A. Benzyl Derivatives

The best known, and most widely used sulphydryl protecting group is the benzyl group. Benzylation takes place by reacting benzyl chloride in the presence of base with the thiol in aqueous or non-aqueous media (reaction 4; R = H)[15,16]. The reaction could take place also with the sodium



(4)

mercaptide using liquid ammonia as solvent[17]. The protecting group is removed by reductive cleavage with sodium in liquid ammonia[17,18,19]. In cases in which the benzyl thioether is insoluble in liquid ammonia, reductive cleavage can be achieved by using sodium in boiling butanol[20] or sodium in boiling ethanol[21]. It is of importance to note that sometimes desulphurization occurs during the cleavage with sodium in liquid ammonia[22].

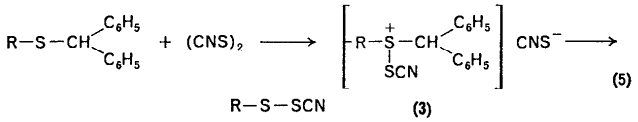
Due to the large lowering of the Pd or Pt catalyst efficiency caused by the sulphur which is present in a thioether form, the reductive cleavage of the benzyl group from the thioether cannot be achieved by catalytic hydrogenation. It has been shown that sufficient catalyst efficiency is retained for the reductive cleavage of the *p*-nitrobenzyl group, presumably due to the labilization of the CH_2—S bond by the strong inductive effect of the nitro group. The *p*-nitrobenzyl protecting group, which is introduced by reacting *p*-nitrobenzyl chloride with the thiol (reaction 4; R = NO_2), is removed by hydrogenation under atmospheric pressure, using 10% Pd on charcoal as a catalyst[23]. It has been shown that this reaction is not a general one and it does not take an unequivocal course since e.g. S-*p*-nitrobenzyl-cysteine gives S-*p*-aminobenzyl-cysteine and similarly benzyloxycarbonyl-S-*p*-nitrobenzyl-cysteinylglycine gives benzyloxycarbonyl-S-*p*-aminobenzyl-cysteinylglycine[24]. Recently it has been shown that the *p*-aminobenzyl group could be cleaved from the thioether by using 10% $HgSO_4$ solution in 5% H_2SO_4 (Hopkin's reagent)[25]. Thus the *p*-nitrobenzyl group could be removed in a two-step reaction involving first reduction to the corresponding *p*-aminobenzyl derivative and then removal of the *p*-aminobenzyl group by acidic $HgSO_4$ solution.

While the benzyl protecting group is stable towards acidic cleavage under normal conditions, introduction of a methoxy group at the *p* position will increase its tendency to acidic cleavage. Thus the *p*-methoxybenzyl group which is introduced in the usual manner (reaction 4; R = OCH_3)[26] is removed by means of trifluoroacetic acid[26] or anhydrous hydrogen fluoride[27].

B. Diphenylmethyl Derivatives

Reaction of thiols with diphenylmethyl chloride gives the diphenylmethyl (or benzhydryl) thioethers[28]. It has been shown that the thioether could be obtained in high yield by reacting the thiol with diphenylmethanol in the presence of BF_3 etherate[29]. The diphenylmethyl protecting group is removed either by trifluoroacetic acid or *via* reductive cleavage using sodium in liquid ammonia[28]. The diphenylmethyl thioether could also be cleaved by thiocyanogen using trifluoroacetic acid–acetic acid as a

solvent[30]. One pathway for this reaction may be *via* formation of a sulphonium salt intermediate (3) which can eject a stabilized carbonium ion and sulphenylthiocyanate, the latter reacting further with another molecule of thioether or with free thiol to yield the disulphide (reaction 5). The formation of free thiols from sulphenylthiocyanates directly or *via* the disulphide is discussed in Chapter 4. An alternative possibility is that the protecting group is split in the acidic solvent and the free thiol thus formed reacts with thiocyanogen to give sulphenylthiocyanate (reaction 6).



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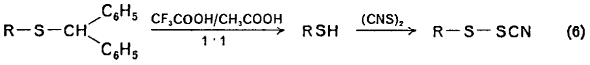

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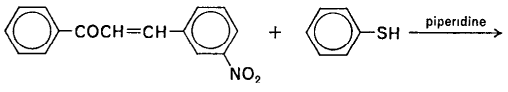
Another protecting group which could be included in this class is a thioether obtained by reacting the thiol with *m*-nitrobenzalacetophenone in the presence of piperidine (reaction 7); the protecting group is removed



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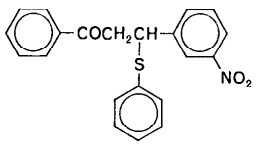


by treatment with basic lead acetate[31]. This group is used to protect the sulphydryl moiety of thiophenol and substituted thiophenols during electrophilic substitution reactions on the benzene rings[31].

C. Triphenylmethyl Derivatives

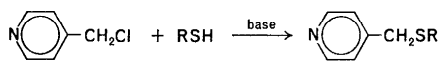
The triphenylmethyl (trityl) derivatives are obtained by reacting the appropriate thiol with triphenylmethyl chloride[28,32,33], or with triphenyl

methanol and BF_3 etherate[29]. Similarly to the diphenylmethyl thioether, the triphenylmethyl thioether is cleaved by sodium in liquid ammonia to give the free thiol[34], however, contrary to the diphenylmethyl thioether, the triphenylmethyl group could also be cleaved from the thioether by heavy metal ions. Moreover, trityl thioethers are more susceptible to acid hydrolysis as well as to thiocyanogen oxidation than the corresponding diphenylmethyl derivatives.

Although the removal of the triphenylmethyl group by trifluoroacetic acid[28] and hydrogen chloride in chloroform[33] is reported, there are cases in which acidic cleavage (e.g. by means of trifluoroacetic acid, hydrogen bromide in glacial acetic acid, *p*-toluene sulphonic acid) indeed removed the protecting group but the product obtained did not possess any free sulphydryl group[35,36]. It seems that the best acidic reagent to use is hydrochloric acid in aqueous acetic acid[36]. The heavy metal ions used for the removal of the triphenylmethyl group from the thioether are Ag^+ and Hg^{2+}. Initially, methanolic silver nitrate solution in the presence of pyridine was used[28]. Later it has been shown that there are cases in which better cleavage yields are obtained by using mercuric acetate[35,36], in other cases silver nitrate gives the best results[37] and in some cases both reagents give about the same yields[38]. It seems that the cleavage yield depends upon the whole molecule in question, and the metal of choice could be found only experimentally. The triphenylmethyl moiety is removed from the thioether very easily by oxidation with thiocyanogen in the presence of sodium acetate. The sulphenylthiocyanate which is obtained reacts with free thiol or with another molecule of the thioether to form unsymmetrical or symmetrical disulphides which can be reduced later to the free thiol[39]. The removal of the triphenylmethyl group by the thiocyanogen is so easy that it can even be removed in the presence of a diphenylmethyl thioether, without any cleavage of the latter compound[40].

D. Picolyl Derivatives

Contrary to catalytic hydrogenation which usually fails in the presence of thiols or thioethers, electrolytic reduction at a mercury cathode takes place without difficulty[41]. Among the thioethers which could be cleaved by electrolytic reduction are the 4-picolyl thioethers. These derivatives are obtained by reacting the free thiol with freshly distilled 4-picolyl chloride in the presence of base. The thioether is completely stable towards acidic



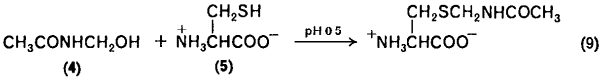
(8)

cleavage, and no cleavage could be detected after its storage for 7 days in trifluoroacetic acid or in hydrogen bromide in acetic acid. The protecting group could be removed by electrolytic reduction at a mercury cathode in 0·5N sulphuric acid solution[42].

This protecting group was recently used in the synthesis of L-cystinyl-bis-glycine[42].

E. Acetamidomethyl Derivatives

The acetamidomethyl thioether is obtained by reacting a 10% excess of acetamidomethanol (**4**) with the thiol at pH 0·5. The protecting group is very stable in the pH range of 0–13 as well as towards concentrated strong



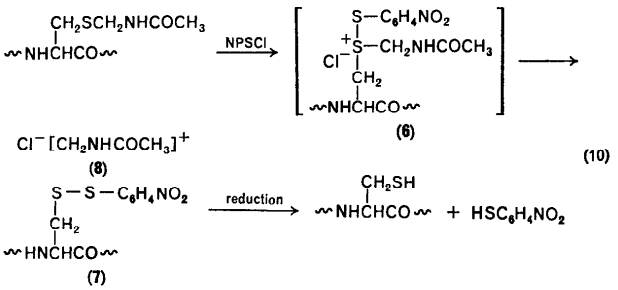
(4) (5) (9)

acids (e.g. trifluoroacetic acid, hydrogen bromide in glacial acetic acid, anhydrous hydrofluoric acid). It is removed from the thioether by using two equivalents of mercuric ions at pH 4[43].

In the case of cysteine (**5**) the product obtained is contaminated by cystine and also by thiazolidine carboxylic acid (obtained by reaction of cysteine and formaldehyde, the latter arising from hydrolytic decomposition of acetamidomethanol, see section VI). However, the product could easily be purified by using ion exchange columns[44]. On the other hand, anhydrous conditions should avoid the decomposition of the acetamidomethanol and indeed a reaction using hydrogen fluoride as a solvent results in quantitative yield of the pure product[45].

An elegant method for the cleavage of the protecting group has been discussed recently[46]. It is based on the observation that sulphenyl halides are reacting with unsymmetrical thioethers giving disulphides among other products, depending upon the structure of the thioethers. Reaction of 2-nitrophenylsulphenyl chloride (NPSCl) with acetamidomethyl cysteine residue would form a thiosulphonium ion (**6**) which decomposed to the mixed disulphide derivative (**7**) and to (**8**). The thiol function is then regenerated from the disulphide derivative by the usual reduction procedure (see section II).

The reaction of sulphenyl halides with thioethers seems to be a general procedure for the cleavage of a thiol protecting group, provided that a stable cation could be ejected from the thiosulphonium ion intermediate. Thus the thioether linkages between the haem group and the cysteine



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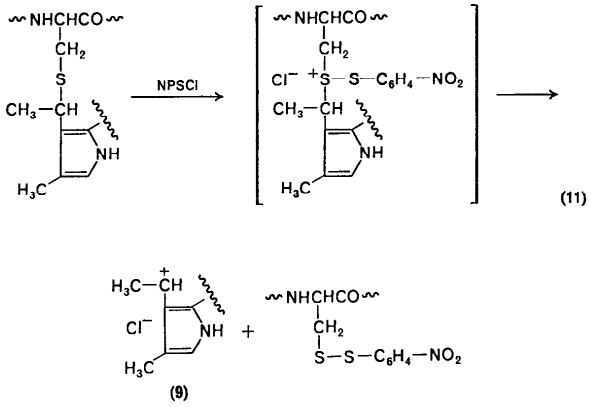
residue in horse heart cytochrome C was rapidly and quantitatively cleaved by 2-nitrophenylsulphenyl chloride[47]. The cleavage is successfully effected due to the easy formation of the carbonium ion (**9**), stabilized by the conjugated porphyrin system. This haem cleavage procedure is a very useful alternative to the available methods[48].

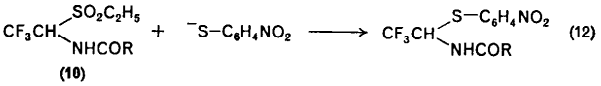


(11)

F. β, β, β-Trifluoro-α-acylaminoethyl Derivatives

Only one work has been reported using this protecting group[49]. The thioether is obtained by an exchange reaction of the thiol with β,β,β-trifluoroethyl-α-ethanesulphonyl-α-N-acylamine (**10**). In the case of

R = $C_6H_5CH_2O$, the protecting group is removed by hydrogen bromide in acetic acid followed by adjusting the pH to 9–10. While some protected

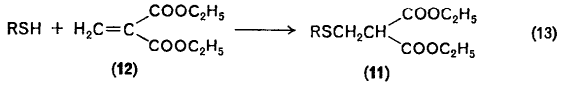


(12)

cysteine derivatives were prepared[49], the removal of the protecting group from those derivatives is not reported. Some more work should be carried out on this protecting group before it gains any use.

G. β, β-Diethoxycarbonylethyl Derivatives

Another protecting group which has not yet gained a wide use is the β,β-diethylcarbonylethyl group. The thioether (**11**) is obtained by the addition of the thiol to diethyl methylenemalonate (**12**). The protecting



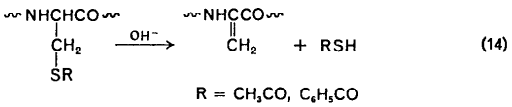
(13)

group is stable towards acidic reagents (e.g. trifluoroacetic acid, hydrogen bromide in acetic acid) but is cleaved by 1N KOH solution *via* β-elimination[50]. This protecting group was used to protect the thiol group of cysteine during the synthesis of glutathione[51].

IV. THIOESTERS

A. Acetyl and Benzoyl Derivatives

The acetyl and benzoyl derivatives are obtained by the reaction of the corresponding acyl chloride with the thiol[52]. These thioesters are in effect 'active esters' prone to attack by nucleophiles in general and very susceptible to dilute base. The protecting groups are removed completely by very dilute alkali within 20 min, but with dilute ammonia solution only 50% cleavage occurs during the same time. The hydrolytic cleavage is accompanied by a β-elimination as a side-reaction, especially in cysteine derivatives (reaction 14). This side-reaction can be avoided in low



(14)

molecular weight peptides when the protecting group is removed by methanolysis with sodium methoxide in methanol[52]. Since the extent of β-elimination is related to the polarity of the solvent used, it has been shown that in the case of large peptides which are soluble only in highly polar solvents β-elimination occurs during methanolysis too[53].

The acetyl and benzoyl thioesters are stable towards aqueous acids as well as towards trifluoroacetic acid. While the benzoyl group is stable towards 2N hydrochloric acid in glacial acetic acid, the acetyl group is cleaved under those conditions[52].

B. Benzyloxycarbonyl Derivatives

These derivatives are obtained by reacting benzyloxycarbonyl chloride with a thiol[54]. Contrary to the N-benzyloxycarbonyl derivatives they are stable towards hydrogen bromide in acetic acid[52,55] but they are cleaved by phosphonium iodide in acetic acid[54] or by boiling trifluoroacetic acid[52]. The protecting group is removed by methanolysis and ammonolysis, but those cleavage reactions proceed much slower than in the case of the corresponding acyl derivatives. The protecting group is removed very easily by ammonolysis using concentrated aqueous ammonia solution[52] as well as by methanolysis using fivefold excess of sodium methoxide.

C. Urethane Derivatives

The best known protecting group of this class is the ethylcarbamoyl which is obtained by the reaction of ethyl isocyanate with the thiol[56]. The protecting group is stable in acidic and neutral solutions but is

$$C_2H_5NCO + HSR \longrightarrow \underset{H}{C_2H_5NCOSR} \qquad (15)$$

cleaved easily in basic solution (e.g. aqueous and anhydrous ammonia solution, dilute sodium hydroxide solution, dilute sodium methoxide solution in methanol)[57]. No β-elimination could be detected when ethylcarbamoyl cysteine derivatives were treated with 1N sodium hydroxide solution to yield the unprotected cysteine derivatives[57]. This is contrary to the behaviour of the corresponding benzoyl and acetyl derivatives where considerable β-elimination is observed. It has been shown recently that the ethylcarbamoyl moiety is removed by treatment with heavy metal ions (Hg^{2+}, Ag^{+})[58].

An interesting protecting group of this class is the β-(N-acyl-N-methylaminoethyl)carbamoyl group. The isocyanate (**13**), which reacts

with the mercaptan to give the thiourethane, is obtained *via* the following route[59].

$$CH_2{=}CHCN + CH_3NH_2 \longrightarrow CH_3NHCH_2CH_2CN \xrightarrow{Ba(OH)_2} CH_3NHCH_2CH_2COOH \xrightarrow{RY}$$

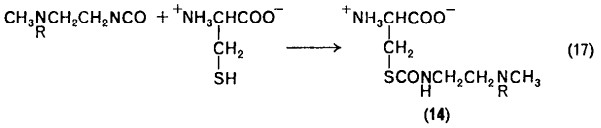
$$\underset{R}{CH_3NCH_2CH_2COOH} \xrightarrow{HN_3} \underset{R}{CH_3NCH_2CH_2CON_3} \xrightarrow{\Delta} \underset{R}{CH_3NCH_2CH_2NCO} \qquad (16)$$

(13)

$R = C_6H_5CH_2OCO$, $Y = Cl$.

$R = (CH_3)_3COCO$, $Y = N_3$.

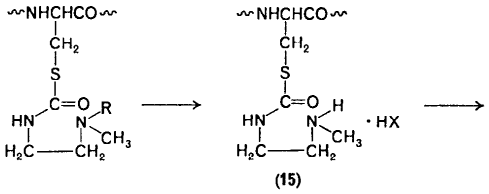
Reaction of **13** with cysteine will give β-(N-acyl-N-methylaminoethyl) carbamoyl cysteine (**14**). The acyl group, which is a urethane-type



(17)

(14)

protecting group of the β-amino group, is removed by the usual means[60] to give the ammonium salt (**15**). Upon neutralization of the salt there is an intermolecular nucleophilic attack of the β-amino group on the carbonyl group, followed by cleavage and formation of the free mercaptan



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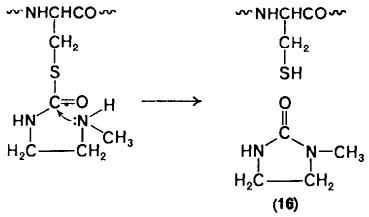


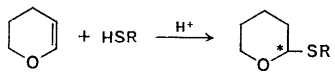
(16)

and 1-methyl-2-imidazolidone (**16**). This protecting group was recently used to protect the thiol group of cysteine during the synthesis of oxytocine[59].

V. SEMITHIOACETALS

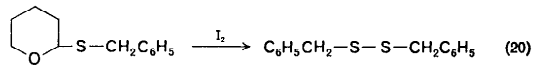
A. Tetrahydropyranyl Derivatives

The tetrahydropyranyl derivatives are prepared by reacting the free thiol with 2,3-dihydropyrane in the presence of acid as a catalyst[61,62]. A disadvantage in using this protecting group is the introduction of a new asymmetric centre (*) to the molecule. The protecting group is removed by hydrolysis with very dilute acid[61], by the action of aqueous



(19)

silver nitrate solution[63], or by reaction with iodine[64]. In the latter case the disulphide (which could be reduced to the free thiol) is obtained, e.g. benzyl tetrahydropyranyl sulphide reacts with iodine to give dibenzyl disulphide. A similar cleavage is observed by the action of thiocyanogen:



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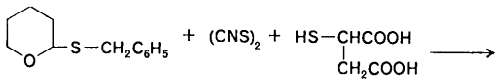
benzyl tetrahydropyranyl sulphide reacts with thiocyanogen in the presence of $ZnCl_2$ and mercaptosuccinic acid to form the disulphide (**17**)[64]. The cleavage of this sulphide by electrophiles is presumably due to the



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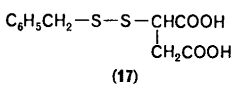
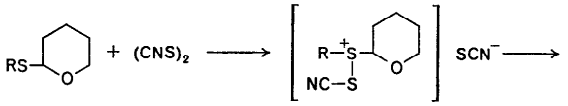


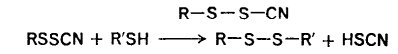
(**17**)

fact that the intermediate sulphonium salt (**18**) can eject a stabilized carbonium ion (see sections III.B, III.E) and the sulphenyl thiocyanate

reacts further with thiols or certain sulphides to produce unsymmetrical or symmetrical disulphides.

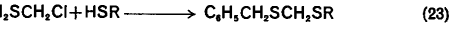


(**18**) (22)



B. Benzylthiomethyl and Phenylthiomethyl Derivatives

These derivatives were obtained by the reaction of various thiols with benzylthiomethyl chloride in methanol[65]. It was found that products obtained by this route are difficult to purify and the method of choice now



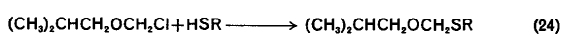
(23)

is reduction of a symmetrical disulphide by means of sodium in liquid ammonia, followed by addition of the freshly distilled benzylthiomethyl chloride[66]. The protecting group is stable in acidic media (e.g. hydrogen bromide in acetic acid) but is removed by mercuric acetate solution in 80% formic acid[66]. The usefulness of this group as a thiol protecting group was demonstrated in the synthesis of glutathione[67].

The phenylthiomethyl ($C_6H_5SCH_2$—) protecting group is obtained and removed in an identical way to that of the benzylthiomethyl group[66].

C. Isobutyloxymethyl Derivatives

Isobutyloxymethyl derivatives are obtained by reaction of isobutyloxymethyl chloride with thiols[66]. These are more sensitive to acid than the corresponding benzylthiomethyl derivatives. The isobutyloxymethyl



(24)

group is cleaved by hydrogen bromide in acetic acid[66], BF_3 etherate or trifluoroacetic acid[68], but it is stable towards 2N hydrochloric acid in 50% acetic acid[68] or 12N hydrochloric acid in acetone[68]. The isobutyloxymethyl sulphide is decomposed to some extent by 2N sodium hydroxide[66], but it is stable towards hydrazine hydrate in boiling ethanol[68].

The protecting group can be cleaved by thiocyanogen similarly to the triphenylmethyl, diphenylmethyl and tetrahydropyranyl groups[68].

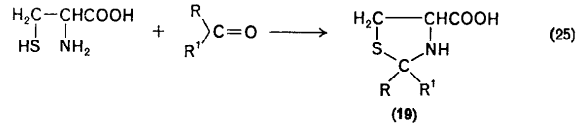
The reactivity of the isobutyloxymethyl group towards thiocyanogen lies between that of the triphenylmethyl and the diphenylmethyl group. The difference in reactivity is not sufficient to allow the selective oxidative cleavage of the triphenylmethyl group in the presence of the isobutyloxymethyl moiety, or the cleavage of the isobutyloxymethyl group in the presence of the diphenylmethyl moiety[68].

VI. HETEROCYCLIC RINGS

A. Thiazolidine Derivatives

Cysteine and cysteine derivatives (like other β-aminothiols) react with aldehydes and ketones to form thiazolidine derivatives[69].

The best known derivatives of this class are thiazolidinecarboxylic acid (**19**, $R = R^1 = H$) (or thioproline) and 2,2-dimethylthiazolidinecarboxylic acid (**19**, $R = R^1 = CH_3$) which are formed by the reaction of



(25)

(19)

cysteine with formaldehyde or acetone respectively[69-71]. The protecting group can be removed by mild acid hydrolysis[69-71]. In the case of thiazolidinecarboxylic acid, oxidation with iodine yields the disulphide which can be easily reduced to the free mercaptan[69]. The protecting group can be removed from 2,2-dimethylthiazolidinecarboxylic acid by aqueous mercuric chloride solution[70,71].

VII. ACKNOWLEDGEMENTS

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VIII. REFERENCES

1. C. B. Anfinsen, *J. Polymer Sci.*, **49**, 31 (1961).
2. R. Cecil and J. R. McPhee, *Adv. Prot. Chem.*, **14**, 272 (1959).
3. For references see A. Schoberl and A. Wagner in *Methods in Organic Chemistry*, (Houben Weyl), Vol. 9, Georg Thieme, Stuttgart, 1955, p. 59.
4. F. Yoneda, K. Suzuki and Y. Nitta, *J. Amer. Chem. Soc.*, **88**, 2328 (1966).
5. J. H. Freisheim and F. M. Huennekens, *Biochemistry*, **8**, 2271 (1969).
6. M. Sokolovsky, D. Harell and J. F. Riordan, *Biochemistry*, **8**, 4740 (1969).

7. J. B. Mudd and T. T. McManus, *Arch. Biochem. Biophys.*, **132**, 237 (1969).
8. For references see A. Schoberl and A. Wagner in *Methods in Organic Chemistry*, (Houben Weyl), Vol. 9, Georg Thieme, Stuttgart, 1955, p.23.
9. W. W. Cleland, *Biochemistry*, **3**, 480 (1964).
10. M. E. Levison, A. S. Josephson and D. M. Kirschenbaum, *Experientia*, **25**, 126 (1969).
11. D. S. Tarbell and D. P. Harnish, *Chem. Rev.*, **49**, 1 (1951).
12. R. Adams and A. Ferretti, *J. Amer. Chem. Soc.*, **81**, 4939 (1959).
13. W. Truce and D. P. Tate, *Abstracts of Papers 132nd A.C.S. Meeting*, September 1957, p. 43-P.
14. S. Shaltiel, *Biochem. Biophys. Res. Commun.*, **29**, 178 (1967).
15. M. D. Armstrong and J. D. Lewis, *J. Org. Chem.*, **16**, 749 (1951).
16. M. Frankel, D. Gertner, H. Jacobson and A. Zilkha, *J. Chem. Soc.*, 1390 (1960).
17. H. S. Loring and V. du Vigneaud, *J. Biol. Chem.*, **111**, 385 (1935).
18. R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
19. J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 800 (1952).
20. W. I. Patterson and V. duVigneaud, *J. Biol. Chem.*, **111**, 393 (1935).
21. K. Hofmann, A. Bridgwater and A. E. Axelrod, *J. Amer. Chem. Soc.*, **71**, 1253 (1949).
22. P. G. Katsoyannis, *Am. J. Med.*, **40**, 652 (1966).
23. C. Berse, R. Boucher and L. Piche, *J. Org. Chem.*, **22**, 805 (1957).
24. M. A. Ondetti and M. Bodanszky, *Chem. Ind. (London)*, 697 (1962).
25. M. D. Bachi and K. J. Ross-Petersen, *J. Org. Chem.*, **37**, 3550 (1972).
26. S. Akabori, S. Sakakibara, Y. Shimonishi and Y. Nobuhara, *Bull. Chem. Soc. Japan*, **37**, 433 (1964).
27. S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Japan*, **38**, 1412 (1965).
28. L. Zervas and I. Photaki, *J. Amer. Chem. Soc.*, **84**, 3887 (1962).
29. R. G. Hiskey and J. B. Adams, Jr., *J. Org. Chem.*, **30**, 1340 (1965).
30. R. G. Hiskey and M. A. Harpold, *Tetrahedron*, **23**, 3923 (1967).
31. A. H. Herz and D. S. Tarbell, *J. Amer. Chem. Soc.*, **75**, 4657 (1953).
32. L. Zervas and D. M. Theodoropoulos, *J. Amer. Chem. Soc.*, **78**, 1359 (1956).
33. G. Amiard, R. Heymes and L. Velluz, *Bull. Soc. Chim. France*, 698 (1956).
34. D. Theodoropoulos, *Acta Chem. Scand.*, **13**, 383 (1959).
35. F. I. Carroll, H. M. Dickson and M. E. Wall, *J. Org. Chem.*, **30**, 33 (1965).
36. R. G. Hiskey, T. Mizoguchi and H. Igeta, *J. Org. Chem.*, **31**, 1188 (1966).
37. R. G. Hiskey and J. B. Adams, Jr., *J. Org. Chem.*, **31**, 2178 (1966).
38. L. Zervas, I. Photaki, A. Cosmatos and D. Borovas, *J. Amer. Chem. Soc.*, **87**, 4922 (1965).
39. R. G. Hiskey and D. N. Harpp, *J. Amer. Chem. Soc.*, **87**, 3965 (1965).
40. R. G. Hiskey, T. Mizoguchi and E. L. Smithwick, Jr., *J. Org. Chem.*, **32**, 97 (1967).
41. P. M. Scopes, K. B. Walshaw and G. T. Young, *J. Chem. Soc.*, 782 (1965).
42. A. Gosden, D. Stevenson and G. T. Young, *Chem. Comm.*, 1123 (1972).
43. D. F. Veber, J. D. Milkowski, R. G. Denkewalter and R. Hirshmann, *Tetrahedron Letters*, 3057 (1968).
44. P. Hermann and E. Schreier in *Peptides* (1972), Proceedings of the 12th European Peptide Symposium (Ed. H. Hanson and H. Jakubke), North Holland, Amsterdam, 1973, p. 126.

45. D. F. Veber, R. D. Milkowski, S. L. Varga, R. G. Denkewalter and R. Hirschmann, *J. Amer. Chem. Soc.*, **94**, 5456 (1972).
46. A. Fontana in *Peptides* (1972), Proceedings of the 12th European Peptide Symposium (Ed. H. Hanson and H. Jakubke), North Holland, Amsterdam 1973, p. 122.
47. A. Fontana, F. M. Veronese and F. Boccu, *FEBS Letters*, **32**, 135 (1973).
48. E. Margolias and A. Schejter, *Adv. Prot. Chem.*, **21**, 114 (1966).
49. F. Weygand, W. Steglich, I. Lengyel and F. Fraumberger, *Chem. Ber.*, **99**, 1932 (1966).
50. T. Wieland and A. Sicber, *Ann. Chem.*, **722**, 222 (1969).
51. T. Wieland and A. Sieber, *Ann. Chem.*, **727**, 121 (1969).
52. L. Zervas, I. Photaki and N. Ghelis, *J. Amer. Chem. Soc.*, **85**, 1337 (1963).
53. R. G. Hiskey, R. A. Upham, G. M. Beverly and W. C. Jones, Jr., *J. Org. Chem.*, **35**, 513 (1970).
54. A. Berger, J. Noguchi and E. Katchalski, *J. Amer. Chem. Soc.*, **78**, 4483 (1956).
55. M. Sokolovsky, M. Wilchek and A. Patchornik, *J. Amer. Chem. Soc.*, **86**, 1202 (1964).
56. D. L. Ross, C. G. Skinner and W. Shive, *J. Med. Chem.*, **3**, 516 (1961).
57. St. Guttmann, *Helv. Chim. Acta*, **49**, 83 (1966).
58. H. T. Storey, J. Beacham, S. F. Cernosek, F. M. Finn, C. Yanaihara and K. Hofmann, *J. Amer. Chem. Soc.*, **94**, 6170 (1972).
59. G. Jager and R. Geiger in *Peptides* (1972), Proceedings of the 12th European Peptide Symposium (Ed. H. Hanson and H. Jakubke), North Holland, Amsterdam 1973, p. 90.
60. Y. Wolman, *Protection of the Amino Group*, Section III in *The Chemistry of the Amino Group* (Ed. S. Patai), Interscience, London, 1968, p. 682.
61. F. Kipnis and J. Ornfelt, *J. Amer. Chem. Soc.*, **73**, 822 (1951).
62. R. G. Hiskey and W. P. Tucker, *J. Amer. Chem. Soc.*, **84**, 4789 (1962).
63. G. F. Holland and L. A. Cohen, *J. Amer. Chem. Soc.*, **80**, 3765 (1958).
64. R. G. Hiskey and W. P. Tucker, *J. Amer. Chem. Soc.*, **84**, 4794 (1962).
65. P. J. E. Pimlott and G. T. Young, *Proc. Chem. Soc.*, 257 (1958).
66. P. J. E. Brownlee, M. E. Cox, B. O. Handford, J. C. Marsden and G. T. Young, *J. Chem. Soc.*, 3832 (1964).
67. R. Camble, R. Purkayastha and G. T. Young, *J. Chem. Soc. (C)*, 1219 (1968).
68. R. G. Hiskey and J. T. Sparrow, *J. Org. Chem.*, **35**, 215 (1970).
69. S. Ratner and H. T. Clarke, *J. Amer. Soc.* **59**, 200 (1937).
70. F. E. King, J. W. Clark-Lewis and R. Wade, *J. Chem. Soc.*, 880 (1957).
71. J. C. Sheehan and D. H. Yang, *J. Amer. Chem. Soc.*, **80**, 1158 (1958).

CHAPTER **15**

Rearrangements involving thiols

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I. INTRODUCTION

This chapter deals with rearrangement reactions which involve thiols either as starting materials or as products. However, because of the high reactivity of thiols in both nucleophilic and free radical reactions, they are actually involved in many cases as transient species only. Such cases, in which the intermediacy of thiols is evident or highly probable, are also included here.

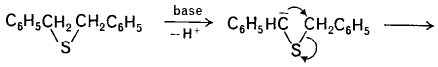
Only few rearrangements involving thiols, particularly those which are of synthetic importance or are related to biochemical processes, have been thoroughly investigated. It was not the aim of this chapter to unearth and list all the rearrangements which have been reported in the literature, but rather to group and describe, with illustrative examples, the most important types.

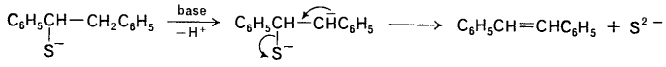
II. GROUP MIGRATIONS FROM AND ONTO THIOLS

A. Alkyl Migrations

1. Sulphur to carbon

Migrations of alkyl groups from oxygen to negatively charged carbon (Wittig rearrangement) are well known[1]. The analogous rearrangement of sulphides, which would lead to isomeric thiols, has not yet been observed. A Wittig-like mechanism was however used to explain the formation of stilbene from dibenzyl sulphide and strong base, assuming the intermediacy of the thiol (**1**) which eliminates sulphide ion[2].

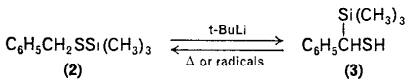




(1)

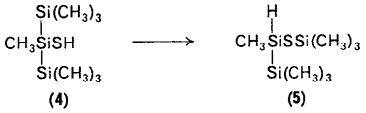
Trialkylsilyl groups, on the other hand, migrate very readily under Wittig conditions. Benzylthiotrimethylsilane (**2**) on treatment with tert-butyllithium is rapidly converted to α-trimethylsilyl toluene-α-thiol (**3**) in almost quantitative yield.

The reverse rearrangement (**3**→**2**) occurs on heating **3** at 190°C under the influence of radical catalysis. A similar rearrangement, which involves



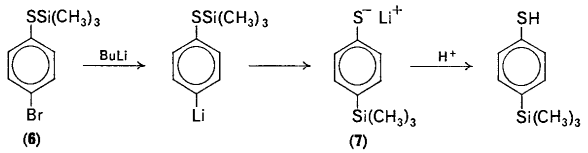
(2) (3)

radical induced migration of trialkylsilyl groups from silicon to sulphur (**4**→**5**) was also reported[4].



(4) (5)

Migration of trialkylsilyl and germanyl groups from sulphur to aromatic carbon was also observed[5]. Lithiation of 4-bromo-S-trimethylsilylbenzenethiol (**6**) yields the lithium salt of 4-trimethylsilylbenzenethiol (**7**). The mechanism has not been investigated and it has not been established whether an intra- or intermolecular process is involved.

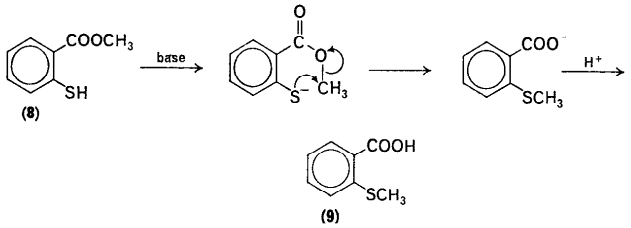


(6) (7)

2. Sulphur to oxygen

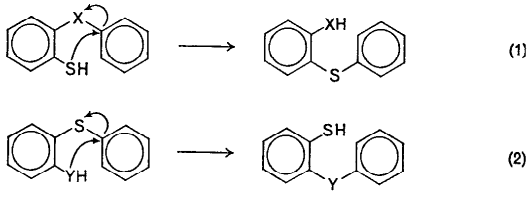
Oxygen—alkyl bonds are easily cleaved by thiol salts[6]. An intramolecular reaction of this type would result in migration of an alkyl group from oxygen to sulphur. Indeed, treatment of methyl 2-mercaptobenzoate (**8**) with alkali gives 73% of 2-methylthiobenzoic acid (**9**)[7].

With benzylamine, **8** yields the benzylamide of **9**, probably through dehydration of the benzylamine salt.



B. *Aryl Migrations (the Smiles Rearrangement)*

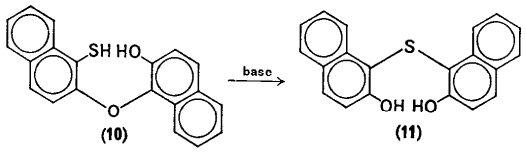
The Smiles rearrangement is defined as an aromatic nucleophilic displacement in which the nucleophile and the leaving group are joined, usually by being *ortho*-substituents on an aromatic ring. The result is a migration of an aryl group from one heteroatom to another. The thiol group can be involved in the Smiles rearrangement either as the nucleophile (equation 1) or as the displaced group (equation 2).



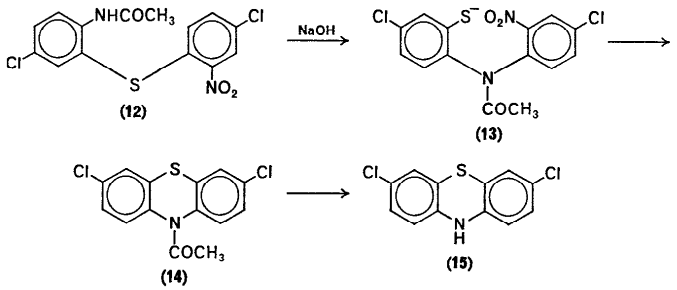
A recent review[8] describes in detail the mechanistic and synthetic aspects of the reaction and also presents a tabular survey of all Smiles rearrangements which appeared in the literature.

Because of the high nucleophility of the thiol group and its anion, it is to be expected that reactions of the type shown in equation (1) would be of a wide scope. However, only a small number of examples were reported, mostly by Smiles[9], and all involve the conversion of mercaptodiaryl ethers to hydroxydiaryl sulphides. In this manner 2-hydroxy-1′-mercapto-1,2′-dinaphthyl ether (**10**) yielded 2,2′-dihydroxy-1,1′-dinaphthyl ether (**11**).

Reactions of the type shown in equation (**2**) have attracted much more attention, as they present the easiest and the most direct synthetic route to the medically important phenothiazines[10]. Almost all the rearrangements reported in the literature are of 2-acylamino-2′-nitrodiphenyl sulphides which yield phenothiazines on treatment with base. A typical



one[11] is that of the sulphide (**12**). Its rearrangement led initially to the thiol salt (**13**) and subsequently the thiol group displaced the nitro group to give **14**. The acyl group is usually hydrolysed under the reaction conditions and the phenothiazine (**15**) was obtained in one step. Isolation of the intermediate N-arylphenothiazines was reported in several cases[12,13].

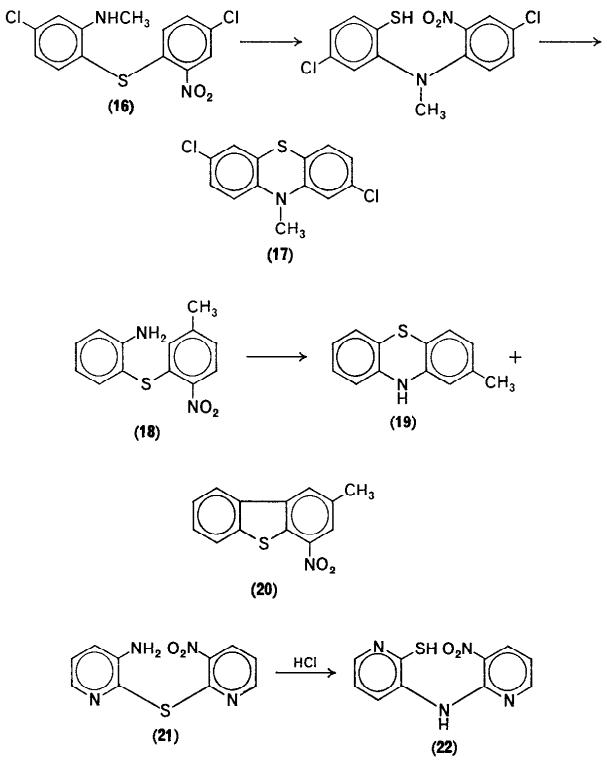


Although the phenothiazines could have been formed by a direct displacement of the nitro group by the amide, the positions of the substituents in the products establish the mechanism shown and the intermediacy of thiols.

A closely related reaction is the rearrangement of N-alkylaminodiaryl sulphides, which yield N-alkylphenothiazines on heating in boiling quinoline or aniline[14]. Compound **17** was thus obtained from **16** (57% yield).

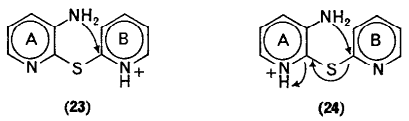
Contrary to previous reports it was recently found[15] that 2-amino-2′-nitrodiphenyl sulphide (**18**) also rearranged on heating at 190°C alone or in dimethylacetamide to give the phenothiazine **19**. The dibenzothiophen **20** was also formed as a by-product.

The rearrangement of pyridyl sulphides is particularly interesting, as it was found to occur also under acidic conditions[16]. 3-Amino-3′-nitro-2,2′-dipyridyl sulphide (**21**) gives 2-mercapto-3′-nitro-3,2′-dipyridylamine (**22**) in nearly quantitative yield on short treatment with hydrochloric acid.



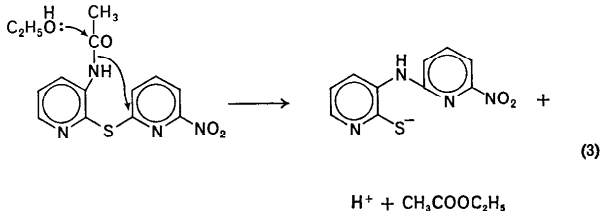
(16) (17) (18) (19) (20) (21) (22)

The catalytic effect of the acid can be explained by protonation of either pyridine ring. Protonation of ring B (**23**) makes it more susceptible to nucleophilic attacks, while protonation of ring A (**24**) stabilizes the leaving group.



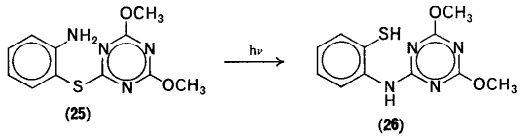
(23) (24)

The thermal rearrangement of dipyridyl sulphides proceeds much easier than that of diphenyl sulphides and is highly solvent-dependent: it is rapid in boiling ethanol, slower in water and does not occur at all in benzene or dimethylsulphoxide[16]. It was also observed that 2-acylamino-pyridyl sulphides rearrange faster than the corresponding 2-amino derivatives. These facts suggest solvent participation such as shown in equation 3.



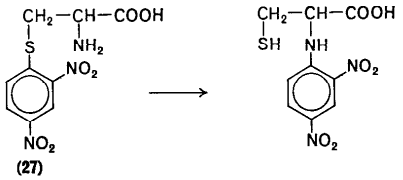
(3)

The photochemical Smiles rearrangement was also reported recently. An example involving a thiol is the conversion of **25** to **26** on irradiation in ethanol[17].

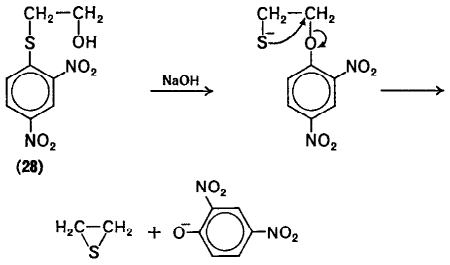


(25) (26)

Migrations of 2,4-dinitrophenyl groups from aliphatic thiols are also known. Migration to nitrogen occurs[18] in the cysteine derivative **27** at pH 7, and migration to oxygen in compound **28** on treatment with base[19].



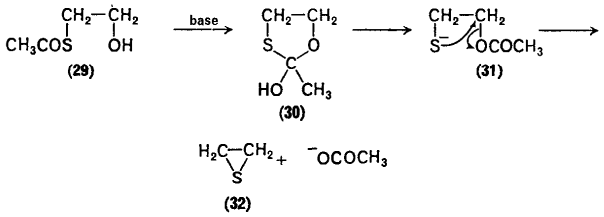
(27)



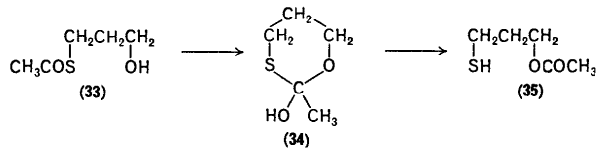
C. Acyl Migrations

1. Sulphur to Oxygen

Acetyl transfer from thiol to hydroxyl groups occurs readily, under basic conditions, in the series $RCOS(CH_2)_nOH$ when n is 2 or 3, but not when n is 4. S-Acetylmercaptoethanol (**29**) thus yields thiiran (**32**) as a result of acetate ion elimination from the rearranged product **32**[20,21].



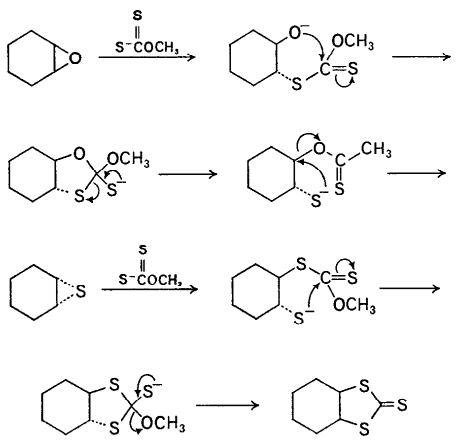
S-Acetyl-3-mercaptopropanol (**33**) yields, under the same conditions, 3-mercaptopropyl acetate (**35**) which is stable and isolatable.



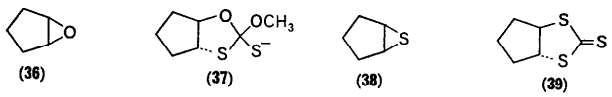
The importance of the distance between the thiol and the hydroxy groups implicates intermediate ring formations (**30** and **34**) during the migrations. It can be expected that a five-membered ring intermediate would provide the optimum ring size for the transfer, and indeed compound **29** rearranges 30 times faster than **33**[23].

A kinetic study of the rearrangement of **33** to **35** showed that the equilibrium constant is 56 (at 39°C, 0·3 ionic strength), corresponding to a difference of free energy of 2500 kcal/mole between esters and thiol esters[24].

A similar rearrangement which involves migration of the thionoalkoxy group is assumed to occur during the reaction of xanthate salts with epoxides, which leads to trithiocarbonates[25]. The proposed mechanism[26,27] is presented in equation (4).



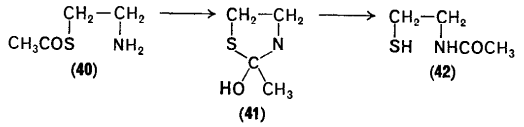
Evidence for the mechanism is provided by the fact that cyclopentene oxide (**36**) does not react[28], as its rearrangement would require the existence of the intermediate **37** which possesses two *trans*-fused five-membered rings. The thiiran **38**, however, reacts smoothly and gives **39**. This striking difference in behaviour can probably be attributed mainly



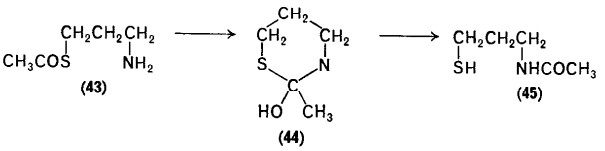
to the much greater nucleophilicity of the thiol anion, but ring closure may also be facilitated by an enhanced ease of deformation of bond angles when oxygen is replaced by sulphur.

2. Sulphur to Nitrogen

The migration of an acyl group from a thiol to an amino group is analogous to the migration to hydroxyl described above, and proceeds through the corresponding cyclic intermediates. S-Acetyl-2-aminoethanethiol (**40**) rearranges in this manner very readily to 2-acetamidoethanethiol (**42**) *via* the thiazolidine **41** and S-acetyl-3-aminopropanethiol (**43**) rearranges to 3-acetamidopropanethiol (**45**) via **44**[29].

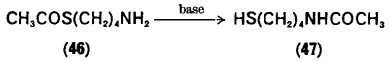


(40) (41) (42)

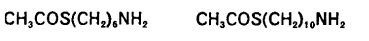


(43) (44) (45)

These rearrangements take place even in acidic media and, as expected, the rearrangement of **40** proceeds much faster than that of **43** (at pH 5, rate ratio 1 : 100). The rearrangement of S-acetyl-4-aminobutanethiol (**46**) to **47**, which would require a seven-membered ring intermediate, occurs at a measurable rate only at pH > 8 and is much slower. On increasing the distance between the thiol and the amino group, no rearrangement was observed and treatment of **48** or **49** with base results only in hydrolysis of the acetyl group



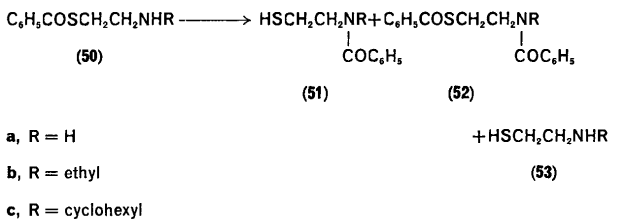
(46) (47)



(48) (49)

A kinetic study on the reaction **40** → **41** → **42**[34,35] confirmed the intermediacy of **41**. All the reaction steps were assumed to be equilibria, and equilibrium constants and rate constants were determined. Of particular interest is the pH effect. The migration rate exhibits an inverse dependence on hydrogen ion concentration at low pH (2·5–3), a plateau region (pH 3–4·5) which is ascribed to general base catalysis by H_2O and then general base catalysis by OH^- at higher pH (> 5)[23]. A detailed mechanism which accounts for all the data and includes protonation equilibria was proposed[23].

S-Benzoyl-2-aminoethanethiol and its N-alkyl derivatives (**50**) rearrange immediately on liberation from the hydrobromide salts[30]. However, besides the expected 2-benzamidoethanethiols (**51**) small amounts of N,S-dibenzoyl-?-aminoethanethiols (**52**) were also obtained, which indicates that the intramolecular acyl migrations were followed by transthiolesterifications[31] from the starting materials **50** to the rearranged products **51**. The transesterifications must be accompanied by elimination of 2-aminoethanethiols (**53**) and although attempts to isolate **53a** and **53b** from the reactions of **50a** and **50b** failed, **50c** afforded all three products



(50) (51) (52)

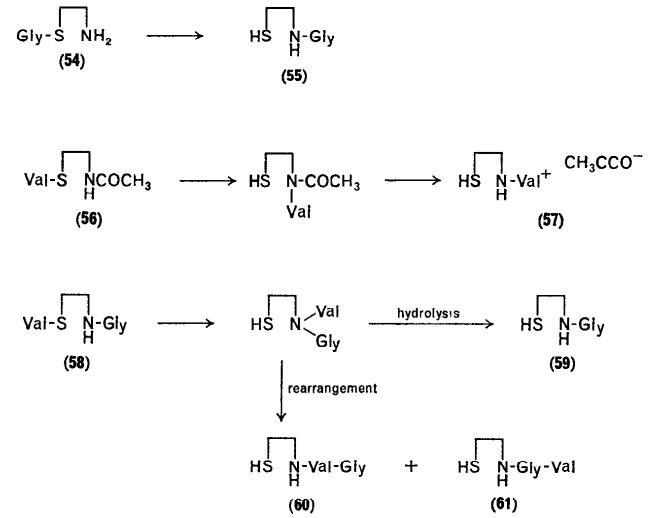
a, R = H

b, R = ethyl

c, R = cyclohexyl

+ HSCH₂CH₂NHR (53)

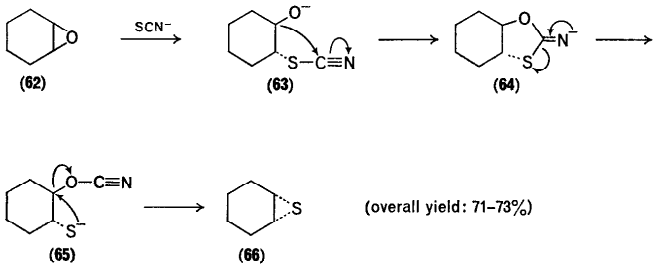
Applications by the rearrangement to peptides were studied by Wieland[29,32,33]. Migrations of α-aminoacyl groups are very rapid and the rearrangement of S-glycylcystamine **54** to the N-glycyl derivative (**55**) was complete at pH 5·2 in 2 min. Under basic conditions α-aminoacyl groups migrate from sulphur even to amides, to give diacylimines. These undergo a very facile hydrolysis and lose one acyl group (**56** → **57**), or if possible rearrange further *via* N → N acyl migrations to give tripeptides. 5-Valyl-N-glycylcystamine (**58**) for example yielded a mixture which contained N-glycylcystamine (**59**), N-valylglycylcystamine (**60**) and N-glycylvalylcystamine (**61**).



D. Migrations of Nitrogen-containing Species

1. Cyano group migrations

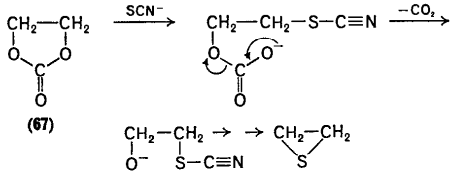
One of the most important syntheses of thiirans[36] is the direct conversion of oxirans to thiirans with thiocyanate salts[37]. The mechanism proposed[38,39] is opening of the oxirane (**62**) to hydroxy thiocyanate (**63**), rearrangement to mercapto cyanate (**65**) through the cyclic intermediate **64** and finally elimination of cyanate ion to give the thiiran **66**.



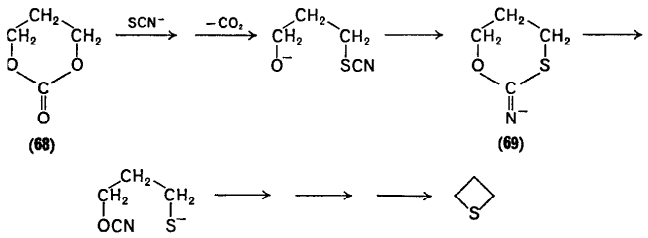
This mechanism was substantiated by isolation of the *p*-nitrobenzoyl derivative of the cyclic intermediate **64**, and by the observation that inversion occurred at each asymmetric carbon[40], as the mechanism demands that the resultant thiiran possess a configuration opposite to that of the starting oxirane.

Cyclopentane oxide does not react under customary reaction conditions because of the considerable strain required to form a cyclic intermediate analogous to **64**. However, on employing harsher conditions 20% of the corresponding thiiran could be obtained[41].

A modification of the above reaction, which proceeds via the same intermediate, is the reaction of thiocyanate salts with ethylene carbonates (**67**)[42].



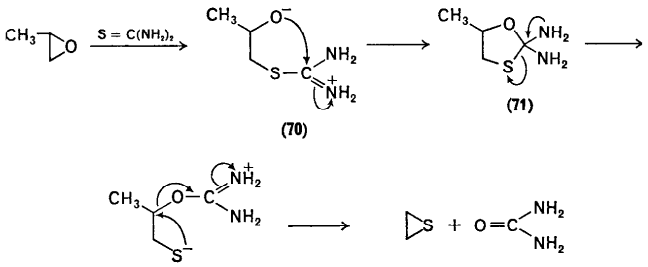
Propylene carbonates (**68**) react as well to give thietans[43], thus the rearrangement can proceed also *via* the six-membered ring intermediate **69**.



2. Amidino group migrations

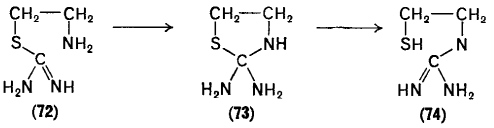
This rearrangement is assumed to occur during the conversion of oxirans to thiirans by reaction with thiourea[44] (equation 5).

The β-hydroxyisothiouronium salt **70** can be isolated in the presence of acid[44,45] and react further on addition of base. The importance of the cyclic intermediate **71** is evident from the failure of cyclopentene oxide to react.



(70) (71)

In a similar manner the amidino group migrates[46] from S to N. S-(2-Aminoethyl)isothiouronium salts (**72**) rearrange, at neutral pH, to 2-mercaptoethylguanidine (**74**). Both starting materials and products are in this case isolatable as hydrobromide salts. The reaction was shown[47] to involve the cyclic intermediate **73**.



(72) (73) (74)

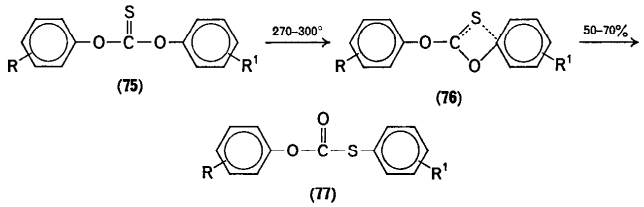
S-(3-aminopropyl)isothiouronium salts rearrange in the same manner to (3-mercaptopropyl)guanidines through a six-membered cyclic intermediate[46].

III. REARRANGEMENTS OF THE O-THIOACYL SYSTEM TO THE S-ACYL SYSTEM

A. Rearrangements which Proceed through a Four-membered Cyclic Transition State

The first reported rearrangement of this type was the thermal isomerization of diarylthioncarbonates (**75**) to diarylthiolcarbonates (**77**)[48,49].

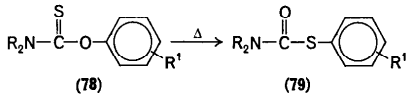
An examination of a large series of compounds[50] showed that when R and R′ are electron-withdrawing substituents, rate accelerations are experienced, and in unsymmetrically substituted thioncarbonates the rearrangement occurs primarily in the direction of the ring bearing the more electron-withdrawing substituents. The reaction thus originates from the nucleophilic character of the sulphur. A kinetic study[51] showed that



(75) (76) (77)

the reaction is first order and all these data indicate that the rearrangement consists of an aromatic nucleophilic displacement in which the ring migrates from O to S via a four-membered cyclic transition state (**76**). Since this mechanism requires no solvation, the reaction could be carried out also in the gas phase (440°C, short periods) and yields were improved[52]. The reaction can serve as an efficient preparation method for aromatic thiols by hydrolysis of the products **77**.

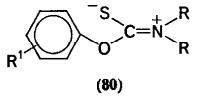
N,N-Dialkylthioncarbamates (**78**) rearrange similarily to thiolcarbamates (**79**). This isomerization is faster and proceeds at lower temperatures and in higher yields (usually above 90%)[53,54].



(78) (79)

The four-membered cyclic mechanism is supported by substituent effects and kinetic results[53,55]. No crossover products were found on heating a mixture of two thioncarbamates.

Steric rate enhancement due to hindered rotation was found to be present in ortho-substituted compounds in this series[56]. The relatively low temperature required results from increased nucleophilicity of the sulphur, since the polarization is assisted by the dialkylamino group, towards the zwitterionic form **80**.



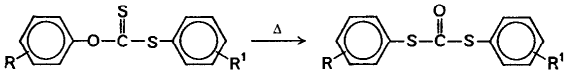
(80)

A stronger electron-donating inductive effect of R should promote the nucleophilic character of the sulphur further, and indeed the rearrangement rate was found to increase in the order[55]:

$$R = i\text{-}C_4H_9 > n\text{-}C_3H_7 > C_2H_5 > CH_3$$

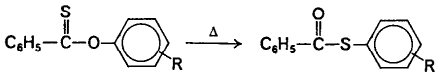
The reaction was utilized for the synthesis of aromatic thiols[53] and sulphonic acids[57] by hydrolysis or oxidation of the rearranged products.

The heating of xanthates usually results in β-elimination and formation of olefins (Chugaev reaction[58]). However, when there is no β-hydrogen at the alcohol moiety, rearrangement takes place. A kinetic study of the influence of substituents on the reaction rate of a series of diaryl xanthates to diaryl dithiocarbonates (**81**→**82**) again indicates a four-membered cyclic transition state[59]. A similar transition state is indicated, by the same



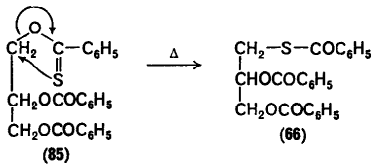
(**81**) (**82**)

kind of evidence, for the rearrangement of aryl thiobenzoates (**83**) to aryl thiolbenzoates (**84**)[60].



(**83**) (**84**)

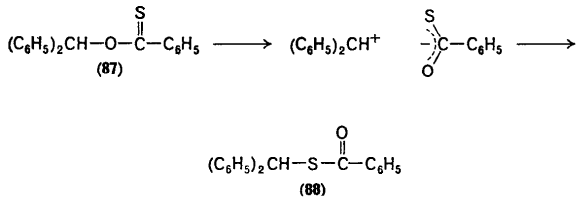
The rearrangement of alkyl thiobenzoates had also been reported in certain cases[61]. An application of this reaction is the thermal conversion of thionesters of glycerol to esters of thioglycerol (**85**→**86**)[62].



(**85**) (**66**)

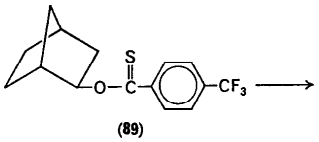
B. *Rearrangements which Proceed through Dissociation and Return*

O-Alkyl thionesters rearrange easily to thiolesters when the alkyl group can form relatively stable carbonium ions. Benzhydryl thioncarbonate (**87**) yields **88** on heating in ethanol[63] and the suggested mechanism is a dissociation to a ion pair, the return of which occurs *via* the sulphur because of the greater nucleophility of the sulphur compared to the oxygen.

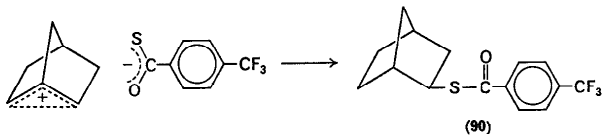


(**87**) (**88**)

A study of the rearrangement of the optically active exo-norbornyl thiobenzoate **89** to **90** showed that the rate of racemization was equal to the rate of the disappearance of **89**. This indicates that no return *via* the oxygen occurs[64].

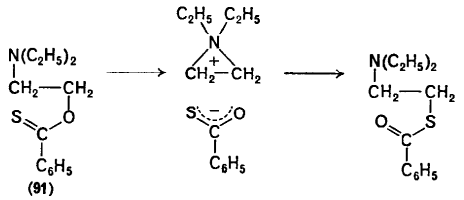


(**89**)



(**90**)

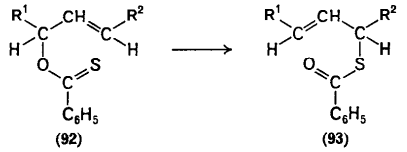
The dissociation, and hence the rearrangement, may be caused by neighbouring group participation in the formation of the cation. An example is the rearrangement of the thionester **91**[65].



(**91**)

C. Rearrangement of Allyl Thionesters

Allyl thionbenzoates (**92**) rearrange[66] to allyl thiolbenzoates (**93**), accompanying an allyl shift, on heating to 100°C.



(92) (93)

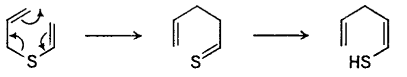
This isomerization is very little influenced by the medium and occurs only ca. ten times faster in acetic acid than in cyclohexane. This low sensitivity to the ionizing power of the solvent indicates that allyl thionesters rearrange by a mechanism which involves very little change in charge separation between the ground state and the transition state. Thus a cyclic concerted mechanism is more probable than dissociation to ion pairs. This conclusion was confirmed by deuterium isotope effect measurements[67].

Allyl thioncarbamates[68] and allyl xanthates[65] also rearrange easily in the same manner.

IV. THE THIO-CLAISEN REARRANGEMENT

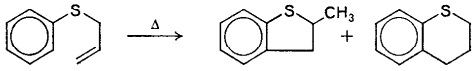
A. The Rearrangement of Allyl Aryl Sulphides

The thio-Claisen rearrangement is a [3,3]sigmatropic process, which consists of synchronous cleavage of a carbon—sulphur bond and formation of a new carbon—carbon bond (equation 6).



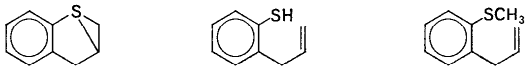
(6)

The thiols formed usually do not survive under the reaction conditions and cyclize to five- and six-membered rings. Heating of allyl phenyl sulphide (**94**) in high boiling amines[69,70] or carboxylic acids[71] yields 2-methyl-2,3-dihydrobenzothiophene (**95**) and thiachroman (**96**). The two products are not interconvertible under these conditions.



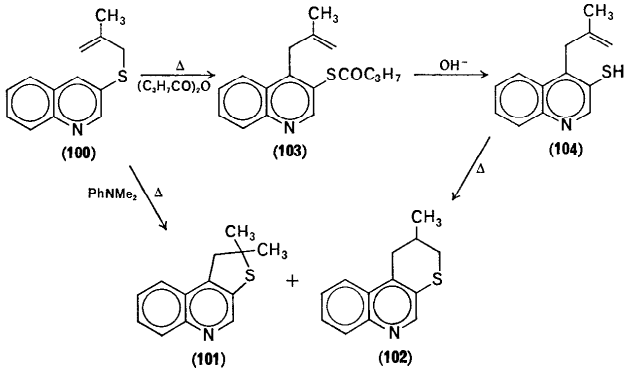
(94) (95) (96)

Although a different reaction pathway, which involves the thiiran **97** as intermediate, has been proposed[72,73], the intermediacy of 2-allylbenzenethiol (**98**) and therefore the correctness of the concerted mechanism has been rigorously established. In the presence of methyl iodide the methylthio derivative **99** was isolated[74]. Furthermore, compound **98** was synthesized independently[72] and was shown to undergo a facile cyclization to give both **95** and **96**. When cyclized under the rearrangement conditions, the proportions of the products from **98** were identical with those obtained directly from **94**[74].



(97) (98) (99)

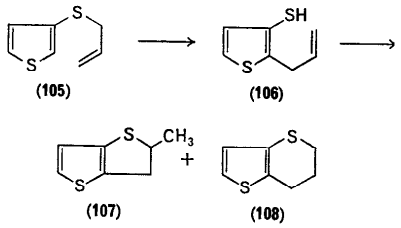
Definitive evidence for the sole intermediacy of 2-allylarenethiols was obtained from work on the rearrangements of allyl quinolyl sulphides[75–77]. For example, 3-methallylquinolyl sulphide (**100**), which rearranges in dimethylaniline to **101** and **102**, gave in butyric anhydride quantitative yield of the butyryl derivative (**103**) of the Claisen product **104**. Compounds **100** and **104** yielded **101** and **102** in the same proportions when heated under identical conditions.



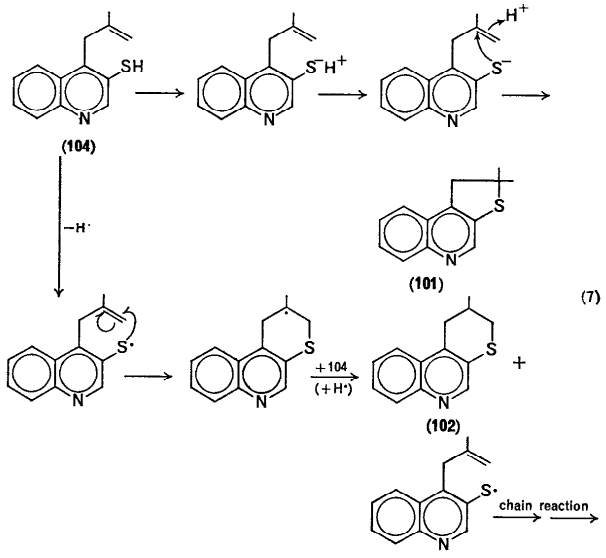
(100) (103) (104)

(101) (102)

Similar results were observed in the thiophene series[78]. In the rearrangement of allyl 3-thienyl sulphide (**105**) to **107** and **108**, the intermediate 2-allylthiol **106** has been isolated for the first time directly from the reaction mixture.



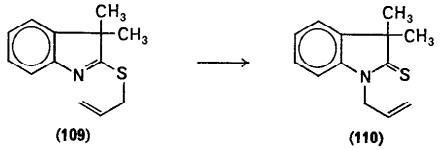
The five-membered ring products result from the normal (Markownikoff) internal addition of the thiol group to the double bond, whereas the six-membered ring products result from abnormal (anti-Markownikoff) addition, which is characteristic of a free radical process. The formation of both heterocycles thus indicates competitive thermally induced heterolytic and homolytic fissions of the thiol S—H bond (equation 7). The cyclization mechanisms were verified[79] by a detailed examination of the thermal behaviour of **104**. Product **102** was formed almost exclusively



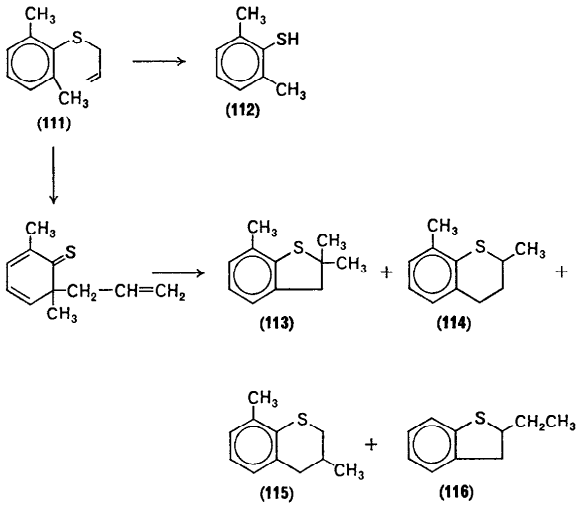
(7)

under free radical initiation. The intervention of free radical intermediates was also evident from e.s.r. monitoring of the reaction.

Cyclization does not occur when the thione initially formed in the rearrangement cannot tautomerize to the corresponding enethiol. The indolenine **109** for example rearranges to **110** which shows no tendency to cyclize[80].

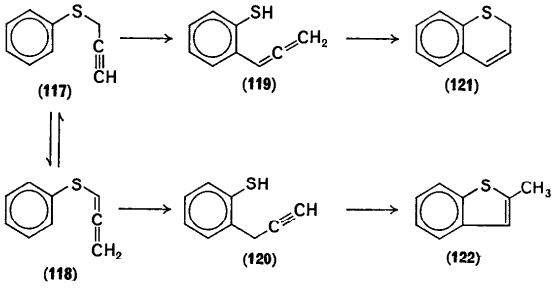


When the two *ortho*-positions are blocked, no *para*-Claisen products are observed. Heating of allyl 2,6-dimethylphenyl sulphide (**111**) yields a cleavage product **112** and four cyclic materials (**113–116**) which probably result from *ortho*-rearrangement followed by 1,3- and 1,4-methyl migrations[81].

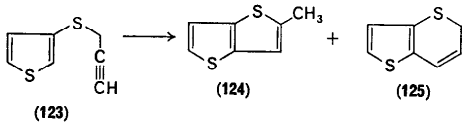


B. The Rearrangement of Prop-2-ynyl Aryl Sulphides

This reaction also yields both five and six-membered cyclic products, but has been shown to consist of two parallel processes[82]. Prop-2-ynyl phenyl sulphide (**117**) on heating initially isomerizes to phenyl allenyl sulphide (**118**) and both **117** and **118** undergo the thio-Claisen rearrangement to the allenic (**119**) and acetylenic (**120**) thiols respectively. Subsequent cyclization yields the final products, 2H-thiachromene (**121**) and 2-methylbenzothiophene (**122**).



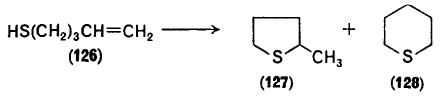
The rearrangement of prop-2-ynyl-3-thienyl sulphide (**123**) proceeds in the same manner to give **124** and **125**[83].



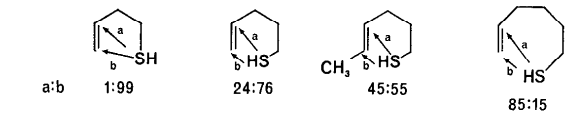
V. INTERNAL ADDITIONS, ELIMINATIONS AND RING-CHAIN TAUTOMERISMS

A. Intramolecular Additions to Double Bonds

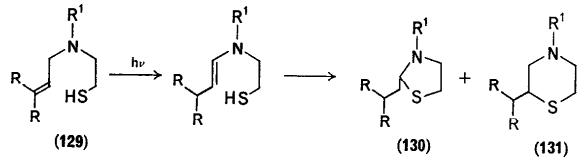
The cyclization of *ortho*-allylbenzenethiols has already been discussed in connection with the thio-Claisen rearrangement. It has been shown that ionic and free radical processes operate simultaneously to give five- and six-membered heterocycles respectively[79]. Similar dual pathways were observed in the cyclization of 5-mercapto-1-pentene (**126**) which gave both **127** and **128**[84].



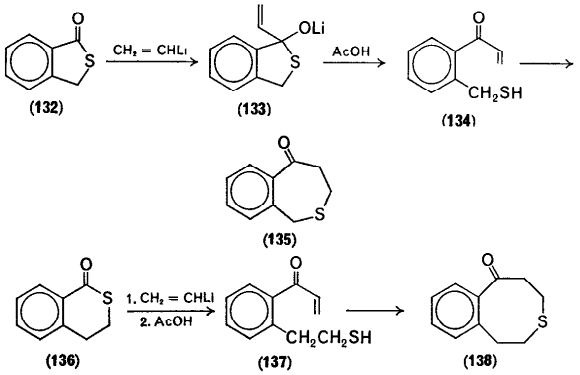
Free radical cyclization (Bz_2O_2 catalysis) of a series of simple mercaptoolefins gave the following product ratios[85].



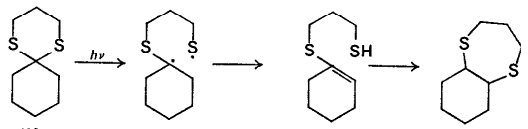
Different results were observed in nitrogen-containing chains. Ultraviolet irradiation of N-allyl-2-aminoethanethiols (**129**) gave mainly thiazolidines (**130**) when R was H and hexahydrothiazines (**131**) when R was methyl. These results can be rationalized by assuming an initial double-bond migration towards the nitrogen[86].



Reaction routes which include internal additions of thiols have been proposed for several rearrangement reactions. Addition of vinyllithium to thiophthalide (**132**) yielded, after acidification, 4,5,6,7-tetrahydro-2H-benzo[c]thiepin-5-one (**135**). The probable course is ring opening of the adduct **133** to the thiol **134** which cyclizes by conjugate addition to give **135**. The thiolactone **136** yielded **138** in an analogous manner *via* the thiol **137**[87].



Another example is the photolysis of mercaptoles. Compound **139** yielded the rearranged product **140** (*cis-trans* mixture, ratio 8 : 1). Its formation is best explained by a homolytic scission of the C—S bond, followed by hydrogen atom transfer and addition of the thiol to the double bond[88].



(139) (140)

B. Intramolecular Additions to Triple Bonds

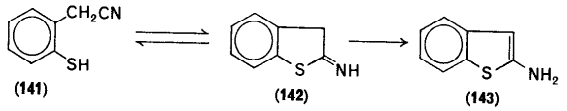
Cyclization of a series of acetylenic thiols under various conditions gave the following results[89] (Table 1, in %).

Although the polymerization was extensive in most cases, the analysis of the cyclic products clearly shows that in terminal acetylenes nucleophilic reaction leads mainly to exo-methylene-heterocycles, while free radical cyclization leads to unsaturated rings.

The cyclization of *ortho*-prop-2-ynylbenzenethiol has already been discussed in connection with the thio-Claisen rearrangement[82].

C. Cyclization and Ring-chain Tautomerism of Cyanothiols

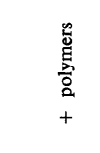
Cyanothiols cyclize irreversibly in cases in which the product exists predominately in its enamino form. *o*-Mercaptobenzylcyanide (**141**) thus cyclized to **142** which tautomerized to **143** and was shown to exist only in a cyclic form[90].



(141) (142) (143)

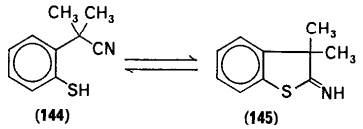
Compound **144**, on the other hand, cyclized to **145** in which the methyl substituents at position 3 preclude tautomerism to an enamine, and indeed its exists in the cyclic form only in neutral or acidic media. However, in basic solution it reacts as the open chain form **144**, and can be S-alkylated or oxidized to the corresponding disulphide[91].

TABLE 1. Cyclization of acetylenic thiols



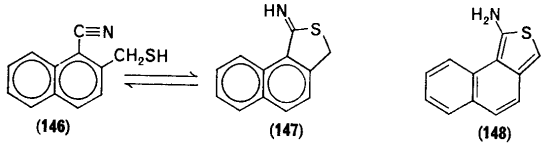
	R	Cyclization method					
$n = 2$	H	hν	traces	—	—	44	
$n = 3$	H	Nu	—	25	—	not determined	
	H	Δ	10	—	3	47	
	H	Bz_2O_2	15	—	6	56	
	H	hν	48	traces	traces	43	
	CH_3	hν-acetone	35	traces	traces	43	
	C_4H_5	hν	4	17	10	28	
		hν	—	75	—	10	
$n = 4$	H	Nu	—	20	—	not determined	
	H	Δ	5	traces	—	80	
	H	Bz_2O_2	28	traces	—	70	
	H	hν	38	traces	—	52	
	H	hν-acetone	51	traces	—	44	
$n = 5$	H	hν	23	—	—	51	
$n = 6$	H	hν	8	—	—	39	

hν, ultraviolet irradiation.
hν-acetone, ultraviolet irradiation with acetone as sensitizer.
Δ, prolonged heating in cyclohexane.
Bz_2O_2, short heating; Bz_2O_2 added for free radical initiation.
Nu, nucleophilic cyclization by heating with NaOH.



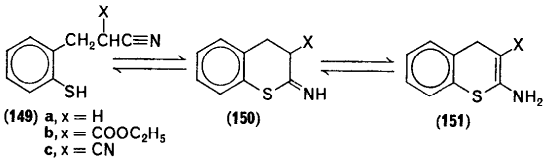
(144) (145)

The same ring-chain tautomerism is exhibited by the pair **146** ⇋ **147**. The cyclic form **147** exists as the imino tautomer, as aromatic stability would be disrupted by the *o*-quinoidal structural requirement of the enamino tautomer **148**[92].



(146) (147) (148)

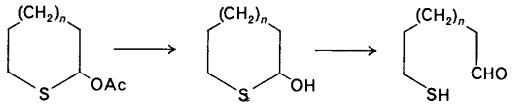
In corresponding six-membered systems it was shown that **151b** and **151c** are the predominant forms, as a result of conjugation with the substituent X, and therefore they do not show tautomerism with the open forms **149**. It was speculated by the authors[93] that if X would be a hydrogen, the cyclic form might exist as **150a**, and would be in tautomerism with **149a**. However, this could not be demonstrated, due to the failure to synthesize **149a** or **150a**.



(149) a, x = H
b, x = $COOC_2H_5$
c, x = CN
(150) (151)

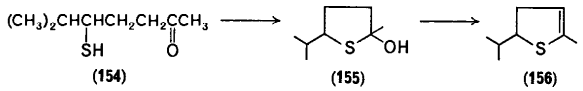
D. Ring-chain Tautomerism of Mercaptoaldehydes and Mercaptoketones

The order of stability of the cyclic form of hemithioacetals relative to the open chain form is parallel to that observed for hydroxyaldehydes[94]. 2-Hydroxytetrahydrothiophen (**152a**) and 2-hydroxyhexahydrothiopyran (**152b**) (prepared from the corresponding acetates) were shown, by spectral evidence, to exist in their cyclic forms both in the neat state and in their solutions in organic solvents. In aqueous solutions, however, they are converted into the tautomeric mercaptoaldehydes **153a** and **153b** and can be titrated as thiols with aqueous iodine. The seven-membered ring **152c**, on the other hand, exists as such only in the solid state, but shows spectral properties ascribable to the open form **153c** in chloroform solution, indicating that tautomerism occurs in this case very readily[95].



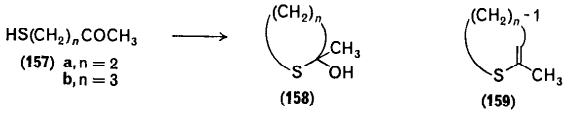
(152) a, n = 0
b, n = 1
c, n = 2
(153)

Similar results were obtained with mercaptoketones. The thiol **154** was prepared from open-chain precursors and cyclized spontaneously with loss of water to **156**. The possible intermediate **155** could not be isolated[96].



(154) (155) (156)

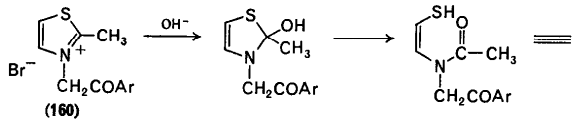
The unsubstituted mercaptoketones **157a** and **157b** were never isolated and isolation attempts led to the unsaturated heterocycles **159a** and **159b**, probably *via* the hemithioacetals **158a** and **158b**[96,97].



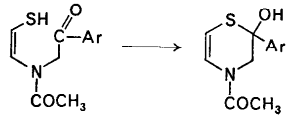
(157) a, n = 2
b, n = 3
(158) (159)

Internal thiol–carbonyl interactions were extensively investigated in the field of thiosugars, and were applied to the syntheses of the thiofuranose[98], and thiopyranose[99,100] and thioseptanose[101,102] systems.

Ring opening and closure involving mercaptoketones were assumed to occur during the unexpected formation of 4-acetyl-2,3-dihydro-2-hydroxy-2-phenyl-4H-1,4-thiazine (**161**), on treating 2-methyl-3-phenacylthiazolin bromide (**160**) with base[103].

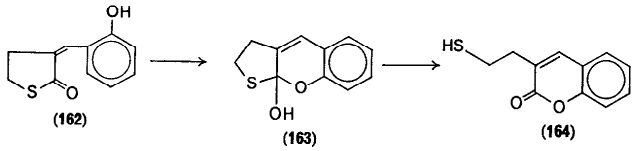


(160)



(161)

Another interesting example exists in the photochemical rearrangement of α-(*o*-hydroxybenzylidene)-γ-butyrothiolactone (**162**) to 3-(2-mercaptoethyl) coumarin (**164**). The rearranged product **163** is stabilized by ring opening, as the carbonyl group formed becomes a part of the coumarin system[104].

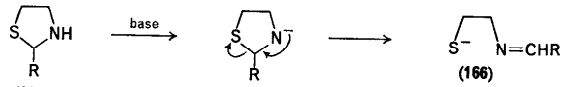


(162) (163) (164)

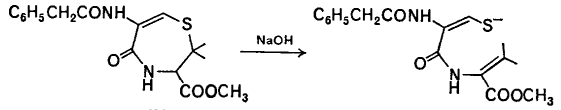
E. Ring Openings of Cyclic Sulphides to Unsaturated Thiols

1. β-Eliminations

This reaction has been observed mainly in nitrogen-containing systems. The proton elimination can occur either from a β-nitrogen atom or from a β-carbon. 2-Arylthiazolidines (**165**) are thus opened to the iminothiols **166**[105] and the tetrahydro-1,4-thiazepine **167** is opened to the enethiol **168** which is unstable, but could be trapped. The corresponding

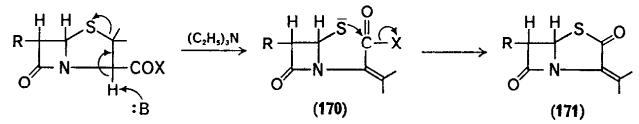


(165) (166)



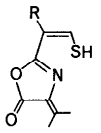
(167) (168)

perhydrothiazepine is opened by base at a much slower rate, and the resulting thiol is isolatable[106]. Rearrangements of this type are most common in penicillin chemistry. An example is the rearrangement of penicillins with an activated carboxyl group **169** to anhydropenicillins (**171**), in which the first step is β-elimination to the thiol (**170**)[107].



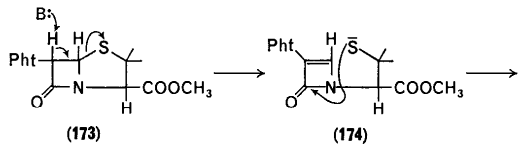
(169) (170) (171)

The intermediate **170** could, in certain cases, be trapped as its acyl derivative[108]. In another instance the carboxyl group reacted with the lactam ring leading to the oxazole **172**[109].



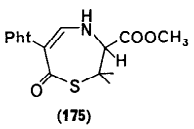
(172)

Another type of penicillin rearrangement is that which starts with proton elimination from C-6. Such mechanism explains the epimerization at C-6 on treating phthaloylpenicillin methyl ester (**173**) with sodium hydride[110]. The first step is the opening of the thiazole ring to the thiol salt **174** which recyclizes to **173** with epimerization. Support for this mechanism was provided by the isolation of the thiazepine **175** as a by-product[111].



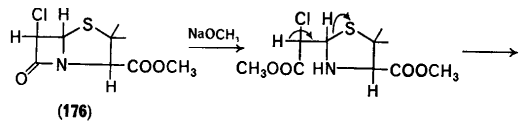
(173) (174)

Pht = Phthalimido

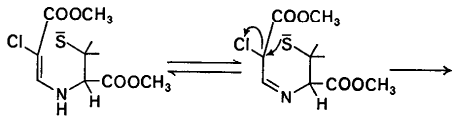


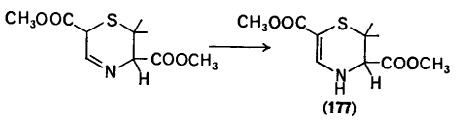
(175)

Another interesting penicillin rearrangement which involves β-elimination and a thiol intermediate is that of methyl 6α-chloropenicillate (**176**) to the 1,4-thiazine **177**, through the pathway shown[112]. Ring opening



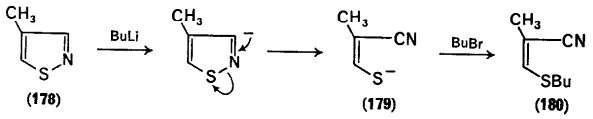
(176)





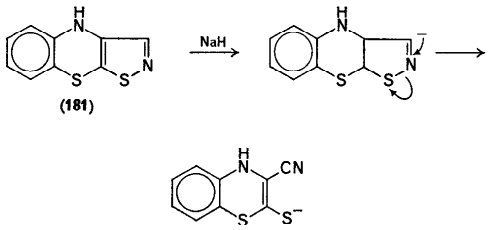
(177)

by β-elimination occurs also in the fully aromatic isothiazole system. During the lithiation of 4-methylisothiazole (**178**), which occurred mainly at position 5, the nitrile **180** was also formed as a by-product. The mechanistic pathway is probably lithiation at position 3, ring opening to the thiol salt **179** and alkylation by butyl bromide present in the reaction mixture[113].



(178) (179) (180)

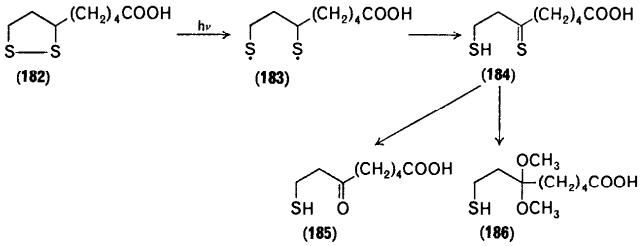
Similar openings were observed on treating condensed isothiazoles (**181**) with base[114].



(181)

2. Homolytic fissions followed by hydrogen transfer

The results of several photolysis reactions of sulphur-containing rings can be rationalized by postulating this process. One example is the photolysis of lipoic acid (**182**)[115] which yielded **185** (in water) or **186** (in methanol). The proposed mechanism is a homolytic scission of the S—S bond to the diradical **183** and migration of the tertiary hydrogen atom as a radical, to form the thionthiol **184** which reacts with the solvent. A similar mechanism which involves a primary homolytic cleavage of a C—S bond was assumed to occur in the photolysis of mercaptols[88].



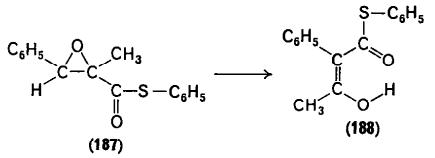
(182) (183) (184) (185) (186)

VI. MISCELLANEOUS REARRANGEMENTS

A. Migration of a Thiol Ester Group

The only case in which this type of migration occurs is the acidolytic ring opening of epoxides[116]. Phenyl α-methyl-*trans*-β-phenylthiolglycidate (**187**) gave, upon treatment with boron trifluoride etherate, 45% yield of the enol tautomer of phenyl α-phenylacetothiolacetate (**188**).

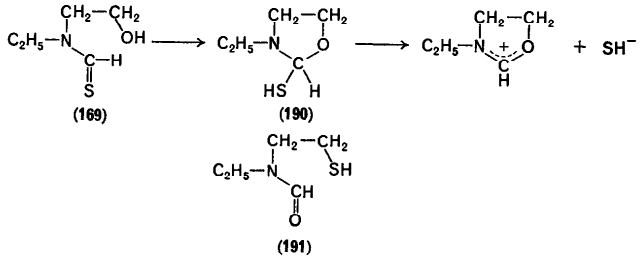
The tendency of the thiol ester group to migrate in this particular case is not surprising, since the unusual migration of the carbethoxy group in the corresponding glycidic ester was observed previously[117].



(187) (188)

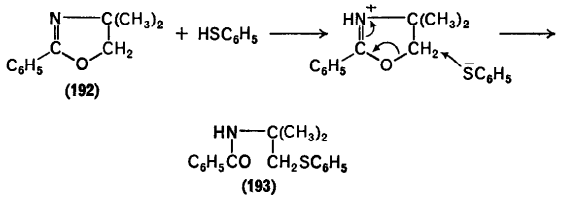
B. Dissociation and Return of the Hydrosulphide Ion

A rearrangement which proceeds through this mechanism was observed during the preparation of N-(β-hydroxyethyl)-N-ethyl-thioformamide (**189**). An isomer, N-(β-mercaptoethyl)-N-ethylformamide (**191**), was obtained as a side product and it was shown[118] that **191** was formed from **189**. The probable course is a cyclization of **189** to 1-ethyloxazolidine-2-thiol (**190**) and attack of the hydrosulphide ion at C-4. This mechanism is



(189) (190) (191)

supported by a previous report on the ring opening of oxazolidines by thiols (**192** → **193**)[119].



(192) (193)

VII. REFERENCES

1. U. Schollkopf, *Angew. Chem. Int. Ed.*, **9**, 763 (1970).
2. T. J. Wallace, H. Pobiner, J. E. Hofmann and A. Schriesheim, *J. Chem. Soc.*, 1271 (1965).
3. A. Wright, D. Ling, P. Boudjouk and R. West, *J. Amer. Chem. Soc.*, **94**, 4784 (1972).
4. C. G. Pitt and M. S. Fowler, *J. Amer. Chem. Soc.*, **90**, 1928 (1968).
5. A. R. Bassindale and D. R. M. Walton, *J. Organometal Chem.*, **25**, 389 (1970).
6. J. C. Sheehan and G. D. Daves, *J. Org. Chem.*, **29**, 2006 (1964).
7. J. C. Grivas and K. C. Navada, *J. Org. Chem.*, **36**, 1520 (1971).
8. W. M. Truce, E. M. Kreider and W. W. Brand, *Org. React.*, **18**, 99 (1970).
9. L. A. Warren and S. Smiles, *J. Chem. Soc.*, 914 (1931).
10. C. O. Okafor, *Intern. J. of Sulfur Chem.*, **6B**, 237 (1971).
11. K. J. Farrington and W. K. Warburton, *Aust. J. Chem.*, **8**, 545 (1955).
12. C. F. Wight and S. Smiles, *J. Chem. Soc.*, 340 (1935).
13. H. L. Yale and F. Sowinski, *J. Amer. Chem. Soc.*, **80**, 1651 (1958).
14. K. Fujii, *J. Pharm. Soc. Japan*, **77**, 3 (1957).
15. F. A. Davis and R. B. Wetzel, *Tetrahedron Letters*, 4483 (1969).
16. O. R. Rodig, R. E. Collier and R. K. Schlatzer, *J. Org. Chem.*, **29**, 2652 (1964).
17. K. Matsui, N. Maeno and S. Suzuki, *Tetrahedron Letters*, 1467 (1970).
18. H. P. Burchfield, *Nature*, **181**, 49 (1959).
19. C. C. J. Culvenor, W. Davies and W. E. Savige, *J. Chem. Soc.*, 4480 (1952).
20. L. W. C. Miles and L. N. Owen, *J. Chem. Soc.*, 817 (1952).
21. J. S. Harding and L. N. Owen, *J. Chem. Soc.*, 1528 (1954).
22. J. S. Harding and L. N. Owen, *J. Chem. Soc.*, 1536 (1954).
23. R. B. Martin and R. I. Hendrick, *J. Amer. Chem. Soc.*, **84**, 106 (1962).
24. J. P. Jencks, S. Cordes and J. Carrioulo, *J. Biol. Chem.*, **235**, 3608 (1960).
25. C. C. J. Culvenor, W. Davies and K. Pausacker, *J. Chem. Soc.*, 1050 (1946).
26. A. M. Creighton and L. N. Owen, *J. Chem. Soc.*, 1024 (1960).
27. D. A. Lightner and C. Djerassi, *Tetrahedron*, **21**, 583 (1965).
28. S. M. Iqbal and L. N. Owen, *J. Chem. Soc.*, 1030 (1960).
29. T. Wieland and H. Hornig, *Annalen*, **600**, 12 (1956).
30. C. J. M. Stirling, *J. Chem. Soc.*, 4524 (1958).
31. G. S. Sasin, P. R. Shaeffer and R. Sasin, *J. Org. Chem.*, **22**, 1183 (1957).
32. T. Wieland, E. Bokelman, L. Bauer, H. U. Lang and H. Lam, *Annalen*, **583**, 129 (1953).
33. T. Wieland, H. U. Lang and D. Liebsch, *Annalen*, **597**, 227 (1955).
34. R. B. Martin, S. Lowey, E. L. Elson and J. T. Edsall, *J. Amer. Chem. Soc.*, **81**, 5089 (1959).
35. R. B. Martin and A. Parcell, *J. Amer. Chem. Soc.*, **83**, 4830 (1961).
36. M. Sonder, *Chem. Rev.*, **66**, 297 (1966).
37. H. R. Snyder, J. M. Stewart and J. B. Ziegler, *J. Amer. Chem. Soc.*, **69**, 2672 (1947).
38. M. G. Ettlinger, *J. Amer. Chem. Soc.*, **72**, 4792 (1950).
39. E. E. Van Tamelen, *J. Amer. Chem. Soc.*, **73**, 3444 (1951).
40. C. C. Price and P. F. Kirk, *J. Amer. Chem. Soc.*, **75**, 2396 (1953).

41. L. Goodman and R. B. Baker, *J. Amer. Chem. Soc.*, **81**, 4924 (1959).
42. S. Searles, H. R. Hays and E. F. Lutz, *J. Org. Chem.*, **27**, 2832 (1962).
43. S. Searles, H. R. Hays and E. F. Lutz, *J. Org. Chem.*, **27**, 2828 (1962).
44. F. G. Bordwell and H. M. Andersen, *J. Amer. Chem. Soc.*, **75**, 4959 (1953).
45. R. Ketcham and V. P. Shah, *J. Org. Chem.*, **28**, 229 (1963).
46. D. G. Doherty, R. Shapira and W. T. Burnett, *J. Amer. Chem. Soc.*, **79**, 5667 (1957).
47. J. X. Khym, R. Shapira and D. G. Doherty, *J. Amer. Chem. Soc.*, **79**, 5663 (1957).
48. A. Schönberg and L. Vargha, *Ber.*, **63**, 178 (1930).
49. A. Schönberg, L. Vargha and W. Paul, *Annalen*, **483**, 107 (1930).
50. H. R. Al-Kazimi, D. S. Tarbell and D. Plant, *J. Amer. Chem. Soc.*, **77**, 2479 (1955).
51. D. H. Powers and D. S. Tarbell, *J. Amer. Chem. Soc.*, **78**, 70 (1956).
52. H. Kwart and E. R. Evans, *J. Org. Chem.*, **31**, 410 (1966).
53. M. S. Newman and H. A. Karnes, *J. Org. Chem.*, **31**, 3980 (1966).
54. J. D. Edwards and M. Pianka, *J. Chem. Soc.*, 7338 (1965).
55. K. Miyazaki, *Tetrahedron Letters*, 2793 (1968).
56. H. M. Relles and G. Pizzolato, *J. Org. Chem.*, **33**, 2249 (1968).
57. J. E. Cooper and J. M. Paul, *J. Org. Chem.*, **35**, 2046 (1970).
58. H. R. Nace, *Organic Reactions*, **12**, 57 (1962).
59. Y. Araki, *Bull. Chem. Soc. Japan*, **43**, 252 (1970).
60. Y. Araki and A. Kaji, *Bull. Chem. Soc. Japan*, **43**, 3214 (1970).
61. S. A. Karjala and S. M. McElvain, *J. Amer. Chem. Soc.*, **55**, 2966 (1933).
62. E. J. Hedgley and N. H. Leon, *J. Chem. Soc.* (*C*), 467 (1970).
63. S. G. Smith, *Tetrahedron Letters*, 979 (1962).
64. S. G. Smith and J. P. Petrovich, *Tetrahedron Letters*, 3363 (1964).
65. T. Taguchi, Y. Kawazoe, K. Yoshihira, H. Kanayama, M. Mori, K. Tabata and K. Harono, *Tetrahedron Letters*, 2717 (1965).
66. S. G. Smith, *J. Amer. Chem. Soc.*, **83**, 4285 (1961).
67. K. D. McMichael, *J. Amer. Chem. Soc.*, **89**, 2943 (1967).
68. D. L. Garmaise, A. Uchiyama and A. F. McKay, *J. Org. Chem.*, **27**, 4509 (1962).
69. H. Kwart and C. M. Hackett, *J. Amer. Chem. Soc.*, **84**, 1754 (1962).
70. C. Y. Meyers, C. Rinaldi and L. Bonoli, *J. Org. Chem.*, **28**, 2440 (1963).
71. H. Kwart and M. H. Cohen, *Chem. Comm.*, **319** (1968).
72. H. Kwart and E. R. Evans, *J. Org. Chem.*, **31**, 413 (1966).
73. H. Kwart and M. H. Cohen, *J. Org. Chem.*, **32**, 3135 (1967).
74. H. Kwart and J. L. Schwartz, *Chem. Comm.*, **44** (1969).
75. Y. Makisumi and A. Marubayashi, *Tetrahedron Letters*, 1971 (1969).
76. Y. Makisumi and T. Susatani, *Tetrahedron Letters*, 1975 (1969).
77. Y. Makisumi and A. Marubayashi, *Tetrahedron Letters*, 2449 (1969).
78. J. Z. Mortensen, B. Hedegaard and S. O. Lawesson, *Tetrahedron*, **27**, 3831 (1971).
79. Y. Makisumi and A. Marubayashi, *Tetrahedron Letters*, 2453 (1969).
80. B. W. Bycroft and W. Landon, *Chem. Comm.*, 168 (1970).
81. H. Kwart and M. H. Cohen, *Chem. Comm.*, 1296 (1968).
82. H. Kwart and T. J. George, *Chem. Comm.*, 433 (1970).
83. L. Brandsma and D. Schuijl-Laros, *Rec. Trav. Chim.*, **89**, 110 (1970).

84. C. Walling and M. S. Pearson, *J. Amer. Chem. Soc.*, **86**, 2263 (1964).
85. J. M. Surzur, M. P. Crozet and C. Dupuy, *Compt. Rend.*, **264C**, 610 (1967).
86. J. M. Surzur and M. P. Crozet, *Compt. Rend.*, **268C**, 2109 (1969).
87. W. C. Lumma, G. A. Durta and C. A. Vocker, *J. Org. Chem.*, **35**, 3442 (1970),
88. J. D. Willett, J. R. Grunwell and G. A. Berchtold, *J. Org. Chem.*, **33**, 2297 (1968).
89. J. M. Surzur, C. Dupuy, M. P. Crozet and M. Aimar, *Compt. Rend.*, **269C**, 849 (1969).
90. G. W. Stacy, F. W. Villaescusa and T. E. Wollner, *J. Org. Chem.*, **30**, 4074 (1965).
91. G. W. Stacy and T. E. Wollner, *J. Org. Chem.*, **32**, 3028 (1967).
92. G. W. Stacy, A. J. Papa, F. W. Villaescusa and S. C. Ray, *J. Org. Chem.*, **29**, 607 (1964).
93. G. W. Stacy, D. L. Eck and T. E. Wollner, *J. Org. Chem.*, **35**, 3495 (1970).
94. C. D. Hurd and W. H. Saunders, *J. Amer. Chem. Soc.*, **74**, 5342 (1952).
95. J. M. Cox and L. N. Owen, *J. Chem. Soc.* (*C*), 1130 (1967).
96. L. Bateman and R. W. Glazebrook, *J. Chem. Soc.*, 2834 (1958).
97. T. Bacchetti and A. Fiecchi, *Gazz. Chim. Ital.*, **83**, 1037 (1953).
98. L. N. Owen and P. L. Ragg, *J. Chem. Soc.* (*C*), 1291 (1966).
99. T. J. Adley and L. N. Owen, *J. Chem. Soc.* (*C*), 1287 (1966).
100. R. L. Whistler, M. S. Feather and D. L. Ingles, *J. Amer. Chem. Soc.*, **84**, 122 (1962).
101. J. M. Cox and L. N. Owen, *J. Chem. Soc.* (*C*), 1121 (1967).
102. R. L. Whistler and C. S. Campbell, *J. Org. Chem.*, **31**, 816 (1966).
103. D. J. Adam and M. Wharmby, *Tetrahedron Letters*, 3063 (1969).
104. H. Zimmer, *Tetrahedron Letters*, 5435 (1968).
105. G. W. Stacy and P. L. Strong, *J. Org. Chem.*, **32**, 1487 (1967).
106. N. J. Leonard and R. Y. Ning, *J. Org. Chem.*, **32**, 677 (1967).
107. S. Wolfe, J. C. Godfrey, C. T. Holdrege and Y. G. Perron, *Canad. J. Chem.*, **46**, 2549 (1968).
108. J. P. Clayton, *J. Chem. Soc.* (*C*), 2123 (1969).
109. S. Kukolja, R. D. G. Cooper and R. B. Morin, *Tetrahedron Letters*, 3381 (1969).
110. S. Wolfe and W. S. Lee, *Chem. Comm.*, 242 (1968).
111. O. K. J. Kovacs, B. Erkstram and B. Sjoberg, *Tetrahedron Letters*, 1863 (1969).
112. I. McMillan and R. J. Stoodley, *Tetrahedron Letters*, 1205 (1966).
113. R. G. Mietich, *Canad. J. Chem.*, **48**, 2006 (1970).
114. M. P. L. Caton and R. Slack, *J. Chem. Soc.* (*C*), 1402 (1968).
115. P. R. Brown and J. O. Edwards, *J. Org. Chem.*, **34**, 3131 (1969).
116. J. Wemple, *J. Amer. Chem. Soc.*, **92**, 6694 (1970).
117. S. P. Singh and J. Kagan, *J. Amer. Chem. Soc.*, **91**, 6198 (1969).
118. W. Walter and G. Maerten, *Annalen*, **715**, 35 (1968).
119. H. L. Wehrmeister, *J. Org. Chem.*, **28**, 2587 (1963).

CHAPTER 16

Thiols as nucleophiles

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I. INTRODUCTION

The thiols act as nucleophiles in two basic types of reaction, involving either substitution or addition to a multiple bond such as C=C

$$RSH + AB \longrightarrow RSA + HB \quad (1)$$

or

$$RS^- + AB \longrightarrow RSA + B^- \quad (2)$$

$$RSH + \rangle C{=}C\langle \longrightarrow RSC{-}CH \quad (3)$$

In reactions of the type 1 the HB generated may fracture the S—A bond formed; for example the silicon—sulphur bond in H_3SiSCF_3 is susceptible to fracture by HI.

$$H_3SiSCF_3 + HI \longrightarrow H_3SiI + CF_3SH$$

The substitution reactions discussed in this review will be restricted primarily to the thiolate anion, RS^- acting as a nucleophile. This may be present initially when a metal thiolate, such as silver(I) or lead(II), is employed, or may be generated in solution in the presence of a base such as sodium hydroxide or trimethylamine. The acidity of the thiol is important if the RS^- anion acts as a nucleophile in a neutral thiol solution. Thiolate nucleophiles can be obtained in non-aqueous solution by treatment of thiol esters, such as CH_3COSR, with strong non-nucleophilic bases[1], or by hydrolysis of thiourea derivatives[2].

The substitution type reaction is not restricted to substitution at a carbon atom, either aliphatic or aromatic, but includes the main group and transition elements. Several examples will be given of the varieties of the use of thiolates as nucleophiles, and although most of these reactions are

general, some of the illustrative examples will be drawn from the chemistry of halogenated thiols, in which the author is particularly interested. The review will generally be restricted to monofunctional thiols, and usually excludes dithiols, thio acids, etc.

Various reviews have been written on parts of this topic and these will be referred to at appropriate places in the text. The alkoxide nucleophiles have been investigated considerably more than the thiolate nucleophiles, and conversely selenolates significantly less than thiolates. In general the order of nucleophilic strength increases in the series alcohols, thiols and selenols, although sulphur-containing nucleophiles are generally less basic than their oxygen analogues.

The nucleophilic reactivities towards cations of several nucleophiles has been reviewed[3]. A parameter N_+ which is characteristic of the nucleophile system and independent of the cation has been defined as

$$\log [K_n/K_{H_2O}] = N_+$$

where K_n is the rate constant for reaction of a cation with a specific nucleophilic system (i.e. a given nucleophile in a given solvent), K_{H_2O} is the rate constant for reaction of the same cation with water in water. This generalization can successfully be applied to the reactions of various nucleophiles with various cations. It has been suggested that the N_+ values are related to the solvation energies of the nucleophiles[4]. In all the reactions studied, values of N_+ are highest for the benzenethiolate anion. Comparable values for the reactions of nucleophiles with *p*-nitro-(Malachite Green) are, solvent in brackets, MeOH (MeOH), 0·5; MeO^- (MeOH), 7·5; N_3^- (MeOH), 8·5; CN^- (DMSO), 8·6; PhS^- (MeOH), 10·7; PhS^- (DMSO), 13·1. Unfortunately data are not currently available to correlate analogous oxygen, sulphur and selenium nucleophiles by this method.

A considerable range and variety of thiols have been employed as nucleophiles. Some thiols are unstable in basic solution, but can be employed as their thiolate salts. Examples of this type of thiol include trifluoromethanethiol and pentafluorobenzenethiol. The trifluoromethanethiolate anion readily loses fluoride in solution in an irreversible reaction[5], but the mercury derivative, $Hg(SCF_3)_2$, effectively acts as a source of

$$(SCF_3)^- \longrightarrow CSF_2 + F^-$$

nucleophilic trifluoromethanethiolate ions. The pentafluorobenzenethiolate anion decomposes in basic solution in air. The reaction probably proceeds initially with the oxidation of the thiolate to the disulphide, which is then

attacked nucleophilically by the thiolate.

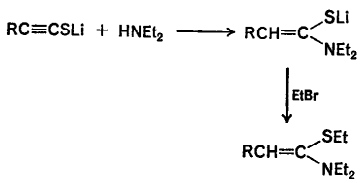
$$2\ C_6F_5S^- \xrightarrow{air} C_6F_5SSC_6F_5 + 2\ e$$

$$2\ C_6F_5S^- + C_6F_5SSC_6F_5 \longrightarrow C_6F_5SC_6F_4SSC_6F_4SC_6F_5 + 2\ F^-$$

The product, termed perfluoropoly(phenylene sulphide)[6], has been characterized by chemical analysis and molecular weight[7].

Some thiolates, such as pentachlorobenzenethiolate, show no nucleophilic reactivity[8].

Other interesting thiols that have been studied include the silylalkanethiols, such as $(EtO)_3SiCH_2CH_2CH_2SH$[9], and $(Me_3SiO)_2MeSi(CH_2)_3SH$[10]. A series of syntheses based on the alkynethiolates has been reported[11]. In some reactions the C≡C bond is retained, but in others it reacts, e.g.



The stereochemistry of the thiol is important. Steric effects have been used to explain the differences in rates of reactivity of RC_6H_4SH (R = H, 2-*t*-Bu, 4-*t*-Bu) in addition reactions with N-ethyl maleimide or displacement of $2,4\text{-}(O_2N)_2C_6H_3S^-$ from $2,4\text{-}(O_2N)_2C_6H_3SSEt$[12].

In some circumstances the electrophiles studied are susceptible to the thiolate anion causing both substitution or addition. An example is $HC{\equiv}CCMeHalCO_2Et$[13]. In this case the thiolate can also act as a reducing agent. The reducing properties of the thiols will only be commented on when it is incidental to substitution or addition. The reducing power of thiolates, however, means that the electrophiles employed generally do not contain a group that is readily reduced, such as the nitro group. When simultaneous substitution and addition occur, the reaction will be discussed in the substitution section, particularly in compounds containing C≡C bonds.

This review is divided into two main sections; substitution reactions and addition reactions. Sometimes the classification of a particular reaction is somewhat arbitrary. Dealkylation reactions, some of which can superficially appear to be neither substitution nor addition reactions, are basically substitution reactions and a section is devoted to these reactions, including both aliphatic and aromatic examples.

II. SUBSTITUTION REACTIONS

A. Aliphatic Substitution

1. Introduction

Simple thiolate substitution of an aliphatic compound can be represented by the equation

$$RX + R^1S^- \longrightarrow RSR^1 + X^-$$

where the group X may be a halogen, methoxy (discussed mainly under dealkylation), methanesulphonate, tosyl, etc.

Examples of the reactions of alkyl and acyl halides are[14]:

$$CH_2Cl_2 + Pb(SC_6F_5)_2 \longrightarrow CH_2(SC_6F_5)_2 + PbCl_2$$

$$ClCH_2CH_2Cl + Pb(SC_6F_5)_2 \longrightarrow (CH_2SC_6F_5)_2 + PbCl_2$$

$$2\ PhCOCl + Pb(SC_6F_5)_2 \longrightarrow 2\ PhCOSC_6F_5 + PbCl_2$$

An inert solvent is usually used but liquid ammonia has been used in the reaction of alkyl chlorides with sodium hydrogen sulphide[15,16]. The compound $(PhS)_3CH$ was formed in the reaction of the benzenethiolate anion and dibromocarbene, prepared from $PhHgCBr_3$ in benzene at 80°C. The postulated initial step was the addition of the electrophile Br_2C: to the sulphur nucleophile, forming an anion intermediate which picked up a proton yielding $PhSCHBr_2$. Subsequent nucleophilic replacement of bromine by the thiolate gave the product[17], $(PhS)_3CH$.

Polymers are formed when dithiols react with dihaloalkanes. Condensation of $p\text{-}HSCH_2C_6H_4CH_2CH(Me)C_6H_4CH_2SH\text{-}p$ with dihaloalkanes gives polymers[18] such as $H(SCH_2C_6H_4CH_2CH(Me)C_6H_4CH_2S(CH_2)_m)_nHal$. Two different monofunctional high molecular weight chlorides (R^1Cl and R^2Cl) react with the difunctional thiol, $(CH_2SH)_2$, in the presence of triethylamine to give primarily the symmetrical bisulphides, $(R^1SCH_2)_2$ and $(R^2SCH_2)_2$, and only very small yields of the unsymmetrical bisulphide $R^1SCH_2CH_2SR^2$[19].

Recently copper(I) salts including thiolates have been studied as nucleophiles. Copper(I)butanethiolate and copper(I)cyanide in DMF did not react with *t*-butyl chloride or benzyl chloride, but halogenoaromatic compounds react under similar conditions. When the reactions were repeated in the presence of thiourea or quinoline, the expected products, di-*t*-butyl sulphide, valeronitrile and phenylacetonitrile, were obtained. The thiourea or quinoline probably act as ligands and bind strongly to the copper, forming the ion $(CuL_4)^+$, leaving the counterion (e.g. BuS^- from CuSBu) available for normal nucleophilic attack[20].

2. Reactions with electrophiles of the type $RM(CH_2)_nX$ and $RMCHX_2$

a. Displacement of halogen. Alkylthio-substituted acetic acids can be obtained from monochloracetic acid and a thiol[21],

$$ArOCH_2SH + ClCH_2CO_2H \longrightarrow ArOCH_2SCH_2CO_2H$$

Derivatives of 1,1,1-trifluoroacetone may be prepared similarly[22],

$$CF_3COCH_2Cl + RSH \xrightarrow{Et_3N} CF_3COCH_2SR \quad (R = Et, Bu, Ph)$$

Organotin derivatives containing the $RSCH_2Sn(IV)$ group can readily be obtained in the reactions of RSNa and $BrCH_2Sn\lessgtr$ or $RSCH_2Li$ and $ClSn\lessgtr$, the former method being preferred. Compounds containing both Sn—S and Sn—C bonds can be prepared[23],

$$RSNa + (BrCH_2)_2SnBr_2 \longrightarrow (RSCH_2)_2Sn(SR)_2$$

A similar reaction involves replacement of the Cl in $RR^1NP(O)(CH_2Cl)_2$ and $(ClCH_2)_2P(O)OPh$ or tosyl in $4\text{-}MeC_6H_4SO_3CH_2P(O)Ph_2$ with $(SR^2)^-$,[24,25]. Substituted trialkylphosphine oxides or sulphides, $(RSCH_2CH_2)_3PX$ (X = O, S) can be prepared analogously[26,27], from a thiolate anion and $(ClCH_2CH_2)_3PX$.

b. Displacement of sulphonyl groups. Ready replacement of the methanesulphonate group by the benzenethiolate group from bismethanesulphonates of 3-arylthiopropane-1,2-diols, 2-arylthiopropane-1,3-diols and 1-arylthiopropane-2-ols has been reported[28,29].

$$p\text{-}MeSC_6H_4SCH_2CH(OSO_2Me)CH_2(OSO_2Me) + PhS^- \longrightarrow p\text{-}MeSC_6H_4SCH_2CH(SPh)CH_2(SPh)$$

The reaction proceeded via a direct S_N2 substitution except when rearrangement occurred, which was only partially observed in the reaction

$$p\text{-}MeOC_6H_4SCH_2CH(Me)OSO_2Me + PhS^- \longrightarrow p\text{-}MeOC_6H_4SCH_2CH(Me)SPh + p\text{-}MeOC_6H_4SCHMeCH_2(SPh)$$

The cyclic intermediate $\overset{+}{C}H(Me)SArCH_2X^-$ is postulated. It is impossible to detect whether rearrangement or direct substitution occurred in the reaction

$$PhSCH_2CH(OSO_2Me)Me + SPh^- \longrightarrow PhSCH(Me)CH_2SPh + MeSO_3^-$$

The nitro groups were reduced in derivatives of 2,4-dinitrobenzene.

The trifluoromethanesulphonyl group is displaced in *p*-tolylsulphonylmethyltrifluoromethanesulphonate in the S_N2 reaction with nucleophiles, such as benzenethiolate[30],

$$p\text{-}MeC_6H_4SO_2CH_2O_3SCF_3 + PhS^- \longrightarrow p\text{-}MeC_6H_4SO_2CH_2SPh + CF_3SO_3^-$$

In the reaction of 2,2-dialkyl-3-(tosyloxy)propionaldehydes with benzene- or methane-thiolates the tosyl group is displaced and it is postulated that the attack originates at the carbon atom of the carbonyl group[31].

$$Me_2C(CHO)CH_2OTs + RS^- \longrightarrow Me_2C(CHO)CH_2SR$$

The carbon—sulphur bond is fractured in the reaction of *p*-toluenesulphonyl cyanide with sodium ethanethiolate in ethanol. Other thiols, not thiolates, can also fracture the carbon—sulphur bond[32].

$$p\text{-}TolSO_2CN + EtS^- \longrightarrow EtSCN$$

3. Reactions of electrophiles of the type $Ar(CH_2)_nX$

The chlorine kinetic isotope effect in nucleophilic displacement at saturated carbon in *para*-substituted benzyl chlorides, with thiolate and analogous oxygen nucleophiles, has been examined[33]. The reactions proceed via a concerted transition state.

$$R'S^- + RCl \longrightarrow [R'S \sim R \sim Cl] \longrightarrow RSR' + Cl^-$$

As the *para*-substituent changes from more electron donating to more electron withdrawing, the relative importance of bond breaking and bond making in the transition state alters. With methoxide and benzenethiolate nucleophiles the chlorine isotope effect (K_{35}/K_{37}) increases in the order $p\text{-}NO_2 < H < p\text{-}MeO$, indicating greater bond breaking as the *para*-substituent becomes more electron withdrawing. For both oxygen and sulphur nucleophiles the isotope effect decreases with increase in basicity, PhO^- *vs* MeO^-, and PhS^- *vs* *n*-BuS^-, indicating less bond cleavage at the transition state with the stronger nucleophile. In comparison between oxygen and sulphur the reaction is slower with the oxygen nucleophiles (presumably owing to solvation) and the isotope effect is smaller, suggesting not only that the bond breaking is less, but also that the oxygen is a stronger nucleophile.

In a Hammett equation study of the reactions of 1,1-diaryl-2,2,2-trichloro- and 1,1-diaryl-2,2-dichloro-ethane with benzenethiolate, two types of reactions were observed. For one type, the ρ-value for the benzenethiolate-promoted dehydrochlorination of $(p\text{-}XC_6H_4)_2CHCCl_3$ in

ethanol at 65°C was 2·11, while for the S_N2 substitution of benzenethiolate for chlorine in $(p\text{-}XC_6H_4)_2CHCHCl_2$ forming $(p\text{-}XC_6H_4)_2C{=}CHSPh$ as the sole organic product[34], the ρ-value was 0·41:

$$(p\text{-}XC_6H_4)_2CHCHCl_2 + 2\ PhS^- \longrightarrow (p\text{-}XC_6H_4)_2C{=}CHSPh + 2\ Cl^- + PhSH$$

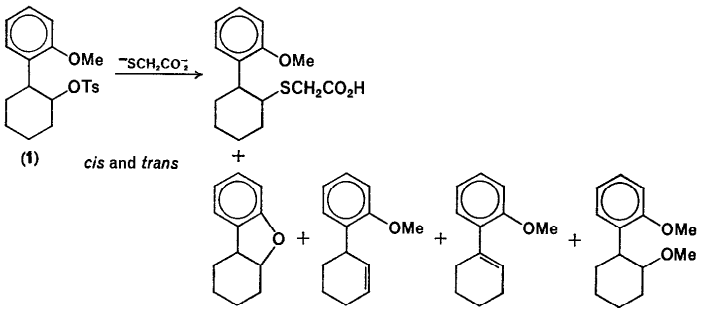
The potential insecticide $m\text{-}RSCH_2C_6H_4O_2CNHMe$ (R = Me, Et, *i*-Pr) can be prepared from methyl isocyanate and $m\text{-}RSCH_2C_6H_4OH$, which in turn is obtained from $m\text{-}ClCH_2C_6H_4OH$[35],

$$m\text{-}ClCH_2C_6H_4OH + EtSH \xrightarrow[MeONa]{MeCN} m\text{-}EtSCH_2C_6H_4OH$$

Heterocyclic derivatives can be used as electrophiles. The hydrochloride of 3-chloromethylpyridazine reacted with sodium benzenethiolate in toluene, replacing the Cl by SPh[36].

4. Reactions with cyclic compounds

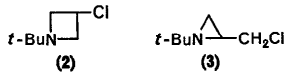
Replacements of substituents by a thiolate group occurs in several cyclic compounds. Several products are found in the reaction of 2-phenylcyclohexyl-*p*-toluenesulphonate (**1**) with the dipotassium salt of mercaptoacetic acid in methanol, corresponding to simple replacement, neighbouring



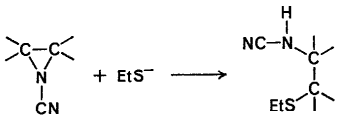
group replacement forming a furan derivative, elimination forming an olefin and solvolysis. The actual products depend on the reactant ratio, anion : tosylate; the furan is formed when the ratio is 2 : 1, but at 50 : 1 simple displacement occurs. The tosyl group is more readily replaced than the aromatic methoxy[37].

In the nitrogen heterocyclic systems, 1-*t*-butyl-3-chloroazetide (**2**) and 1-*t*-butyl-2-chloromethylarizidine (**3**) react with thiolate anions giving

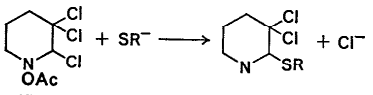
simple replacement of the chlorine, although other nucleophiles give



partial hydrolysis and cyanide converts (**3**)→(**2**) (as its cyanide)[38]. The ethanethiolate anion and various other nucleophiles have been used for ring opening of N-cyanoaziridine in steroids, such as 2β,3β-(cyanoimino)-cholestane[3]:



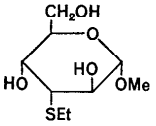
Displacement of the 2-chlorine of 2,3,3-trichloro-1-acetylpiperidine (**4**) occurs with various nucleophiles, including alkoxides and thiolates[40],



(**4**)
R = Me, Et, *i*-Pr, Bu, *sec*-Bu, Ph

Cleavage of the C—N bond occurs when 1-[β-(phenylsulphonyl) ethyl]-piperidine hydrochloride or methiodide is treated with aromatic thiols in aqueous dioxane and sulphonyl sulphides are formed. An elimination–addition mechanism is proposed[41].

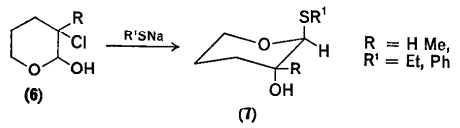
Treatment of methyl 3,4,6-tri-O-acetyl-2-bromo-2-deoxy-β-D-glucopyranoside with ethanethiol/sodium methoxide and methanol gives 100% yield of methyl 3-S-ethyl-3-thio-β-D-altropyranoside (**5**). The S-benzyl



(**5**)

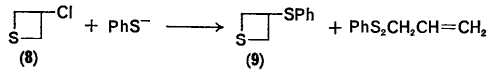
analogue is prepared similarly[42]. In the reactions of chlorohydrin derivatives (**6**) with thiolates the chlorine is also replaced by thiolate giving,

for instance, *trans*-3-hydroxy-2-ethylthio-tetrahydropyran (**7**). When R = H, the product is 45% diaxial and 55% diequatorial[43].



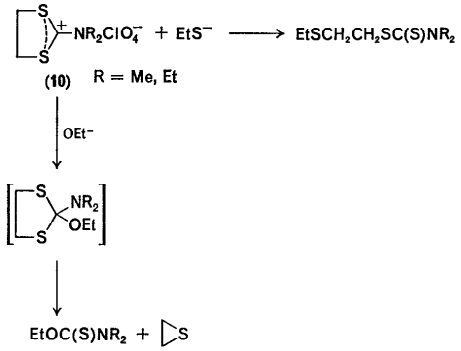
(6) (7)

The ring is partially fractured in the treatment of 3-chlorothietane (**8**) with the benzenethiolate anion. A mixture containing 30% of phenyl-3-thietanyl sulphide (**9**) and $PhS_2CH_2CH{=}CH_2$ was obtained. The latter



(8) (9)

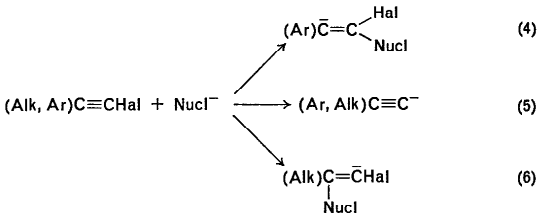
was also prepared from PhSCl and $HSCH_2CH{=}CH_2$. The reaction probably proceeds[44] via the formation of the cations $H_2C{=}CHCH_2S^+$ and  . The C—S bond is fractured in 2-dialkylamino-1,3-dithiolium perchlorate (**10**) when treated with the ethane-thiolate anion in DMF. Quite different products are found with the ethoxide ion as a nucleophile, involving attack on the 2-carbon atom as opposed to attack on the 4-carbon atom with the ethanethiolate anion[45].



5. Reactions with RC≡CX, R¹R²C=CR³X and R¹R²C=NX

The reactions described in this section will be concerned primarily with the replacement of a group X with a group RS, and not addition reactions.

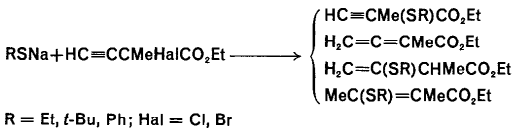
a. Alkyne derivatives. Three different routes are proposed for the reaction of acetylenes with nucleophiles[46],



The intermediates react further to give (Alk,Ar)C≡CNuCl. With thiolate nucleophiles (EtS^- and PhS^-), Hal = Cl, Br, I, the mechanism is restricted to attack on the halogen (5), but attack on the carbon is also observed in the reaction of EtS^- and ArC≡CCl. The second-order rate constants in methanol–water mixtures for *meta*- and *para*-substituted 1-bromo-2-phenylacetylenes correlate well with Hammett σ constants; $\rho = 1{\cdot}15$. A linear correlation was also observed between $\log K_2$ and pK_a of the corresponding thiols.

The rate constants for the reaction of $p\text{-}ZC_6H_4C{\equiv}CHal$ (Z = Me, H, Cl; Hal = Cl, Br) with $p\text{-}MeC_6H_4S^-Na^+$ in DMF, forming $p\text{-}ZC_6H_4C{\equiv}CSC_6H_4SMe\text{-}p$ have been measured. Attempts to trap the ion $p\text{-}ZC_6H_4C{\equiv}C^-$ were unsuccessful. These results have, however, been interpreted differently from those presented in the previous paragraph, and an addition–elimination mechanism is favoured, involving the formation of $p\text{-}ZC_6H_4\bar{C}{=}CHal(SC_6H_4Me\text{-}p)$ and fast elimination of Hal^- to give the product[47].

Various products were obtained from the reaction of sodium thiolate with the acetylene derivative $HC{\equiv}CCMeHalCO_2Et$ (Hal = Cl,Br), where the thiolate replaced the halogen, acted as a reducing agent or added across an acetylenic or ethylenic bond[13].



R = Et, *t*-Bu, Ph; Hal = Cl, Br

The proportion of the reduction products, especially when Hal = Br, increased with the basicity of the nucleophile and its concentration.

The reactions of 1,3-dihalopropynes (**11**) with nucleophiles, amines and thiols have been studied[48]. Heterocyclic thiols are used as potassium salts in aqueous methanol.

$$Hal^1C{\equiv}CHCH_2Hal^2 + RS^- \longrightarrow Hal^1C{\equiv}CCH_2SR$$

(11)

(Hal¹ = Cl, Hal² = I(**a**); Hal¹ = Hal² = I(**b**) or Br)

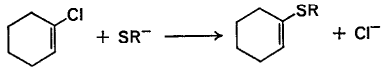
The reactions of **11a** or **11b** with other thiols RSH (R = ethyl, *i*-butyl, phenyl, or benzyl), in the presence of potassium hydroxide, however, gave deiodination and the corresponding dialkyl, diphenyl or dibenzyl disulphide, which could in most cases be isolated quantitatively,

$$IC{\equiv}CCH_2I + PhCH_2S^- + PhCH_2SH \longrightarrow HC{\equiv}CCH_2I + (PhCH_2S)_2 + I^-$$

The only thiols forming iodoacetylenic sulphides were heterocyclic thiols having a tautomeric thiolactam structure.

b. Alkene derivatives. Substitution reactions of nucleophiles with ethylenic substrates have been recently reviewed, and the similarity with aromatic nucleophilic substitution emphasized. The possible mechanisms of these reactions have been discussed[49,50].

A simple example of substitution in a vinyl halide is the preparation of thiol derivatives of 1-cyclohexene from the thiol and sodamide in THF with chloro-1-cyclohexene (**12**)[51].



(12)

Vinyl bromides react with copper(I) thiolates, both aliphatic and aromatic, to give vinyl sulphides. Vinyl bromides studied include β-bromostyrene and 1-bromo-2-methyl-1-propene. This method of synthesis of thioethers is claimed to be superior to that using sodium thiolates and most other reported methods[52]. 1,2-Dibromoethylene gives a mixture of *cis* (18%) and *trans* (42%) 1,2-diphenylthioethylene with copper(I) benzenethiolate, but with copper(I) ethanethiolate ethylthioacetylene is formed with the elimination of hydrogen bromide[52],

$$BrHC{=}CHBr + CuSEt \longrightarrow HC{\equiv}CSEt + HBr + CuBr$$

When substitution occurs in an ethylene derivative, it is of interest to observe whether the original configuration is retained. Several reactions of ethylene compounds where configuration is retained have been examined. Some are shown below.

$$HalCH{=}CHCO_2Et + EtS^- \longrightarrow EtSCH{=}CHCO_2Et\ ^{53}\quad (Hal = Cl, Br, I)$$

$$R^1(R^2S)C{=}CR^1(OSO_2R^3) + R^4SH \longrightarrow R^1(R^2S)C{=}CR^1(SR^4) + R^3SO_2H\ ^{54}$$

In the former reaction, mixtures of isomers are formed when the ethoxide ion is used as a nucleophile. In the latter reaction, when a thiolate instead of a thiol is used as the nucleophile, the electropositive carbon of the trinitrobenzene residue is attacked forming a sulphide and ketone[54].

$$Ph(p\text{-}ClC_6H_4S)C{=}C(Ph)OSO_2R + p\text{-}ClC_6H_4S^- \longrightarrow Ph(p\text{-}ClC_6H_4S)CHCOPh + p\text{-}ClC_6H_4SR$$

(R = 2,4,6-$(NO_2)_3C_6H_2$)

In some reactions such as

$$(PhSO_2)HC{=}CFH + PhS^- \longrightarrow (PhSO_2)HC{=}C(SPh)H + F^-$$

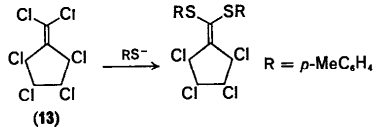
the *trans* reactant gives the *trans* product, but the *cis* reactant gives *cis* and *trans* products in a 3 : 1 ratio[55].

When several halides are present, as in trifluorochloroethylene, replacement of a fluorine with a thiolate occurs:

$$CF_2{=}CFCl + PhSNa \longrightarrow (PhS)CF{=}CFCl + NaF$$

Butanethiol reacts similarly with $CF_2{=}CFHal$ (Hal = Cl, Br), and $CF_2{=}CCl_2$ forming $BuSCF{=}CFHal$ and $BuSCF{=}CCl_2$ respectively[56]. In the compound $AcNHCH{=}CCl_2$ the butanethiolate ion can replace one of the chlorine atoms or add across the double bond, forming $AcNHCH{=}CCl(SBu)$ and $AcNHCH(SBu)CHCl_2$ respectively[57].

Other interesting examples of this type of reaction include that of hexachlorofulvene (**13**) with $p\text{-}MeC_6H_4S^-$ in the presence of triethylamine[56].



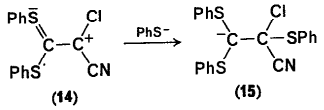
(13)

Various acrylonitrile derivatives have been examined. The configuration is retained in the reaction of 3-halomethacrylonitriles with sodium

ethanethiolates, and an addition–elimination mechanism is proposed[59]. 3,3-Di(thiophenyl)acrylonitriles can be prepared in the reaction

$$Cl_2C{=}CR(CN) + 2\ PhS^- \longrightarrow (PhS)_2C{=}CR(CN) \quad (R = H, Me, CN, Ph)$$

When R = Cl, the product is mainly $(PhS)_2C{=}C(Cl)CN$, together with trace amounts of $(PhS)_2C{=}C(SPh)CN$. Displacement of the α-Cl is unusual. This has been attributed to the high nucleophilic character of the anion, increased positive charge on the α-carbon atom (**14**) and stabilization of the intermediate (**15**) by the benzenethiol group[60]. Reactions of



(**14**) (**15**)

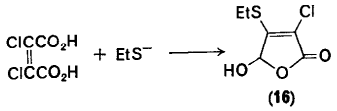
other acrylic acid derivatives with thiolates can give nucleophilic replacement, or the thiol can be oxidized to the disulphide[61].

$$MeC(R){=}CBrCO_2Et + PhSNa \longrightarrow MeC(R){=}C(SPh)CO_2Et \quad (R = EtS, t\text{-}BuS, PhS, EtO)$$

$$MeC(SEt){=}CBrCO_2Et + EtS^-/EtSH \longrightarrow MeC(SEt){=}CHCO_2Et + EtSSEt + Br^-$$

The rate constants for the addition of butanethiol to ethyl acrylate have been measured by iodometry over a wide pH range. Below pH 4 it is assumed that the reaction is initiated by the neutral molecule, but at pH > 7 the anion BuS^- started the reaction[62].

Cyclization occurs when dichloro- and dibromo-maleic acids react with thiols in the presence of triethylamine forming 2-halo-3-mercaptomalealdehydic (**16**) derivatives[63], e.g.



(**16**)

c. Imine derivatives. Displacements in compounds having C=N bonds have been observed. One of the simplest types of reaction is that of $ClCH_2CNO$, which can be converted into *o*-(thiocyanomethylthio)-benzamide, an antibacterial, by refluxing with the sodium salt of

o-mercapto-benzamide[64]. Iminoboranes react with thiols forming alkylthio iminoboranes[65],

$$Cl_3C(Br)C{=}NBMeBr + RSH \longrightarrow Cl_3CC(SR){=}NH\cdot BMeBr_2 \xrightarrow{Et_3N} Cl_3CCSR{=}Br_2(Me)B\cdot NEt_3 \quad (NH)$$

The bromine on the boron may react further

$$Cl_3CC(SPh){=}NH\cdot BMeBr_2 + PhSH \longrightarrow Cl_3CC(SPh){=}N(H)\cdot BMeBr(SPh) \xrightarrow{\text{Distillation, } -HBr} Cl_3CC(SPh){=}N{-}B(Me)SPh$$

Similar reactions are observed with 1,1-dibromo-4-(*p*-nitrophenyl)- and -(*p*-chlorophenyl)-2,3-diazabuta-2,3-dienes and aliphatic or aromatic thiols, resulting from the thiolysis of one or two bromine atoms[66],

$$p\text{-}O_2NC_6H_4CH{=}N{-}N{=}CBr_2 + p\text{-}ClC_6H_4SH \xrightarrow[C_6H_6]{NEt_3} p\text{-}O_2NC_6H_4CH{=}N{-}N{=}CBr(SC_6H_4Cl\text{-}p)$$

B. Aromatic Substitution

1. Introduction

Aromatic nucleophilic substitution with a thiolate anion can be represented as

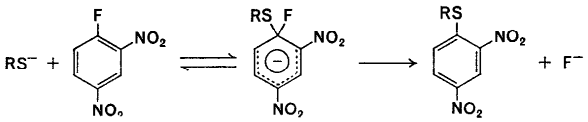
$$ArX + RS^- \longrightarrow ArSR + X^-$$

where X^- is usually a stable ion, such as a halide. When more groups or atoms that may be replaced are present initially multiple substitution can occur. Nucleophilic aromatic substitution is usually discussed in the annual volumes of *Organic Reaction Mechanisms*. Reviews of aromatic nucleophilic substitution usually mention *inter alia* the thiolate as a nucleophile. One review has been devoted to the behaviour of sulphur reagents in nucleophilic aromatic substitution[67], and also discusses substitution in benzenethiazoles. A book has also been published on nucleophilic substitution[68].

While the reactions of the corresponding oxygen-containing nucleophiles have been studied in considerable detail, there is a relative paucity of data

on the thiolates as nucleophiles in aromatic substitution. Few kinetic data are available. Any data show that the RS^- is a better nucleophile than its oxygen analogues, although this has been questioned[67].

The reactions of various halonitrobenzenes with thiolates have been studied in more detail. A comprehensive review of the activating effects of the nitro group in aromatic substitution[69] covers the literature up to the middle of 1967. This review discusses primarily the displacement of halogen, although displacement of other groups such as hydrogen, nitro, alkoxy, aryloxy, and sulphonate are also considered. The relative rates of the reaction of 1-X-2,4-dinitrobenzenes with piperidine in MeOH at 0°C decreases in the series $F \gg NO_2 \gg OSO_2C_6H_4CH_3\text{-}p \gg SOC_6H_5 \sim Br \sim Cl > SO_2C_6H_5 \sim OC_6H_4NO_2\text{-}p > I$. A similar sort of series can be expected when the thiolate anion is used as a nucleophile. The reaction of nitro compounds with nucleophiles occurs primarily via an addition–elimination mechanism, involving a Meisenheimer complex.



Obvious variables in such a reaction are the stereochemistry of the entering group, the stability of the intermediate Meisenheimer complex, and the effect of the leaving group. A thermochemical approach concluded that the decomposition of the Meisenheimer complex was rate determining[70], however, this is not in accord with the leaving group lability[71]. As cleavage of the carbon—fluorine bond is acid catalysed, it has been concluded that the rate-determining step is the formation of the Meisenheimer complex rather than its decomposition[72]. Substitution of 2,4-dinitrochlorobenzene with 2,3,5,6-tetrafluorobenzene thiolate gives replacement of the chlorine[73]. A detailed discussion of the thermodynamics of the reaction of MeS^- and PhS^- with 1-X-2,4-dinitrobenzene has been reported[68,70].

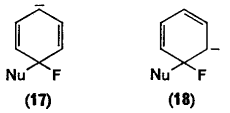
The nucleophilic activity is $PhS^- > MeS^-$ for the reaction with 1-iodo-2,4-dinitrobenzene, but $MeS^- > PhS^-$ for *p*-fluoronitrobenzene[68]. Data on the reaction of substituted halogenobenzothiazoles show that there are appreciable steric effects in the cases of α branching (methyl > ethyl > *i*-propyl > *t*-butyl), whereas β and ω branching do not cause any steric effect and influence the reaction rates only slightly because of their typical electronic effects[67]. The mobility of the leaving halogen, derived from kinetic data with various halogenonitrobenzenes, is $F > Cl > Br > I$[74].

The intermediate Meisenheimer complexes have been reviewed[75,76], and

the work of Crampton is important in this area[77,78,79]. Further reference should be made to the chapter in this book by M. R. Crampton.

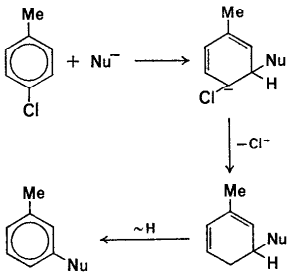
When substitution occurs in polyhalogenated aromatic compounds, such as the pentafluorobenzene derivatives, C_6F_5X, the extent of the replacement of F or X by the nucleophile and the product orientation must be determined.

A detailed study of the orientation and reactivity in the nucleophilic replacement reactions of aromatic polyhalo-compounds has been published[80]. This involves study of the stability of the Wheland type intermediates (**17**, **18**) where Nu is a nucleophile. The formation of *meta*



(17) (18)

products with a nucleophile may be rationalized by the scheme involving a carbene intermediate[81],



Most activating groups cause primarily *para* substitution but some *ortho* substitution may occur. Deactivating groups, such as NH_2, O^-, or S^- will cause *meta* substitution[68]. The solvent plays an important role in determining the relative amounts of *ortho* and *para* substitution. Solvents with dielectric constant lower than about 30 cause some *ortho* substitution, whereas solvents of dielectric constant greater than 30 cause almost exclusive *para* substitution. This has been attributed to increasing ionic dissociation of the nucleophile in the higher dielectric constant solvents[82,83]. Presumably the formation of *meta* substitution products in solvents of

low dielectric constant does not involve the formation of the thiolate anion as an active entity.

Thiolates can also cause dehalogenation of various halogen compounds, such as 2-bromo-3-nitro-thiophene[84] and 2- and 4-halo-1-naphthols[85].

2. Substitution in hexahalobenzenes

Pentafluoro- and pentachloro-benzenethiols can readily be prepared by the reaction of a hydrogen sulphide anion, SH^-, with hexafluoro- and hexachlorobenzene respectively[6,86]. No dithiols can be produced in this reaction. Due to the basic medium employed the thiol formed will be present as its thiolate anion, which is not readily attacked further nucleophilically. Using hexafluorobenzene and excess hydrogen sulphide perfluoropoly(phenylene sulphide) may be isolated[6]. When the hydrogen sulphide anion is replaced by a thiolate as a nucleophile, multiple replacement of fluorine or chlorine can occur. The products of these reactions can be summarized:

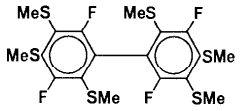
$$C_6Hal_6 + SR^- \longrightarrow C_6Hal_5SR \quad (19)$$
$$C_6Hal_4(SR)_2 \quad (20)$$
$$C_6Hal_3(SR)_3 \quad (21)$$
$$C_6Hal_2(SR)_4 \quad (22)$$
$$C_6Hal(SR)_5 \quad (23)$$
$$C_6(SR)_6 \quad (24)$$

The reaction of hexafluorobenzene with various nucleophiles (R = Me[87], Et[87], Ph[87,73], *p*-HC_6F_4[73], *p*-$NH_2C_6F_4$[73]) in ethylene glycol and/or pyridine as a solvent has been studied. The products obtained are **19**, **20** and **22**. The compounds **21**, **23** and **24** have not been isolated, but **21** must be present as intermediate in the conversion of **20** to **22**. The orientation of the products has been deduced from ^1H and ^{19}F n.m.r. spectra[87], or chemical oxidation and Raney nickel degradations[73]. The compound **20** has the two RS groups *para*, whereas the compounds $C_6F_2(SMe)_4$, $C_6F_2(SEt)_4$, $C_6F_2(SMe)_2(SPh)_2$, and $C_6F_2(SPh)_4$ have the two fluorines *para*[8,73]. When 2-mercaptoethanol was used as a nucleophile, the sulphur atom rather than the oxygen acted as the nucleophile and 1,2,4,5-tetrafluoro-3,5-bis-2-hydroxyethylthiobenzene was isolated[88].

This work has also been extended to decafluorobiphenyl where each ring is substituted once or three times:

$$C_6F_5C_6F_5 + SR^- \longrightarrow RSC_6F_4C_6F_4SR \quad (25)$$
$$(RS)_3C_6F_4C_6F_4(SR)_3 \quad (26)$$

The predominant product is **25** when R = Et or Ph, but when R = Me, the mono- and tri-substituted products are formed. The orientation of **25** is *p*-$(RS)C_6F_4C_6F_4(SR)$-*p*[87] and that of **26** is probably[87]



Substitution of hexachlorobenzene with various nucleophiles has also been studied [87,89]. No monosubstituted products were isolated.

$$C_6Cl_6 + SR^- \longrightarrow p\text{-}(RS)_2C_6Cl_4 + p\text{-}Cl_2C_6(SR)_4$$

The orientation of the disubstituted product has been deduced by alternate synthesis, whereas that of *p*-$Cl_2C_6(SR)_4$ has only been derived intuitively[89]. Attempts to use the $C_6Cl_5S^-$ anion as a nucleophile to form the sulphide $(C_6Cl_5)_2S$ have failed[8].

The obvious extension of this work to hexabromobenzene has been investigated, where it is found that the SMe^- anion will not react[8]. Study of the reactions of other nucleophiles with hexabromobenzene leads to photodebromination and some nucleophilic substitution[90]. Pentabromobenzenethiol has recently been prepared from the pentabromophenyl Grignard reagent and sulphur[91].

A somewhat analogous system is pentafluoropyridine where substitution with hydrogen sulphide anion, or benzenethiolate, occurs *para* to the nitrogen. The thiol formed reacts with pentafluoropyridine to give the corresponding sulphide[83,92]. 2,3,5,6-Tetrachloropyridine thiol is prepared similarly from pentachloropyridine and the hydrogen sulphide anion in ethylene glycol[93].

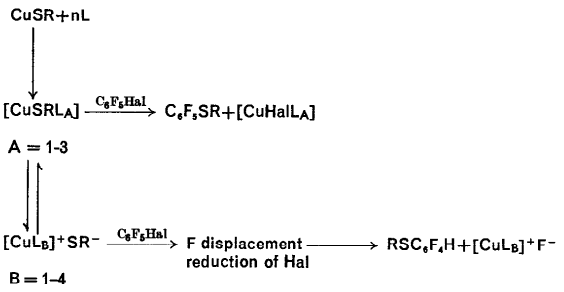
3. Substitution in mixed hexahalobenzenes

For the series of monosubstituted halopentafluorobenzenes such as C_6F_5Hal, it is of interest to observe which halogen is replaced initially.

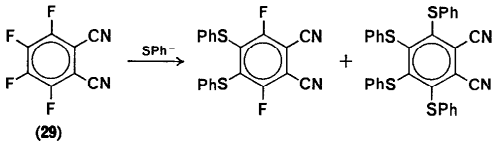
Bis(pentafluorophenyl)sulphide, $(C_6F_5)_2S$, may be prepared from bromopentafluorobenzene and copper(I) pentafluorobenzenethiolate in DMF[94]. The use of the copper salt eliminates the need to generate the pentafluorobenzenethiolate anion, $C_6F_5S^-$, in basic solution. The copper-assisted nucleophilic displacement reactions of halopentafluorobenzenes have been studied[95]. The reaction of CuSBu with C_6F_5Br gave two products

$$C_6F_5Br + CuSBu \longrightarrow C_6F_5SBu + BuSC_6F_4H$$
$$(27) \qquad (28)$$

The ratio of the products depended on the solvent employed. In DMF product **27** was formed exclusively, whereas product **28** involving halogen reduction was formed in various solvents in the presence of thiourea, although thiourea alone does not react with bromopentafluorobenzene. In similar experiments using chloropentafluorobenzene no reaction occurred in the absence of thiourea, but when it was added exclusive fluorine replacement occurred without chlorine reduction. With iodopentafluorobenzene and copper(I) benzenethiolate and urea, rapid reduction of the iodine occurred together with multiple fluorine replacement resulting in the formation of 2,4-difluoro-1,3,5-tris(phenylthio)benzene; pentafluorobenzene gave essentially the same products under the same conditions. The formation of product **27** without further substitution suggests that species such as $C_6F_5(Br)(SBu)$ may be ligated to the copper. A reaction scheme has been postulated involving the participation of the solvent, and the thiolate anion acting as a reducing agent,



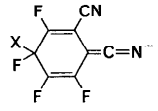
Nucleophilic substitution of tetrafluorophthalonitrile (**29**) with the benzenethiolate anion gives replacement of two or four fluorine atoms, but not the nitrile groups.



(29)

In solvent water the tetrasubstituted product is formed, but in methanol the ratio of disubstituted to tetrasubstituted is about 8 : 1 [96]. The formation of 4- and 5-disubstitution product rather than the anticipated 3- and 6- is similar to that observed in analogous reactions, and may be due to the

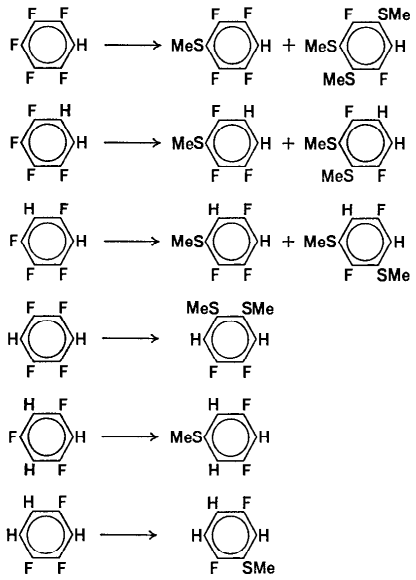
formation of a more stable *para* than *ortho* intermediate (**30**). The orientation of the product has been deduced from its ^{19}F n.m.r. spectrum.



(30)

4. Substitution in halobenzenes

The reactions of various fluorobenzenes with thiolate anions have been investigated in ethylene glycol/pyridine mixtures. The results are shown below using the methanethiolate anion as a nucleophile[8].



Product orientations have been deduced from ^1H and ^{19}F n.m.r. No reaction occurred with any difluorobenzene or fluorobenzene. Under these conditions the maximum substitution observed requires there to be two fluorine atoms still in the nucleus. Changing the solvents it is possible

to replace the fluorine in fluorobenzene or bromine in bromobenzene by a thiolate group, for example in HMPA/THF solvent mixtures using EtSNa, BuSNa or PhSNa in the presence of $NaNH_2$, the sulphides C_6H_5SR are formed[97-100]. An ideal solvent was found to be HMPA–THF in the ratio 1 : 5[99]. Similar reactions involving replacement of one or two aromatic halogens with potassium benzenethiolate or potassium thioresorcinolate have been observed in pyrrolidine as solvent[101].

Reaction of pentafluorobenzene with copper(I) benzenethiolate gives 2,4-difluoro-1,3,5-tris(phenylthio)benzene. This orientation is not inconsistent with the ^{19}F n.m.r.[95]. The fluorine *para* to the hydrogen in pentafluorobenzene has been replaced by a variety of nucleophiles, such as $p\text{-}HC_6F_4S^-$ forming $p\text{-}HC_6F_4SC_6F_4H\text{-}p$[73].

No thiolate substitution of *p*-dichlorobenzene, 1,2,4,5-tetrachlorobenzene, or pentachlorophenylanisole in alcohol was observed[89] Substitution of 2,3,4- and 2,4,5-trichlorobenzonitrile, 2,3-, 2,5- and 3-4-dichlorobenzonitrile and *o*- and *p*-chlorobenzonitriles with sodium hydrogen sulphide in liquid ammonia afforded the cyanothiophenols. Preferential replacement of the *p*-Cl was observed. *Meta*-chlorobenzonitrile did not undergo nucleophilic substitution under these conditions, but was rather hydrolysed by the water present in the NaSH [102].

In the naphthalene derivatives 1-fluoro- and 1-bromo-naphthalenes and 2-fluoro- and 2-bromo-naphthalenes reacted with *n*-butanethiolate in DMSO to give good yields of *n*-butyl 1-naphthyl sulphide and *n*-butyl 2-naphthyl sulphide respectively. *t*-Butanethiolate reacted similarly[103].

5. Substitution in miscellaneous polyhalogenated aromatics

The reaction of nitro and amino fluorobromobenzenes of the type $o\text{-}XC_6F_4Br$ and $p\text{-}XC_6F_4Br$ where $X = NO_2$ or NH_2 with the pentafluoro benzenethiolate anion, in its copper(I) salt, resulted in the replacement of the bromine[82]. The pentafluorobenzenethiolate anion or the anion of 2,3,5,6-tetrafluoro-4-mercaptopyridine, replaced the fluorine *ortho* or *para* to the nitro group in nitropentafluorobenzene. *Para* substitution only occurred in solvents of high dielectric constant, such as DMF and acetonitrile, whereas in solvents of low dielectric constant, such as ether, mixed replacement of *ortho*- and *para*-fluorine was observed[82, 83]. Increasing ionization of the thiol is postulated to cause predominantly *para* substitution.

6. Substitution in monohalogenated benzene derivatives

This section includes compounds such as 1-fluoro-2-nitrobenzene, where the fluorine atom is activated by the nitro group. The reactions of halonitrobenzenes with thiolate nucleophiles have been reviewed[69]. The

fluorine atom may easily be replaced in 1-fluoro-2-nitrobenzene by 2,3,5,6-tetrafluorobenzenethiolate forming *o*-nitrophenyl-2,3,5,6-tetrafluorophenyl sulphide, but polymerization of the pentafluorobenzenethiolate anion occurred when it was employed as the nucleophile[104]. Replacement of halogen in the cyclic derivatives such as 1- and -2-fluoro- and -chloro-anthraquinones[105], and various halo-1,2,3-benzothiazoles[106], is also observed.

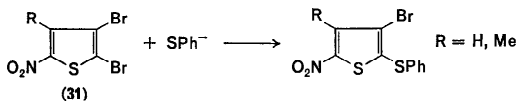
Considerable use has been made of the copper(I) benzenethiolate and butanethiolate in the preparation of thioethers. A large series of compounds of general formula $(RS)_nX$, $n > 1$, R = Ph or Bu, and X is an aryl group, have been prepared from the copper(I) thiolates and aryl halides (aryl bromides only reacted with the butanethiolate)[52, 107].

7. Substitution in heterocyclic compounds

This type of reaction is essentially similar to that of replacement of an aromatic halogen by a thiolate group. Halogen compounds studied include 3,4-dimethyl-5-bromo-2(N,N-dimethylaminomethylene)-2H-pyrrole[108] and chlorofuro-[2,3-d]pyridazines[109]. Copper(I) alkylthiolates have been used to form thioethers with 2-bromothiophene, 2-bromopyridine and 2-bromofuroic acid, the latter with concomitant decarboxylation[52].

The rate and activation parameters have been determined for the reaction of potassium methanethiolate with various 2-fluoro- and bromo-pyridines. Although an *ortho*-methyl group did not activate the 2- position in 2-bromo- or 2-fluoro-pyridine towards attack by the methanethiolate ion, deactivation of the *ortho* rather than the *para* position was observed. At 110°C for the bromo-compounds $K_0\text{-Me} : K_p\text{-Me} = 3{\cdot}9$, while $K_0\text{-Br} : K_p\text{-Br} = 2{\cdot}2$. The results have been compared with those obtained using methoxide and benzenethiolate anions in methanol. The relative rates observed in HMPA are the same as those in methanol[110]. Thiophenol reacts faster than its anion with a bromopyridine, in methanol, due to a rapid acid–base pre-equilibrium in which the pyridine is protonated. An *o*-MeO substituent accelerates the replacement of Br, and a small increase is also noted on going from MeOH to DMSO as solvent[111].

In 2,3-dibromo-5-nitrothiophenes (**31**) the 2-bromo group is replaced by the benzenethiolate anion



(31)

The *meta* methyl group increases the reactivity towards nucleophiles of the 2-bromine by increasing the Reinheimer and Bunnett effect of the 3-bromine on the activated 2-bromine[112].

Nucleophilic substitution of 2-chloro-4,6-bis(isopropylamino)-S-triazine with sodium methanethiolate in methanol gave prometryne (**32**) in 90% yield. The reaction is second order and the activation energies were 20·26 and 27·24 kcal/mole in *i*-propanol and methanol respectively[113,114].

(32)

8. Substitution of groups other than halogen

The rate constants for the replacement of various groups X in *p*-$XC_6H_4SO_2CF_3$ by NaSPh in methanol decreased in the order

$$X = SO_2CH_3 > NO_2 > F > Cl$$[115].

The element effect of atoms or groups increased with increasing activation and polarizability of the aromatic system.

Nitro groups in heterocyclic compounds can be replaced by thiolate groups. 5-Phenylmercapto-2-furaldehyde is obtained from 5-nitro-2-furaldehyde and benzenethiolate. Thiolates will not, however, react with halogenofurfural[116]. One nitro group in 3,4-dinitrothiophene may be replaced by a benzenethiolate group, but rearrangement occurs and phenyl-2-(4-nitrothienyl) sulphide is formed[117]. Sodium benzenethiolate or benzeneselenate gives replacement of either one but not both of the nitro groups in 2,3-dinitrothiophene[118].

Displacement of a thiolate group occurs in 2-methylthio- and 2-ethylthio-4[1(3)H] pyrimidines at the 2 position in greater than 70% yield, using a thiol in basic solution. A 5-halo and 6-amino substituent hindered the reaction but a 1-methyl or 6-hydroxy group facilitated it by influencing the tautomerism[119].

C. Dealkylation Reactions

A dealkylation reaction can be defined as the removal of an alkyl group, and its subsequent replacement by hydrogen, or the removal of an alkyl group from an ammonium salt with the formation of an amine, e.g.

$R_2\overset{+}{N}Me_2Cl^- + PhS^-Na^+ \longrightarrow R_2NMe$ (reference 120)

$p\text{-}CH_3C_6H_4OCH_2\overset{+}{N}Me_3I^- + HSR \longrightarrow p\text{-}CH_3C_6H_4OCH_2SR$ (reference 121)

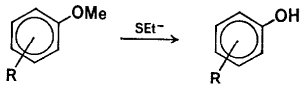
$Et_3\overset{+}{M}e\overset{+}{N}(CH_2)_2\overset{+}{N}MeEt_2\,2\,I^- + PhSH \longrightarrow PhSMe$ (reference 121)

The method can be used preparatively. Other examples include the selective demethylation of triethylmethylammonium chloride with sodium benzenethiolate[122]. A somewhat analogous reaction is observed in the reaction of alkoxytri(dimethylamine)phosphonium chloride (**33**) with thiolates forming a phosphine oxide and sulphide[23].

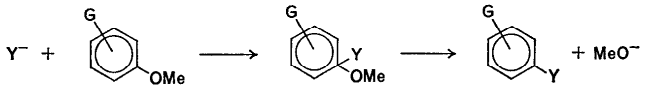
$$PhCH_2OP^+(NMe_2)_3Cl^- + PhSH(Et_3N) \xrightarrow{60°C} OP(NMe_2)_3 + PhCH_2SPh + Et_3NH^+Cl^-$$

(33)

The method is not restricted to group V derivatives and can easily be applied to oxygen esters and ethers. The use of various nucleophiles in this type of reaction has been discussed[124]. The main advantage of this technique for the demethylation of ethers with ethanethiolate in a solvent such as DMF is that a relatively low temperature is required and the group R may be acid sensitive[124-126].



The thiolate is generated *in situ* from sodium hydride and the corresponding thiol[124]. Aryl methyl ethers with strong electron-withdrawing substituents (G) require milder conditions for cleaving the ether linkage, but these compounds are also likely to suffer substitution of the aromatic carbon with strong carbon nucleophiles[127].



Using methyl ethers of di- and tri-hydric phenols, selective mono-demethylation occurs, e.g. resorcinol monomethyl ether is obtained from resorcinol dimethyl ether and sodium ethanethiolate in DMF. An exception is pyrogallol trimethyl ether which afforded pyrogallol 1-monomethyl ether in high yield[124]. Methylene ethers, such as methylenedioxybenzene, can be quantitatively converted to catechol, via the intermediate formation of ethyl *o*-hydroxyphenoxymethyl sulphide[126].

This method, using ethanethiolate, has been extended to esters[126]. The cleavage of methyl esters by lithium propanethiolate in HMPA, an S_N2 reaction, has been reported. The lithium salt reacts very much faster than the sodium salt[128]. The benzenethiolate and propanethiolate anions have

also been used in the conversion of esters to the corresponding acid or its sodium salt[129,130]. Examples include the conversion of *p*-anisate into *p*-hydroxybenzoic acid and methyl *p*-chlorophenoxyacetate to *p*-chlorophenol[126]. The latter is an example of the cleavage of an aryloxyacetate. However hydrolysis of *p*-nitrophenylacetate with both simple and polyfunctional thiols proceeds at a rate dependent upon the thiolate ion concentration. The initial products are *p*-nitrophenol and the thiol ester. Thermodynamic parameters E_a, ΔH^*, ΔF^* and ΔS^* have been found to be 8·0, 7·4, 16·7 kcal/mole and −30·7 e.u. respectively for the reaction of cysteine with *p*-nitrophenylacetate (29·6°C)[131].

$$R^1COR + R^2S^- \rightleftharpoons \left[R^1C(O^-)(OR)SR^2\right] \rightleftharpoons RO^- + R^1CSR^2$$

The two methoxy groups in amide acetals can be replaced by a dithiol forming 1,3-dithiolanes (**34**),

$$RC(OMe)_2NMe_2 + HSCH_2CH_2SH \longrightarrow$$

(**34**)

Replacement of only one methoxy group is found in the reaction of DMF-dimethyl sulphate mixture (presumably forming $HC(OMe)_2NMe_2$) with sodium ethanethiolate,

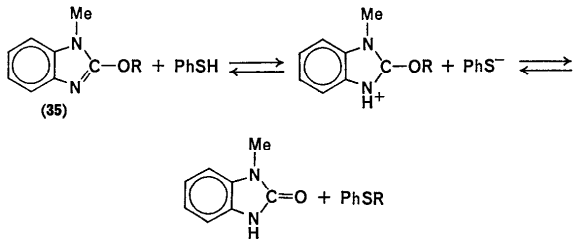
$$HC(OMe)_2NMe_2 + EtS^- \longrightarrow Me_2NCH(OMe)SEt + OMe^-$$

but thiols themselves displace both methoxy groups[132]. Other formamide mercaptals have also been used to form amide mercaptals, where R_2N = piperidine and R^1 = Me, C_6H_{13}, C_7H_{15} and $PhCH_2$[133].

$$R_2NCH(OMe)_2 + R'SH \longrightarrow R_2NCH(SR')_2$$

An interesting extension of this type of reaction is the transalkylation reaction between 2-alkoxy-1-methylbenzimidazole (**35**) and benzenethiol. The kinetics of this reaction indicate a rapid acid–base equilibrium, followed by an S_N2 attack at the ether saturated carbon by the PhS^- ion[134].

A somewhat analogous reaction is observed in the reaction of the mixed anhydride, acetic formic anhydride, with thiophenol in pyridine, where 93% of the thioformate, HCOSPh, and 7% of the thioacetate, MeCOSPh, are formed[135].



(**35**)

The thiolate anion acts both as a dealkylating agent and a reducing agent with $p\text{-}CH_2{=}CHOC_6H_4NO_2$. The yields of the various products are shown.

$$p\text{-}CH_2{=}CHOC_6H_4NO_2 \xrightarrow[\text{abs. EtOH}]{\text{NaSBu}} \begin{cases} 50\%\ BuSC_6H_4N(O){=}NC_6H_3SBu\text{-}p \\ 3\%\ BuSC_6H_4N{=}NC_6H_4SBu\text{-}p \\ 25\%\ p\text{-}CH_2{=}CHOC_6H_4N(O){=}NC_6H_4OCH{=}CH_2\text{-}p \end{cases}$$

When this reaction was studied under electrophilic conditions with the thiol in Et_2O/SO_2 or in a sealed tube with a free radical initiator, different reactions ensued, including addition across the C=C bond[136].

D. Reactions with Main Group Elements

1. Introduction

Thiols and thio-β-diketone derivatives of the elements have been reviewed, and compared with the alkoxides[137]. The alkali and alkaline earth metal salts of the thiols are probably ionic and can be prepared in numerous ways. In the aqueous phase, the excess water is removed by azeotropic distillation with toluene[138,139]. Alternatively using other solvents, salts or solvated salts can be isolated[7,140,141]. The crystal structures of the alkali metal thiolates, MSMe (M = Li, Na, K), have been reported and are of the same type as the corresponding alkoxides[142].

The thiol derivatives of the other main groups elements are often prepared from their halides using the thiol in the presence of a hydrogen halide acceptor or by using a metal thiolate, such as lead, where R is a main group element.

$$RHal + R'SH + Et_3N \longrightarrow RSR' + Et_3NH^+Hal^-$$

$$2\ RHal + Pb(SR')_2 \longrightarrow 2\ RSR' + PbHal_2$$

2. Group II

Few thiolate derivatives of beryllium are known. Di(*t*-butylthio)triberyllium tetra-*t*-butoxide, $(t\text{-BuS})_2Be_3(OBu\text{-}t)_4$, has been obtained from dichlorotriberyllium tetra-*t*-butoxide, $Cl_2Be_3(OBu\text{-}t)_4$, and lithium butanethiolate[143]. Other beryllium thiolates are prepared by reaction of a thiol with dialkylberyllium or dialkyneberyllium and do not involve a thiolate anion as an intermediate[144,145]. Various other compounds such as thiomagnesium alkyls[146] and dimethyl(methylthio)aluminum[147] are obtained analogously.

3. Boron

Reviews have been published about the problems and results of boron–sulphur chemistry[148], and organic boron–sulphur compounds[149]. The trialkylthio- or arylthioboranes can readily be prepared from boron trihalide and a metal thiolate:

$$3\ Pb(SC_6F_5)_2 + 2\ BCl_3 \longrightarrow 2\ B(SC_6F_5)_3 + 3\ PbCl_2 \quad \text{(reference 14)}$$

$$3\ Hg(SCF_3)_2 + 2\ BBr_3 \longrightarrow 2\ B(SCF_3)_3 + 3\ HgBr_2 \quad \text{(reference 150)}$$

In the latter reaction the mixed products $Hal_xB(SCF_3)_{3-x}$ (x = 1,2; Hal = Cl, Br) can also be isolated. Mixed arylakylthioboranes such as bis(ethylthio)phenylborane may be prepared analogously from dichlorophenylborane and lead ethanethiolate[151], or using the thiol in the presence of triethylamine[152]:

$$BrBMe_2 + HSPh + NEt_3 \longrightarrow PhSBMe_2 + Et_3\overset{+}{N}HBr^- \quad \text{(reference 152)}$$

Interesting new compounds of the type $M[RS(BH_3)_2]$ have recently been reported to be formed in the reaction of a metal thiolate with diborane in THF. The compound $K[EtS(BH_3)_2]$ has been isolated and some of its reactions studied[153].

$$KSEt + B_2H_6 \longrightarrow K[EtS(BH_3)_2]$$

4. Group IV

The reactions of thiolates with various carbon compounds are discussed elsewhere in this chapter.

Thiol derivatives of silicon, germanium, tin and lead can readily be prepared from a halide, usually chloride, and a thiol in the presence of a hydrogen halide acceptor or a metal thiolate. Various illustrative examples

are shown below:

$$H_3SiBr + NaSMe \longrightarrow MeSSiH_3 + H_4Si + \text{solids} \quad \text{(reference 154)}$$

$$H_3MI + NaSPh \longrightarrow H_3MSPh + NaI \quad (M = Si, Ge) \quad \text{(references 155, 156)}$$

$$Pb(p\text{-}SC_6F_4C_6F_4S\text{-}p) + Ph_3MCl \longrightarrow p\text{-}Ph_3MSC_6F_4C_6F_4SMPh_3\text{-}p \quad (M = Sn, Pb) \quad \text{(reference 157)}$$

$$Ph_3MHal + C_6F_5SH + Py \longrightarrow Ph_3MSC_6F_5 + PyH^+Cl^- \quad \text{(reference 158)}$$

$$GeCl_4 + 4\ RSH + 4\ NH_3 \longrightarrow Ge(SR)_4 + 4\ NH_4Cl\downarrow \quad \text{(reference 159)}$$

$$(ArO)_nSiCl_{4-n} + Ar'SH + Et_3N \longrightarrow (ArO)_nSi(SAr')_{4-n} \quad \text{(reference 160)}$$

Thiols can displace ammonia from silizanes[7].

$$(Me_3Si)_2NH + 2\ C_6F_5SH \longrightarrow 2\ Me_3SiSC_6F_5 + NH_3$$

The silicon analogue of the methanethiolate and methaneselenate anions, H_3SiS^- and H_3SiSe^-, are formed in the reaction of trisilylamine and hydrogen sulphide or selenide,

$$(SiH_3)_3N + H_2Y \longrightarrow (H_3Si)_2Y + NH_4^+(YSiH_3)^- \quad (Y = S, Se)$$

The trimethylammonium salt can also be formed.

$$4\ Me_3NH(HS) + 3\ SiH_3Br \longrightarrow 3\ Me_3NHBr + (SiH_3)_2S + 2\ H_2S + Me_3NH(SSiH_3)$$

The salts of the anion H_3SiS^- are stable at room temperature[161]. Similar anions Ph_3MS^- (M = Ge, Sn, Pb), presumably present in the lithium derivatives Ph_3MSLi, are well characterized and have been used in the synthesis of unsymmetrical sulphides[102].

$$Ph_3MSLi + Ph_3M'Cl \longrightarrow Ph_3MSM'Ph_3 \quad (M \text{ and } M' = Ge, Sn, Pb)$$

The derivatives such as $Et_2Sn(SNa)_2$ can be prepared from Et_2SnCl_2 and Na_2S, and react with chloro compounds to give the corresponding organotin thiol derivative[163],

$$Et_2Sn(SNa)_2 + 2\ BzCl \longrightarrow Et_2Sn(SBz)_2 + 2\ NaCl$$

The compound $(RSCH_2)_2Sn(SR)_2$ can be obtained by replacement of bromine bonded to carbon and tin in $(BrCH_2)_2SnBr_2$ by its reaction with the sodium thiolate RSNa [23].

5. Group V

The thiol-substituted amines such as sulphenamides and tris-(alkanesulphenyl) amines are often prepared from sulphenyl chlorides and ammonia[164,165], and never from nitrogen trichloride and a thiolate anion or a thiol. Chloramines react with thiols to produce symmetrical disulphides[166].

$$Me_2NCl + 2\,PhSH \longrightarrow PhSSPh + Me_2NH_2Cl$$

However methyl-N-chlorobenzimidate (**36**) and benzenethiol form N-benzoylbenzenesulphenamide (**37**). The reaction may proceed through the formation of the unknown PhCON=SHPh as an intermediate[167].

$$\underset{\textbf{(36)}}{PhC(OMe)=NCl} + PhSH \longrightarrow \underset{\textbf{(37)}}{PhCONH(SPh)} + MeCl$$

The kinetics of the reaction of diazonium ions $XC_6H_4N_2^+$ with benzenethiolate anions show that initially the *syn*-diazo thioether is formed rapidly, which is followed by the slower *syn-anti* isomerism. Only in the cases of *p*-nitro- and *p*-cyano-benzenediazonium ions is it possible to distinguish between the first and second reactions. Using benzenediazonium ion and the *p*-Me- and *p*-OCH_3-substituted ions with benzenethiolate, first-order kinetics were observed over the entire range of the reaction. It is postulated that there the rate-determining step is formation of the *syn*-diazothioether, followed by its rapid isomerization to the *anti*-diazothioether[168].

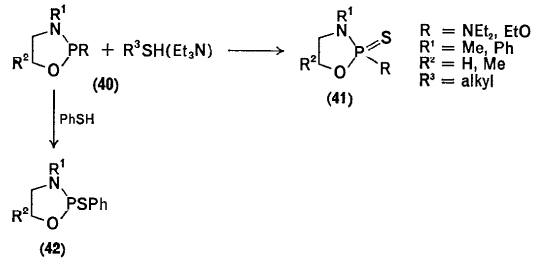
The simple alkyl and aryl-thio phosphorus derivatives, $(RS)_3P$, $(RS)_3PO$ and $(RS)_3PS$, can readily be prepared from phosphorus trichloride (or phosphorus pentachloride), phosphoryl chloride or thiophosphoryl chloride, and the corresponding lead thiolate[14,140,169]. Partially substituted compounds, such as $Cl_xP(SCF_3)_{3-x}$, are sometimes formed[170]. Substituted derivatives $R_2P(SR^1)$ and $RP(SR^1)_2$ can be prepared from the corresponding halide and lead thiolate[14,140,171]. Various mixed fluorophosphoranes, such as $MePF_2(SEt)_2$, can be prepared from $MePF_4$ and ethanethiol or its sodium salt[172].

p-Nitrophenyl methylphosphonic acid (**38**) reacts with thiolate nucleophiles leading to the formation of thiophosphonic esters (**39**), although the

$$\underset{\textbf{(38)}}{MeP(O)(OC_6H_4NO_2\text{-}p)O^-} + RS^- \xrightarrow{pH\ \sim 11} \underset{\textbf{(39)}}{MeP(O)(SR)O^-} + p\text{-}O_2NC_6H_4O^-$$

formation of some disulphide complicates the reaction[173]. Thiophosphites are also formed in the reaction of sodium thiolates or thiol/triethylamine with acetyl phosphite[174].

Reactions involving fracture of P—O or P—N bonds and displacement of EtO and Et_2N groups in various 1,3,2-oxaazaphospholanes (**40**) (R = EtO, Et_2N) with thiols in the presence of triethylamine have been examined[175]. Aliphatic thiolates used their sulphur in reaction with **40** to form 50–60% oxaazaphospholane 2-sulphide (**41**), whereas benzene thiol formed 78% 2-phenylthio-N-phenyl-1,3,2-oxaazaphospholane (**42**) (R^1 = Ph; R^2 = H). Similar derivatives (**40**; R = R^3S) are readily prepared from **40** when R = Cl, on treatment with a thiol in the presence of triethylamine[175].



The thiolate group may be added to phosphorus acting as a ligand, for example in the preparation of (ethyldimethylthiophosphinite)pentacarbonyl molybdenum[176],

$$(ClR_2P)Mo(CO)_5 + EtSH \xrightarrow[Et_3N]{\text{hexane}} (EtSMe_2P)(CO)_5Mo + Et_4NHCl$$

The thiol derivatives of arsenic can be prepared by similar methods to those used for phosphorus[14,141]. Various mixed derivatives such as BuPhAsSPr can be prepared from BuPhAsI and PrSNa in absolute ethanol[177]. Displacement of an OEt group may occur in PhAsCl(OEt), but this reaction may involve rearrangement of an unstable intermediate PhAs(OEt)SBu[178].

$$2\,PhAsCl(OEt) + 2\,BuSH + 2\,Et_3N \longrightarrow PhAs(SBu)_2 + PhAs(OEt)_2 + 2\,Et_3\overset{+}{N}HCl^-$$

Derivatives of heterocyclic arsenic compounds can be prepared, e.g.

when X = O and R = Cl, the reaction with PhSNa gave R = SPh, but when X = S the reaction with PhSNa in benzene gave $As(SPh)_3$, along with ethylene arsenite[178].

Derivatives of antimony(III), $Sb(SR)_3$, can be prepared analogously[14,141], or from antimony trichloride and thiols in the presence of ammonia[179]. Antimony(V) derivatives have been prepared[180], e.g.

$$Me_3SbCl_2 + 2\,MeSH + 2\,Et_3N \xrightarrow{-60^\circ} Me_3Sb(SMe)_2 + 2\,Et_3\overset{+}{N}HCl^-$$

These compounds are thermally unstable, decomposing to Me_3Sb and MeSSMe. The unstable Me_4SbSR analogues can be prepared from pentamethylantimony and a thiol at low temperature[181].

Bismuth thiolates can be prepared in reactions similar to those used to prepare metal thiolates[7].

6. Group VI

Attempts to prepare compounds of the type RSOSR containing a single-bonded system RS—O—SR failed, and possible rearrangement of this as an unstable intermediate occurred[182].

$$4\,CF_3SCl + 2\,Ag_2O \longrightarrow CF_3SSCF_3 + CF_3SO_2SCF_3 + 4\,AgCl$$

The reactions of chlorine monoxide with thiols or thiolates have not been investigated.

The thiolate anion can play a very important role in the thiol–disulphide interchange.

Various derivatives of sulphur may be prepared by the reaction of sulphur monochloride, sulphur dichloride or sulphenyl halides with thiolates; the products depend on the reactant stoichiometry.

$$(C_6F_5S)_2Pb + SCl_2 \longrightarrow C_6F_5SSSC_6F_5 + PbCl_2 \quad \text{(reference 183)}$$

$$Ph_3CSH + SCl_2 \xrightarrow{-78^\circ C} Ph_3CSSCl + HCl \quad \text{(reference 184)}$$

$$(C_6F_5S)_2Pb + S_2Cl_2 \longrightarrow C_6F_5SSSSC_6F_5 + PbCl_2 \quad \text{(reference 183)}$$

$$(EtS)_2Pb + 2\,C_6F_5SBr \longrightarrow 2\,C_6F_5SSEt + PbBr_2 \quad \text{(reference 185)}$$

$$RSH + ClCOSCl \longrightarrow RSSCOCl + HCl \quad \text{(reference 186)}$$

Symmetrical disulphides are formed in the reaction of a thiol with an azide in the presence of copper(I). The reaction probably proceeds through the formation of a sulphenamide which is decomposed by the thiol[187].

$$2R^1SH + RN_3 \longrightarrow RNHSR^1 + N_2$$
$$\downarrow R^1SH$$
$$RNH_2 + R^1SSR^1$$

Unsymmetrical disulphides can be formed by the decomposition of a sulphenamide with a thiol[188–190].

$$RSNEt_2 + PhSH \longrightarrow RSSPh + Et_2NH \quad (R = Me, Ph) \quad \text{(reference 188)}$$

The cleavage of the N—S bond in N(thiosulphenyl)phthalimide with thiols yields an unsymmetrical trisulphide[189,191]. Unsymmetrical disulphides are also formed in the thiolate anion fracture of the C—S bond in ethyl thiocyanate in DMF; small amounts, less than 10%, of the symmetrical disulphides are formed[192].

$$EtSCN + RS^- \longrightarrow EtSSR + CN^- \quad (R = n\text{-}C_8H_{17}, PhCH_2, Ph)$$

Attempts to prepare derivatives of sulphur(IV) or sulphur(VI) by the reaction of thionyl or sulphonyl chloride with lead thiolate failed, as the sulphur(II) derivative and sulphur dioxide were formed[14,182],

$$2\,(C_6F_5S)_2Pb + 2\,SOCl_2 \longrightarrow 2\,[C_6F_5S{\cdot}SO{\cdot}SC_6F_5] + 2\,PbCl_2$$
$$\downarrow$$
$$2\,C_6F_5SSC_6F_5 + SO_2 + S$$

$$(CF_3S)_2Hg + SO_2Cl_2 \longrightarrow [CF_3S{\cdot}SO_2{\cdot}SCF_3] + HgCl_2$$
$$\downarrow$$
$$CF_3SSCF_3 + SO_2$$

Other reactions of thiolate anions with sulphur(IV) and sulphur(VI) include the reaction with arylsulphonylsulphones

$$n\text{-}BuS^- + ArS(O)S(O)_2Ar \longrightarrow n\text{-}BuSS(O)Ar + ArSO_2^- \quad \text{(reference 193)}$$

and the reaction with the trithionate ion,

$$PhS^- + {}^-O_3SSSO_3^- \xrightarrow{K_a} PhS{-}SSO_3^- + SO_3^{2-}$$
$$\swarrow K_c \qquad K_b \searrow SO_3^{2-}$$
$$S_2O_3^{2-} + PhSSPh \xrightarrow[SO_3^{2-}]{K_d} PhSSO_3^- + S_2O_3^{2-}$$

The rate-determining step is K_a, and added formaldehyde eliminates the K_b and K_d paths, leaving PhSSPh[194].

Very few thiolate derivatives of selenium, and virtually none of tellurium, are known. Attempts to prepare $R_2Se(SC_6F_5)_2$ or $Me_2Te(SC_6F_5)_2$ from the dialkyl (or aryl) selenium dichloride, dimethyltellurium dichloride and lead pentafluorobenzenethiolate resulted in the formation of the disulphide, $C_6F_5SSC_6F_5$, and the dialkyl (or aryl) selenium, R_2Se, or Me_2Te. The chlorides Se_2Cl_2 and $TeCl_2$ yielded only the disulphide and selenium or tellurium[14]. Tellurium—sulphur and —selenium bonds have been formed in the reaction of organotellurium bromides with benzenethiol or benzeneselenol[195],

$$o\text{-}HCOC_6H_4TeBr + PhMH \longrightarrow o\text{-}HCOC_6H_4TeMPh \quad (M = S, Se)$$

7. Group VII

Attempts to prepare simple sulphenyl fluorides from thiolates and fluorine have not been reported, but are unlikely to be successful due to the oxidizing powers of fluorine[196] or chlorine monofluoride[197] causing oxidation of the sulphur(II)

$$(CF_3)_2S + F_2 \xrightarrow{-78°C} (CF_3)_2SF_2 \quad \text{(reference 196)}$$

Attempts to prepare trifluoromethanesulphenylfluoride resulted in the formation of trifluoromethylsulphur trifluoride and bis(trifluoromethyl)-disulphide[198].

Conversely sulphenyl chlorides can readily be prepared by the action of chlorine on a metal thiolate.

$$(C_6F_5S)_2Pb + 2\,Cl_2 \longrightarrow 2\,C_6F_5SCl + PbCl_2 \quad \text{(reference 185)}$$

Sulphenyl bromide can be obtained analogously in solution, but removal of the solvent caused decomposition[185],

$$(C_6F_5S)_2Pb + 2Br_2 \longrightarrow 2[C_6F_5SBr] + PbBr_2$$

$$2[C_6F_5SBr] \xrightarrow{\text{removal of solvent}} C_6F_5SSC_6F_5$$

$$2[C_6F_5SBr] + Pb(SEt)_2 \longrightarrow C_6F_5SSEt$$

The thiolate anion is quantitatively oxidized by iodine to the disulphide[185], and this method, involving the formation of an unstable sulphenyl iodide, is the basis of the iodometric determination of mercapto groups in a number of compounds[199].

$$(C_6F_5S)_2Pb + 2\,I_2 \longrightarrow 2\,[C_6F_5SI] + PbI_2$$

$$2\,[C_6F_5SI] \longrightarrow C_6F_5SSC_6F_5 + I_2$$

The thiolate anion is an intermediate in the oxidation of a thiol by iodine[200].

E. Reactions with Transition Metal Derivatives

1. Simple transition metal derivatives

This section will be primarily restricted to the derivatives and reactions of monofunctional thiols. The dithiol derivatives of the transition metals are a rapidly expanding area of research and have been reviewed several times recently[201-204]. Other polyfunctional thiols, such as monothioglycol, (with Ag(I)[205] and In(III)[206]), α-mercaptopropionic acid[207,208] and thioethanolamine[209,210], have been extensively studied and will not be discussed further. It is however noteworthy that interesting complexes of the type Ag_2SR^+, AgSR and $Ag(SR)_2^-$[205], $In(SR)_n^{(3-n)+}$ (n = 1, 2, 3, 4; R = $HOCH_2CH_2$)[208], and $\{Cd[NiL_2]_2\}^{2+}$ and $\{Ag[NiL_2]_2\}^+$ (L = H_2NCH_2-CH_2SH)[209] are formed.

Simple transition metal mercaptides, such as $Ni(SR)_2$ or $Hg(SR)_2$, are usually prepared by reactions not involving the thiolate anion as a nucleophile[7,141,211]. Occasional use is made of thiolates, for instance in the preparation of chromium(III) methanethiolate, where sodium methanethiolate was reacted with chromium chloride in excess of dimethyl disulphide under dry nitrogen and irradiated to yield the desired product, which can also be prepared by other photochemical methods[212]. Cobalt thiolates, $[Co(SR)_2]_n$, may be prepared from cobalt acetate in methanol with a basic solution of the thiol[213]. Some biochemical applications of thiolate anions are important. The binding of thiols to Co(II) corrins has been studied by e.s.r. where it has been shown that the thiols, thiolates and sulphides bind to the cobalt. The binding of Co(II) B_{12} complexes to thiols and sulphides will necessitate a re-examination of the methyl-transferring enzymes in which thiols are known to be important[214].

The continuous oxidation of thiols involved in the sweetening of light naphtha, with air to the disulphides using cobalt phthalocyanine complexes as catalysts, involves the formation of a stable complex between the thiolate ions and the metallocyanine catalyst[215].

The kinetics of various reactions involving thiolates and platinum complexes have been studied. The rate of reaction of *trans*-$[Pt(py)_2Cl_2]$

with nucleophiles Y is given by the equation

$$\text{rate} = K_1[\text{complex}] + K_2[\text{complex}][Y]$$

A reactivity sequence $PhS^- \gg MeO^- > N_3^-$ etc. was deduced[216]. The kinetic behaviour of *trans*-$[Pt(PEt_3)_2RCl]$ (R = Ph, *o*-Tol) with different entering groups, including PhS^-, has been examined. The rate was found to be independent of the reagent concentration in solvents (solv) such as methanol or DMSO[217].

$$\textit{trans}\text{-}[Pt(PEt_3)_2(o\text{-Tol})Cl] \underset{}{\overset{\text{slow}\ +\text{solv}}{\rightleftharpoons}} \text{intermediate} \overset{+Y}{\rightleftharpoons} \textit{trans}\text{-}[Pt(PEt_3)_2(o\text{-Tol})Y] + Cl^-$$

2. Complex ions

Various complex ions of the type $[M(SR)_2]^-$ and $[M(SR)_4]^{2-}$ have been reported. These complexes can be formed readily when R = Ph[218], C_6F_5[218-220], C_6Cl_5[140,221] and the metal M may be Co(II), Pd(II), Pt(II), Zn(II), Cd(II), Hg(II), Cu(I), Ag(I) or Au(I). The complex ions are usually prepared by the reaction of an alkali metal thiolate with an appropriate metal salt. The anions may be isolated as their salts with potassium, tetramethyl- or tetrabutyl-ammonium, or tetraphenyl-arsonium cations. The electronic spectra of these systems have been analysed[218,222] and the nature of the bonding discussed[223]. The SC_6F_5 ligand is intermediate between NCO^- and NCS^- in the spectrochemical series, about the same as I^- in the nephelauxetic (cloud expanding) series (reflecting the decreasing covalency of the ligands), and the optical electronegativity $\chi_{opt}(SC_6F_5)$ is 2·5~2·6[222]. However other data have been interpreted to give slightly different spectrochemical and nephelauxctic series[218]. The data for several ligands have been discussed and various deductions made. High ligand electro-negatives, χ_L, are associated with high coordination numbers and high complex symmetries, whereas ligands with lower values of χ_L promote lower coordination numbers and distorted symmetries. This has been rationalized in terms of the charge balance requirements of the metal ion and the covalence of the metal ligand bond[223].

Similar complex ions, stabilized as the tetraalkyl ammonium salts, have been prepared from tetrafluorobenzene-1,2-dithiol (H_2tfdt). The complex ions formed were $[Mtfdt_2]^-$ (M = Fe(III), Co(III), Ni(III)), and $[Mtfdt_3]^{2-}$ (M = Mo(IV) and Pt(IV))[224].

3. Organometallic compounds

This section is concerned primarily with organometallic transition metal complexes.

Cyclopentadienyltitanium thiolates have been prepared in benzene solution from the corresponding chloride and several thiols in the presence of triethylamine in good yields[225].

$$(\pi\text{-}C_5H_5)_2TiCl_2 + 2\ RSH + 2\ Et_3N \longrightarrow (\pi\text{-}C_5H_5)_2Ti(SR)_2 + 2\ Et_3NH^+Cl^-$$

The compound $(\pi\text{-}C_5H_5)_2Ti(SR)_2$ (R = Me, Ph) has also been prepared from $(\pi\text{-}C_5H_5)_2TiCl_2$ and NaSR[226]. Attempts to prepare $(\pi\text{-}C_5H_5)_2Ti(SCF_3)_2$ from $(\pi\text{-}C_5H_5)_2TiCl_3$ and $AgSCF_3$ resulted in the formation of $(\pi\text{-}C_5H_5)_2TiF_2$[227], and several unsuccessful attempts have been made to prepare $(\pi\text{-}C_5H_5)_2Ti(SC_6F_5)_2$[228].

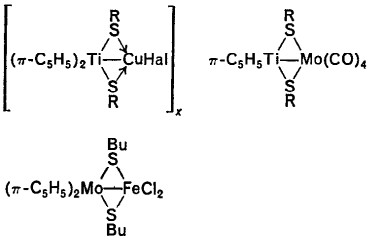
The extremely unstable mono-π-cyclopentadienyltitanium tri(benzenethiolate) has also been reported[229],

$$\pi\text{-}C_5H_5TiCl_3 + 3\ HSPh + 3\ NEt_3 \longrightarrow \pi\text{-}C_5H_5Ti(SPh)_3 + 3\ Et_3NH^+Cl^-$$

If a 1 : 1 reactant stoichiometry is used, the stable compound $\pi\text{-}C_5H_5TiCl_2(SPh)$ is readily isolated and can be purified by vacuum sublimation. The derivatives of zirconium, $(\pi\text{-}C_5H_5)_2Zr(SPh)_2$ and $(\pi\text{-}C_5H_5)_2Zr(SePh)_2$, have been prepared analogously from $\pi\text{-}C_5H_5ZrCl_2$ and the thiol or selenol in the presence of triethylamine[230].

Various other analogous compounds, such as $(\pi\text{-}C_5H_5)_2Nb(SR)_2$ (R = Me, Ph[231]) and $(\pi\text{-}C_5H_5)_2M(SR)_2$ (M = Mo, W[232]) can be obtained from the corresponding chloride and sodium thiolate.

The compounds of the type $(\pi\text{-}C_5H_5)_2M(SR)_2$ (M = Ti, Mo, W, Nb) have been found to have extremely interesting properties[231,233,239]. They can act as bidentate ligands forming complexes, some of which may contain metal—metal bonds, e.g.



Various other organometallic thiolate complexes may be formed by using thiolates.

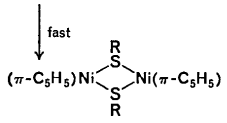
$$[(\pi\text{-}C_5H_5)Ni(n\text{-}Bu_3P)_2]^+Cl^- + Na^+SR^- \rightarrow [(\pi\text{-}C_5H_5)Ni(n\text{-}Bu_3P)SR] + NaCl + n\text{—}Bu_3P$$

where the thiolate anion can be derived from aliphatic or aromatic thiols[240, 241]. Dithiol derivatives can also be obtained[242].

$$2[(\pi\text{-}C_5H_5)Ni(n\text{-}Bu_3P)_2]^+Cl^- + NaS(CH_2)_nSNa \longrightarrow$$
$$(\pi\text{-}C_5H_5)(n\text{-}Bu_3P)NiS(CH_2)_nSNi(n\text{-}Bu_3P)(\pi\text{-}C_5H_5) \quad (n = 2, 4, 6)$$

Other reactions involving thiols, such as the reaction

$$(\pi\text{-}C_5H_5)_2Ni + HSR \longrightarrow (\pi\text{-}C_5H_5)NiSR + C_5H_6$$



which has been studied kinetically, do not involve the thiolate anion, but rather the thiol itself, the sulphur of which bonds initially to the nickel[243].

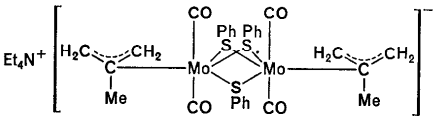
Various CF_3S derivatives have been prepared using silver trifluoromethanethiolate, and its reactions with certain norbornadiene and tetraphenylcyclobutadienemetal complexes studied[244]. The reaction of the norbornadiene derivative $C_7H_8PtCl_2$, with $AgSCF_3$ in dichloromethane solution resulted in the replacement of both chlorine atoms with CF_3S groups to give the white crystalline $C_7H_8Pt(SCF_3)_2$. However, in the analogous reaction of $C_7H_8PdCl_2$ with CF_3SAg, addition of CF_3S groups to the norbornadiene ligand occurred to give two yellow crystalline products, $[(C_7H_8SCF_3)Pd]_2Cl_2$ and $[(C_7H_8SCF_3)Pd]_2(Cl)(SCF_3)$, which are novel nortricyclic derivatives. Reaction of the tetraphenylcyclobutadiene complex $[Ph_4C_4PdBr_2]_2$ with $AgSCF_3$ gave the golden-red $Ph_4C_4Pd(SCF_3)_2$ formulated as a monomeric 16-electron tetraphenylcyclobutadiene complex, but the reaction of $Ph_4C_4Co(CO)_2Cl$ with $AgSCF_3$ gave the binuclear complex $[Ph_4C_4Co(CO)SCF_3]_2$.

The molybdenum complexes $[\pi\text{-}C_5H_5Mo(NO)X]_2$, $[\pi\text{-}C_5H_5Mo(NO)X_2]_2$ and $[\pi\text{-}C_5H_5Mo(NO)(I)(SCH_2Ph)]_2$, X = I, SCH_2Ph, or SPh, have been obtained from the iodide $[\pi\text{-}C_5H_5Mo(NO)I_2]_2$ by reaction with the appropriate thiolate anions under differing conditions[245]. The structures of the analogous chromium compounds $[\pi\text{-}C_5H_5Cr(NO)SPh]_2$ show that the SPh groups act as bridges between the two chromium atoms[246].

Several molybdenum derivatives can be prepared using a thiolate. A stable monomeric π-allyl molybdenum derivative has been obtained by the metathesis[247]:

$$\pi\text{-}C_3H_5Mbipy(CO)_2Cl + TlSC_6F_5 \longrightarrow \pi\text{-}C_3H_5Mbipy(CO)_2SC_6F_5 \quad (M = Mo, W)$$

A dinuclear π-allylmolybdenum complex has been obtained by treatment of its trichloroanalogue with sodium thiolate[248].



Various other mixed cyclopentadienyl carbonyl complexes can be prepared using a thiolate:

$$\pi\text{-}C_5H_5W(CO)_3Cl + NaSR \longrightarrow \pi\text{-}C_5H_5W(CO)_3SR \quad (R = Me, Ph)$$
$$\pi\text{-}C_5H_5W(CO)_3Cl + RSH + Et_3N \longrightarrow$$

(reference 249)

$$\pi\text{-}C_5H_5Fe(CO)_2Br + NaSEt \longrightarrow \pi\text{-}C_5H_5Fe(CO)_2SEt$$ [250]

(reference 250)

4. Carbonyl compounds

Sulphur-containing metal carbonyls have been reviewed, and there is a section concerning mercapto compounds[247]. While several mercapto carbonyl complexes are known which can be prepared from the thiol itself or the disulphide some preparations involve the use of the thiolate anion. The complex ions $[M(CO)_5SC_6F_5]^-$ (M = Cr, Mo, W) can readily be prepared from the pentacarbonyl and sodium pentafluorobenzenethiolate[251]. The square planar complexes, *trans*-$[M(SC_6F_5)(CO)(PPh_3)_2]$ are obtained from thallium(I) pentafluorobenzenethiolate and the complexes $[MCl(CO)(PPh_3)_2]$ (M = Ir, Rh)[252]. The complex $[Ir(SC_6F_5)(CO)(PPh_3)_2]$ will add another mole of pentafluorobenzenethiol in benzene to form $[IrH(SC_6F_5)_2(CO)(PPh_3)_2]$, and also readily adds oxygen, forming $[Ir(SC_6F_5)(O_2)(CO)(PPh_3)_2]$[252].

The yellow diamagnetic anions $[Cr_2(CO)_{10}SR]^-$ (R = H, Me, Et, Ph) are formed on oxidation of aqueous $Na_2[Cr_2(CO)_{10}]$ by RSH, accompanied by the evolution of hydrogen, but when RSH is thio-*p*-cresol the mononuclear anion $[Cr(CO)_5SR]^-$ ($R = C_6H_4Me$) is isolated[253]. The monomeric carbonyl derivatives $M(CO)_5SR^-$ can be prepared by using the mercury thiolates; only a small amount of the dimeric species is obtained [254]. The

$$M_2(CO)_{10}^{2-} + Hg(SR)_2 \longrightarrow 2\,M(CO)_5SR^- + Hg$$

ions $[M_2(CO)_{10}SR]^-$ (M = Cr, Mo, W), stabilized as their bis(triphenylphosphine)iminium derivatives, are obtained in reactions of the type

$$2\,Cr(CO)_6 + SMe^- \xrightarrow[THF]{u.v.} Cr_2(CO)_{10}SMe^- + CO$$

but this reaction does not give as good yields as the reaction of $M(CO)_5Cl^-$ with organotin thiolates[255].

Various carbonyl derivatives containing the SCF_3 group can be obtained using silver trifluoromethanethiolates. Some reactions are summarized below[227]:

$$Mn(CO)_5Br + AgSCF_3 \longrightarrow [CF_3SMn(CO)_4]_2$$

$$Re(CO)_5Br + AgSCF_3 \longrightarrow [CF_3SRe(CO)_4]_2 + CF_3SRe(CO)_5$$

$$\pi\text{-}C_5H_5Fe(CO)_2I + AgSCF_3 \longrightarrow CF_3SFe(CO)_2(\pi\text{-}C_5H_5)$$

$$C_5H_5Fe(CO)_3I + AgSCF_3 \longrightarrow CF_3SFe(CO)_3C_5H_5$$

$$\pi\text{-}C_5H_5Cr(NO)_2Cl + AgSCF_3 \longrightarrow CF_3SCr(NO)_2(\pi\text{-}C_5H_5)$$

$$C_3F_7Fe(CO)_4I + AgSCF_3 \longrightarrow [C_3F_7Fe(CO)_3SCF_3]_2 \quad \text{(reference 256)}$$

Other complexes can be obtained using mercury(II) trifluoromethanethiolate[257].

$$Mn_2(CO)_8P(CF_3)_2I + Hg(SCF_3)_2 \longrightarrow Mn_2(CO)_8P(CF_3)_2SCF_3$$

The compounds $[\pi\text{-}C_5H_5Mo(NO)HalSR]_2$ (Hal = Br, I), $[\pi\text{-}C_5H_5Mo(NO)(SR)]_2$ and $[\pi\text{-}C_5H_5Mo(NO)(SR)_2]_2$ containing bridging sulphur ligands can readily be prepared from $[\pi\text{-}C_5H_5Mo(NO)Hal_2]$ (Hal = Br, I) and the thiol or its sodium salt by replacing one and two halogens respectively[258]. Other dimeric compounds with bridging thiolate groups, such as $[Rh(CO)_2(SPh)]_2$, are readily obtained from benzenethiol and $[Rh(CO)_2Cl_2]^-$ in ethanol. However, analogous compounds such as $[Rh(CO)(SR)_2Hal]$ (Hal = Cl, R = Et, Pr; Hal = Br, R = Et) may be polymeric[259]. Bridging thiolates are also present in the iron compounds, $(CO)_3Fe(SEt)_2Fe(CO)_3$ obtained from $Fe_2(CO)_6(COPh)_2$ and EtSH in hexane[260].

Carbene complexes $(CO)_5CrC(SR)R^1$ (R = Me, Et, Ph; R^1 = Me, Ph) and $(CO)_5WC(SMe)Me$ are readily obtained by nucleophilic displacement of OMe from $(CO)_5MC(OMe)R^1$ (M = Cr, W) with a thiol[261].

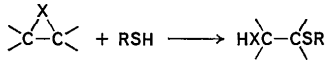
III. ADDITION REACTIONS

A. Introduction

The addition of a thiol or a thiolate to an unsaturated compound A=B can be represented as

$$A{=}B + RSH \longrightarrow RSA{-}BH \text{ or } HA{-}BSR$$

Two products are possible, depending on whether the RS group adds to A or B. This will obviously be affected by the nature of atoms forming the multiple bond and, possibly, by the other groups present in A and B. Addition reactions can occur in cyclic systems, such as epoxides or thioepoxides, involving fracture of the ring



It is, among other things, of interest to ascertain the nature of the addition product.

Most of the addition reactions observed occur by a radical mechanism. This type of reaction has been reviewed[262], and two chapters in this book are concerned with radical reactions of thiols. This discussion will exclude all reactions that occur via the formation of radicals. Considerably less study has been made of ionic additions of thiolates to unsaturated systems than that of radical additions.

B. Reactions with Olefins

Sulphides are formed when a thiol adds onto an olefinic bond. Most of the reactions reported correspond to anti-Markownikoff addition, but this is probably a free radical mechanism, which also occurs in the presence of minute traces of peroxides. With carefully purified reagents in the presence of acid, Markownikoff addition occurs[263a]

$$Me_2C{=}CHMe + RSH \longrightarrow Me_2C(SR)CH_2Me$$

The kinetics of the addition of benzenethiol and substituted benzenethiols to derivatives of phenylvinylsulphone have been studied[264,265]. In 50% aqueous ethanol at 25°C the reaction was second order, first order in the sulphone and in the thiolate anion.

$$XC_6H_4SH \rightleftharpoons XC_6H_4S^- + H^+$$

$$XC_6H_4S^- + YC_6H_4SO_2CH{=}CH_2 \xrightarrow{\text{slow}} YC_6H_4SO_2C^-HCH_2SC_6H_4X$$

$$YC_6H_4SO_2C^-HCH_2SC_6H_4X \xrightarrow{H^+ \text{ fast}} YC_6H_4SO_2CH_2CH_2SC_6H_4X$$

Hammett treatment showed that substitution in the phenyl ring of the sulphone influenced the reaction more than substitution in the thiol, indicating that the transition state resembles a carbanion intermediate[264]. The second-order rate constant for the nucleophilic addition of $p\text{-}MeC_6H_4S^-$ to phenyl vinyl sulphone has been detected at 0–45°C, the energy, free

energy of activation and entropy being 16·0 kcal/mole, 16·6 kcal/mole and −4 e.u. respectively[265].

Allyl alcohol and *n*-BuSH give *n*-BuSCHMeCH$_2$OH in the presence of 5% elementary sulphur as a catalyst and an initial pressure of hydrogen of about 30 atmospheres, but allyl alcohol and *t*-BuSH form *t*-BuS(CH$_2$)$_3$OH under free-radical conditions[266]. The compound MeS(CH$_2$)$_3$SMe has been prepared from allyl chloride, first by MeS$^\cdot$ addition and then MeS$^-$ substitution[266].

Activated thiols will add to *p*-isopropenylphenol in chloroform solution in the presence of *p*-toluenesulphonic acid giving a Markownikoff addition product,

$$p\text{-HOC}_6\text{H}_4\text{CMe}{=}\text{CH}_2 + \text{RSH} \longrightarrow p\text{-HOC}_6\text{H}_4\text{CMe}_2(\text{SR})$$

(R=CH$_2$CO$_2$R', (R'O)$_2$PS, CH$_2$CONHC$_{10}$H$_7$)

Thioacetic acid gave an anti-Markownikoff addition product. Simple thiols did not react even in the presence of catalysts, except under pressure and irradiation. With benzenethiol and *p*-chlorobenzenethiol the unusual addition of the *para*-hydrogen occurred[267].

$$p\text{-HOC}_6\text{H}_4\text{CMe}{=}\text{CH}_2 + \text{C}_6\text{H}_5\text{SH} \longrightarrow p\text{-HOC}_6\text{H}_4\text{CMe}_2\text{C}_6\text{H}_4\text{SH-}p$$

A few other examples of this type of addition, in the presence of a catalyst, are found in the patent literature[267].

Simple Markownikoff addition of thiol to the C=C bond occurs in some carbohydrate derivatives[268], and to dimethyl maleate[269].

C. Reactions with Acetylenes

Thiols do not add less readily to acetylenes than to olefins. The addition occurs at high temperatures in the presence of a base[268a].

$$\text{HC}{\equiv}\text{CH} + \text{R'SH} \longrightarrow \text{CH}_2{=}\text{CH(SR)}$$

A second molecule of thiol may be taken up

$$\text{RSCH}{=}\text{CH}_2 + \text{RSH} \longrightarrow \text{RSCH}_2\text{CH}_2\text{SR}$$

If addition occurs across an acetylenic bond, there is the possibility of formation of *cis* and *trans* isomers. Phenyl acetylene reacts with ethanethiol in the presence of an alkali catalyst at 100–225°C. Progressively larger amounts of the *trans* isomer were formed as the temperature increased, reaching a maximum of 71% *trans* at 200°C. A rapid *cis–trans* isomerism accompanies the vinylation reaction[270].

$$\text{EtSH} + \text{Ph}{=}\text{CH} \longrightarrow (\text{EtS})\text{CH}{=}\text{CHPh}$$

The degree of *trans* stereoselectivity for nucleophilic additions of *p*-toluenethiol derivatives to negatively substituted acetylenic compounds (Y=CN; $SO_2C_6H_4Me$-*p*, $C_6H_4NO_2$-*p*, CO_2Me, $CONH_2$, COMe) in methanol is dependent on the nature of the activating group Y, and

$$\text{HC}{\equiv}\text{CY} + \text{ArS}^- \xrightarrow{\text{MeOH}} \underset{\text{H}}{\overset{\text{ArS}}{\diagdown}}\text{C}{=}\text{C}\underset{\text{H}}{\overset{\text{Y}}{\diagup}} + \underset{\text{H}}{\overset{\text{ArS}}{\diagdown}}\text{C}{=}\text{C}\underset{\text{Y}}{\overset{\text{H}}{\diagup}}$$

decreases where Y is capable of delocalizing the adjacent incipient negative charge[271]. In the tertiary amine-catalysed addition of thiols to ethyl propiolate, it has been shown that the amount of *trans* addition product in the reaction mixture increased as the acidity of the thiol. Similar additions to hexafluoro-2-butyne and trifluoromethylacetylene showed that with both trifluoromethyl-activated acetylenes *trans* addition was predominant. However only 5% *trans* was obtained in the reaction of cyclohexanethiol and trifluoromethyl acetylene[272].

Addition reactions have been studied with various substituted acetylenes such as the Markownikoff additions

$$\text{PhOC}{\equiv}\text{CH} + \text{EtSH} \xrightarrow{\text{Na(NH}_3\text{)}} 52{\cdot}3\%\ \text{H}_2\text{C}{=}\text{C(OPh)SEt}$$

and anti-Markownikoff additions of thiols to phenoxyacetylene, depending on the solvent employed[273],

$$\text{PhOCH}{\equiv}\text{CH} + \text{RSH} \xrightarrow{\text{Et}_2\text{O}} \text{PhOCH}{=}\text{CHSR}\quad (\text{R} = \text{Et, Bu})$$

Addition to trifluoromethylacetylenes has been studied with thiols in the presence of sodium ethoxide or triethylamine[274]:

$$\text{RSH} + \text{XC}{\equiv}\text{CCF}_3 \longrightarrow (\text{RS})\text{XC}{=}\text{CHCF}_3\quad (\text{R} = \text{Et, HOC}_2\text{H}_4\text{, Bu, Ph;}\ \text{X} = \text{Cl, Br, Et, CF}_3\text{, H, Et}_2\text{N})$$

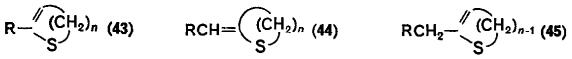
Addition can also occur in systems containing ethylenic and acetylenic bonds, and nucleophilic addition occurs primarily across the acetylenic bond:

$$\text{RSH} + \text{F}_3\text{CC}{\equiv}\text{CCHRCR}^1{=}\text{CR}^2\text{R}^3 \longrightarrow \text{F}_3\text{CCH}{=}\text{C(SR)CHRCR}^1{=}\text{CR}^2\text{R}^3$$

The latter compound isomerizes to $F_3CCH_2C(SR){=}CRCR^1{=}CR^2R^3$. In the free radical addition compounds such as $F_3CC{\equiv}C(CH_2)_3SMe$ were isolated[275].

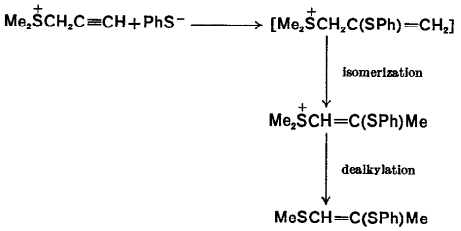
Thiols containing an acetylenic bond may cyclize. The heterocyclization of acetylenic thiols, $RC{\equiv}C(CH_2)_nSH$, has been studied under nucleophilic

and free radical conditions forming products (43), (44) and (45). Compound **44** is the main product of the nucleophilic attack when R = H, and mixtures of all three are formed under free radical conditions[276].



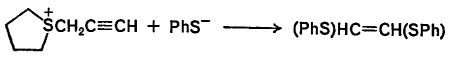
The addition of thiols to acetylenic bonds in compounds with a formal negative or positive charge has been examined. Aromatic thiols react with $HO_2CC{\equiv}CCO_2K$ giving (phenylthio)fumaric acids, which were cyclized in the presence of sulphuric acid to thiachromonecarboxylic acids[276a].

Two products are formed when the benzenethiolate anion reacts with dimethylprop-2-ynylsulphonium bromide, $Me_2\overset{+}{S}CH_2C{\equiv}CH\ Br^-$. The reaction is postulated to proceed through the formation of an allenic system $\overset{+}{S}C{=}C{=}C$. The initial product, not isolated, isomerizes, and may subsequently be dealkylated with excess thiolate:



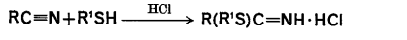
The methanethiolate anion also adds to the ethylenic bond of the dealkylated product and some trisulphide is formed:

A similar reaction is observed with the benzenethiolate anion and 1-(prop-2-ynyl)tetrahydrothiophenium bromide[276b].

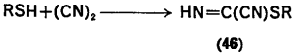


D. Reactions with Nitrile Groups and Azomethine Bonds

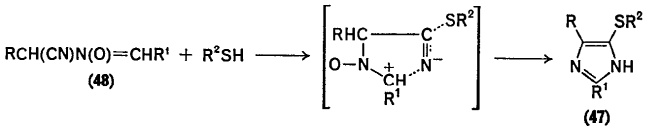
In acidic solution nitriles undergo an addition reaction with thiols forming iminothioesters[263b]



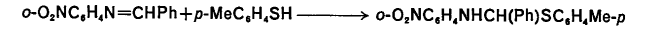
The examples of this type of reaction in the recent literature are somewhat limited. The simplest is the formation of cyanoformimidic acid (**46**) by reaction of cyanogen with a thiol in an inert solvent in the presence of amines or metal hydroxides[277],



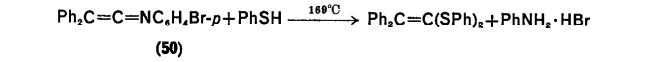
2,4,5-Trisubstituted imidizoles (**47**) can be obtained from thiols and N-(1-cyanoalkyl)alkylidenimine-N-oxides (**48**). This reaction involves a cyclization reaction, proceeding through the initial addition of the thiol to the C≡N bond[278].



Addition of thiols across an azomethine bond occurs resulting in the formation of a carbon—sulphur bond, an example is the formation of N-benzylidene-*o*-nitroaniline (**49**)[279],

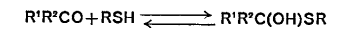


Thiophenol reacts with diphenyl-ketene-(*p*-bromophenyl)imine (**50**) causing reduction of the aromatic bromine and fracture of the C=N bond[280],

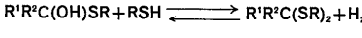


E. Reactions with Carbonyl and Thiocarbonyl Groups

Thiols can react with ketones to give a hemithioacetal:



Further reaction readily gives the thioacetal, although a catalyst is sometimes required[263c]:



Thioacetals may be thermally decomposed to the corresponding thione:

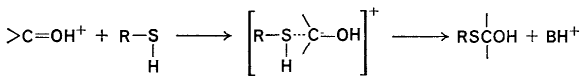
$$R^1R^2C(SR)_2 \longrightarrow R^1R^2CS + R_2S$$

The hemithioacetal can also be reduced by excess thiol to the sulphide[263a]:

$$2\,R^1SH + RCH(OH)SR^1 \longrightarrow RCH_2SR^1 + R^1_2S_2 + H_2O$$

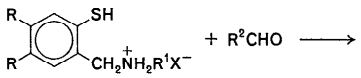
The equilibria between propanethiol and simple carbonyl compounds have been studied in CH_2Cl_2: the resulting α-hydroxysulphides may be converted into the thioacetals where the equilibrium constants are less than 10^2, by addition of an acid catalyst (BF_3 or HCl). Examples of aldehydes and ketones whose values of K are less than 10^2 are MeCHO, Me_2CO; those having K values greater than 10^2 are CCl_3CHO, $(CF_3)_2CO$[281].

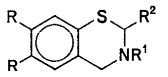
The kinetics of the formation of the hemithioacetal in 50% ethanol–water have shown that the reaction is acid catalysed and does not involve a thiolate anion, probably proceeding via the formation of the protonated ketone[282],



Other studies of rate and equilibrium constants of the formation and breakdown of hemithioacetals (MeCHO + PhSH, or AcSH, or p-$NO_2C_6H_4SH$) reveal a diffusion-controlled rate-determining step, with proton transfer in some sense concerted with cleavage and formation of the C—S bond[283]. A general base-catalysed mechanism involves attack of the RS^- anion on the carbonyl group[284].

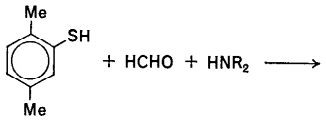
The addition reaction can be utilized synthetically, as is illustrated in the examples where further reaction with amines occurs.

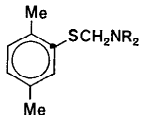




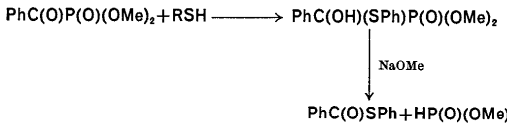
(reference 285)

The phosphorus containing ketone $PhC(O)P(O)(OMe)_2$ does not react with sodium thiolate, but will react with the thiol in the presence of magnesium bromide forming the thioester, upon fracture of the carbon—phosphorus bond[287].

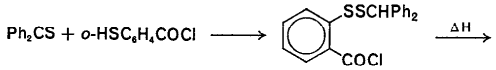


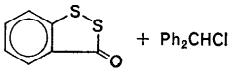


(reference 286)



Analogous reactions occur with thiones, as illustrated by the example[288]:

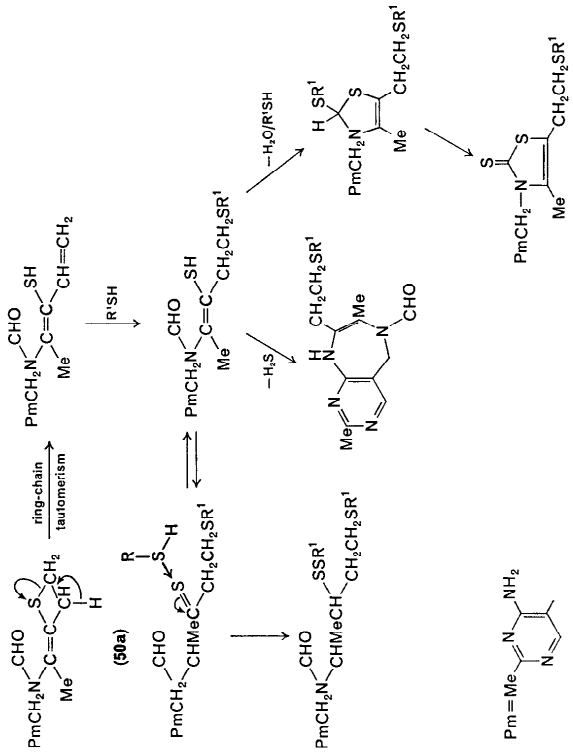




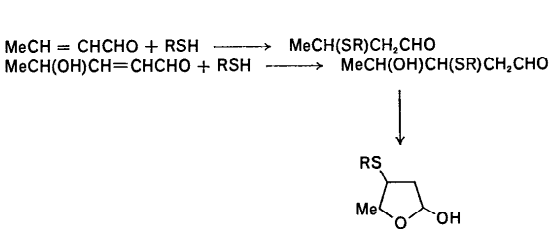
F. *Reactions Involving Conjugated Systems*

With a conjugated system similar to the type C=C—C=X, where X can be C,N,O, it is of interest to observe where addition of a thiol occurs. The majority of reactions involve addition across the C=C bond, but exceptions are found.

Several products are obtained from the reaction of thiols with thiamine anhydride (**50a**). A conjugated system is postulated as an intermediate with initial 1,2 addition. The reaction products depend subtly on the pH[289].

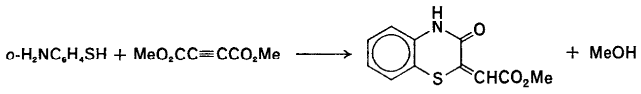


Reactions of the conjugated aldehydes, crotonaldehyde and 4-hydroxy-2-pentenal, with a C=C—C=O bond system, in aqueous solution with thioglycollic acid, either as its sodium salt or ethyl ester, give addition across the C=C bond. The 4-hydroxy-2-pentenal adduct cyclizes to the hemiacetal,

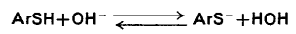


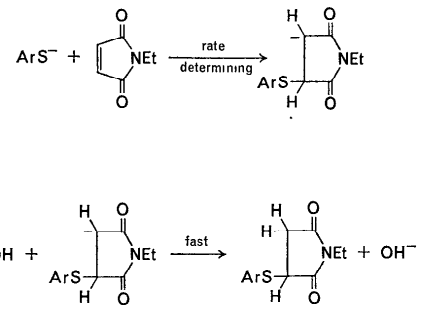
The kinetics have been studied and show that with thioglycollic acid derivatives between pH 1·5 and 2·5 the RS^- ion and RSH react, but at pH > 2·5 the RS^- anion is the reactive entity. A reaction mechanism has been derived[290].

1,4-Addition of thiols in basic solution to the C=C—C=O bond system in α,β unsaturated ketones, such as 4-benzylidene-1-butyl-pyrrolidine-2,3-dione, has been observed, forming with benzenethiol in piperidine, 1-butyl-3-hydroxy-4(α-phenylthiobenzyl)-3-pyrrolin-2-one[291]. Addition of thiols primarily to the C=C bond in C=C—C=O systems in quinones and lactones has been observed[292,293]. The reactions were studied in neutral or alkaline solution and probably involve attack by the thiolate anion. In compounds containing both carbonyl or carboxyl groups and acetylenic triple bonds, addition occurs primarily across the acetylene bond. Cyclization of the initial product so formed is also observed[294].



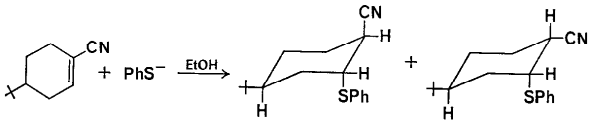
The addition of thiols to N-ethylmaleimide within the pH range 5–7 in 95% ethanol has been studied[295]. The reaction proceeds via the mechanism





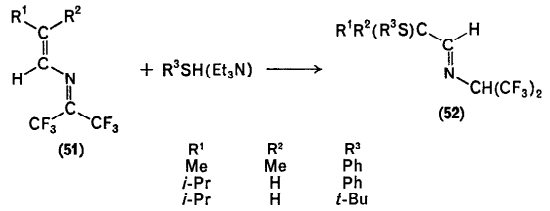
Attack by the neutral thiol could not be detected. The rate of attack of *ortho*-alkyl-substituted benzenethiolate anions upon the olefinic bond is sensitive to the bulk of the alkyl group. Two effects can be distinguished:
(1) inihibition of solvation of the thiolate anion, which increases its nucleophilicity (rate accelerating), and
(2) steric interference between the thiolate nucleophile and the olefin in the transition state (rate retarding). Net steric acceleration is observed in the nucleophilic addition to an activated double bond of *o*-*t*-butylbenzenethiolate which is an order of magnitude more reactive than the other alkylbenzenethiols studied. The implications of these results as regards hydrophobic bulk effects in enzymatic reactions involving mercaptide functions have been discussed[295].

The addition of the benzenethiolate anion to 4-*t*-butyl-1-cyanocyclohexene, containing formally a —C=C—C≡N bond system, occurs across the C=C bond. In ethanol two products are obtained both containing axial phenylthio groups, but in THF some equatorial SPh is also formed[296],

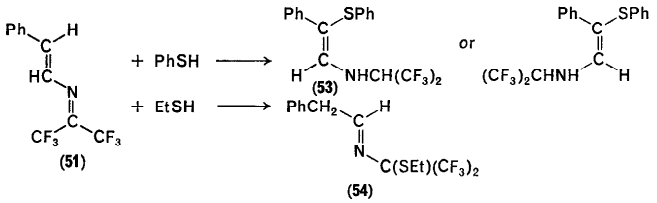


Addition reactions occur in C=C—C=N conjugated systems. Various products are formed in the reaction of thiols, in the presence of triethylamine, with N-[1,1,1,3,3,3-hexafluoroisopropylidene]-2,2-dialkylvinylamine (**51**)[297]. 1,4-Addition, forming products of orientation (**52**), occurs

with *t*-butanethiol and benzenethiol:



The reaction of benzenethiol with **51** when R^1 = Ph and R^2 = H gives an enamine **53**; a differently orientated 1,4-addition product **54** is obtained with ethanethiol.



Addition across a conjugated C=N—C=O system is the mechanism postulated for the reaction of the 2,2-dichlorovinylamine derivatives of RCONHCH=CCl_2 with BuSH in the presence of a small amount of alkaline, although the reaction appears superficially to be addition across a C=C bond:

$$MeCONHCH{=}CCl_2 + BuSH \longrightarrow MeCONHCH(SBu)CHCl_2$$

Initially a conjugated C=N—C=O system is formed and the thiol gives 1,4-addition[298]:

$$Cl_2C{=}CHNHCOMe \longrightarrow [CHCl_2CH{=}N{-}C(Me){=}O] \xrightarrow{BuSH/KOH} CHCl_2CH(SBu)N{=}C(Me)OH \longrightarrow CHCl_2CH(SBu)NHCOMe$$

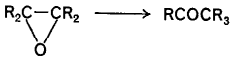
G. Reactions with Alkylene Oxides and Sulphides

Alkylene oxides undergo ring-opening reactions with a wide variety of substances:

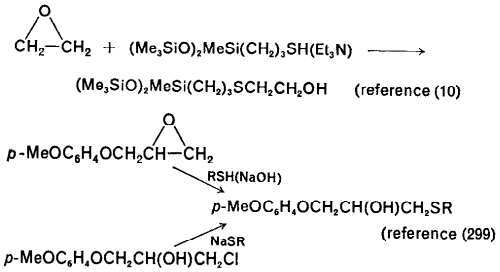
$$RCH{-}CH_2 + HA \longrightarrow RCHOHCH_2A \text{ or } RCH(A)CH_2OH$$

Under basic or neutral conditions when R is an electron-donating group, the main product is that formed by attack at the least substituted carbon atom, namely $RCHOHCH_2A$. The A^- ion probably attacks before the C—O bond is completely broken. Thiols react to form hydroxythioethers.

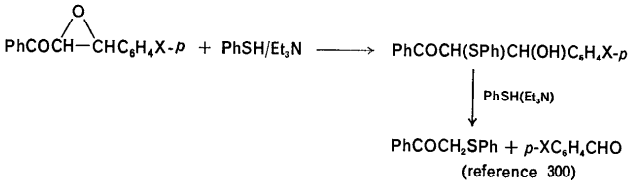
The alkylene oxides are thermally unstable and form the isomeric aldehydes or ketones:



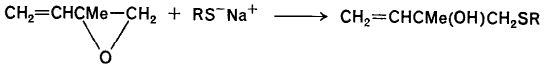
The products of the reactions of thiols or thiolate ions with alkylene oxides may correspond to addition across the C—O bond, or reactions of the isomeric aldehyde or ketone if the temperature is sufficiently high. Simple addition is observed in reactions such as



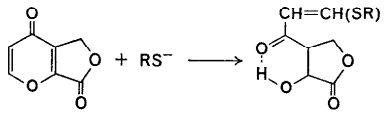
In other reactions both the C—O and C—C ring bonds are cleaved, and the intermediate product corresponding to addition across the C—O bond can be isolated:



The C—O ring is opened by thiolates, in preference to addition to the carbon—carbon double bond in the butylene derivative[301].

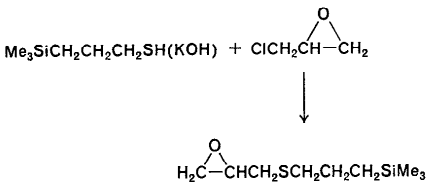


Large epoxide rings may also be opened by hydrogen sulphide[302,303]. The C—O bond is broken when dehydroisopatuline (**55**) reacts with thiolates[304], forming hydroxy-3-(*trans*-3-mercaptoacryloyl)but-2-en-4-olides (**56**).

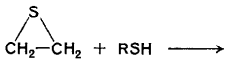


(**55**) (**56**)

In compounds containing both an epoxide ring and an aliphatic chloride, such as epichlorohydrin, the thiolate reacts preferentially with the aliphatic chlorine[305]

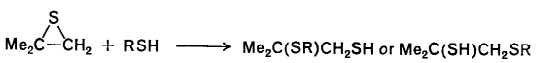


Various examples are known where an alkylene sulphide ring system is fractured by a thiol or thiolate, the reactions are essentially similar to those of the oxygen analogues. The thiol generated in the initial reaction may react further with the remaining cyclic sulphide[306].

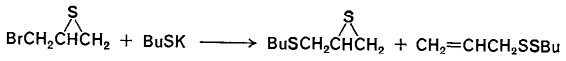


50-70% $RSCH_2CH_2SH$ and 20-30% $RSCH_2CH_2SCH_2CH_2SH$

Thiols can react with alkylene sulphides to form two products[263d],

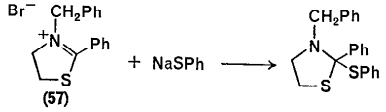


While thioepichlorhydrin reacts with potassium butanethiolate without ring fracture to form butylthioglycidyl sulphide, the products of the reaction with thioepibromohydrin include butylthioglycidyl sulphide and some of the disulphide, $CH_2{=}CHCH_2SSBu$, formed by fracture of the alkylene sulphide ring and subsequent dehydrobromination[307].



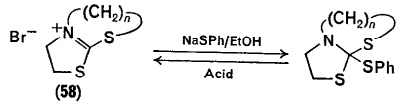
H. Reactions with Cyclic Compounds

Thiols will add across the C=N bond in cyclic systems, such as 3-benzyl-2-phenylthiazolinum bromide (**57**)[308],:



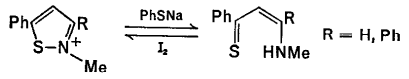
(**57**)

Similar addition occurs in the bicyclic compound (**58**):



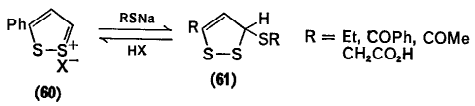
(**58**)

The benzenethiolate anion acts primarily as a reducing agent with 2-alkylisothiazolium salts (**59**); simple aliphatic thiols did not react[309].



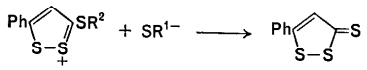
(**59**)

However, with 5-phenyl-1,2-dithiolium cation (**60**), unlike the isothiazolium cations, simple 5-adducts (**61**) were formed with a range of sulphur nucleophiles:



(**60**) (**61**)

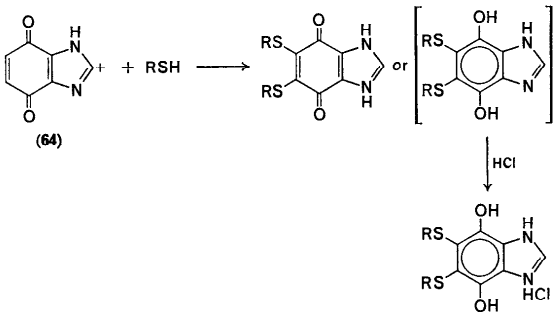
A similar 3-adduct was formed by only the ethanethiolate ion with 3,5-diphenyl-1,2-dithiolium salts. Other thiols, except ethanethiol, which did not react, convert to 3-alkylthio-5-phenyl-1,2-dithiolium cations (**62**) into 1,2-dithiole-3-thione (**63**).



(**62**) (**63**)

This is probably not a simple demethylation as no S-methylated nucleophiles were detected. The thione is also produced in the reaction of benzenethiolate anion with the 1,2-dithiolium cation with no S-alkyl substituent in the 3-position[309].

Two products, a disubstituted quinone or hydroquinone, are formed exclusively in the reaction of thiols with 4,7-benzimidazoledione (**64**) in methanol. The quinone is formed exclusively by aliphatic thiols and the hydroquinone when R=Ph, *p*-Tol, or $HOCH_2CH_2$[310].



(**64**)

IV. REFERENCES

1. G. E. Wilson and J. G. Riley, *Tetrahedron Lett.*, 379 (1972).
2. Yu. N. Kukushkin, V. V. Sibirskaya, S. D. Banzargashieva and O. I. Arkhangel'skaya, *Zh. Neorg. Khim.*, **17**, 1695 (1972); *Chem. Abstr.*, **77**, 96389 (1972).
3. C. D. Ritchie, *Accounts Chem. Res.*, **5**, 348 (1972).
4. C. D. Ritchie and P. O. I. Virtanen, *J. Amer. Chem. Soc.*, **94**, 4966 (1972)
5. F. Jellinek, *Proc. Chem. Soc.*, 319 (1959).
6. P. Robson, M. Stacey, R. Stephens and J. C. Tatlow, *J. Chem. Soc.*, 4754 (1960).
7. M. E. Peach, *Can. J. Chem.*, **46**, 2699 (1968).

8. M. E. Peach and A. M. Smith, *J. Fluorine Chem.*, in press.
9. G. A. Gornowicz and J. L. Speier, *Mech. React. Sulfur Compounds*, **3**, 53 (1968).
10. S. R. Wendel, *U.S. Pat.*, 3,691,222; *Chem. Abstr.*, **78**, 4355 (1973).
11. J. F. Arens, L. Brandsma, P. J. W. Schuijl and H. E. Wijers, *Quart. Rep. Sulfur Chem.*, **5**, 1 (1970).
12. D. S. Garwood and D. C. Garwood, *Tetrahedron Lett.*, 4959 (1970); *J. Org. Chem.*, **37**, 3804 (1972). (See also ref. 295.)
13. M. Verny, *Bull. Soc. Chim. Fr.*, 1942 (1970).
14. M. E. Peach and H. G. Spinney, *Can. J. Chem.*, **49**, 644 (1971).
15. Y. Takikawa and S. Takizawa, *Technol. Rep. Iwate Univ.*, **5**, 67 (1971); *Chem. Abstr.*, **77**, 125838 (1972).
16. Y. Takikawa and S. Takizawa, *Iwate Daigaku Kogakubu Kenkyu Hokoko*, **24**, 85 (1971); *Chem. Abstr.*, **77**, 151589 (1972).
17. S. D. Saraf, *J. Natur. Sci. Math.*, **11**, 127 (1971); *Chem. Abstr.*, **77**, 113948 (1972).
18. Z. A. Sadykhov and S. A. Gambarova, *Dokl. Akad. Nauk Azerb. SSR*, **27**, 35 (1971); *Chem. Abstr.*, **77**, 61415 (1972).
19. T. Mukaiyama, T. Endo, Y. Kojima and T. Sato, *J. Amer. Chem. Soc.*, **94**, 7575 (1972).
20. J. Burdon, P. L. Coe, C. R. Marsh and J. C. Tatlow, *J. Chem. Soc., Perkin Trans.*, **1**, 639 (1972).
21. F. A. Rustamov, G. K. Abdullaev, E. A. Agamalieva and D. Sultanova, *Uch. Zap. Azerb. Gos. Univ. Ser. Khim. Nauk*, 65 (1968); *Chem. Abstr.*, **72**, 110944 (1970).
22. V. G. Noskov and L. Z. Soborovskii, *Zh. Org. Khim.*, **7**, 2221 (1971); *Chem. Abstr.*, **76**, 13762 (1972).
23. R. D. Brasington and R. C. Poller, *J. Organomet. Chem.*, **40**, 115 (1972).
24. W. Wegener and P. Scholz, *Z. Chem.*, **12**, 137 (1972).
25. W. Wegener and P. Scholz, *Z. Chem.*, **11**, 20 (1971).
26. L. Maier, *Phosphorus*, **1**, 111 (1971); *Chem. Abstr.*, **76**, 34345 (1972).
27. L. Maier, *Phosphorus*, **1**, 245 (1972); *Chem. Abstr.*, **77**, 75267 (1972).
28. M. S. Khan and L. N. Owen, *J. Chem. Soc., Perkin Trans.*, **1**, 2060 (1972).
29. M. S. Khan and L. N. Owen, *J. Chem. Soc., Perkin Trans.*, **1**, 2067 (1972).
30. K. Hovius and J. B. F. N. Engberts, *Tetrahedron Lett.*, 2477 (1972).
31. K. Lucas, P. Weyerstahl, H. Marschall and F. Nerdel, *Chem. Ber.*, **104**, 3607 (1971).
32. A. M. van Leusen and J. C. Jagt, *Tetrahedron Lett.*, 967 (1970).
33. E. P. Grimsrud and J. W. Taylor, *J. Amer. Chem. Soc.*, **92**, 739 (1970).
34. D. J. McLennan and R. J. Wong, *J. Chem. Soc., Perkin Trans.*, **2**, 279 1972).
35. H. Hoffmann, A. Doerken and I. Hammann, *Ger. Offen.*, 2,034,539; *Chem. Abstr.*, **76**, 85580 (1972).
36. K. Yu Novitskii, N. K. Sadovaya, E. F. Kas'yanova and L. K. Semina, *Khim. Geterotsikl Soldin*, 412 (1970); *Chem. Abstr.* **73**, 25385 (1970).
37. S. K. Core and F. J. Lotspeich, *J. Org. Chem.*, **36**, 399 (1971).
38. V. R. Gaertner, *J. Org. Chem.*, **35**, 3952 (1970).
39. K. Ponsold and W. Ihn, *Tetrahedron Lett.*, 4121 (1972).
40. H. Boehme and H. Dehmel, *Arch. Pharm. (Weinheim)*, **304**, 403 (1971); *Chem. Abstr.* **75**, 76541 (1971).

41. R. Andrisano, A. Angeloni, P. de Maria, A. Fini and G. Salvadori, *Ric. Sci.*, **39**, 660 (1969); *Chem. Abstr.*, **73**, 25259 (1970).
42. T. Van Es, *Cabohyd. Res.*, **11**, 282 (1969).
43. G. Descotes and D. Sinou, *Bull. Soc. Chim. Fr.*, 4116 (1971).
44. B. Arbuzov and O. N. Nuretidinova, *Izv. Akad. Nauk SSSR, Ser, Khim.*, 2594 (1971); *Chem. Abstr.*, **76**, 12668 (1972).
45. T. Nakai and M. Okawara, *Bull. Chem. Soc. Japan*, **43**, 1864 (1970).
46. M. C. Verploegh, L. Donk, H. J. T. Bos and W. Drenth, *Recl. Trav. Chim. Pays-Bas*, **90**, 765 (1971).
47. P. Beltrame and P. L. Beltrame, *Gazz. Chim. Ital.*, **102**, 164 (1972); *Chem. Abstr.*, **77**, 18857 (1972).
48. F. Kai and S. Seki, *Meiji Seika Kentya Nempo*, **10**, 32 (1968); *Chem. Abstr.*, **72**, 31696 (1970).
49. G. Modena, *Accounts Chem. Res.*, **4**, 73 (1971).
50. Z. Rappoport, *Advan. Phys. Org. Chem.*, 7, 1 (1969).
51. P. Caubère and J. J. Brunet, *Bull. Soc. Chim. Fr.*, 2418 (1970).
52. R. Adams and A. Ferretti, *J. Amer. Chem. Soc.*, **81**, 4927 (1959).
53. J. Biougne and F. Theron, *C. R. Acad. Sci. C*, **272**, 858 (1971).
54. G. Capozzi, G. Melloni and G. Modena, *J. Chem. Soc.* (*C*), 2625 (1970).
55. G. Marchese and F. Naso, *Chim. Ind.* (*Milan*), **53**, 760 (1971); *Chem. Abstr.*, **75**, 98077 (1971).
56. R. Sauvetre and J. F. Normant, *Bull. Soc. Chim. Fr.*, 3202 (1972).
57. A. N. Mirskova, E. F. Zorina and A. S. Atavin, *Zh. Org. Khim.*, **8**, 1150 (1972); *Chem. Abstr.*, **77**, 125911 (1972).
58. E. T. McBee, E. P. Wesseler, R. Hurnaus and T. Hodgins, *J. Org. Chem.*, **37**, 1100 (1972).
59. D. V. Gardner and D. E. McGreer, *Can. J. Chem.*, **48**, 2104 (1970).
60. R. L. Soulen, D. B. Clifford, F. F. Crim and J. A. Johnston, *J. Org. Chem.*, **36**, 3386 (1971).
61. M. Verny, *Bull. Soc. Chim. Fr.*, 1946 (1970).
62. D.-S. Kwon and T. -R. Kim, *Daehan Hwahak Hwoejee*, **16**, 232 (1972); *Chem. Abstr.*, **77**, 139121 (1972).
63. F. Zanker and F. Reicheneder, *Ger. Offen.*, 2,032,709; *Chem. Abstr.*, **76**, 140252 (1972).
64. M. Watanabe, K. Taki and T. Murayama, *Japan Pat.*, 7,208,804; *Chem. Abstr.*, **77**, 5209 (1972).
65. A. Mellcr and W. Maringgelc, *Monatsh. Chem.*, **102**, 121 (1971).
66. J. Donovan, J. Cronin, F. L. Scott and A. F. Hegarty, *J. Chem. Soc., Perkin Trans.*, **2**, 1050 (1972).
67. G. Bartoli, L. Di Nunno, L. Forlani and P. E. Todesco, *Int. J. Sulfur Chem. Part C*, **6**, 77 (1971).
68. J. Miller, *Aromatic Nucleophilic Substitution*, Elsevier, Amsterdam and New York, 1968.
69. T. J. DeBoer and I. P. Dirkx in *Chem. Nitro Nitroso Groups* (Ed. H. Feuer), Interscience Publishers, New York, 1969, p. 487.
70. D. L. Hill, K. C. Ho and J. Miller, *J. Chem. Soc.* (*B*), 299 (1966).
71. J. F. Bunnett and W. D. Merritt, *J. Amer. Chem. Soc.*, **79**, 5967 (1957).
72. J. F. Bunnett and N. S. Nudelman, *J. Org. Chem.*, **34**, 2038 (1969).

73. P. Robson, T. A. Smith, R. Stephens and J. C. Tatlow, *J. Chem. Soc.*, 3692 (1963).
74. J. Miller and H. W. Yeung, *J. Chem. Soc., Perkin Trans.*, **2**, 1553 (1972).
75. M. R. Crampton, *Adv. Phys. Org. Chem.*, 7, 211 (1969).
76. M. J. Strauss, *Chem. Rev.*, **70**, 667 (1970).
77. M. R. Crampton, *J. Chem. Soc. (B)*, 1208 (1968).
78. M. R. Crampton, *J. Chem. Soc. (B)*, 2112 (1971).
79. M. R. Crampton and M. El Ghariani, *J. Chem. Soc (B)*, 1043 (1971).
80. J. Burdon, *Tetrahedron*, **21**, 3373 (1965).
81. W. Pritzkow, *Z. Chemie*, **10**, 330 (1970).
82. G. G. Yakobson, G. G. Furin, L. S. Kobrina and N. N. Vorozhtsov, *J. Gen. Chem. USSR*, **37**, 1221 (1967).
83. L. S. Kobrina, G. G. Furin and G. G. Yakobson, *J. Gen. Chem. USSR*, **38**, 505 (1968).
84. G. Guanti, C. Dell'Erba and D. Spinelli, *Gazz. Chim. Ital.*, **100**, 184 (1970); *Chem. Abstr.*, **73**, 13939 (1970).
85. M. Bosco, V. Calo, F. Ciminale, L. Forlani, L. Lopez, N. Efisio and P. E. Todesco, *Gazz. Chim. Ital.*, **101**, 685 (1971); *Chem. Abstr.*, **76**, 58649 (1972).
86. M. Blazejak and J. Haydn, *U.S. Pat.*, 3,560,573; *Chem. Abstr.*, **74**, 76174 (1971). Y. Takikawa, *Kogyo Kagaku Zacchi.*, **70**, 1384 (1967); *Chem. Abstr.*, **68**, 59210 (1968).
87. K. R. Langille and M. E. Peach, *J. Fluorine Chem.*, **1**, 407 (1971/2).
88. J. Burdon, V. A. Damodaran and J. C. Tatlow, *J. Chem. Soc.*, 763 (1964).
89. M. Kulka, *J. Org. Chem.*, **24**, 235 (1959).
90. I. Collins and H. Suschitzky, *J. Chem. Soc (C)*, 2337 (1969).
91. C. F. Smith, G. J. Moore and C. Tamborski, *J. Organometal. Chem.*, **42**, 257 (1972).
92. R. E. Banks, R. N. Haszeldine, D. R. Karsa, F. E. Pickett and I. M. Young, *J. Chem. Soc (C)*, 1660 (1969).
93. E. Ager, B. Iddon and H. Suschitzky, *J. Chem. Soc (C)*, 193 (1970).
94. L. J. Belf, M. W. Buxton and G. Fuller, *J. Chem. Soc.*, 3372 (1965).
95. J. Burdon, P. L. Coe, C. R. Marsh and J. C. Tatlow, *J. Chem. Soc., Perkin Trans.*, **1**, 763 (1972).
96. J. M. Birchall, R. N. Haszeldine and J. O. Morley, *J. Chem. Soc. (C)*, 456 (1970).
97. P. Caubère, *Bull. Soc. Chim. Fr.*, 3446 (1967).
98. P. Caubère, *Bull. Soc. Chim. Fr.*, 3451 (1967).
99. P. Caubère and M.-F. Hochu, *Bull. Soc. Chim. Fr.*, 2854 (1969).
100. P. Caubère and B. Loubinoux, *Bull. Soc. Chim. Fr.*, 3008 (1968).
101. Monsanto Co., *Brit. Pat.* 1,201,222; *Chem. Abstr.*, **73**, 109489 (1970).
102. Y. Takikawa and S. Takizawa, *Technol. Rep. Iwate Univ.*, **5**, 59 (1971); *Chem. Abstr.*, **77**, 101077 (1972).
103. J. S. Bradshaw, J. A. South and R. H. Hales, *J. Org. Chem.*, **37**, 2381 (1972).
104. G. G. Yakobson, G. G. Furin and L. S. Kobrina, *J. Gen. Chem. USSR*, **37**, 1217 (1967).
105. M. V. Shternshis and S. M. Shein, *Zh. Org. Khim.*, **8**, 1684 (1972); *Chem. Abstr.*, **77**, 139112 (1972).

106. J. H. Davies, E. Haddock, P. Kirby and S. B. Webb, *J. Chem. Soc. (C)*, 2843 (1971).
107. K. Adams, W. Reifschneider and M. D. Nair, *Croat. Chem. Acta*, **29**, 277 (1957); *Chem. Abstr.*, **53**, 16145 (1959).
108. H. von Dobeneck, T. Messerschmitt, E. Brunner and U. Wunderer, *Justus Liebigs Ann. Chem.*, **751**, 40 (1971).
109. M. Robba, M. C. Zaluski, B. Roques and M. Bonhomme, *Bull. Soc. Chim. Fr.*, 4004 (1969).
110. A. J. Newmann, Jr., *Diss. Abs. Int. B*, **32**, 6928 (1972).
111. R. A. Abramovitch, F. Helmer and M. Liveris, *J. Org. Chem.*, **34**, 1730 (1969).
112. D. Spinelli, G. Consiglio and C. A. Corrao, *Tetrahedron Lett.*, 4021 (1972).
113. D. H. Sim and S. Y. Jo, *Hwahah Kwa Hwahah Kongop*, 292 (1971); *Chem. Abstr.*, **77**, 125544 (1972).
114. D. H. Sim and S. Y. Jo, *Choson Minhujuui Inmin Konghwaguk Kwahagwon Tongbo*, 16 (1972); *Chem. Abstr.*, **77**, 139990 (1972).
115. S. M. Shein and K. V. Solodova, *Zh. Org. Khim.*, **6**, 1465 (1970); *Chem. Abstr.*, **73**, 98184 (1970).
116. F. Lieb and K. Eiter, *Justus Liebigs Ann. Chem.*, **761**, 130 (1972).
117. C. Dell'Erba, D. Spinelli and G. Leandri, *Gazz. Chim. Ital.*, **99**, 535 (1969); *Chem. Abstr.*, **72**, 12464 (1970).
118. C. Dell'Erba and G. Guanti, *Gazz. Chim. Ital.*, **100**, 223 (1970); *Chem. Abstr.*, **73**, 3721 (1970).
119. G. M. Kheifets, N. V. Khromov-Borisov and L. A. Gavrilova, *Zh. Org. Khim.*, **7**, 199 (1971); *Chem. Abstr.*, **74**, 99975 (1971).
120. L. F. Fieser and M. Fieser in *Reagents for Organic Synthesis*, Vol. I, Wiley, New York, 1967, p. 1106.
121. S. Hayashi, M. Furukawa, Y. Fujino, N. Ishii and Y. Kamijo, *Chem. Pharm. Bull. Tokyo*, **20**, 15 (1972).
122. M. Mori, *Kagaku No Ryoiki*, **25**, 872 (1971); *Chem. Abstr.*, **75**, 140376 (1971).
123. B. Castro and C. Selve, *Bull. Soc. Chim. Fr.*, 2296 (1971).
124. G. I. Feutrill and R. N. Mirrington, *Aust. J. Chem.*, **25**, 1719 (1972).
125. G. I. Feutrill and R. N. Mirrington, *Tetrahedron Lett.*, 1327 (1970).
126. G. I. Feutrill and R. N. Mirrington, *Aust. J. Chem.*, **25**, 1731 (1972).
127. B. Koutek and K. Setinek, *Collect. Czech. Chem. Commun.*, **33**, 866 (1968).
128. P. A. Bartlett and W. S. Johnson, *Tetrahedron Lett.*, 4459 (1970).
129. J. C. Sheehan and G. D. Davies, *J. Org. Chem.*, **29**, 2006 (1964).
130. W. R. Vaughan and J. B. Baumann, *J. Org. Chem.*, **27**, 739 (1962).
131. J. R. Whitaker, *J. Amer. Chem. Soc.*, **84**, 1900 (1962).
132. D. Olschwang, *Bull. Soc. Chim. Fr.*, 3354 (1971).
133. F. M. Stoyanovich, I. A. Ivanova and B. P. Fedorov, *Bull. Soc. Chim. Fr.*, 2013 (1970).
134. P. Dembech, A. Ricci, G. Seconi and P. Vivarelli, *J. Chem. Soc. (B)*, 2299 (1971).
135. P. C. Bax and W. Stevens, *Recl. Trav. Chim. Pays-Bas*, **89**, 265 (1970).
136. M. F. Shostakovskii, A. Kh. Filippova, E. I. Dubinskaya, V. K. Voronov and E. I. Brodskaya, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **90** (1972); *Chem. Abstr.*, 77, 5090 (1972).

137. R. C. Mehrotra, V. D. Gupta and D. Sukhani, *Inorg. Chim. Acta Rev.*, **2**, 111 (1968).
138. H. E. Jones, *Ger. Offen.*, 1,804,266; *Chem. Abstr.*, **72**, 54727 (1970).
139. H. E. Jones, *U.S. Pat.*, 3,663,624; *Chem. Abstr.*, **77**, 74822 (1972).
140. C. R. Lucas and M. E. Peach, *Inorg. Nucl. Chem. Lett.*, **5**, 73 (1969).
141. C. R. Lucas and M. E. Peach, *Can. J. Chem.*, **48**, 1869 (1970).
142. E. Weiss and U. Joergens, *Chem. Ber.*, **105**, 481 (1972).
143. R. A. Andersen, N. A. Bell and G. E. Coates, *J. Chem. Soc., Dalton Trans.*, 577 (1972).
144. G. E. Coates and A. H. Fiswick, *J. Chem. Soc. (A)*, 635 (1968).
145. G. E. Coates and B. R. Francis, *J. Chem. Soc. (A)*, 160 (1971).
146. G. E. Coates and J. A. Heslop, *J. Chem. Soc. (A)*, 631 (1968).
147. D. J. Brauer and G. D. Stucky, *J. Amer. Chem. Soc.*, **91**, 5462 (1969).
148. M. Schmidt and W. Siebert, *Allg. Prakt. Chem.*, **22**, 263 (1971).
149. R. H. Cragg and M. F. Lappert, *Organometal. Chem. Rev.*, **1**, 43 (1966).
150. A. Hass and M. Haeberlein, *Chem. Ztg.*, **96**, 412 (1972).
151. R. H. Cragg, *J. Chem. Soc. (A)*, 2962 (1968).
152. H. Vahrenkamp, *J. Organometal. Chem.*, **28**, 167 (1971).
153. J. J. Mielcarek and P. C. Keller, *Chem. Comm.*, 1090 (1972).
154. C. Glidewell, *J. Chem. Soc. (A)*, 823 (1971).
155. C. Glidewell and D. W. H. Rankin, *J. Chem. Soc. (A)*, 753 (1969).
156. J. T. Wang and C. H. van Dyke, *Inorg. Chem.*, **7**, 1319 (1968).
157. K. R. Langille and M. E. Peach, *Can. J. Chem.*, **48**, 1474 (1970).
158. W. E. Davidson, K. Hills and M. C. Henry, *J. Organometal Chem.*, **3**, 285 (1965).
159. R. C. Mehrotra, V. D. Gupta and D. Sukhani, *J. Inorg. Nucl. Chem.*, **29**, 83 (1967).
160. A. M. Kuliev, N. S. Kyazimov and G. A. Zeinalov, *Zh. Obsch. Khim.*, **39**, 557 (1969); *Chem. Abstr.*, **71**, 49441 (1969).
161. H. F. Angus, S. Cradock and E. A. V. Ebsworth, *Inorg. Nucl. Chem. Lett.*, **5**, 717 (1969).
162. H. Schumann and I. Schumann-Ruidisch, *J. Organometal Chem.*, **18**, 355 (1969).
163. G. H. Reifenberg and W. J. Considine, *Brit. Pat.*, 1,173,466; *Chem. Abstr.*, **72**, 43846 (1970).
164. P. Sartori and A. Golloch, *Chem. Ber.*, **103**, 3936 (1970).
165. P. Sartori and A. Golloch, *Chem. Ber.*, **104**, 967 (1971).
166. H. H. Sisler, N. K. Kotia and R. H. Highsmith, *J. Org. Chem.*, **35**, 1742 (1970).
167. A. J. Papa, *J. Org. Chem.*, **35**, 2837 (1970).
168. C. D. Ritchie and P. O. I. Virtanen, *J. Amer. Chem. Soc.*, **94**, 1589 (1972).
169. R. A. Shaw and M. Woods, *Phosphorus*, **1**, 41 (1971).
170. H. J. Emeléus and H. Pugh, *J. Chem. Soc.*, 1108 (1960).
171. R. A. N. McLean, *Inorg. Nucl. Chem. Lett.*, **5**, 745 (1969).
172. G. I. Drozd, S. Z. Ivin and M. A. Sokal'skii, *Zh. Obsch. Khim.*, **39**, 1177 (1969); *Chem. Abstr.*, **71**, 50072 (1969).
173. E. J. Behrman, M. J. Biallas, H. J. Brass, J. O. Edwards and M. Isaks, *J. Org. Chem.*, **35**, 3069 (1970).
174. E. E. Nifat'ev and I. V. Fursenko, *Vestn. Mosk. Univ., Khim.*, **12**, 245 (1971); *Chem. Abstr.*, **75**, 48598 (1971).

175. A. N. Pudovik, M. A. Pudovik, S. A. Terent'eva and V. E. Bel'skii, *Zh. Obsch. Khim.*, **41**, 2407 (1971); *Chem. Abstr.*, **76**, 140598 (1972).
176. C. S. Kraihanzel and C. M. Bartish, *J. Organometal. Chem.*, **43**, 343 (1972).
177. M. P. Osipova, G. Kamai and N. A. Chadalra, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1326 (1969); *Chem. Abstr.*, **71**, 81485 (1969).
178. N. A. Chadaeva, G. Kh. Kamai and K. A. Mamakov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1726 (1971); *Chem. Abstr.*, **76**, 25387 (1972).
179. R. C. Mehrotra, V. D. Gupta and S. Chatterjee, *Aust. J. Chem.*, **21**, 2929 (1968).
180. H. Schmidbaur and K. H. Mitschke, *Chem. Ber.*, **104**, 1842 (1971).
181. H. Schmidbaur and H. K. Mitschke, *Chem. Ber.*, **104**, 1837 (1971).
182. A. Haas and M. E. Peach, *Z. Anorg. Allg. Chem.*, **338**, 299 (1965).
183. M. E. Peach, *Int. J. Sulfur Chem.*, **8**, 27 (1973).
184. D. N. Harpp and D. K. Ash, *Int. J. Sulfur Chem., Part A*, **1**, 211 (1971).
185. R. J. Neil, M. E. Peach and H. G. Spinney, *Inorg. Nucl. Chem. Lett.*, **6**, 509 (1970).
186. D. L. J. Clive and C. V. Denyer, *Chem. Comm.*, 773 (1972).
187. T. Saegusa, Y. Ito and T. Shimizu, *J. Org. Chem.*, **35**, 2979 (1970).
188. D. A. Armitage, M. J. Clark and C. C. Tso, *J. Chem. Soc. Perkin Trans.*, **1**, 680 (1972).
189. D. N. Harpp, D. K. Ash, T. G. Back, J. G. Gleason, B. A. Orwig, W. F. van Horn and J. P. Sneyder, *Tetrahedron Lett.*, 3551 (1970).
190. K. S. Boustany and A. B. Sullivan, *Tetrahedron Lett.*, 3547 (1970).
191. A. B. Sullivan and K. Boustany, *Int. J. Sulfur Chem., Part A*, **1**, 207 (1971).
192. V. I. Dronov and N. V. Pokaneshchikova, *J. Org. Chem. (USSR)*, 2233 (1970).
193. J. L. Kice and J. D. Campbell, *J. Org. Chem.*, **36**, 2288 (1971).
194. R. D. Ritter and J. H. Krueger, *J. Amer. Chem. Soc.*, **92**, 2316 (1970).
195. J. L. Piette, R. Lysy and M. Renson, *Bull. Soc. Chim. Fr.*, 3559 (1972).
196. E. W. Lawless, *Inorg. Chem.*, **9**, 2796 (1970).
197. D. T. Sauer and J. M. Shreeve, *Chem. Comm.*, 1679 (1970).
198. C. T. Ratcliffe and J. M. Shreeve, *J. Amer. Chem. Soc.*, **90**, 5403 (1968).
199. M. E. Peach, *Int. J. Sulfur Chem.*, **8**, 151 (1973).
200. J. P. Danehy, *Int. J. Sulfur. Chem., Part C*, **6**, 159 (1971).
201. J. A. McCleverty, *Prog. Inorg. Chem.*, **10**, 49 (1968).
202. D. Coucouvanis, *Prog. Inorg. Chem.*, **11**, 233 (1970).
203. R. Eisenberg, *Progr. Inorg. Chem.*, **12**, 295 (1970).
204. J. A. McCleverty, Med. Tech. Publ. Co. Int. Rev. Sci., *Inorg. Chem., Ser. One* 1972, **2**, 301 (1972).
205. K. Tunaboylu and G. Schwarzenbach, *Helv. Chim. Acta*, **54**, 2166 (1971).
206. K. Tunaboylu and G. Schwarzenbach, *Helv. Chim. Acta*, **55**, 2065 (1972).
207. S. K. Srivastava and H. L. Nigam, *Curr. Sci.*, **41**, 601 (1972).
208. S. K. Srivastava, P. C. Srivastava and H. L. Nigman, *Indian J. Chem.*, **10**, 223 (1972); *Chem. Abstr.*, **77**, 52919 (1972).
209. S. A. Grachev, L. I. Shchelkunova and Yu. A. Makashev, *Zh. Neorg. Khim.*, **17**, 1364 (1972); *Chem. Abstr.*, **77**, 42569 (1972).
210. S. A. Grachev, L. I. Shchelkunova, Yu. A. Makeshev and J. S. Burzina, *Zh. Neorg. Khim.*, **17**, 1949 (1972); *Chem. Abstr.*, **77**, 118911 (1972).
211. E. W. Abel and B. C. Crosse, *J. Chem. Soc. (A)*, 1377 (1966).

212. D. A. Brown, W. K. Glass and B. Kumar, *J. Chem. Soc.* (*A*), 1510 (1969).
213. L. Markó and G. Bor, *J. Organometal. Chem.*, **3**, 162 (1965).
214. S. Cockle, H. A. O. Hill, S. Ridsdale and R. J. P. Williams, *J. Chem. Soc. Dalton Trans.*, 297 (1972).
215. Universal Oil Products, *Fr. Pat.* 1,595,726; *Chem. Abstr.*, **74**, 78073 (1971).
216. R. G. Pearson, H. Sobel and J. Songstad, *J. Amer. Chem. Soc.*, **90**, 319 (1968).
217. G. Faraone, V. Ricevuto, R. Romeo and M. Trozzi, *Inorg. Chem.*, **9**, 1525 (1970).
218. B. R. Hollebone and R. S. Nyholm, *J. Chem. Soc.* (*A*), 332 (1971).
219. W. Beck, K. H. Stetter, S. Tadros and K. E. Schwarzhans, *Chem. Ber.*, **100**, 3944 (1967).
220. R. S. Nyholm, J. F. Skinner and M. H. B. Stiddard, *J. Chem. Soc.* (*A*), 38 (1968).
221. C. R. Lucas, M. E. Peach and K. K. Ramaswamy, *J. Inorg. Nucl. Chem.*, **34**, 3267 (1972).
222. W. Beck, W. P. Fehlhammer, K. H. Stetter and S. Tadros, *Chem. Ber.*, **100**, 3955 (1967).
223. B. R. Hollebone, *J. Chem. Soc.* (*A*), 481 (1971).
224. A. Callaghan, A. J. Layton and R. S. Nyholm, *Chem. Commun.*, 399 (1969).
225. H. Köpf and M. Schmidt, *Z. Anorg. Allg. Chem.*, **340**, 139 (1965).
226. S. A. Giddings, *Inorg. Chem.*, **6**, 849 (1967).
227. R. B. King and N. Welcman, *Inorg. Chem.*, **8**, 2540 (1969).
228. M. E. Peach and H. G. Spinney, unpublished observation.
229. H. Köpf and B. Block, *Z. Naturforsch. b*, **23**, 1534 (1968).
230. H. Köpf, *J. Organometal. Chem.*, **14**, 353 (1968).
231. W. E. Douglas and M. L. H. Green, *J. Chem. Soc., Dalton Trans.*, 1796 (1972).
232. M. G. Harriss, M. L. H. Green and W. E. Lindsell, *J. Chem. Soc.* (*A*), 1453 (1969).
233. A. R. Dias and M. L. H. Green, *J. Chem. Soc.* (*A*), 1951 (1971).
234. A. R. Dias and M. L. H. Green, *J. Chem. Soc.* (*A*), 2807 (1971).
235. G. R. Davies and B. T. Kilbourn, *J. Chem. Soc.* (*A*), 87 (1971).
236. T. S. Cameron, C. K. Prout, G. V. Rees, M. L. H. Green, K. K. Joshi, G. R. Davies, B. T. Kilbourn, P. S. Braterman and V. A. Wilson, *Chem. Commun.*, 14 (1971).
237. P. S. Braterman, V. A. Wilson and K. K. Joshi, *J. Chem. Soc.* (*A*), 191 (1971).
238. P. S. Braterman, V. A. Wilson and K. K. Joshi, *J. Organometal. Chem.*, 31, 123 (1971).
239. P. S. Braterman and V. A. Wilson, *J. Organometal. Chem.*, **31**, 131 (1971).
240. M. Sato and T. Yoshida, *J. Organometal. Chem.*, **39**, 389 (1972).
241. M. Sato, F. Sato, N. Takemota and K. Iida, *J. Organometal. Chem.*, **34**, 205 (1972).
242. F. Sato, T. Yoshida and M. Sato, *J. Organometal. Chem.*, **37**, 381 (1972).
243. P. C. Ellgen and C. D. Gregory, *Inorg. Chem.*, **10**, 980 (1971).
244. R. B. King and A. Efraty, *Inorg. Chem.*, **10**, 1376 (1971).
245. J. A. McCleverty and T. A. James, *J. Chem. Soc.* (*A*), 1068 (1971).
246. A. T. McPhail and G. A. Sim, *J. Chem. Soc.* (*A*), 1858 (1968).
247. E. W. Abel and B. C. Crosse, *Organometal. Chem. Rev.*, **2**, 443 (1967).

248. H. D. Murdoch and R. Henzi, *J. Organometal. Chem.*, **5**, 552 (1966).
249. R. Havlin and G. R. Knox, *Z. Naturforsch. b*, **21**, 1108 (1966).
250. M. Ahmad, R. Bruce and G. R. Knox, *J. Organometal. Chem.*, **6**, 1 (1966).
251. W. Beck and S. Tadros, *Z. Anorg. Allg. Chem.*, **375**, 231 (1970).
252. M. H. B. Stiddard and R. E. Townsend, *J. Chem. Soc.* (*A*), 2719 (1970).
253. H. Behrens, E. Lindner and S. Birkle, *Z. Anorg. Allg. Chem.*, **369**, 131 (1969).
254. W. J. Schlientz and J. K. Ruff, *Inorg. Chem.*, **11**, 2265 (1972).
255. J. K. Ruff and R. B. King, *Inorg. Chem.*, **8**, 180 (1969).
256. R. B. King, *J. Amer. Chem. Soc.*, **85**, 1584 (1963).
257. J. Grobe, *Z. Anorg. Allg. Chem.*, **331**, 63 (1964).
258. J. A. McCleverty and D. Seddon, *J. Chem. Soc., Dalton Trans.*, 2588 (1972).
259. J. V. Kingston and G. R. Scollary, *J. Inorg. Nucl. Chem.*, **33**, 4373 (1971).
260. V. Kiener and E.-O. Fischer, *J. Organometal. Chem.*, **42**, 447 (1972).
261. E.-O. Fischer, M. Leupold, C. G. Kreiter and J. Müller, *Chem. Ber.*, **105**, 150 (1972).
262. K. Griesbaum, *Angew. Chem., Int. Ed. Engl.*, **9**, 273 (1970).
293. E. E. Reid, *Organic Chemistry of Bivalent Sulfur*, Chemical Publishing Co. Inc., New York. (a) Vol. 2, p. 13 (1969). (b) Vol. 4, p. 55 (1962). (c) Vol. 3, p. 148 (1960). (d) Vol. 3, p. 11 (1970).
264. P. DeMaria and A. Fini, *J. Chem. Soc.* (*B*), 2335 (1971).
265. P. DeMaria and M. Falzone, *Chim. Ind.* (*Milan*), **54**, 791 (1972); *Chem. Abstr.*, **77**, 151198 (1972).
266. K. S. Boustany and A. Jacot-Guillarmod, *Chimia*, **23**, 331 (1969); *Chem. Abstr.*, **71**, 112211 (1969).
267. F. Wolf and H. Finke, *Z. Chem.*, **12**, 180 (1972).
268. J. M. J. Tronchet and J. M. Boureois, *Helv. Chim. Acta*, **54**, 1718 (1971).
269. Sh. S. Kuliev, *Azerb. Khim. Zh.*, 74 (1970); *Chem. Abstr.*, **75**, 63054 (1971).
270. B. A. Trofimov, S. V. Amosova, A. S. Atavin, G. A. Kalabin, N. K. Gusarova and M. V. Ivanov, *Izv. Akad. Nauk SSSR Ser. Khim.*, 1947 (1971); *Chem. Abstr.*, **76**, 13687 (1972).
271. W. E. Truce and G. J. W. Tichenor, *J. Org. Chem.*, **37**, 2391 (1972).
272. T. G. Frey, *Diss. Abs., Int. B*, **32**, 3251 (1971).
273. A. I. Borisova, A. K. L. Filippova, V. K. Voronov and M. F. Shostakovskii, *Izv. Akad. Nauk SSSR, Ser. Khim*, 2498 (1969); *Chem. Abstr.*, **72**, 66538 (1970).
274. N. I. Gazieva, A. I. Shchekotikhin and V. A. Ginsburg, *Zh. Org. Khim.*, 7, 1815 (1971); *Chem. Abstr.*, **76**, 3349 (1972).
275. Yu. I. Porfir'eva, V. A. Konotopov and A. A. Petrov, *Zh. Org. Khim.*, **5**, 1914 (1969); *Chem. Abstr.*, **72**, 54622 (1970).
276. J. M. Surzur, C. Dupuy, M. P. Crozet and N. Aimar, *C. R. Acad. Sci., Ser. C*, **269**, 849 (1969).
276a R. Hazard and J. King, *Brit. Pat.*, 1, 291,865, *Chem. Abstr.*, **78**, 43276 (1973).
276b J. W. Batty, P. D. Howes and C. J. M. Stirling, *J. Chem. Soc., Perkin Trans.*, **1**, 59 (1973).
277. W. Gruber and P. Quis, *Ger. Offen.*, 1,543,424; *Chem. Abstr.*, **72**, 89822 (1970). *Ger. Offen.*, 1,231,235; *Chem. Abstr.*, **66**, 46116 (1967).
278. M. Masui, K. Suda, M. Yamauchi and C. Yijima, *J. Chem. Soc., Perkin Trans.*, **1**, 1955 (1972).
279. R. Marshall and D. M. Smith, *J. Chem. Soc.* (*C*), 3510 (1971).

280. M. W. Barker, S. C. Lauderdale and J. R. West, *J. Org. Chem.*, **37**, 3555 (1972).
281. L. Field and B. J. Sweetman, *J. Org. Chem.*, **34**, 1799 (1969).
282. L. Fournier, A. Natat, G. Lamaty, and J. P. Roque, *Recl. Trav. Chim. Pays-Bas*, **91**, 1015 (1972).
283. R. E. Barnett and W. P. Jencks, *J. Amer. Chem. Soc.*, **91**, 6758 (1969).
284. G. E. Leenhard and W. P. Jencks, *J. Amer. Chem. Soc.*, **88**, 3982 (1966).
285. J. Szabo, I. Varga, E. Vinkler and E. Barthos, *Acta Chim. (Budapest)*, **72**, 213 (1972); *Chem. Abstr.*, **76**, 153687 (1972).
286. A. M. Kuliev, A. K. Kyazim-Zadc, and K. Z. Guseinov, *Dokl. Akad. Nauk Azerb. SSR*, **27**, 20 (1972); *Chem. Abstr.*, **77**, 34071 (1972).
287. I. Shahak and J. Peretz, *Isr. J. Chem.*, **9**, 35 (1971).
288. H. Tokunaga, T. Kawashima and N. Inamoto, *Bull. Chem. Soc. Jap.*, **45**, 2220 (1972).
289. A. Takamizawa, K. Hirai and T. Ishiba, *Tetrahedron Lett.*, 437 (1970).
290. H. Esterbauer, *Monatsh. Chem.*, **101**, 782 (1970).
291. J. K. Sugden, J. E. Hogan and N. J. Van Abbe, *J. Chem. Soc. (C)*, 3875 (1971).
292. W. S. Powell and R. A. Heacock, *Experientia*, **28**, 124 (1972); *Chem. Abstr.*, **76**, 140396 (1972).
293. S. M. Kupchan, T. J. Giacobbe, I. S. Krull, A. M. Thomas, M. A. Eakin and D. C. Fessler, *J. Org. Chem.*, **35**, 3539 (1970).
294. Y. Maki and M. Suzuki, *Chem. Pharm. Bull.*, **20**, 832 (1972); *Chem. Abstr.*, **77**, 61922 (1972).
295. D. Semenow-Garwood, *J. Org. Chem.*, **37**, 3797 (1972).
296. R. A. Abramovitch, M. M. Rogić, S. S. Singer and N. Venkateswaran, *J. Org. Chem.*, **37**, 3577 (1972).
297. K. Bürger, G. George and J. Fehr, *Justus Liebigs Ann. Chem.*, **757**, 1 (1972).
298. A. N. Mirskova, E. F. Zorina and A. S. Atavin, *Zh. Org. Khim.*, **7**, 2221 (1971); *Chem. Abstr.*, **76**, 13715 (1972).
299. A. M. Kuliev, S. B. Bilalov, Z. E. Aliev and S. M. Agaeva, *Dokl. Akad. Nauk Azerb. SSR*, **27**, 24 (1971); *Chem. Abstr.*, **77**, 19287 (1972).
300. S. Ukai, K. Hirose, T. Hattori, M. Kayano, and C. Yamamoto, *Yakugaku Zasshi*, **92**, 278 (1972); *Chem. Abstr.*, **77**, 34207 (1972).
301. G. I. Zaitseva and V. M. Albitskaya, *Zh. Org. Khim.*, **5**, 612 (1969); *Chem. Abstr.*, **71**, 21638 (1969).
302. E. Tobler, *Ind. and Eng. Chem. Product Res. and Develop.*, **8**, 415 (1969).
303. W. Umbach, R. Mehren and W. Stein, *Fette, Seifen, Anstrichm.*, **71**, 199 (1969); *Chem. Abstr.*, **71**, 49175 (1969).
304. A. Caudet, J. Conquelet and R. Vessière, *C. R. Acad. Sci. Ser. C*, **272**, 107 (1971).
305. M. G. Voronkov and Z. I. Mikhailov, *Zh. Obsch. Khim.*, **42**, 615 (1972); *Chem. Abstr.*, **77**, 88583 (1972).
306. M. A. Korshunov, R. G. Kuzovleva and I. V. Furaeva, *Prom. Sin. Kauch. Nauch-Tekh. Sb.*, **No. 7**, 7 (1970); *Chem. Abstr.*, **77**, 125821 (1972).
307. O. N. Nuretdinova and B. A. Arbuzov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 550 (1972); *Chem. Abstr.*, **77**, 101303 (1972).
308. A. D. Clark and P. Sykes, *J. Chem. Soc. (C)*, 103 (1971).
309. P. Sykes and H. Ullah, *J. Chem. Soc., Perkin Trans.*, **1**, 2305 (1972).
310. L. C. March and M. M. Joullié, *J. Heterocycl. Chem.*, **7**, 249 (1970).

CHAPTER 17

Oxidation of thiols

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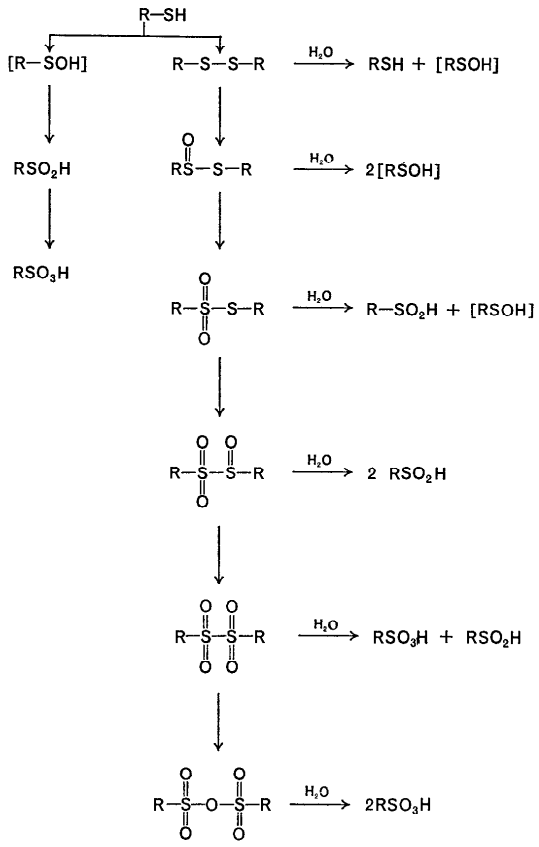
I. INTRODUCTION

Aliphatic and aromatic thiols are oxidized by a variety of reagents to disulphides and to higher oxidation products depending on the specific reaction conditions (Scheme 1).

The two oxidation chains are not as separate as indicated in the scheme since a number of interconversions are possible. They may be thought



SCHEME 1

to occur *via* the hydrolytic products which are shown on the right side of the Scheme. The sulphenic acid has been reported in brackets since its very high reactivity does not permit isolation except in very special cases (see section III.B).

Most of the reactions indicated in the scheme are reversible eventually through appropriate derivatives; however, true equilibria among pairs of the above-mentioned products are quite rare.

In this chapter we shall mainly deal with the oxidation of thiols to disulphides (equation 1).

$$2\,RSH \xrightarrow{[0]} RSSR + H_2O \qquad (1)$$

The subsequent stages of oxidation will be dealt with only in limited and specific cases. Attention has been mainly focused on the most commonly used chemical oxidizing agents.

Electrochemical and photochemical oxidations are also briefly discussed but for more comprehensive reviews on these subjects see the relevant chapters in this volume.

The literature, which is not comprehensively reviewed, has been covered up to the middle of 1972. References to some later published papers have been also made.

II. ELECTROCHEMICAL OXIDATION

Studies on the formally simple equilibrium (2) meet severe difficulties.

$$2\,RSH \rightleftharpoons RSSR + 2\,H^+ + 2\,e^- \qquad (2)$$

Polarographic studies (dropping mercury electrode, D.M.E.) have been limited by the chemical intervention of the mercury[1] whereas more recent work with noble metals electrodes has been hampered by absorption and/or passivation phenomena[2].

Much attention has been devoted to systems of biological interest and electrochemical methods for quantitative analysis of thiol and disulphide groups in simple organic compounds as well as in proteins have been reported[3-8].

The polarography of thiols is characterized by an anodic wave which often is well defined[1,9-11] although, as for instance in the case of cysteine, the shape of the polarogram depends strongly on pH and buffer[12]. Kolthoff and Barnum[12] showed that the anodic wave of cysteine is due to the formation of mercurous mercaptide (HgSR), i.e. to the oxidation of the electrode and not of the thiol. Complications may arise when the reaction product is insoluble in the medium and covers the electrode[12].

Thiols are also oxidized at a platinum electrode but at more positive potentials (see below).

The oxidation of the mercury electrode, the anodic potential of which is decreased by salt formation, appears to be quite general[1,9].

The values of $E_{\frac{1}{2}}$ do not change very much with the nature of the thiol at pH values high enough to ensure that all the thiols are in the anionic form[9] as shown in Table 1.

TABLE 1. Half-wave potential[a] and pK_a of mercaptans at pH 11·5[9]

Thiol	$E_{\frac{1}{2}}$ (volts)[b]	pK_a
2-Mercaptoethylguanidine	−0·508	8·8
2-Mercaptopropylguanidine	−0·534	9·4
2-Mercaptoethylamine	−0·560	10·75
2-Mercaptoethanol	−0·537	9·6
Thioglycollic acid	−0·580	10·68
Cysteine	−0·580	10·28
Glutathione	−0·480	9·12

[a] At the dropping mercury electrode (D.M.E.).
[b] Referred to standard calomel electrode (S.C.E.).

The reduction of disulphides on D.M.E., the reverse of equation (2), appears to be a simpler reaction and in some cases a single cathodic wave was observed which behaves as required by a reversible process[9,10]; however, in many other cases evidence for irreversible processes was found[1,9,10]. Moreover, in some conditions cystine[1,13] as well as other disulphides[14] present a pre-wave.

Whereas the wave at higher potential appears to be due to a diffusion-controlled process, the pre-wave, as also shown by oscillographic polarography studies[13,14], depends on the absorption and reaction of the disulphide at the electrode.

The following equations were proposed to explain the process:

$$RSSR + Hg \longrightarrow RSSR \cdot Hg\ (ads.) \quad (3)$$

$$RSSR \cdot Hg \longrightarrow (RS)_2Hg \quad (4)$$

$$(RS)_2Hg + 2\,e^- + 2\,H^+ \longrightarrow 2\,RSH \cdot Hg \quad (5)$$

$$2\,RSH \cdot Hg \longrightarrow 2\,RSH + Hg\ (desorb.) \quad (6)$$

On platinum or gold electrodes, aqueous solutions of disulphides are not reduced and only the oxidation of thiols could be studied.

It was observed that cysteine as well as other thiols[2,12,15,16] is oxidized, by a one-electron process, to cystine and the latter is further oxidized, probably, to cysteic acid. Strong absorption phenomena were observed.

In DMF solutions the redox reaction benzenethiol-diphenyl disulphide (equation 2, R = Ph) could be studied by cyclic voltammetry on an inert electrode from both directions. The results obtained indicate that the reactions are 'irreversible' and that the acid hydrogen of benzenethiol is converted to molecular hydrogen.

The authors[16] proposed that the following reactions occur at an inert electrode in solvents like DMF:

$$2\,RSH - 2\,e^- \longrightarrow RSSR + 2\,H^+ \quad (2)$$

$$2\,RS^- - 2\,e^- \longrightarrow RSSR \quad (7)$$

$$RSSR + 2\,e^- \longrightarrow 2\,RS^- \quad (8)$$

$$2\,RSH + 2\,e^- \longrightarrow 2\,RS^- + H_2 \quad (9)$$

It is noteworthy that diphenyl disulphide in the stated conditions[16] is not further oxidized, contrary to what is observed with cystine[2,12,15] in aqueous solutions.

However, at higher potentials the disulphide can be oxidized: in acetonitrile with sodium perchlorate as supporting electrolyte, diphenyl disulphide is oxidized to benzenesulphonic acid[17]. Possibly, in this case the perchlorate ion does intervene in a chemical reaction subsequent to the anodic process.

All schemes proposed for the oxidation of thiols to disulphides in a more or less explicit way imply the formation of thiyl radicals as intermediates.

The absence of any reaction of these radicals with the solvent suggests that the dimerization occurs at the electrode surface in a very fast process.

III. CHEMICAL OXIDATION

A. *Oxidation by Peroxidic Compounds*

The oxidation of thiols by hydrogen peroxide, alkyl hydroperoxides as well as peroxyacids is a well-known reaction in its qualitative aspects, but very little mechanistic study has been carried out[18,19].

The initially formed product is in most cases the corresponding disulphide, which can be easily oxidized further by excess oxidant.

A particular example of overoxidation is the oxidative desulphurization of heteroaromatic thiols by hydrogen peroxide which may lead to the

formation of the corresponding hydrocarbon[20, 21] or hydroxy derivative[22, 23] depending upon the reaction conditions.

Because of the easy overoxidation, these reactions are scarcely used for preparative purposes. However, the oxidation of thiols to disulphides by peroxides attracted some interest in the patent literature connected with the general problem of hydrocarbon sweetening. More recently interest was revived by the suggested use of hydrogen peroxide as a selective oxidant and control of sulphide odours in sewage treatments and similar applications[24].

Aliphatic and aromatic thiols are easily oxidized to disulphides in aqueous or alcoholic solutions under both acid and alkaline conditions[25, 26]. Higher molecular weight thiols are better oxidized as copper salts[27, 28]. Particularly in the presence of aliphatic amines the oxidation is easily carried out also in hydrocarbon solvents[29].

In hydrocarbons, and more generally in aprotic solvents, lower molecular weight aliphatic peracids are quite effective in oxidizing thiols to disulphides.

A mechanistic study[18, 30] of the oxidation of *o*-mercapto-phenylacetic acid in water in the pH range 2·44–7·17 by hydrogen peroxide showed that the rate of reaction (r) was independent of the thiol concentration, first order in H_2O_2 and inversely proportional to the square root of hydrogen ion concentration (equation 10).

$$r = k[H_2O_2]/[H^+]^{\frac{1}{2}} \qquad (10)$$

The suggestion that the reaction was catalysed by traces of heavy metal ions was advanced on the basis of the acceleration observed on addition of ferrous ions and the depression of rates when EDTA in excess was added. In this case the kinetic expression also changed becoming first order in thiol concentration (equation 11).

$$r = k[H_2O_2]\,[RSH]/[H^+]^{\frac{1}{2}} \qquad (11)$$

Further thorough studies would be necessary to define in detail the mechanism of these reactions. The limited evidence available is, however, consistent with the reasonable assumption that the reaction proceeds by a radical chain mechanism[31], probably initiated by heavy metal ions and involving thiyl radicals following a scheme similar to that proposed for the oxidation of mercaptans by molecular oxygen (see section IV).

The quite abundant literature on the oxidation of thiol groups in compounds of biological interest like glutathione, cysteine, etc., which has been recently reviewed[32], is also in line with the above conclusions.

B. Oxidation by Halogens

The products of the oxidation of thiols by halogens vary with the halogen and with the reaction medium.

In aqueous solvents chlorine and bromine react with thiols to give sulphonyl halides or sulphonic acids[33–36] (equations 12 and 13).

$$RSH + 3\,X_2 + 2\,H_2O \longrightarrow RSO_2X + 5\,HX \qquad (12)$$

$$RSH + 3\,X_2 + 3\,H_2O \longrightarrow RSO_3H + 6\,HX \qquad (13)$$

The same compounds are obtained starting with disulphides and there is evidence that at least in some conditions the latter are intermediates in the reaction (see below).

Under anhydrous conditions the following reactions have been observed[37] (equation 14–17):

$$RSH + X_2 \longrightarrow RSX + HX \qquad (14)$$

$$RSX + X_2 \rightleftharpoons RSX_3 \qquad (15)$$

$$RSX + RSH \longrightarrow RSSR + HX \qquad (16)$$

$$RSSR + X_2 \rightleftharpoons 2\,RSX \qquad (17)$$

Excess of halogen forms sulphur trihalides (equation 15). In the case of arylsulphur trichlorides the equilibrium is shifted to the left by increasing the temperature; with aliphatic derivatives containing a methylene group linked to sulphur the decomposition of the trichloride may lead to the formation of α-chlorinated sulphenyl chlorides[33] (equation 18).

$$RCH_2{-}SCl_3 \longrightarrow \underset{\displaystyle Cl}{\overset{\displaystyle |}{RCH{-}SCl}} + HCl \qquad (18)$$

Careful hydrolysis of alkyl or aryl sulphur trihalides, in particular trichlorides, yields either sulphinic acid or sulphinyl halide[33, 38, 39]. The latter is obtained in good yields by reacting the trihalide with the stoichiometric amount of acetic acid[40] (equation 19).

$$RSX_3 + CH_3COOH \longrightarrow RSOX + CH_3COX + HX \qquad (19)$$

The reaction of thiols with halogens in aprotic not nucleophilic solvents can be, possibly, represented as in equation (20).

$$R{-}SH + X_2 \rightleftharpoons \underset{\displaystyle X}{\overset{\displaystyle |}{[R{-}\overset{+}{S}{-}H}} + X^-] \longrightarrow RSX + HX \qquad (20)$$

Although there are no mechanistic studies in this area, schemes equivalent to equation (20) have been proposed for the halogenolysis of sulphides and other bivalent sulphur compounds[41-47]. The reaction goes to completion to the right with chlorine and bromine, but takes a more complex course with iodine. With fluorine the reaction yields higher oxidation products with extensive fluorination at the hydrocarbon moiety[48].

Sulphenyl halides are very prone to nucleophilic attack[33,35,49] (equation 21) and in particular excess mercaptan reacts with them to give the corresponding disulphide (equation 16).

$$RSX + NuH \longrightarrow RSNu + HX \qquad (21)$$

NuH = R_2NH, HSCN, ROH, RSH, etc.

The hydrolysis of sulphenyl halides is believed to form sulphenic acids (equation 22). These compounds, however, have never been isolated in this reaction; rather thiolsulphinate esters are formed by fast reaction of sulphenic acids with sulphenyl halides[50-53] (equation 23).

Disproportionation of sulphenic acids has also been suggested as a possible route for the formation of these compounds[49] (equation 24). The hydrolysis of sulphenyl halides under not carefully controlled conditions and particularly in concentrated solutions lead to disulphides and thiolsulphonates[54-59] because of the easy disproportionation of thiolsulphinates (equation 25).

$$RSX + H_2O \longrightarrow [RSOH] + HX \qquad (22)$$

$$[RSOH] + RSX \longrightarrow RS(=O)-S-R + HX \qquad (23)$$

$$2\,[RSOH] \longrightarrow R-S(=O)-S-R + H_2O \qquad (24)$$

$$2\,RS(=O)-S-R \longrightarrow R-S-S-R + RS-SO_2-R \qquad (25)$$

Some anthraquinone-sulphenic and -disulphenic acids and 1-methyluracil-4-sulphenic acid have been prepared by different routes[60-63]. In these compounds intramolecular hydrogen-bonded and tautomeric structures are suggested to stabilize the sulphenic derivatives[64-66]. Chemical and n.m.r. evidence for the existence of an aliphatic sulphenic acid in the

thermal decomposition of di-*t*-butyl sulphoxide has been reported[67] (equation 26).

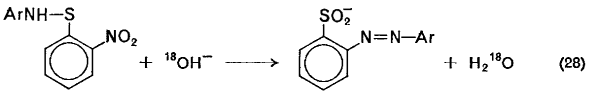
$$(CH_3)_3C-S(=O)-C(CH_3)_3 \longrightarrow (CH_3)_3CSOH + (CH_3)_2{=}CH_2 \qquad (26)$$

Sulphenyl halides have been considered for a long time as a source of sulphenyl cations[49] (equation 27). However, unambiguous evidence on free sulphenyl cations is scarce and somewhat contradictory.

$$RSX \rightleftharpoons RS^+ + X^- \qquad (27)$$

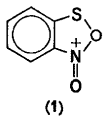
The substitutions at sulphenyl sulphur so far studied in detail occur *via* bimolecular mechanism[54] except, possibly, the very special case of 2,4,6-trimethoxybenzenethiol arylsulphonate which was reported to undergo unimolecular solvolysis[68].

On the other hand, evidence on the formation of a cationic species, thought to be the sulphenyl cation, by dissolving 2,4-dinitrobenzene sulphenyl chloride in concentrated sulphuric acid has been obtained[69-70]. However, the nature of the cation is not certain[71]. Moreover, the substrate chosen is, perhaps, not quite typical. Strong interaction between the *o*-nitro group and the sulphenyl sulphur are in fact shown by X-ray analysis of methyl *o*-nitrobenzenesulphenate ester[72] and also by the oxygen transfer from nitrogen to sulphur observed in the alkaline rearrangement of 2-nitrobenzenesulphenyl anilides[73] (equation 28).



(28)

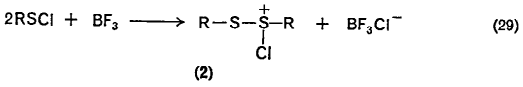
This suggests that in the special case of *o*-nitrobenzene derivatives and similar species the cation formed might have the cyclic structure (**1**).



(1)

Finally, it has been reported that sulphur dichloride and trichloromethanesulphenyl chloride give 1 : 1 and 2 : 1 complexes with Lewis acids ($SbCl_5$, $AlCl_3$, $FeCl_3$) with a salt-like behaviour[74,75]. The instability of the complexes made a full characterization unfeasible.

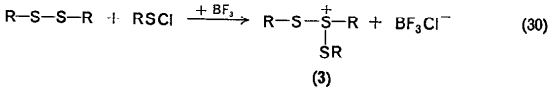
Relevant to this point is the recent finding[76,77] that methane and ethane sulphenyl chloride form by addition of either BF_3 or SbF_5 in liquid SO_2 a dimeric cationic species (**2**) described as follows (equation 29):



(29)

(2)

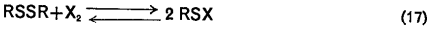
The same species seems to be formed in fluorosulphonic acid and 100% sulphuric acid as well[77]. Preliminary results also indicate that reaction 29 occurs with aromatic sulphenyl chlorides. The tendency of sulphur compounds to give species like (**2**) seems quite general: for example, disulphide and sulphenyl chloride in FSO_3H or 100% H_2SO_4 and in SO_2 with BF_3 or SbF_5 give a species analogous to (**2**)[77] (equation 30).



(30)

(3)

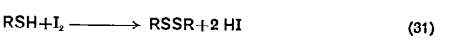
Furthermore, ions similar to (**3**) are postulated as intermediates in the interchange reaction of disulphides and sulphenyl chlorides[78,79], and intermediates like (**2**) should be involved in the reaction of disulphide with halogens (equation 17) which has to be considered an equilibrium reaction.



(17)

Equilibrium (17) is completely shifted to the right for X = Cl and largely to the left with X = I. The case of bromine is, as usually, intermediate. As a matter of fact very few sulphenyl iodides are known[80,81]. Apparently only sterically hindered derivatives are able to exist and also their stability, which may be reasonably great in dilute solution, is very low in concentrated solution or as pure material.

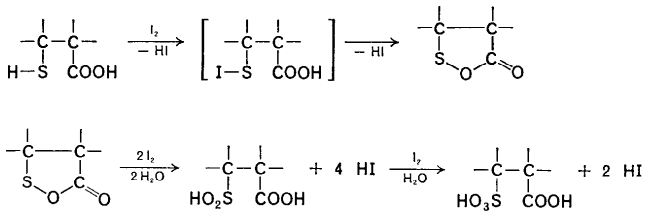
Since equilibrium (17) is shifted almost completely to the left in the case of X = I whereas reaction (14) goes to completion even with iodine, a method to titrate thiols based on the reaction in equation (31) has been



(31)

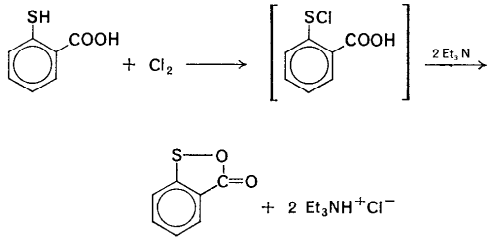
widely used. However, care has to be taken to use the appropriate conditions of pH and dilution to avoid overoxidation of the disulphide which may be a quite serious cause of error[82]. Thiols containing a β-carboxyl group are particularly susceptible to consume more than the iodine

required by equation (31). It was suggested that the carboxyl group intramolecularly attacks the initially formed sulphenyl iodide to form a sulphenic anhydride which may undergo further oxidation at sulphur (Scheme 2)[82,83]. This mechanism seems likely also in view of the recent



SCHEME 2

evidence of trapping *o*-sulphenobenzoic acid anhydride by reaction of *o*-mercaptobenzoic acid with chlorine in the presence of triethyl amine[84] (equation 32).



(32)

C. Oxidation by Dimethyl Sulphoxide and Other Sulphoxides

The oxidizing power of dimethyl sulphoxide (DMSO) as well as of other sulphoxides is well known and has been recently reviewed[85].

Yiannios and Karabinos[86] reported that thiols were selectively oxidized by DMSO to the corresponding disulphides in high yield with the concomitant reduction of DMSO to dimethyl sulphide (equation 33). Further

(33)

studies, mainly by Wallace and coworkers[87–91] confirmed these early results. They studied the reaction of several thiols with DMSO and TMSO

(tetramethylene sulphoxide) in large excess and in the absence of solvent. In the stated conditions second-order kinetics[89] and strong catalysis by added amines (Table 2) were observed[90].

TABLE 2. Effect of amines on the oxidation rate of 1-dodecanethiol by TMSO at 100°C [90]

Amine	pK_a [a]	k, s^{-1}	Rel. rate
—	—	$7{\cdot}58\times10^{-6}$	1
N,N-Dimethylaniline	5·1	$1{\cdot}15\times10^{-5}$	1·5
2,6-Dimethylpyridine	6·6	$1{\cdot}64\times10^{-5}$	2·2
1-*n*-Dodecylamine	10·6	$6{\cdot}42\times10^{-4}$	84·4
Tri-*n*-butylamine	11·4	$2{\cdot}04\times10^{-3}$	269

[a] Reference 92.

These authors showed[89] that the rate of oxidation depends on the acidity of thiol and a correlation between the estimated pK_a of them and the energy of activation was suggested (Table 3).

The oxidation rates depend also on the structure of sulphoxide[89] (Table 4). As shown in Figure 1, a linear correlation of $\log k_{obs}$ with the recently evaluated pK_a in water of sulphoxides[93,94] does hold.

TABLE 3. Effect of thiol acidity on the oxidation with TMSO at 100°C [89]

Thiol	pK_a	k, s^{-1}	Rel. rate	E_a, kcal/mole
1-Dodecanethiol	13·5	$7{\cdot}8\times10^{-6}$	1	19·4
α-Toluenethiol	10·5	$1{\cdot}9\times10^{-4}$	25	13·7
o-Toluenethiol	8	$6{\cdot}6\times10^{-3}$	850	6·2
Benzenethiol	7	$4{\cdot}0\times10^{-2}$	5186	4·9

TABLE 4. Effect of sulphoxide basicity in the oxidation of α-toluenethiol at 100°C [89]

Sulphoxide	pK_a	$\log k$ [a]	Rel. rate
Diphenyl sulphoxide	−2·54[b]	−5·91	1
Phenyl methyl sulphoxide	−2·27[b]	−5·11	6·22
DMSO	−1·80[b]	−4·40	33·3
TMSO	−1·31[c]	−3·71	159

[a] k, sec^{-1}.
[b] Reference 93.
[c] Reference 94.

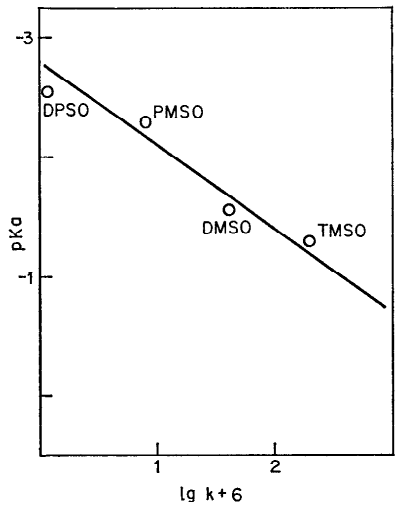
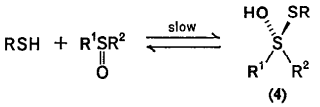


FIGURE 1. Correlation between the oxidation rates at 100°C of α-toluenethiol and the pK_a of the sulphoxides. pK_a values taken from the literature: diphenyl sulphoxide (DPSO), phenyl methyl sulphoxide (PMSO) and dimethyl sulphoxide (DMSO), reference 93; tetramethylene sulphoxide (TMSO), reference 94.

The authors[87–91] proposed that the slow step of the reaction is the formation of the adduct (**4**) (equation 34) followed by a fast reaction with a second molecule of thiol (equation 35). Similar mechanisms have been



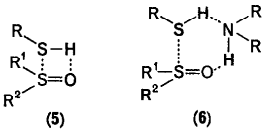
(34)

(35)

proposed for other sulphoxide-promoted oxidations[95–99] and the recent isolation of stable tetracoordinate sulphur compounds[100–103] makes this hypothesis quite likely.

However, the detailed mechanism could be more complicated as it is, in part, suggested by the phenomena of base and acid catalysis observed[90].

It may well be, as suggested[90], that four-centre (**5**) and five-centre (**6**) transition states are involved for the uncatalysed and amine-catalysed



(5) (6)

reactions, respectively. Alternatively an acid–base interaction of the reagents (equation 36) to give an ion pair, followed by collapse of the latter to the adduct (**4**) (equation 37) could be postulated. This

$$RSH + R_2SO \rightleftharpoons RS^- + R_2\overset{+}{S}OH \quad (36)$$

$$RS^- + R_2\overset{+}{S}OH \rightleftharpoons RS-\underset{R}{\overset{R}{\underset{|}{\overset{|}{S}}}}-OH \quad (37)$$

(4)

resembles the mechanisms proposed for some nucleophilic substitutions by thiols of 2-chlorobenzimidazole[104] and chloroquinolines[105].

The reaction of (**4**) with the thiol to give the products (equation 35) may also be more complicated than depicted[106]. However, any hypothesis would be highly speculative in the absence of more detailed kinetic studies.

The oxidation of thiols with sulphoxides presents several attractive features like the simplicity of the reaction, the high yield and the selectivity of disulphide formation. It has to be noticed, however, that tertiary thiols do not react with sulphoxides or they give very little disulphide even in the presence of amine catalysts. Reaction temperatures higher than 100°C give rise to extensive decomposition[91].

An interesting synthetic application of this reaction is the recovery of optically active sulphoxides from racemates when an optically active thiol is oxidized with more than the stoichiometric amount of the sulphoxide[107].

D. Oxidation by Other Organic Chemicals

Several organic compounds may oxidize thiols to disulphides or to products of further oxidation in a variety of experimental conditions. We shall briefly deal in this section with some of the more characteristic cases.

1. Diethyl azodicarboxylate

Diethyl azodicarboxylate oxidizes thiols to disulphides, in the dark at room temperature, with concomitant formation of diethyl hydrazodicarboxylate[108, 109] (equation 38).

$$EtOC(=O)-N=N-C(=O)OEt + 2RSH \longrightarrow RS-SR + EtOC(=O)-\underset{H}{N}-\underset{H}{N}-C(=O)OEt \quad (38)$$

The reaction may also be carried out in refluxing anhydrous solvents. In Table 5 the results obtained for the oxidation of several thiols are

TABLE 5. Oxidation of thiols to disulphides with diethyl azodicarboxylate[109]

Thiol	Solvent	Temperature[a]	Time, h	Yield, %
Ethanethiol	None	R	48	90
2-Propanethiol	None	R	72	70
2-Propene-1-thiol	None	R	0·5	90
1-*n*-Dodecanethiol	Benzene	B	5	95
t-Dodecanethiol	Benzene	B	10	70
Benzenethiol	None	R	24	90
4-Nitrobenzenethiol	Ethanol	B	8	—
2-Aminobenzenethiol	Benzene	B	4	67
2-Naphthalenethiol	Chloroform	B	5	87
2-Mercaptobenzothiazole	Benzene	B	0·5	95

[a] R = room temperature; B = refluxing solvent.

reported. It was reported[110] that triphenylphosphine catalyses the reaction. Formation of a charge transfer complex (**7**) with the azo derivative as formulated below (equation 39) was suggested. It seems likely that radicals or radical ions intervene in the reaction.

$$EtOC(=O)-N=N-C(=O)OEt + Ph_3P \longrightarrow [EtOC(=O)-N=N-C(=O)OEt] \rightarrow [?] \xrightarrow{2RSH}$$

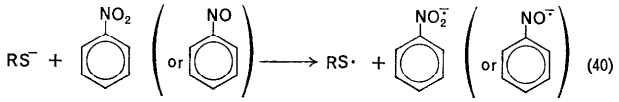
(7)

$$RSSR + Ph_3P + EtOC(=O)-\underset{H}{N}=\underset{H}{N}-C(=O)OEt \quad (39)$$

2. Nitroso and nitro-compounds

In basic medium thiols are oxidized to disulphides by nitrobenzene or nitrosobenzene[111-112] which are reduced mainly to azoxy and azobenzene.

E.s.r. experiments indicate the presence of stable radical anions derived by electron transfer from the thiol anion to the nitro or nitroso group (equation 40).



(40)

Other species may oxidize thiols to disulphides following a similar route. Among them azodicarbonamide[111], maleic anhydride[111] and 4-nitropyridine N-oxide[112] seem to be the most reactive ones.

3. Iodosobenzene

In refluxing dioxane, iodosobenzene and benzenethiol give rise to the formation of diphenyl disulphide in fairly good yield (76%)[113] (equation 41).

$$C_6H_5IO + 2\,PhSH \longrightarrow C_6H_5I + PhSSPh + H_2O \quad (41)$$

Although extensive studies have not been made on this reaction, it may represent a general and convenient method for thiol oxidation.

4. Trimethylsulphoxonium iodide

When benenethiol reacts with trimethylsulphoxonium iodide in dimethylformamide at 100°C, phenyl methyl sulphide, diphenyl disulphide and dimethyl sulphide are formed[114].

The reaction seems to be quite complex. Formation of a labile adduct between the oxonium salt and the thiol is suggested (equation 42).

$$(CH_3)_3\overset{O}{\overset{\|}{S}}{}^{+}I^- + PhSH \longrightarrow [(CH_3)_3\overset{O}{\overset{\|}{S}}{}^{+}PhS^-] + HI \quad (42)$$

Decomposition of this intermediate would lead to dimethyl sulphoxide and phenyl methyl sulphide (equation 43). Diphenyl disulphide should

$$[(CH_3)_3\overset{O}{\overset{\|}{S}}{}^{+}PhS^-] \longrightarrow CH_3\overset{O}{\overset{\|}{S}}CH_3 + PhSCH_3 \quad (43)$$

arise either from the reaction of the thiol with dimethyl sulphoxide (see section III.C) or from oxidation by iodine (see section III.B) generated in

the reduction of dimethyl sulphoxide by hydrogen iodide[93] (equations 44 and 45).

$$(CH_3)_2SO + 2\,HI \longrightarrow (CH_3)_2S + H_2O + I_2 \quad (44)$$

$$2\,PhSH + I_2 \longrightarrow PhSSPh + 2\,HI \quad (45)$$

5. Halogen transfer agents

Several 'positive halogen' compounds, ZHal, like N-halo-succinimide N-chlorobenzotriazole, dichloroiodobenzene, etc., react with thiols to give sulphenyl halides or disulphides depending on the relative ratios of the reagents[87] (equations 46 and 47).

$$ZHal + RSH \longrightarrow ZH + RSHal \quad (46)$$

$$ZHal + 2\,RSH \longrightarrow ZH + HHal + RSSR \quad (47)$$

Among these compounds, 2,4,4,6-tetrabromo-cyclohexa-2,5-dienone has been reported to be particularly selective[115].

E. *Oxidation by Metal Ions and Oxides*

Ions and oxides of transition metals which may exist in different valence states have been shown to oxidize thiols. Most of the studies so far available on this topic deal with the oxidation by ferric ions; careful investigations with many other metals have been carried out as well. The catalytic effect of these metal ions on the auto-oxidation of thiols has been pointed out (see section IV). The intervention of metals in a number of redox enzymes in which the metal is bound to a thiol group at the active site of the enzyme has been also suggested.

1. Ferric ion

Complexes of Fe^{3+} as $Fe(CN)_6^{3-}$ and ferric octanoate, $[Fe(Oct)_3]$ quantitatively oxidize thiols to disulphides in the absence of oxygen (equation 48). This reaction has been largely employed in the synthesis of synthetic rubber[116].

$$2\,RSH + 2\,Fe^{3+} \longrightarrow RSSR + 2\,Fe^{2+} + 2\,H^+ \quad (48)$$

Oxidation of thiols by $Fe(Oct)_3$ has been carried out in acetone and xylene[117]. Kinetic studies indicate that the reaction follows a second-order rate law. It is suggested that disulphide arises from dimerization of thiyl radicals which are formed in the rate-determining reaction of thiol with $Fe(Oct)_3$ (equations 49, 50).

$$2\,RSH + 2\,Fe(Oct)_3 \longrightarrow 2\,RS\cdot + 2\,Fe(Oct)_2 + 2\,OctH \quad (49)$$

$$2\,RS\cdot \longrightarrow RSSR \quad (50)$$

The intermediacy of such radicals is exemplified by the reaction in the presence of alkenes. In this case formation of sulphide, probably arising from a chain reaction, is observed (equations 49, 51, 52). At constant

$$RSH + Fe(Oct)_3 \longrightarrow RS\cdot + Fe(Oct)_2 + OctH \quad (49)$$

$$RS\cdot + R'CH{=}CH_2 \longrightarrow RSCH_2{-}\dot{C}HR' \quad (51)$$

$$RSCH_2{-}\dot{C}HR' + RSH \longrightarrow RSCH_2CH_2R' + RS\cdot \quad (52)$$

alkene and mercaptan concentrations the ratio of disulphide to sulphide formation decreases with decreasing metal ion concentration (Table 6).

TABLE 6. Effect of ferric octoanate concentration on the ratio disulphide : sulphide in the oxidation of 1-*n*-dodecanethiol[a] in the presence of 1-*n*-dodecene[117]

1-*n*-Dodecene / 1-*n*-Dodecanethiol	1-*n*-Dodecanethiol / $Fe(Oct)_3$	Dodecyl disulphide / Dodecyl sulphide
10	1	21
10	4	5·4
10	10	1·4
10	20	0·07

[a] 1-*n*-Dodecanethiol 0·2 M in xylene at 35°C.

It is suggested that this is related to the increased rate of formation and consequently the greater steady concentration of thiyl radicals at higher metal concentration which makes the dimerization reaction faster than the sequence of reactions leading to the sulphide.

Oxidation of thiols by $Fe(CN)_6^{3-}$ in alkaline and acid medium has been studied[118-122]. In both cases disulphide is the oxidation product; however, the reaction mechanism markedly differs. In the pH range 7–10·8 the rate of oxidation of *n*-octanethiol is pH dependent and exhibits a first-order dependence on $Fe(CN)_6^{3-}$, thiol and OH^-[118].

Cyanide ion depresses the rate but at higher cyanide concentration the rate of oxidation is practically independent from it.

Owing to the observed order in OH^- and since the rate increases with the pH, thiol anion is believed to be the reactive species.

Different mechanisms are proposed for this reaction depending upon the presence of added cyanide. A mechanism similar to that outlined in equations (49) and (50) is suggested for the oxidation in the presence of added cyanide, i.e. slow formation of thiyl radicals and fast formation of disulphide *via* dimerization of the radicals or further oxidation of them to

a cationic species (equation 53) which is neutralized by thiolate anion (equation 54).

$$RS\cdot + Fe(CN)_6^{3-} \longrightarrow RS^+ + Fe(CN)_6^{4-} \quad (53)$$

$$RS^+ + RS^- \longrightarrow RSSR \quad (54)$$

In the absence of added cyanide ion, a reversible substitution of a CN^- by an RS^- residue in the ferric complex has been postulated to be rate determining (equation 55).

Rapid decomposition of the sulphur-containing complex generates thiyl radical and pentacoordinate Fe^{2+} complex which reacts with the CN^- to give the ferrocyanide complex (equations 56 and 57).

Disulphide is then formed according to equation (50) or (53) and (54). Kinetic studies[119-122] of acid oxidation of thiols by ferricyanide, suggest

$$Fe(CN)_6^{3-} + RS^- \underset{\text{slow}}{\rightleftharpoons} [Fe(CN)_5RS]^{3-} + CN^- \quad (55)$$

$$[Fe(CN)_5RS]^{3-} \xrightarrow{\text{fast}} Fe(CN)_5^{3-} + RS\cdot \quad (56)$$

$$Fe(CN)_5^{3-} + CN^- \xrightarrow{\text{fast}} Fe(CN)_6^{4-} \quad (57)$$

that the reaction mechanism is quite complex. The rate law shows a second-order dependence on the $Fe(CN)_6^{3-}$ concentration and first on that of the thiol[119-121]. Inhibition by small amounts and catalysis by higher concentration of $Fe(CN)_6^{4-}$ is observed; the rate of oxidation is also dependent on the initial ferricyanide concentration and on the pH.

Several mechanisms[119-122] have been proposed for the acid oxidation of thiols by ferricyanide ions but since they are not fully established, we will not report them in detail.

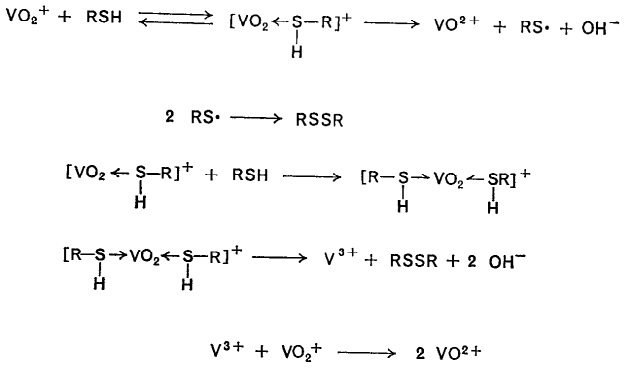
2. Other metal ions

Like ferric ions, other heavy metal ions in their higher oxidation states react with thiols to give the corresponding disulphides. Quite frequently complexation of thiols with the metal occurs followed by a one-electron transfer to give thiyl radicals which dimerize to disulphide. This is the case, for example, with Ce^{4+}, Co^{3+} and V^{5+} ions in acid solution[123-125].

The homolitic nature of such reactions was confirmed by an e.s.r. study of the Ce^{4+} oxidation of several thiols which showed the presence of thiyl radicals among other radical species. Thus primary thiols give a 1 : 2 : 1 triplet signal, secondary a 1 : 1 doublet and tertiary a single absorption line[126].

The nature and the stability of the complex formed depends upon the metal[123-125]. In the V^{5+} oxidation for instance, kinetic evidence and

formation of more than one mole of base suggest the intervention of two different complexes both leading to the disulphide but following separate paths[125] (Scheme 3).



SCHEME 3

The importance of the nature and stability of the complexes between metal ions and thiols is clearly indicated in the case of the oxidation with Mo^{5+} and Mo^{6+} of thioglycollic acid, cysteine and glutathione[127,128].

A detailed study shows that the kinetic equation may change with pH and with metal concentration as well as with the particular thiol. Indeed the mechanism of the reaction is not unique although some of the differences of the reaction features could be explained on the basis of different stability and nature of the complexes formed in the early stages of the reaction.

Other reaction paths are available at least in some special cases. For instance in the oxidation with manganic acetylacetonate[129], disulphide is believed to arise from reaction of a sulphenium ion with the thiol (equation 58) which implies that further oxidation of thiyl radicals to

$$RSH + RS^+ \longrightarrow RSSR + H^+ \quad (58)$$

sulphenium ion is faster than dimerization. The intervention of thiyl radicals has been ruled out by the absence of addition products when the reaction is carried out in the presence of alkenes.

The difference in mechanism between the Fe^{3+} and the Mn^{3+} oxidation of thiols is probably due to the powerful ability of the latter in oxidizing the radical first formed[130].

Oxidation by cupric complexes in non-polar media is a more complex reaction, as shown by the formation of sulphide together with disulphide[117]. The former may arise from cupric thiolate (equation 59) or *via* desulphurization of the disulphide by copper ions.

$$Cu(SR)_2 \longrightarrow CuS + RSR \quad (59)$$

Lead tetraacetate is also able to oxidize thiols at low temperature to disulphides[131–134].

High yield of disulphide is obtained when one mole of lead tetraacetate is allowed to react with two moles of thiol[132] (equation 60).

$$2\,RSH + Pb(OAc)_4 \longrightarrow RSSR + Pb(OAc)_2 + 2\,AcOH \quad (60)$$

When the lead salt–thiol ratio is 0·25, lead mercaptide is formed together with disulphide and acetic acid[134] (equation 61).

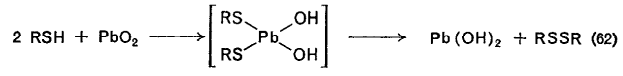
$$4\,RSH + Pb(OAc)_4 \longrightarrow RSSR + Pb(SR)_2 + 4\,AcOH \quad (61)$$

Higher temperature and the presence of alcohols would cause further oxidation of the disulphide and formation of sulphinic esters[133].

3. Metal oxides

A large variety of metal oxides like MnO_2, PbO_2, CrO_3, Fe_2O_3, Co_2O_3, CuO[134–136] oxidize thiols to disulphides at low temperature in chloroform or xylene solution.

In the oxidation by lead dioxide, formation of an intermediate by addition of two molecules of thiol to the metal oxide has been suggested[134]. It may give the disulphide by decomposition (equation 62), or generate an intermediate lead tetramercaptide which decomposes giving disulphide (equations 63 and 64).



$$[(RS)_2Pb(OH)_2] + RSH \longrightarrow [Pb(SR)_4] + 2\,H_2O \quad (63)$$

$$[Pb(SR)_4] \longrightarrow RSSR + Pb(SR)_2 \quad (64)$$

Manganese dioxide is the most effective oxidizing agent among the above-mentioned oxides.

The nature of such reactions has been checked for MnO_2, Fe_2O_3, Co_2O_3, by carrying out the oxidation in the presence of an alkene. Formation of large amounts of thiol addition products to the double bond suggests intermediacy of thiyl radicals.

It was also observed that the rate of stirring affects the rate of the oxidation which suggests that the reaction is a diffusion-controlled process. Under these circumstances the greater ability of MnO_2 in oxidizing thiols is probably due to surface effects and more favourable absorption of thiols[136].

IV. OXIDATION BY MOLECULAR OXYGEN

The easy oxidation of thiols on exposure to air is well known as is the sensitivity of this reaction to catalysts[137] like metal ions, u.v. light and other initiators of radical reactions. It is also known that autooxidation of thiols is accelerated by bases.

The interest in this reaction from the industrial (sweetening of crude petroleum) and biological points of view notwithstanding, the mechanism of the autooxidation of thiols is not as yet satisfactorily understood.

We shall attempt in this section to review critically the more significant contributions, with the interpretations of the phenomena as offered by the authors.

A. *Catalysis by Strong Bases*

Cullis and coworkers[138] studied the oxidation of ethanethiol in aqueous alkaline solution under constant pressure of oxygen. They observed low reproducibility of the oxygen uptake rates even when careful precautions were taken to avoid the presence of adventitious impurities. Under their conditions (EtSH 0·3–0·5 M; NaOH 0·5–2 M; the base always in excess) the stoichiometry of the reaction was found, in agreement with other authors[137, 139, 140], to be:

$$4\,RSH + O_2 \longrightarrow 2\,RSSR + 2\,H_2O \qquad (65)$$

Dependence on the first power of both the oxygen pressure and the base concentration was also observed. The order in thiol was found to be about one at the beginning, decreasing to zero as the reaction progressed. The oxygen uptake rates were faster at the beginning and reached a stationary value after 20–30% reaction. Apparently the change in order with respect to thiol as well as the change in rate depends on the disulphide formed. Indeed, disulphide added at zero time suppresses the typical features of the initial reaction (Table 7). It is not clear which is the effect of disulphide. It is insoluble in water and hence a two-phase system results as soon as minor amounts of this product is formed. Partition of thiol between the two phases may be important and, possibly, be involved in the observed order in the base. With the minimum of base added, however, the thiol

should be already fully in the anionic form and hence an excess of base should not affect the rates.

The authors[138] emphasize the point that they cannot exclude even in their conditions that trace metal catalysis may still be active. Indeed the addition of sequestering agents like EDTA (ethylenediamine tetra-acetic

TABLE 7. Effect of diethyl disulphide and metal ion sequestering agents on the oxidation of ethanethiol[a 138]

[EtSH], M	[Compound added]	M	Initial rate, mole l^{-1} s^{-1}	Final steady rate, mole l^{-1} s^{-1}
0·5	—	—	$1·7 \times 10^{-8}$	$1·0 \times 10^{-6}$
0·5	EDTA[b]	0·1	$3·3 \times 10^{-8}$	$2·0 \times 10^{-6}$
0·5	EN[c]	0·1	Undetectable	$1·1 \times 10^{-6}$
0·5	EtSSEt	0·5	—	$2·0 \times 10^{-6}$
0·0	EtSSEt	0·5	—	—[d]
0·5	EtSSEt	0·5	—	$1·1 \times 10^{-6}$
0·0	KCN + EtSSEt	0·25 + 0·5	—	—[d]
0·5	KCN	0·25	$1·2 \times 10^{-6}$	$2·3 \times 10^{-7}$ [e]

[a] Oxygen pressure 700 mm Hg; temperature, 30°C.
[b] EDTA, ethylenediaminetetra-acetic acid.
[c] EN, ethylenediamine.
[d] Oxygen uptake *ca.* 0.
[e] Oxygen uptake after 10 days *ca.* 300%.

acid) and EN (ethylenediamine) causes contradictory results. Furthermore, added cyanide ion gives slower rates of oxygen uptake, and the reaction no longer yields disulphide but products of more profound oxidation (Table 7).

The same authors[138] studied, in the same conditions, the oxidation of a number of thiols and found the following sequence of reactivity:

$$n\text{-HexSH} > i\text{-BuSH} > n\text{-BuSH} > \text{EtSH} > \text{PhCH}_2\text{SH} > s\text{-BuSH} > \text{PhSH} > t\text{-BuSH}$$

The sequence does not appear simple. Steric effects could, perhaps, be responsible for the low reactivity of *t*-BuSH and electronic effects for that of benzenethiol. However, the authors' suspicions that the sequence could be partially determined by different amounts of adventitious catalytic impurities deserves careful attention.

The first-order dependence of the initial rate on thiol concentration as well as the base catalysis would indicate that thiolate ions play a particular role in the reaction.

Wallace and coworkers[140-143] had reached similar conclusions by studying the oxidation rates of several thiols. They also observed that the solvent has a quite large effect, which, in a general way, may be explained on the same basis. As shown in Table 8, the rate increases quite steadily on passing from alcoholic to non-protic and to dipolar aprotic solvents.

TABLE 8. Solvent effect on the oxidation rate of *n*-butanethiol[140]

Solvent	k, s^{-1} [a]	Rel. rate
Methanol	$5{\cdot}4\times10^{-5}$	1
Tetrahydrofuran	193×10^{-5}	36
Dioxane	482×10^{-5}	89
Diglyme	538×10^{-5}	100
Dimethylacetamide	1560×10^{-5}	289
Dimethylformamide	1795×10^{-5}	332

[a] 23·5°C, constant oxygen pressure 1 atm.

From data on relative rates of oxidation in methanol, ethanol and *t*-butanol in the presence of the corresponding alkoxides (Table 9) a correlation of the rate of oxidation with the pK_a of the alcohol was inferred[140]. However, on changing the cation, large variations in rates were observed (Table 9), strongly suggesting that ion-pairing phenomena are involved.

TABLE 9. Oxidation of *n*-butanethiol in alcoholic solvents at 23·5°C by molecular oxygen (1 atm)[140]

Alcohol	Base[a]	pK_a	k, s^{-1}
Methanol	NaOMe	15·5[b]	$5{\cdot}4\times10^{-5}$
Methanol	KOMe	—	$52{\cdot}2\times10^{-5}$
Ethanol	NaOEt	15·9[b]	$9{\cdot}6\times10^{-5}$
t-Butanol	NaOBu-*t*	19·2[c]	$35{\cdot}0\times10^{-5}$
t-Butanol	KOBu-*t*	—	$57{\cdot}8\times10^{-5}$
t-Butanol	RbOBu-*t*	—	$321{\cdot}7\times10^{-5}$
t-Butanol	CsOBu-*t*	—	$798{\cdot}3\times10^{-5}$

[a] Two-fold excess in respect to *n*-butanethiol.
[b] Reference 144.
[c] Reference 145.

All these facts are interconnected in the sense that both the size of the cation and the cation-solvating power of dipolar aprotic solvents have the effect of disrupting ion pairs and hence rendering the thiolate ion more basic. The protic solvents, on the other hand, by hydrogen-bonding thiolate ions behave in the opposite way.

This latter point is illustrated[142] by the effect of added methanol on the oxidation rates of *n*-butanethiol in dimethylformamide (DMF) and di-(2-methoxyethyl)ether (diglyme) (Table 10).

TABLE 10. Effect of added methanol on the oxidation of *n*-butanethiol in DMF and diglyme at 23·5°C by molecular oxygen (1 atm)[142]

Methanol, %	DMF, %	Diglyme, %	k, s^{-1} [a]	Rel. rate
—	100	—	$1{\cdot}8\times10^{-2}$	334
25	75	—	$6{\cdot}1\times10^{-3}$	114
50	50	—	$1{\cdot}1\times10^{-3}$	21·3
75	25	—	$1{\cdot}5\times10^{-4}$	2·8
90	10	—	$6{\cdot}5\times10^{-5}$	1·2
—	—	100	$5{\cdot}4\times10^{-3}$	100
25	—	75	$2{\cdot}3\times10^{-3}$	43
50	—	50	$6{\cdot}1\times10^{-4}$	11
65	—	35	$1{\cdot}0\times10^{-4}$	1·9
100	—	—	$5{\cdot}4\times10^{-5}$	1

[a] Sodium methoxide as base.

The above results lead the authors[140-142] to propose the following scheme (Scheme 4) for the overall reaction:

$$RSH + B^- \rightleftharpoons RS^- + BH \quad (66)$$

$$RS^- + O_2 \longrightarrow RS\cdot + O_2^{\cdot-} \quad (67)$$

$$RS^- + O_2^{\cdot-} \longrightarrow RS\cdot + O_2^{2-} \quad (68)$$

$$2\,RS\cdot \longrightarrow RSSR \quad (69)$$

$$2\,O_2^{2-} + 2\,BH \longrightarrow \tfrac{1}{2}\,O_2 + 2\,B^- + 2\,OH^- \quad (70)$$

SCHEME 4

This scheme gives rise to some doubts which will be discussed further below. However, we wish to point out that reaction (70) is not essential in its present form since the protonation of O_2^{2-} would give H_2O_2. It, in turn, will be quickly destroyed by excess of mercaptan.

Large excess of base[146] and/or prolonged reaction times causes oxidation beyond the disulphide level in aqueous solutions. This phenomenon is more pronounced in dipolar aprotic solvents[141,147] where sulphonic acids

are produced together with minor amounts of disulphides (Table 11). However, disulphides are again the dominant product when a protic solvent is added (Table 12). No kinetic measurements were made for this

TABLE 11. Effect of solvent, base and temperature on the oxidation of *n*-butanethiol[141]

Solvent	Base[a]	Temperature, °C	Conversion of thiol, mole %, (time, h)	Sulphonic acid, mole %, in product	Disulphide, mole %, in product
HMPA[b]	KOH	23·5	97 (24·5)	95	3
HMPA	KOH	23·5	95 (21·5)	95	1
HMPA	KOH	80	100 (23)	100	—
HMPA	KOH	80	99 (6)	96	1
HMPA	NaOH	23·5	97 (24)	90	8
HMPA	NaOH	80	90 (18·5)	92	1
DMF[b]	KOH	23·5	98 (17·5)	88	9
DMF	NaOH	23·5	94 (18·5)	67	24
Tetra-methyl-urea	KOH	23·5	93 (23)	64	28
Pyridine	KOH	80	83 (18)	20	64

[a] Ratio base/thiol = 4.
[b] HMPA = hexamethylphosphoramide, DMF = dimethylformamide.

TABLE 12. Effect of added water on the product distribution in the oxidation of *n*-butanethiol in HMPA[a] at 23·5°C[141]

H_2O, vol. %	Thiol conversion, %	Sulphonic acid, mole % in product	Disulphide, mole % in product
10	96	54	41
20	99	48	52

[a] HMPA = hexamethylphosphoramide; constant oxygen pressure 1 atm.; ratio KOH/thiol = 4; reaction time = 5 h.

reaction since the systems were always heterogeneous, but based on the rate of oxygen uptake for several mercaptans (Figure 2), the order of reactivity seems to be *n*-butyl > phenyl > 2,2-di-*n*-pentyl-1-hexyl. This parallels the order of reactivity found for the oxidation in hydroxylic solvents[138,142,146]. It was suggested[141,147] that sulphonic acids derive from

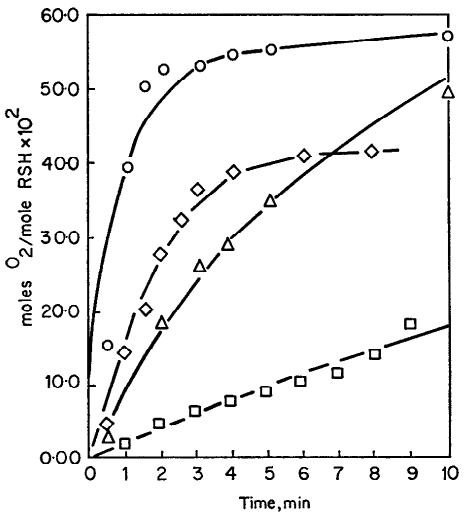


FIGURE 2. Effects of temperature and thiol structure on the oxygen uptake in HMPA (hexamethylphosphoramide)[141]. ○ 1-C_4H_9SH in KOH/HMPA (23·5°); △ C_9H_5SH in KOH/HMPA (23·5°); □ 1-$C_{16}H_{33}SH$ in KOH/HMPA (23·5°); ◇ 1-$C_{16}H_{33}SH$ in KOH/HMPA (80°). Reproduced by permission of the author and editor from *Tetrahedron*, **21**, 2271 (1965).

disproportionation of sulphenate ions formed by nucleophilic displacement at the S—S bond of the disulphide[148] (Scheme 5). This mechanism is

$$RS{-}SR + OH^- \rightleftharpoons RSOH + RS^- \quad (71)$$

$$RSOH + OH^- \rightleftharpoons RSO^- + H_2O \quad (72)$$

$$3\,RSO^- \longrightarrow RSO_3^- + 2\,RS^- \quad (73)$$

SCHEME 5

supported by the fact that disulphide may undergo base-catalysed oxidation in the same solvent system (Table 13) and that increasing amounts of water added to the aprotic solvent markedly favour the formation of disulphide (Table 12 and Figure 3). The protic component of the solvent, decreasing the activity of the base, would inhibit the

TABLE 13. Base-catalysed oxidation of disulphides in HMPA[a] [141]

Disulphide	Temperature, °C	Disulphide conversion, %	Time, h	Sulphonic acid, mole %	Thiol, mole %
Di-*n*-butyl disulphide	23·5	98	41	92	3
Di-*n*-butyl disulphide	80	96	45	97	—
Diphenyl disulphide	23·5	98	22	88	—
Diphenyl disulphide	80	98	22·5	99	—
Di-*o*-tolyl disulphide	80	98	23	98	—

[a] HMPA = hexamethylphosphoramide, ratio KOH/disulphide = 8.

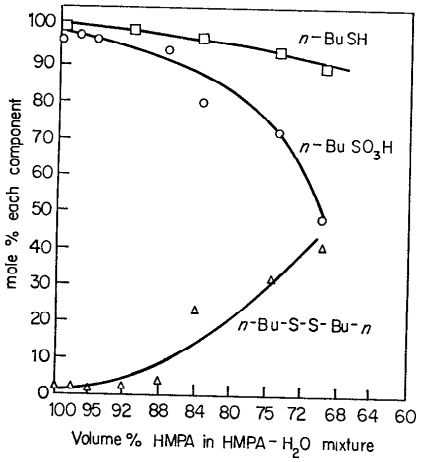


FIGURE 3. Effects of added water on thiol conversion and molar product distribution in the oxidation of *n*-butanethiol in HMPA at 80°C [141]. Ratio KOH/RSH = 4, reaction time 5 h. Reproduced by permission of the author and editor from *Tetrahedron*, **21**, 2271 (1965).

nucleophilic displacement at the disulphide linkage which is responsible for the further oxidation to sulphonic acid.

There is not, however, general agreement with this explanation. Indeed, direct oxidation of mercaptide ion to sulphonic acid was proposed by Berger[149] who considers the formation of disulphide as a side reaction.

Most of the work dealt with the oxidation of *n*-octanethiol but a few other thiols were briefly studied. The reactions were carried out in *t*-butanol with potassium *t*-butoxide as base under the assumption that in this solvent trace metal contaminations are less likely.

The oxidation under 1 atm pressure of oxygen gave sulphinic and sulphonic acids together with variable amounts of disulphide depending on the concentration of the base (Figure 4). Increasing amounts of base

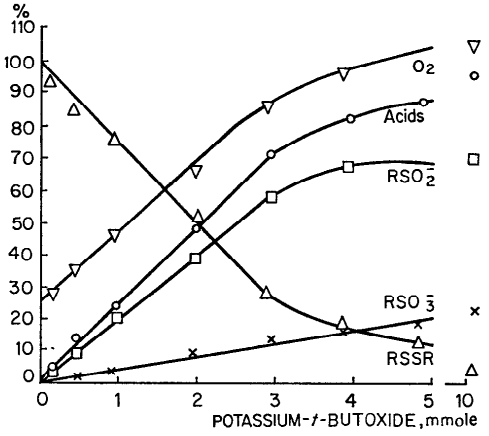


FIGURE 4. Oxidation of *n*-octanethiol in *t*-butanol at 25°C. Dependence of product distribution at complete conversion on potassium *t*-butoxide concentration[149]. *n*-Octanethiol 0·25 M (3 mmoles in 12 ml of *t*-BuOH); the products formed and oxygen uptake are referred to the mercaptan as (mmoles product/mmoles RSH) × 100; 'acids' refer to the sum of RSO_2^- and RSO_3^-. Reproduced by permission from *Rec. Trav. Chim.*, **82**, 773 (1963).

decrease the percentage of disulphide in the final products, thus suggesting a dependence of the distribution of products upon the extent of ionized mercaptan. Formation of disulphide and higher oxidation products are indeed processes which progress at different rates. Oxidation of *n*-octanethiol in the presence of insufficient base shows that in the earlier reaction stages formation of disulphide occurs almost quantitatively. This is even more evident for the oxidation of benzenethiol in which diphenyldisulphide is the only oxidation product up to 20–25% of reaction (Figure 5).

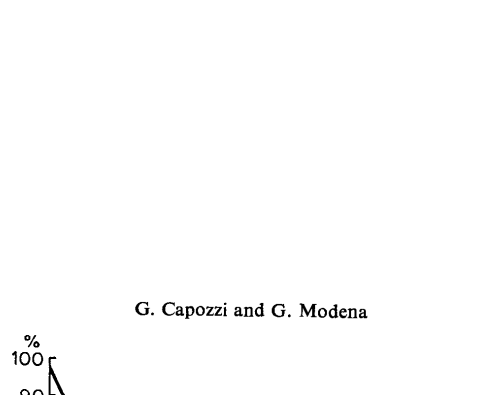


FIGURE 5. Oxidation of benzenethiol in *t*-butanol at 50°C. Distribution of products as a function of time. Benzenethiol 0·17 M; potassium *t*-butoxide 0·11 M; oxygen pressure 1 atm. Reproduced by permission from *Rec. Trav. Chim.*, **82**, 773 (1963).

Catalytic effects on the oxidation of benzenethiol by anthraquinone-1-sulphenic acid, *t*-butyl hydroperoxide and phenyl benzenethiolsulphinate have been observed. It was taken as evidence that sulphenate ion is a key intermediate in the reaction chain leading to the oxidized products. Indeed the above reagents may give rise to the sulphenate ion by ionization or by oxidation (equation 74) or by nucleophilic displacement[54] (equation 75).

$$RS^- + R'OOH \longrightarrow RSO^- + R'OH \qquad (74)$$

$$RS(\text{O})SR + RS^- \longrightarrow RSO^- + RSSR \qquad (75)$$

The overall reaction was rationalized[149] on the basis of Scheme 6. The results reported above and other observations including an analysis of the reaction kinetics lead the author[149] to suggest that the first step is the formation of a peroxysulphenate ion in the triplet state (equation 76) which may react with undissociated thiol, when present, to give, ultimately, disulphide (equation 77).

$$RS^- + O_2 \rightleftharpoons \underset{(\mathbf{8})}{[RSOO^{-*}]} \qquad (76)$$

$$[RSOO^{-*}] + 4\,RSH \longrightarrow RS^- + 2\,H_2O + 2\,RSSR \qquad (77)$$

Alternatively, by an intersystem crossing, **8** gives rise to a peroxysulphenate ion (**9**) which then initiates a chain reaction, probably a short chain, as reported in Scheme 6.

Initiation

$$RS^- + O_2 \rightleftharpoons (\mathbf{8}) \longrightarrow \underset{(\mathbf{9})}{RSOO^-} \qquad (78)$$

$$RSOO^- + RS^- \longrightarrow 2\,RSO^- \qquad (79)$$

Propagation

$$RSO^- + O_2 \longrightarrow RS(\text{=O})\text{—}OO^- \qquad (80)$$

$$RS(\text{=O})\text{—}OO^- + RS^- \longrightarrow RSO^- + RSO_2^- \qquad (81)$$

Termination

$$RS(\text{=O})\text{—}OO^- + RSO^- \longrightarrow 2\,RSO_2^- \qquad (82)$$

$$RS(\text{=O})\text{—}OO^- + RSO_2^- \longrightarrow RSO_2^- + RSO_3^- \qquad (83)$$

$$RSOO^- + RSO_2^- \longrightarrow RSO^- + RSO_3^- \qquad (84)$$

SCHEME 6

The above outlined scheme leads to the conclusion that completely ionized thiols would give exclusively sulphinic and sulphonic acids; nevertheless, the experimental results indicate formation of *ca.* 5% of disulphide in the oxidation of potassium benzenethiolate even with base in large excess. Since formation of disulphide would require the presence of undissociated thiol, other mechanisms must be operative. Again it is possible that the intervention of trace metal catalysis in the oxidation reaction has to be taken into account. Cullis, Hopton and Trimm[138] reported that copper ions in concentrations as low as 10^{-7} M are still active as catalysts and indeed it is very hard to detect metal ions at such low concentrations and to exclude adventitious impurities of this order of magnitude.

Another puzzling point of the mechanisms proposed to explain the autooxidation of thiols in basic solutions (in particular see Scheme 4) is the assumption that mercapto radicals dimerize almost quantitatively without interacting with the solvents in which the reaction was studied.

Although the dimerization of thiyl radicals has been found to be very fast (10^9–10^{10} M^{-1} sec^{-1})[150] the very low concentration of such species could still make the search for an alternative path to disulphide formation rewarding. It may be worth mentioning that Caspari and Granzow[151] observed that mercapto radicals generated by flash photolysis in aqueous solutions give rise to a radical ion, possibly by interaction with an ionized thiol molecule (equation 85).

$$RS\bullet + RS^- \rightleftharpoons RS\overset{\bullet}{\underset{-}{S}}R \qquad (85)$$

Similar radical anions have been observed[150] as transient species in the reaction of various disulphides with hydrated electrons (equation 86) which eventually decay to give thiyl radicals and mercaptide ions (equation 85 from right to left).

$$RSSR + e^- \longrightarrow RS\overset{\bullet}{\underset{-}{S}}R \qquad (86)$$

A related observation was reported by Zweig and Hoffmann[152] who observed a one-electron reduction of naphthalene 1,8-disulphide, contrary to the more usual two-electron reduction of disulphides (see Section II) and also that the radical anion generated from this disulphide with sodium in 1,2-dimethoxyethane has an ESR spectrum characterized by a single line with 1·04 gauss separation from peak to peak, $g = 2{\cdot}0110$. The electrochemical generation of the same radical partially resolves the line into an overlapped 1 : 2 : 1 triplet, $a_H = 0{\cdot}4$ gauss. The lack of coupling of the unpaired electron with the aromatic π system indicates that the electron is localized on sulphur. This, in turn, suggests that disulphide radical ions may be a relatively long-living species and hence reaction intermediates. Indeed, under special experimental conditions[151] or with special geometrical constrictions[152] they live long enough to be physically detected.

B. Catalysis by Aliphatic Amines

Thiols and in particular aromatic thiols are acids strong enough to be partially transformed into their conjugate base by amines. It follows that the oxidation of thiols by molecular oxygen, which is much faster on the anion than on the undissociated thiol (see section III.A), may be catalysed by aliphatic amines acting simply as base (see, however, section III.D).

These catalysts have been used[29] in the oxidation of thiols in hydrocarbon solvents in which amines, but not the more basic alkali hydroxides, are soluble.

The hypothesis that the amine-catalysed oxidation of thiols is a particular case of the more general reaction of oxidation by molecular oxygen of thiolate ions is confirmed by the finding that arene-thiols, which are more acidic and hence more dissociated, are oxidized faster than arylalkane- and alkane-thiols in the presence of amines[143].

A special case of combination of amine catalysis and solvent effect is given[153] by the easy oxidation of aliphatic and aromatic thiols in tetramethylguanidine which acts both as base and as a dipolar aprotic solvent (see Table 14).

TABLE 14. Oxidation of thiols to disulphides in tetramethylguanidine at 23·5°C [a] [153]

Thiol	Disulphide yield, %	Reaction time, h
n-Propanethiol	82	19
i-Propanethiol	82	19
n-Pentanethiol	82	19
Cyclohexanethiol	72	16
α-Toluenethiol	12	43
Benzenethiol	80	19

[a] Constant oxygen pressure 1 atm.

C. Catalysis by Metal Ions

The addition of heavy metal salts to the basic aqueous solution of thiols increases the rate of oxygen uptake[154, 155] as shown in Table 15. It may be easily realized that the catalytic activity varies with the metal ion. The oxidation gives, except for very special cases (see below), only disulphide without any contamination by products of further oxidation (Table 16). The stoichiometric relation of one mole of oxygen for four moles of thiol has always been observed (equation 65).

The results reported in Table 15 have to be considered to be only qualitative; indeed many of the metal ions listed give in the reaction medium slightly soluble oxides and hence formation of precipitates is observed. The addition of thiols to these non-homogeneous solutions causes changes in the amount, colour and possibly nature of the insoluble material. In some cases the nature of the precipitate formed was investigated; in particular $Co(SC_2H_5)_3$, $Pd(SC_2H_5)_2$, $TlSC_2H_5$, $Ni(SC_2H_5)_2$ and $(C_2H_5S)_3Ni(OH)$ were identified in the oxidation of C_2H_5SH catalysed by Co^{2+}, Pd^{2+}, Tl^+ and Ni^{2+} respectively.

TABLE 15. Effect of metal ions on the oxidation of ethanethiol[a] [154]

Metal ion	Salt	Thiol conversion, %, after 1·5 h	$-d[O_2]/dt$ mole $l^{-1} s^{-1}$ [b]
—	—	—	$1·7 \times 10^{-6}$
Ce^{4+}	$(NH_4)_2Ce(NO_3)_6$	12·8	$3·1 \times 10^{-6}$
UO_2^{2+}	$UO_2(NO_3)_2 \cdot 6H_2O$	11·8	$2·9 \times 10^{-6}$
VO^{2+}	$VOSO_4$ + aq·	11·5	$2·6 \times 10^{-6}$
Cr^{3+}	$Cr_2(SO_4)_3 \cdot K_2SO_4 \cdot 24\ H_2O$	6·4	$2·1 \times 10^{-6}$
Mo^{6+}	$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	13·9	$3·2 \times 10^{-6}$
W^{6+}	$Na_2WO_4 \cdot 2\ H_2O$	14·3	$3·4 \times 10^{-6}$
Mn^{2+}	$MnSO_4 \cdot 4\ H_2O$	11·4	$4·6 \times 10^{-6}$
Fe^{2+}	$FeSO_4 \cdot 7\ H_2O$	11·4	$3·6 \times 10^{-6}$
Fe^{3+}	Haemin (Fe = $1·5 \times 10^{-3}$ M)	90·0	$26·8 \times 10^{-6}$
Co^{2+}	$CoSO_4 \cdot 7\ H_2O$	35·7	$12·8 \times 10^{-6}$
Ni^{2+}	$NiSO_4$	45·7	$11·9 \times 10^{-6}$
Pd^{2+}	$PdCl_2$	4·8	$1·5 \times 10^{-6}$
Pt^{4+}	$PtCl_4$	12·2	$2·6 \times 10^{-6}$
Cu^{2+}	$CuSO_4 \cdot 5\ H_2O$	96·7	$26·8 \times 10^{-6}$
Ag^+	$AgNO_3$	5·7	$1·7 \times 10^{-6}$
Zn^{2+}	$ZnSO_4 \cdot 7\ H_2O$	17·5	$3·9 \times 10^{-6}$
Cd^{2+}	$3\ CdSO_4 \cdot 8\ H_2O$	13·9	$3·2 \times 10^{-6}$
Hg^{2+}	$HgCl_2$	6·1	$2·0 \times 10^{-6}$
Al^{3+}	$Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24\ H_2O$	11·8	$3·0 \times 10^{-6}$
Tl^+	Tl_2SO_4	10·3	$2·4 \times 10^{-6}$
Sn^{2+}	$SnCl_2 \cdot 2\ H_2O$	15·4	$2·2 \times 10^{-6}$

[a] Metal ion = 1×10^{-3} M unless otherwise stated; ethanethiol = 0·5 M; NaOH = 2 M; constant oxygen pressure, 700 mm Hg at 30°C.
[b] Rate of oxygen uptake.

TABLE 16. Oxidation of thiols catalyzed by copper, cobalt and nickel sulphate[a] [155]

Thiol	Copper[b]		Cobalt		Nickel	
	90% of reaction, h	Disulphide yield, %[c]	90% of reaction, h	Disulphide yield, %[c]	90% of reaction, h	Disulphide yield, %[c]
EtSH	1	100	4·5	101	4	96
n-BuSH	1·5	101	>10	—	15	102
i-BuSH	1·5	102	6	100	12	99
s-BuSH	2	100	8	98	>10	—
t-BuSH	>10	—	>10	—	>10	—
n-HexSH	1·5	104	5	101	4·5	101
PhSH	>10	—	>10	—	>10	—
$PhCH_2SH$	3	98	>10	—	>10	—

[a] Reaction conditions as in Table 15.
[b] 1×10^{-5} M.
[c] Referred to thiol.

It was suggested[156, 157] that a contribution to the catalysis could be given by undissolved metal complexes. However, a careful study on the effect of these insoluble materials in the case of copper, cobalt and nickel salts did not confirm this hypothesis[155, 158].

In Table 17 the rates of oxygen uptake of solutions containing the precipitates are reported together with those obtained from solutions filtered before and after addition of the thiol.

TABLE 17. Effect of actual dissolved metal on the oxidation of ethanethiol[a] [155, 158]

Metal ion	Initial rate of oxygen uptake, mole $l^{-1} s^{-1}$	Conditions[b]	Metal concentration in solution, M
Cu	$13·2 \times 10^{-6}$	A	10^{-5} [c]
	$13·2 \times 10^{-6}$	B	10^{-5}
	$13·2 \times 10^{-6}$	C	10^{-5}
Co	$10·3 \times 10^{-6}$	A	$1·0 \times 10^{-3}$ [c]
	$7·6 \times 10^{-6}$	B	$8·9 \times 10^{-5}$
	$9·9 \times 10^{-6}$	C	$6·4 \times 10^{-4}$
	$10·2 \times 10^{-6}$	D	$1·0 \times 10^{-3}$
Ni	$15·2 \times 10^{-6}$	A	$1·0 \times 10^{-3}$ [c]
	$3·4 \times 10^{-6}$	B	$1·3 \times 10^{-5}$
	$14·6 \times 10^{-6}$	C	$5·3 \times 10^{-4}$
	$14·8 \times 10^{-6}$	D	$1·0 \times 10^{-3}$

[a] Reaction conditions as in Table 15.
[b] A: no filtration; B: filtration before addition of ethanethiol; C: filtration after addition of thiol; D: metal added as thiol complex.
[c] Concentration of metal ion added.

It is quite clear that precipitates, in this system, do not play any role. The oxygen uptake rates of solutions not filtered (A) and those of solutions filtered before (B) or after addition of the thiol (C) are almost the same within experimental errors. The lower rates observed when the filtration is carried out before addition of thiol (B) could be due to a lower solubility of hydroxides in respect to that of metal mercaptides. This is further confirmed by the fact that addition of metals as thiol complexes gives again the same rate of oxidation (D).

An evaluation of the relative efficiency of the metals listed in Table 15 as catalysts is hindered by several factors. First of all the concentration of the metal ions in solution is not known, except in a few cases (see Table 17); for example, the different rates observed with $FeSO_4$ and haemin complex

(Table 15) could in part be due to different solubility and hence concentration of the two catalysts. A second point is that the highest rates of oxygen uptake reported ($2{\cdot}7 \times 10^{-5}$) are near the diffusion-controlled rates.

In fact with copper salts, rates independent from stirring are obtained only at much lower concentration than those of other metal ions (Table 18). On the same line are the results reported in Table 19 which show that

TABLE 18. Dependence on shake rate of the oxidation of ethanethiol[a] catalysed by copper ions[155]

$[Cu^{2+}]$, M	Shake rate, cycles per minute	Rate of oxygen uptake[b]
10^{-3}	360	0·80
10^{-3}	380	1·23
10^{-4}	310	0·74
	360	0·84
	400	1·57
10^{-5}	310	0·60
	400	0·60

[a] Reaction conditions as in Table 15.
[b] Initial rates, expressed as percentage of final uptake/min.

TABLE 19. Effect of metal concentration on the oxidation of ethanethiol[a] [154]

Metal ion	Salt	Concentration of metal added, M	$-\frac{d[O_2]}{dt}$ mole l^{-1} s^{-1} [b]
Fe^{2+}	$FeSO_4 \cdot 7\,H_2O$	0	$2{\cdot}2 \times 10^{-6}$
		10^{-6}	$2{\cdot}2 \times 10^{-6}$
		10^{-5}	$3{\cdot}0 \times 10^{-6}$
		10^{-4}	$3{\cdot}5 \times 10^{-6}$
		10^{-3}	$5{\cdot}8 \times 10^{-6}$ I
			$3{\cdot}5 \times 10^{-6}$ F
		10^{-2}	$11{\cdot}5 \times 10^{-6}$ I
			$3{\cdot}7 \times 10^{-6}$ F
Fe^{3+}	Haemin	$1{\cdot}5 \times 10^{-3}$	$26{\cdot}8 \times 10^{-6}$
		$0{\cdot}6 \times 10^{-3}$	$11{\cdot}6 \times 10^{-6}$
Mn^{2+}	$MnSO_4 \cdot 4\,H_2O$	10^{-5}	$3{\cdot}2 \times 10^{-6}$
		10^{-4}	$3{\cdot}0 \times 10^{-6}$
		10^{-3}	$3{\cdot}2 \times 10^{-6}$ I
			$4{\cdot}8 \times 10^{-6}$ F
Cr^{3+}	Chrome alum	10^{-5}	$4{\cdot}2 \times 10^{-6}$ I
			$2{\cdot}3 \times 10^{-6}$ F
		10^{-3}	$2{\cdot}0 \times 10^{-6}$

[a] Reaction conditions as in Table 15.
[b] Rate of oxygen uptake. I = initial rate; F = final steady rate.

increasing concentrations of the metals do not increase in the expected way the rates of oxygen uptake; possibly because of saturation effects.

A typical feature often observed is that initial rates differ, and are frequently higher than final steady rates[154] (Figure 6, Table 19).

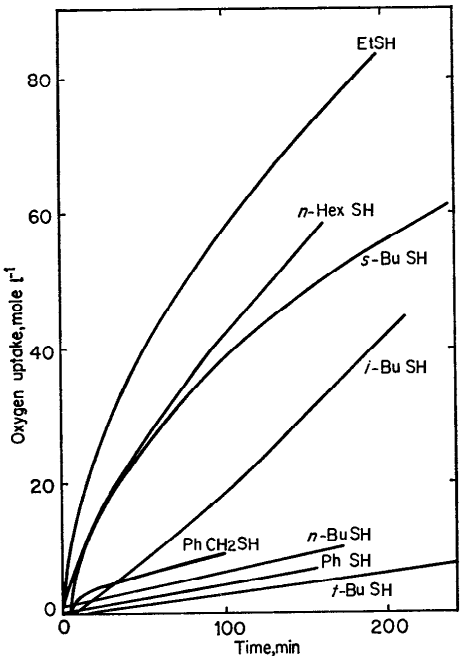


FIGURE 6. Oxidation of thiols catalysed by cobalt sulphate[154]. Reaction conditions as in Table 15. Reproduced by permission of the author and the Society of Chemical Industry from *J. Appl. Chem.*, **18**, 335 (1968).

The authors suggested that this change in rate is linked to the formation of disulphide which could compete with the thiol in coordination to the metal. Indeed the addition of disulphide at the beginning of the reaction depresses the initial rates but does not affect the final steady rate. Since the

disulphide is very little soluble in the reaction medium, its concentration in solution is expected to reach saturation quickly. Other effects due to the formation of a two-phase system could not be ruled out.

Among the most actively studied metals are copper, cobalt and nickel[154, 155] and we shall devote to them a more detailed analysis. The rate of oxidation is zero order in thiol concentration for cobalt (see Figure 6) and for copper ions whereas in the case of nickel, first order in thiol was observed.

However, the rate of oxygen uptake does depend on the thiol: the relative rates vary with different metals but benzenethiol and *t*-butanethiol are always among the least reactive compounds. Probably this is related for benzenethiol to its greater acidity (i.e. its higher oxidation potential) and for *t*-butanethiol to steric hindrance to coordination on the metal.

A detailed analysis of the structural effect of thiols on their rate of oxidation is probably unrewarding because of several uncertainties like partition coefficients of the thiols and metal complexes between the aqueous and disulphide phases and, perhaps more important, the degree of contamination of the solutions by other heavy metals due to the well-known ability of metal mercaptides to distil together with thiols[159]. It has to be noticed that the nominal uncatalysed rate is not as small as could be expected in respect to the catalysed rates and that concentration of copper ions of the order of 1×10^{-5} M is more effective than that of other metals at the 'nominal' concentration of 1×10^{-3} M.

Pertinent to this point is the study of the effect of typical ligands on the rate of oxidation[158, 160]. The results with ethylenediaminetetra-acetic acid (EDTA), ethylenediamine (EN) and CN^- are reported in Table 20.

The complexing agents always reduces the rate of oxygen uptake and the effect of CN^- is particularly large. This latter ion has also the effect of changing the course of the reaction since no disulphide, or at least only traces of it, is formed and more than the stoichiometric amount of oxygen is consumed.

Tests were made to ensure that the increase of oxygen consumption is not due to further oxidation of disulphides which appear to be stable in the reaction conditions even in the presence of CN^-.

The effect of complex ligands like porphirine, phthalocyanine, etc. has been studied by numerous authors particularly on biological systems[32, 143].

Some data on studies with these ligands and simple alkane-thiols[161] are reported in Table 21.

It is interesting to observe that also in these cases, cyanide ion depresses the oxidation rates and that the rates of oxygen uptake with these metal complexes exceed in several cases the limiting rate of oxygen diffusion.

TABLE 20. Effect on the oxidation rates of ethanethiol of several ligands[a 158]

[RSSR] M	[CN^-] M	[EDTA] M	[EN] M	Metal ion[b]	$-\frac{d[O_2]}{dt}$ mole l^{-1} s^{-1} [c]	Final O_2 uptake, % of theoretical	Notes
0	0	0	0	—	$2{\cdot}0 \times 10^{-6}$	101	
0·5	0	0	0	—	$2{\cdot}3 \times 10^{-6}$	—	
0	0·25	0	0	—	$0{\cdot}2 \times 10^{-6}$	302 (at 200 h)	No disulphide formed
0·5	0·25	0	0	—	$1{\cdot}0 \times 10^{-6}$	—	
0·5	0·25	0	0	—	zero	—	No thiol present
0	0	0	0	Cu^{2+}	$18{\cdot}3 \times 10^{-6}$	102	
0	0·25	0	0	Cu^{2+}	$0{\cdot}3 \times 10^{-6}$	—	No disulphide formed
0	0	0·1	0	Cu^{2+}	$11{\cdot}8 \times 10^{-6}$	101	
0	0	0	0·1	Cu^{2+}	$13{\cdot}5 \times 10^{-6}$	—	
0	0	0	0	Co^{2+}	$17{\cdot}7 \times 10^{-6}$ I	99	
					$4{\cdot}3 \times 10^{-6}$ F		
0·5	0	0	0	Co^{2+}	$8{\cdot}8 \times 10^{-6}$ I	—	
					$4{\cdot}4 \times 10^{-6}$ F		
0	0·25	0	0	Co^{2+}	$0{\cdot}6 \times 10^{-6}$	—	No disulphide formed
0	0	0·1	0	Co^{2+}	$7{\cdot}5 \times 10^{-6}$	103	
0	0	0	0·1	Co^{2+}	$0{\cdot}4 \times 10^{-6}$	—	Small amounts of disulphide formed
0	0	0	0	Ni^{2+}	$15{\cdot}3 \times 10^{-6}$	101	
0	0·25	0	0	Ni^{2+}	$1{\cdot}3 \times 10^{-6}$	—	No disulphide formed
0	0	0·1	0	Ni^{2+}	$3{\cdot}0 \times 10^{-6}$	—	No disulphide formed
0	0	0	0·1	Ni^{2+}	$2{\cdot}4 \times 10^{-6}$	99	No disulphide formed

[a] Reaction conditions as in Table 15.
[b] Added as sulphate: 1×10^{-3} M $CoSO_4$ and $NiSO_4$, 1×10^{-6} M $CuSO_4$.
[c] Rate of oxygen uptake. I = initial rate, F = final steady rate.

TABLE 21. Oxidation of ethanethiol[a] catalysed by metal complexes[161]

Metal	Metal complex	Concentration, M	Rate of oxygen uptake, k (mole l^{-1} s^{-1}) $\times 10^6$	
			No KCN	0·25 M KCN
Co	$CoSO_4 \cdot 7\ H_2O$	10^{-3}	10·3	1·1
	Phthalocyanine	$3{\cdot}5 \times 10^{-3}$	47·6	—
	Vitamin B_{12}	10^{-3}	80·4	0·7
Ni	$NiSO_4$ + aq.	10^{-3}	15·1	1·0
Cu	$CuSO_4 \cdot 5\ H_2O$	10^{-5}	13·2	1·2
	Phthalocyanine	$3{\cdot}5 \times 10^{-3}$	5·4	—
Fe	$FeSO_4 \cdot 7\ H_2O$	10^{-3}	4·0	1·1
	Phthalocyanine	$3{\cdot}5 \times 10^{-3}$	14·7	—
	Haemin	10^{-3}	17·1	14·7
—	—	—	1·0	0·2

[a] Reaction conditions as in Table 15.

This could be due to the ability of these complexes to co-ordinate molecular oxygen.

The suggestion was advanced that the metal-catalysed oxidation of thiols in alkaline media is based on an electron transfer from the metal in its higher oxidation state to the thiol *via* an inner-sphere process whenever the thiol may co-ordinate to the metal. Outer-sphere processes are suggested when strong complexing agents prevent the entering of thiol into the co-ordination sphere of the metal[161].

It may be worthwhile to notice that disulphide is formed in quantitative yields when no strong ligands are present otherwise products of further oxidation are obtained.

This puts some doubt on the hypothesis that disulphide formation stems from dimerization of free thiol radicals as indicated in the simplified mechanism (Scheme 7) reported below.

$$2\ M^{n+} + O_2 \longrightarrow 2\ M^{(n+1)} + O_2^{2-}$$

$$2\ RS^- + 2\ M^{(n+1)} \longrightarrow 2\ RS\cdot + 2\ M^{n+}$$

$$2\ RS\cdot \longrightarrow RSSR$$

$$O_2^{2-} + 2\ H_2O \longrightarrow H_2O_2 + 2\ OH^-$$

SCHEME 7

It could be suggested that disulphide is formed within the co-ordination sphere of the metal or in a step concerted with the release of thiyl radicals.

Indeed when, as in the case of cyanide complexes, it is assumed that the oxidation of thiols occurs by an outer-sphere process and hence thiyl radicals are formed as free particles in the solution, disulphide is at the most a minor reaction product and the thiols are oxidized to sulphinic or sulphonic acids.

Most proposed schemes assume that hydrogen peroxide is a by product and that it is consumed in a subsequent probably metal-catalysed fast reaction. Although this cannot be ruled out, it could also be that when the oxygen enters into the co-ordination sphere of the metal it is reduced in successive steps to water rather than released at an intermediate stage of reduction.

D. Catalysis by Organic Redox Systems

Hydroquinone (QH_2) and *p*-phenylenediamine derivatives in basic medium as well as other easily oxidizable species like the reduced forms of several dyes may act as catalysts in the autooxidation of thiols to disulphides.

The rate of oxygen uptake for the oxidation of *n*-hexanethiol in the presence of hydroquinone is characterized by an initial slow rate which increases up to a maximum and then decreases at longer reaction times[162]. The maximum rate at constant oxygen pressure is dependent upon the first power of base and of catalyst concentration (equation 87)

$$\frac{-dO_2}{dt} = k[QH_2]\,[OH^-] \tag{87}$$

The first step of the reaction is assumed to be the oxidation of the hydroquinone anion (QH^-) by the oxygen to generate the semiquinone (QH) (equations 88 and 89).

$$QH_2 + OH^- \rightleftharpoons QH^- + H_2O \tag{88}$$

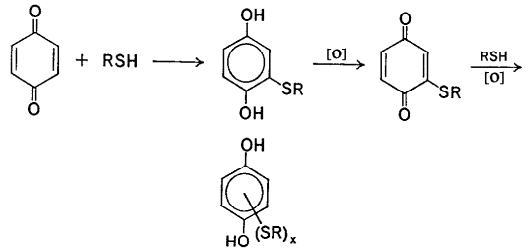
$$QH^- + O_2 \longrightarrow QH + O_2^{\cdot-} \tag{89}$$

The semiquinone then reacts with the thiol to give the corresponding thiyl radical (equation 90) which yields disulphide by dimerization.

$$QH + RS^- \longrightarrow QH^- + RS\cdot \tag{90}$$

The oxidation rates depend on the hydroquinone used as catalyst, but the catalytic power is not directly related to the oxidation rate of the catalyst[163]. However, the two sets of data are obtained in different conditions and in particular at largely different pH, and this could justify the discrepancies observed. Alternatively it is possible that the quinone is

first transformed into its mercapto derivative (equation 91) and that the substituted quinone is the true oxidizing species[162].

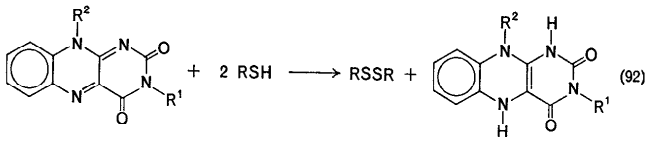


(91)

Studies of the oxidation of thiols with tetrasubstituted quinones not susceptible to further addition would shed light on this problem. Unfortunately data of this kind are not available in the literature.

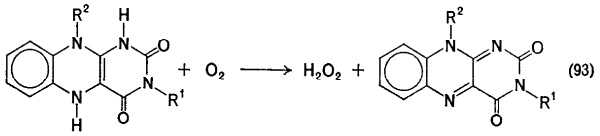
An identical mechanism has been proposed for the oxidation of thiols catalysed by phenylenediamine derivatives[164-166].

Flavine derivatives oxidize thiols to disulphides in the absence of oxygen with formation of dihydroflavines[167] (equation 92).



(92)

The reduced form of this dye may be reoxidized by molecular oxygen with regeneration of the oxidant and formation of hydrogen peroxide which is itself an oxidizing agent toward mercaptans (see section III.A) (equation 93).

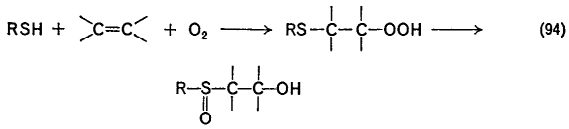


(93)

Other organic redox systems are good catalysts for the oxidation of thiols by molecular oxygen and probably act by similar mechanisms[32].

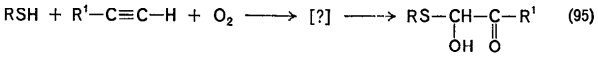
E. Co-oxidation

The autooxidation of thiols in the presence of alkenes takes a quite different course[143,168]. They are in fact oxidized by oxygen to give, possibly by a chain reaction, β-thiohydroperoxides which eventually rearrange to β-sulphinyl alcohols (equation 94). Acetylene derivatives give under the



(94)

same conditions a similar reaction which may be represented by equation (95).

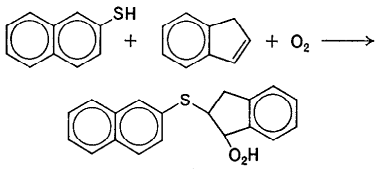


(95)

These reactions are usually called co-oxidation of thiols since an alkene (or an acetylene) is oxidized together with a thiol molecule. It has been reported that the rate of co-oxidation depends on the alkene and on the thiol, with aromatic derivatives reacting faster than the aliphatic ones. Catalysis by typical radical initiators has also been observed[143].

Kharash and coworkers[168] first proposed a hydroperoxysulphide intermediate in the formation of β-sulphinyl alcohols in the co-oxidation of thiols with olefins. This was later confirmed by detection of peroxy compounds[169] in the reaction mixture. Further studies led to the isolation of several hydroperoxysulphides when aromatic thiols were oxidized at low temperatures[170,171].

An example of this class of compounds is the 2-(2-naphthylmercapto)-1-indanyl hydroperoxide (**10**) obtained[170] as a solid, melting at 70°C, by co-oxidation of 2-naphthalenethiol and indene (equation 96).



(96)

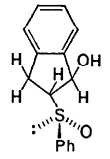
(10)

When the hydroperoxide intermediate formed in the co-oxidation of 2-naphthalenethiol and indene is allowed to decompose in the presence of 2-(4-chlorophenylmercapto)-1-indanol, none of the latter was oxidized. This would suggest an intramolecular transfer of the peroxidic oxygen at the sulphide sulphur.

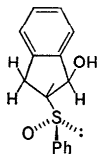
Further evidence on the intramolecular character of the oxygen transfer as well as on the stereochemistry of the co-oxidation process stems from a careful investigation by Szmant and Rigau[172,173] on the reaction of benzenethiol with indene.

They isolated from the reaction and fully characterized three of the four possible diastereoisomeric 2-phenylsulphinyl-1-indanols and prepared the missing isomer by oxidation of *cis*-2-phenylmercapto indanol with hydrogen peroxide or with *m*-chloroperoxybenzoic acid.

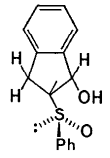
The four stereoisomers (only one enantiomer is reported) are listed below with the relative yields obtained in the co-oxidation in benzene solution.



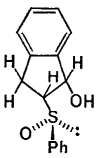
(11) m.p. 101° (60·3%)



(12) m.p. 158° (24·2%)



(13) m.p. 158° (15·6%)



(14) m.p. 146° (0·0%)

These results indicate that a 5·4 : 1 trans/cis mixture of hydroperoxides is formed and hence that in this system the co-oxidation is stereoselective rather than stereospecific as it was earlier suggested[143,169,171].

The formation of only three of the four possible sulphinyl isomers and the ratio in which they are formed appears to be clear evidence of the intramolecular character of the oxidation step.

In fact the molecular models of the *cis* and *trans* phenylmercapto indene hydroperoxides, precursors of compounds **11–14**, show that the *trans* isomer may suffer intramolecular attack at sulphur from both sides through conformations of similar estimated energy and hence compounds **11** and **12** are formed in similar amounts. On the contrary in the case of *cis* hydroperoxide the conformation which would lead to compound **14** by direct oxygen transfer is not accessible requiring that the phenyl be above the indane ring. This may explain why only the *cis* isomer (**13**) is formed.

For the formation of the intermediate hydroperoxide the following mechanism based on a radical chain reaction may be formulated (equations 97–100)[168].

$$RSH \xrightarrow{O_2,\ \text{u.v. light, etc.}} RS\cdot \qquad (97)$$

$$RS\cdot + \rangle C{=}C\langle \longrightarrow RS{-}\overset{|}{C}{-}\overset{|}{C}\cdot \qquad (98)$$

$$RS{-}\overset{|}{C}{-}\overset{|}{C}\cdot + O_2 \longrightarrow RS{-}\overset{|}{C}{-}\overset{|}{C}{-}OO\cdot \qquad (99)$$

$$RS{-}\overset{|}{C}{-}\overset{|}{C}{-}O{-}O_2^{\cdot} + RSH \longrightarrow RS{-}\overset{|}{C}{-}\overset{|}{C}{-}OOH + RS\cdot \qquad (100)$$

When thiols and olefins are co-oxidized in the presence of an aliphatic amine, the end-products are 2-mercaptoethanols, disulphides and water[174,175] (equation 101).

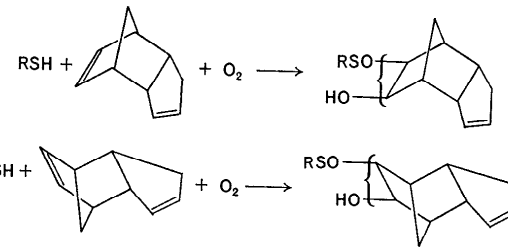
$$3RSH + \rangle C{=}C\langle + O_2 \xrightarrow{R_3N} RS{-}\overset{|}{C}{-}\overset{|}{C}{-}OH + RS{-}SR + H_2O \qquad (101)$$

This reaction may be explained in terms of an amine catalysed oxidation of the thiol[29] by the 2-mercaptoethylhydroperoxy intermediate.

This was confirmed by the observation[174] that the complex of **10** with triethylenediamine oxidizes quantitatively benzenethiol to disulphide.

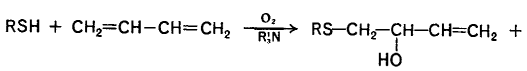
Olefins containing isolated double bonds with different reactivity towards thiyl radicals are selectively co-oxidized at the more reactive unsaturation centre[176]. This is the case of co-oxidation of *endo* and *exo* dicyclopentadienes with 4-chlorobenzenethiol (equations 102, 103). (The bracket indicates that the stereochemistry is unknown.)

Co-oxidation of thiols with 1,3-butadiene, the simplest conjugated diolefin, has been studied in the presence of *t*-butylamine[175]. Products derived from 1,2- and 1,4-addition were observed in the reaction with methane- and ethane-thiols, predominant 1,2-co-oxidation products were formed when benzene or *p*-toluenethiol were used (equation 104).

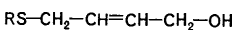


(102)

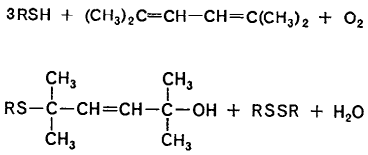
(103)



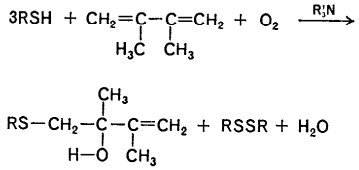
(104)



The 1,2- versus the 1,4-addition to conjugated diolefins also depends on the structure of the diene[175, 177, 178]. 2,5-Dimethyl-2,4-hexadiene gives only 1,4-co-oxidation products[175] (equation 105) whereas 2,3-dimethyl-1,3-butadiene affords 1,2-oxidation products[177] (equation 106).

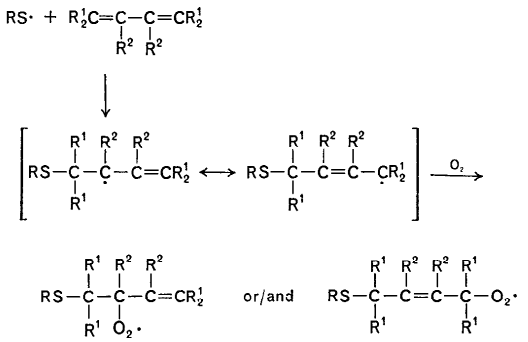


(105)



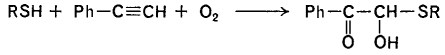
(106)

The scheme suggested for these reactions is similar to that proposed for the co-oxidation of simple alkenes. The thiyl radical attacks one of the terminal carbons to give an allyl radical followed by attack of oxygen at the 2 or 4 carbon depending on the relative stability of the two formal radicals (Scheme 8).



SCHEME 8

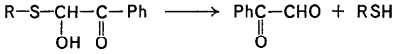
Co-oxidation of thiols and phenylacetylene with oxygen produces phenylglyoxal hemithioacetals[179] (equation 107).



(107)

The reaction occurs more easily than the co-oxidation with olefins. Benzenethiol and phenylacetylene react at reasonable rates even at temperatures below $-70°C$ under u.v. irradiation. At this temperature a peroxidic compound which decomposes above $-10°C$ to give the hemithioacetal is formed.

The products of co-oxidation of thiols and phenylacetylene are unstable and decompose to phenylglyoxal and thiol when vacuum distilled (equation 108).



(108)

The mechanism of the co-oxidation of acetylenes and thiols is not defined; however, a reaction sequence similar to that proposed for the co-oxidation with olefins has been suggested[179].

As reported above, the autooxidation is a quite general and important reaction. Beside the co-oxidation with olefins, which may be an undesired side reaction, oxidation of thiols by molecular oxygen represents a simple method of transforming these unstable compounds characterized by a

quite unpleasant smell into odourless and relatively stable compounds. It may also be a cheap method of synthesis of disulphides although care should be taken to avoid overoxidation. Furthermore, some thiols and their corresponding products of oxidation undergo easy base-promoted α-elimination leading to desulphurized compounds[180-182].

V. PHOTO-OXIDATION

Thiols undergo an easy photolytic reaction (see chapter 10 on photochemistry) which is in fact an oxidation of mercaptans to disulphides (equation 109).

$$2\,RSH \xrightarrow{h\nu} RSSR + H_2 \qquad (109)$$

The instability of thiols to light has been known for a long time[183] and there is a lively interest in the photolytic and radiolytic reactions with high energy radiations of thiols and sulphur compounds in general also because of the problem of biological effects of radiations[32,184,185].

Recent detailed work in the gas phase by Steer and Knight[186,187], largely confirming earlier results[32,184,185,188], showed that the primary photolytic process by irradiation at *ca.* 2500 Å for methane- and ethanethiols is the homolysis of the S—H bond (equation 110) to give thiyl radicals and hydrogen atom. The principal products of the reaction are molecular hydrogen and disulphides. The simple Scheme 9 was proposed for this reaction.

$$RSH \xrightarrow{h\nu} RS\cdot + H\cdot \qquad (110)$$

$$RSH + H\cdot \longrightarrow RS\cdot + H_2 \qquad (111)$$

$$2\,RS\cdot \longrightarrow RSSR \qquad (112)$$

SCHEME 9

Minor amounts of methane and hydrogen sulphide in the methanethiol reaction and of ethane, ethylene and hydrogen sulphide in the ethanethiol reaction were also formed. The authors[186,187] propose that these products are not formed in a primary process, but they derive from reaction of the thiol with a disulphide molecule which has not yet transferred the excess of energy which it contains at the act of formation (Scheme 10).

$$2\,CH_3S\cdot \longrightarrow CH_3SSCH_3^* \qquad (113)$$

$$CH_3SSCH_3^* + CH_3SH \longrightarrow CH_3SSCH_3 + CH_3\cdot + HS\cdot \qquad (114)$$

$$CH_3\cdot + CH_3SH \longrightarrow CH_4 + CH_3S\cdot \qquad (115)$$

$$HS\cdot + CH_3SH \longrightarrow H_2S + CH_3S\cdot \qquad (116)$$

SCHEME 10

In the case of ethanethiol in addition to the processes corresponding to reactions (114)–(116), equation (117) was proposed to explain the formation of ethylene. Reaction (117), because of the larger rearrangement involved, should be slower than the equivalent of reaction (114),

$$C_2H_5SSC_2H_5^* + C_2H_5SH \longrightarrow C_2H_5SSC_2H_5 + C_2H_4 + H_2S \qquad (117)$$

as is in fact observed. Among the evidence presented by the authors[186,187] in favour of the mechanism of formation of hydrogen sulphide and hydrocarbons the decrease of the yields of these products with the pressure of added inert gas is especially convincing.

As far as the primary process (Scheme 9) is concerned the supporting evidence is overwhelming: addition of ethylene, for instance, decreases the yields of hydrogen and disulphide with concomitant formation of ethyl sulphide *via* addition of the thiyl radical to ethylene.

Flash photolysis studies[151] allowed the direct detection of thiyl radicals; these species were also detected by u.v. and e.s.r. when the photolysis was carried out in solid matrices[189-192].

Quite similar processes occur also in aqueous solutions, as well as in other solvents[151,183,184], sometimes complicated, however, by interaction of the radical initially formed (equation 110) with other species present. Indeed the photolysis of thiols has been used as a source of hydrogen atoms to study their reactions with several compounds[193].

Higher molecular weight thiols, particularly secondary and tertiary alkanethiols, may undergo other primary photolytic processes, in particular breaking of the carbon–sulphur bond[184,185]. In the majority of cases, however, the main path seems to be the sulphur–hydrogen bond breaking leading to the formation of thiyl radicals which may undergo in appropriate experimental conditions several reactions besides dimerization to disulphide (section IV). Carbon–sulphur bond fission may also occur when shorter wavelength light is used. Under these conditions more complex phenomena due to the production of particles with excess energy content have also been observed[194-197].

VI. REFERENCES

1. I. M. Kolthoff and J. J. Lingane, *Polarography*, 2nd ed., Vol. 2, Interscience Publishers, New York, 1952, p. 779.
2. S. Pradac and J. Koryta, *J. Electroanal. Chem.*, **17**, 167, 177, 185 (1968).
3. S. Wawzonek, in *Techniques of Chemistry*, Vol. I Pt. IIA (Eds. A. Weissberger and B. W. Rossiter), Wiley, New York, 1971, p. 50.
4. K. Hofmann and R. Hamm, *Z. analyt. Chem.*, **231**, 199 (1967).
5. R. E. Humphrey, C. L. Oleson, G. M. Matula and A. C. Vaught, *Microchem. J.*, **16**, 429 (1971).

6. C. Ambrosino, L. Vancheri, P. Michelin Lausarot and G. Papa, *Ric. Sci.*, **39**, 924 (1969).
7. A. P. Kreshkov and L. B. Oganesyan, *Zhur. analit. Khim.*, **26**, 614 (1971).
8. L. C. Gruen and B. S. Harrap, *Analyt. Biochem.*, **42**, 377 (1971).
9. W. Stricks, J. K. Frischmann and R. G. Mueller, *J. Electrochem. Soc.*, **109**, 518 (1962).
10. W. Stricks and I. M. Kolthoff, *J. Amer. Chem. Soc.*, **74**, 4646 (1952).
11. D. L. Leussing and I. M. Kolthoff, *J. Electrochem. Soc.*, **100**, 334 (1953).
12. I. M. Kolthoff and C. Barnum, *J. Amer. Chem. Soc.*, **62**, 3061 (1940).
13. E. Vianello and E. Fornasari, *Ric. Sci.*, **29**, 124 (1959).
14. S. Roffia and M. A. Raggi, *Ric. Sci.*, **38**, 918 (1968).
15. D. G. Davies and E. Bianco, *J. Electroanal. Chem.*, **12**, 254 (1966).
16. F. Magno, G. Bontempelli and G. Pilloni, *J. Electroanal. Chem.*, **30**, 375 (1971).
17. C. Bontempelli, F. Magno and G. A. Mazzocchin, *J. Electroanal. Chem.*, **42**, 57 (1973).
18. D. S. Tarbell, in *Organic Sulfur Compounds*, Vol. 1 (Ed. N. Kharash), Pergamon Press, New York, 1961, Chap. 10, pp. 97–102.
19. J. G. Wallace, *Hydrogen Peroxide in Organic Chemistry*, E. I. du Pont de Nemours and Co., Electrochemical Dept., Wilmington, Del., U.S.A., 1960.
20. M. Dziewonska and M. Polanska, *Dissertationes Pharm.*, **16**, 507 (1964).
21. W. F. Russel, *U.S. Pat.*, 2,509,453 (1950); *Chem. Abstr.*, **44**, 7885 (1950).
22. R. Kitamura, *J. Pharm. Soc. Japan*, **58**, 29 (1938).
23. R. Kitamura, *J. Pharm. Soc. Japan*, **58**, 816 (1938).
24. W. H. Kibbel, Jr., C. W. Raleigh and J. A. Shepherd, *27th Annual Purdue Industrial Waste Conference*, Purdue University, Lafayette, Indiana, U.S.A. (1972).
25. A. M. Clifford, *U.S. Pat.*, 2,024,567 (1935); *Chem. Abstr.*, **30**, 1264 (1936).
26. A. J. Gracia, *U.S. Pat.*, 2,024,575 (1935); *Chem. Abstr.*, **30**, 1264 (1936).
27. W. A. Schulze and W. W. Crouch, *U.S. Pat.*, 2,415,851 (1947); *Chem. Abstr.*, **41**, 3289 (1947).
28. W. A. Schulze and W. W. Crouch, *U.S. Pat.*, 2,415,852 (1947); *Chem. Abstr.*, **41**, 3290 (1947).
29. A. A. Oswald, F. Noel and A. J. Stephenson, *J. Org. Chem.*, **26**, 3969 (1961).
30. I. Pascal and D. S. Tarbell, *J. Amer. Chem. Soc.*, **79**, 6015 (1957).
31. E. I. Kalinina, E. M. Lukina and V. P. Masleunikov, *Trudy Khim. i khim Tekhnol.*, 190 (1967); *Chem. Abstr.*, **70**, 273 (1969).
32. P. C. Jocelyn, *Biochemistry of the SH group*, Academic Press, London, 1972, Chap. 4, pp. 94–115.
33. I. B. Douglass, in *Organic Sulfur Compounds*, Vol. 1 (Ed. N. Kharasch), Pergamon Press, New York, 1961, Chap. 30.
34. C. R. Russ and I. B. Douglass, in *Sulfur in Organic and Inorganic Chemistry*, Vol. 1 (Ed. A. Senning), M. Dekker Inc., New York, 1971, Chap. 8.
35. P. S. Magee, in *Sulfur in Organic and Inorganic Chemistry*, Vol. 1 (Ed. A. Senning), M. Dekker Inc., New York, 1971, Chap. 9.
36. H. A. Young, *J. Amer. Chem. Soc.*, **59**, 811 (1937).
37. S. R. Sandler and W. Karo, *Organic Functional Group Preparation*, Vol. 12—III, Academic Press, New York, 1972, Chap. 4.
38. I. B. Douglass and D. R. Poole, *J. Org. Chem.*, **22**, 536 (1957).

39. I. B. Douglass and B. S. Farah, *J. Org. Chem.*, **24**, 973 (1959).
40. I. B. Douglass and B. S. Farah, *J. Org. Chem.*, **23**, 330 (1958).
41. N. Kharasch and R. B. Langford, *J. Org. Chem.*, **28**, 1903 (1963).
42. G. Capozzi, G. Melloni and G. Modena, *J. Org. Chem.*, **35**, 1217 (1970).
43. E. Kühle, *Synthesis*, 561 (1970).
44. G. E. Wilson, Jr. and M. G. Huang, *J. Org. Chem.*, **35**, 3002 (1970).
45. H. Kwart, E. N. Givens and C. J. Collins, *J. Amer. Chem. Soc.*, **91**, 5532 (1969).
46. H. Kwart and J. L. Irvine, *J. Amer. Chem. Soc.*, **91**, 5541 (1969).
47. H. Kwart and H. Omura, *J. Amer. Chem. Soc.*, **93**, 7250 (1971).
48. G. A. Silvey and G. H. Cady, *J. Amer. Chem. Soc.*, **72**, 3624 (1950).
49. N. Kharasch, in *Organic Sulfur Compounds*, Vol. 1 (Ed. N. Kharasch), Pergamon Press, New York, 1961, Chap. 32.
50. E. Vinkler and F. Klivényi, *Acta Chim. Acad. Sci. Hung.*, **22**, 346 (1960).
51. L. Di Nunno and G. Scorrano, *Boll. sci. Fac. Chim. ind. Bologna*, **24**, 103 (1966).
52. L. Di Nunno, G. Modena and G. Scorrano, *Ric. Sci.*, **35**, (IIa), 1423 (1965).
53. J. L. Kice and J. P. Cleveland, *J. Amer. Chem. Soc.*, **95**, 104 (1973).
54. J. L. Kice, *Progr. Inorg. Chem.*, **17**, 147 (1972).
55. H. J. Backer and H. Kloosterziel, *Rec. Trav. Chim.*, **73**, 129 (1954).
56. D. Barnard, *J. Chem. Soc.*, 4675 (1957).
57. D. Barnard and E. J. Percy, *Chem. Ind.* (London), 1332 (1960).
58. J. L. Kice, C. G. Venier, G. B. Large and L. Heasley, *J. Amer. Chem. Soc.*, **91**, 2028 (1969).
59. J. L. Kice and J. P. Cleveland, *J. Amer. Chem. Soc.*, **95**, 109 (1973).
60. K. Fries, *Ber.*, **45**, 2965 (1912).
61. T. C. Bruice and R. T. Markiw, *J. Amer. Chem. Soc.*, **79**, 3150 (1957).
62. W. Jenny, *Helv. Chim. Acta*, **41**, 317 (1958).
63. B. C. Pal, M. Uziel, D. G. Doherty and W. E. Cohn, *J. Amer. Chem. Soc.*, **91**, 3634 (1969).
64. N. Kharasch, S. J. Potempa and H. L. Wehrmeister, *Chem. Rev.*, **39**, 269 (1964).
65. H. Z. Lecher and E. M. Hardy, *J. Org. Chem.*, **20**, 475 (1955).
66. P. N. Rylander, *J. Org. Chem.*, **21**, 1296 (1956).
67. J. R. Shelton and K. E. Davis, *J. Amer. Chem. Soc.*, **89**, 718 (1967).
68. H. Kloosterziel and J. H. Wevers, *V Symposium on Organic Sulfur Chemistry*, Lund (Sweden), 1972.
69. N. Kharasch, C. M. Buess and W. King, *J. Amer. Chem. Soc.*, **75**, 6035 (1953).
70. K. C. Malotra and J. K. Puri, *Indian J. Chem.*, **9**, 1409 (1971).
71. E. A. Robinson and S. A. A. Zaidi, *Canad. J. Chem.*, **46**, 3927 (1968).
72. W. C. Hamilton and S. J. La Placa, *J. Amer. Chem. Soc.*, **86**, 2289 (1964).
73. C. Brown, *Chem. Comm.*, 100 (1969).
74. S. N. Nabi, S. Ahmad and S. Ahmad, Jr., *J. Chem. Soc.*, 2636 (1963).
75. S. N. Nabi and M. A. Khaleque, *J. Chem. Soc.*, 3626 (1965).
76. G. Capozzi, V. Lucchini and G. Modena, *Chimica e Industria*, **54**, 41 (1972).
77. G. Capozzi, V. Lucchini and G. Modena, unpublished results.
78. C. G. Moore and M. Porter, *J. Chem. Soc.*, 2890 (1958).

79. F. Pietra and D. Vidali, *J. Chem. Soc.* (*B*), 623 (1970).
80. E. Ciuffarin and G. Guaraldi, *J. Org. Chem.*, **35**, 2006 (1970).
81. L. Field, J. L. Vanhorne and L. W. Cunningham, *J. Org. Chem.*, **35**, 3267 (1970).
82. J. P. Danehy and M. Y. Oester, *J. Org. Chem.*, **32**, 1491 (1967).
83. J. P. Danehy, B. T. Doherty and C. P. Egan, *J. Org. Chem.*, **36**, 2525 (1971).
84. L. Field, P. M. Giles, Jr. and D. L. Tuleen, *J. Org. Chem.*, **36**, 623 (1971).
85. W. W. Epstein and F. W. Sweat, *Chem. Rev.*, **67**, 247 (1967); C. R. Johnson, J. C. Sharp, *Quarterly Report of Sulfur Chemistry*, **4**, 2 (1969).
86. C. N. Yiannios and J. V. Karabinos, *J. Org. Chem.*, **28**, 3246 (1963).
87. T. J. Wallace, *Chem. Ind.* (London), 501 (1964).
88. T. J. Wallace, *J. Amer. Chem. Soc.*, **86**, 2018 (1964).
89. T. J. Wallace and J. J. Mahon, *J. Amer. Chem. Soc.*, **86**, 4099 (1964).
90. T. J. Wallace and J. J. Mahon, *J. Org. Chem.*, **30**, 1502 (1965).
91. T. J. Wallace and H. A. Weiss, *Chem. Ind.* (London), 1558 (1966).
92. D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965.
93. D. Landini, G. Modena, G. Scorrano and F. Taddei, *J. Amer. Chem. Soc.*, **91**, 6703 (1969).
94. F. Di Furia, A. Levi, V. Lucchini and G. Scorrano, unpublished results.
95. W. O. Ranky and D. C. Nelson, in *Organic Sulfur Compounds*, Vol. I (Ed. N. Kharasch), Pergamon Press, New York, 1961, Chap. 17.
96. W. E. Parham and M. D. Bhavsar, *J. Org. Chem.*, **28**, 2686 (1963).
97. S. G. Smith and S. Winstein, *Tetrahedron*, **3**, 317 (1958).
98. A. N. Nesmeyanov, L. S. Isaeva and T. P. Tolstaia, *Doklady. Akad. Nauk. S.S.S.R.*, **151**, 1339 (1963).
99. D. Landini, G. Modena, F. Montanari and G. Scorrano, *J. Amer. Chem. Soc.*, **92**, 7168 (1970), and references therein.
100. D. C. Owsley, G. K. Helmkamp and M. F. Rettig, *J. Amer. Chem. Soc.*, **91**, 5239 (1969).
101. C. R. Johnson and J. J. Rigau, *J. Amer. Chem. Soc.*, **91**, 5398 (1969).
102. I. Kapovits and A. Kalman, *Chem. Comm.*, 649 (1971).
103. I. C. Paul, J. C. Martin and E. F. Perozzi, *J. Amer. Chem. Soc.*, **94**, 5010 (1972), and previous papers.
104. A. Ricci and P. Vivarelli, *J. Chem. Soc.* (*B*), 1280 (1968).
105. G. Illuminati, P. Linda and G. Marino, *J. Amer. Chem. Soc.*, **89**, 3521 (1967).
106. G. Modena, *Int. J. Sulfur Chem.* (*C*), **7**, 95 (1972).
107. K. Balenovic and N. Bregant, *Chem. Ind.* (London), 1577 (1964).
108. F. Yoneda, K. Suzuki and Y. Nitta, *J. Amer. Chem. Soc.*, **88**, 2328 (1966).
109. F. Yoneda, K. Suzuki and Y. Nitta, *J. Org. Chem.*, **32**, 727 (1967).
110. K. Kato and O. Mitsunobu, *J. Org. Chem.*, **35**, 4227 (1970).
111. F. J. Smentowski, *J. Amer. Chem. Soc.*, **85**, 3036 (1963).
112. T. J. Wallace, J. M. Miller, H. Probner and A. Schriesheim, *Proc. Roy. Soc.*, 384 (1962).
113. T. Takaya, H. Enyo and E. Imoto, *Bull. Chem. Soc. Japan*, **41**, 1032 (1968).
114. T. J. Wallace and J. J. Mahon, *Chem. Ind.* (London), 765 (1965).
115. V. Caló, F. Ciminale, G. Lopez and P. E. Todesco, *Int. J. Sulfur Chem., A*, **1**, 130 (1971).

116. B. S. Thyagarajan, *Chem. Rev.*, **58**, 439 (1958).
117. T. J. Wallace, *J. Org. Chem.*, **31**, 3071 (1966).
118. I. M. Kolthoff, E. J. Meehan, M. S. Tsao and Q. W. Choi, *J. Phys. Chem.*, **66**, 1233 (1962).
119. E. J. Meehan, I. M. Kolthoff and H. Kakiuchi, *J. Phys. Chem.*, **66**, 1238 (1962).
120. J. J. Bohning and K. Weiss, *J. Amer. Chem. Soc.*, **82**, 4724 (1960).
121. R. C. Kapoor, O. P. Kachhwaha and B. P. Sinha, *J. Phys. Chem.*, **73**, 1627 (1969).
122. R. C. Kapoor, R. K. Chohan and B. P. Sinha, *J. Phys. Chem.*, **75**, 2036 (1971).
123. J. Hill and A. McAuley, *J. Chem. Soc.* (*A*), 2405 (1968).
124. J. Hill and A. McAuley, *J. Chem. Soc.* (*A*), 156 (1968).
125. W. F. Pickering and A. McAuley, *J. Chem. Soc.* (*A*), 1173 (1968).
126. W. Wolf and J. C. Kertesz and W. C. Landgraf, *J. Magn. Resonance*, **1**, 618 (1969).
127. J. F. Martin and J. T. Spence, *J. Phys. Chem.*, **74**, 3589 (1970).
128. J. F. Martin and J. T. Spence, *J. Phys. Chem.*, **74**, 2863 (1970).
129. T. Nakaya, H. Arabori and M. Imoto, *Bull. Chem. Soc. Japan*, **43**, 1888 (1970).
130. M. J. S. Dewar and T. Nakaya, *J. Amer. Chem. Soc.*, **90**, 7134 (1968).
131. E. J. Bourne, W. M. Corbett, M. Stacey and R. Stephens, *Chem. Ind.* (London), 106 (1954).
132. L. Field and J. E. Lawson, *J. Amer. Chem. Soc.*, **80**, 838 (1958).
133. L. Field, C. B. Hoelzel and J. M. Locke, *J. Amer. Chem. Soc.*, **84**, 847 (1962).
134. T. Mukaiyama and T. Endo, *Bull. Chem. Soc. Japan*, **40**, 2388 (1967).
135. E. P. Papadopulos, A. Jarrar and C. H. Issidorides, *J. Org. Chem.*, **31**, 615 (1966).
136. T. J. Wallace, *J. Org. Chem.*, **31**, 1217 (1966).
137. E. E. Reid, *Organic Chemistry of Bivalent Sulphur*, Vol. I, Chemical Publishing Co. Inc., New York, 1958.
138. C. F. Cullis, J. D. Hopton and D. L. Trimm, *J. Appl. Chem.*, **18**, 330 (1968).
139. C. M. Barringer, *Ind. and Eng. Chem.*, **47**, 1022 (1955).
140. T. J. Wallace and A. Schriesheim, *J. Org. Chem.*, **27**, 1514 (1962).
141. T. J. Wallace and A. Schriesheim, *Tetrahedron*, **21**, 2271 (1965).
142. T. J. Wallace, A. Shriesheim and W. Bartok, *J. Org. Chem.*, **28**, 1311 (1963).
143. A. A. Oswald and T. J. Wallace, in *Organic Sulfur Compounds*, Vol. 2, (Ed. N. Kharash), Pergamon Press, New York, 1965, Chap. 8.
144. P. Ballinger and F. A. Long, *J. Amer. Chem. Soc.*, **82**, 795 (1960).
145. J. Murto, *Acta Chem. Scand.*, **18**, 1043 (1964).
146. J. Xan, E. A. Wilson, L. D. Roberts and N. H. Horton, *J. Amer. Chem. Soc.*, **63**, 1139 (1941).
147. T. J. Wallace and A. Schriesheim, *Tetrahedron Letters*, 1131 (1963).
148. J. P. Danehy and W. E. Hunter, *J. Org. Chem.*, **32**, 2047 (1967).
149. H. Berger, *Rec. Trav. chim.*, **82**, 773 (1963).
150. M. Z. Hoffman and E. Hayon, *J. Amer. Chem. Soc.*, **94**, 7950 (1972), and references cited therein.
151. G. Caspari and G. Granzow, *J. Phys. Chem.*, **74**, 836 (1970).

152. A. Zweig and A. K. Hoffman, *J. Org. Chem.*, **30**, 3997 (1965).
153. T. J. Wallace, N. Jacobson and A. Schriesheim, *Nature*, **201**, 609 (1964).
154. C. F. Cullis, J. D. Hopton, C. J. Swan and D. L. Trimm, *J. Appl. Chem.*, **18**, 335 (1968).
155. J. D. Hopton, C. J. Swan and D. L. Trimm, *Adv. Chem. Ser.*, **75**, 216 (1968).
156. T. J. Wallace, A. Schriesheim, H. Hurwitz and M. B. Glaser, *Ind. and Eng. Chem.* (Process Design), **3**, 237 (1964).
157. T. J. Wallace, A. Schriesheim and H. B. Jonassen, *Chem. Ind.* (London), 734 (1963).
158. C. J. Swan and D. L. Trimm, *J. Appl. Chem.*, **18**, 340 (1968).
159. K. A. Jensen, *Z. anorg. Chem.*, **252**, 227 (1944).
160. C. J. Swan and D. L. Trimm, *Adv. Chem. Ser.*, **76**, 182 (1968).
161. C. F. Cullis and D. L. Trimm, *Discuss. Faraday Soc.*, **46**, 144 (1968).
162. G. H. Meguerian, *J. Amer. Chem. Soc.*, **77**, 5019 (1955).
163. T. H. James, J. M. Snell and A. Weissberger, *J. Amer. Chem. Soc.*, **60**, 2084 (1938).
164. R. H. Rosenwald, *Petrol. Processing*, **6**, 969 (1951).
165. R. H. Rosenwald, *Petrol. Processing*, **11**, 91 (1956).
166. L. M. Rampino and M. J. Gorham, *Petrol. Processing*, **10**, 1146 (1955).
167. M. J. Gibian and D. V. Winkelman, *Tetrahedron Letters*, 3901 (1969).
168. M. S. Kharasch, W. Nudenberg and G. J. Mantell, *J. Org. Chem.*, **16**, 524 (1951).
169. J. F. Ford, R. C. Pitkethly and V. O. Young, *Tetrahedron*, **4**, 325 (1958).
170. A. A. Oswald, *J. Org. Chem.*, **24**, 443 (1959).
171. A. A. Oswald, *J. Org. Chem.*, **26**, 842 (1961).
172. H. H. Szmant and J. J. Rigau, *Tetrahedron Letters*, 3337 (1967).
173. H. H. Szmant and J. J. Rigau, *J. Org. Chem.*, **37**, 447 (1972).
174. A. A. Oswald, F. Noel and G. Fisk, *J. Org. Chem.*, **26**, 3974 (1961).
175. A. A. Oswald, K. Griesbaum and B. E. Hudson, Jr., *J. Org. Chem.*, **28**, 2351, 2355 (1963).
176. A. A. Oswald and F. Noel, *J. Org. Chem.*, **26**, 3948 (1961).
177. A. A. Oswald, B. E. Hudson, Jr., G. Rodgers and F. Noel, *J. Org. Chem.*, **27**, 2439 (1962).
178. W. A. Thaler, A. A. Oswald and B. E. Hudson, Jr., *J. Amer. Chem. Soc.*, **87**, 311 (1965).
179. K. Griesbaum, A. A. Oswald and B. E. Hudson, Jr., *J. Amer. Chem. Soc.*, **85**, 1969 (1963).
180. T. J. Wallace, J. E. Hofmann and A. Schriesheim, *J. Amer. Chem. Soc.*, **85**, 2739 (1963).
181. T. J. Wallace, H. Pobiner and A. Schriesheim, *J. Org. Chem.*, **29**, 888 (1964).
182. T. J. Wallace, H. Pobiner, J. E. Hofmann and A. Schreisheim, *J. Chem. Soc.*, 1271 (1965).
183. W. E. Haines, G. L. Cook and J. S. Ball, *J. Amer. Chem. Soc.*, **78**, 5213 (1956).
184. J. C. Calvert and J. N. Pitts, Jr., *Photochemistry*, Wiley, New York, 1966, pp. 488–492.
185. D. C. Neckers, *Mechanistic Organic Photochemistry*, Reinhold Publishing Corp., New York, 1967, pp. 276–279.

186. R. P. Steer and A. R. Knight, *J. Phys. Chem.*, **72**, 2145 (1968).
187. R. P. Steer and A. R. Knight, *Canad. J. Chem.*, **47**, 1335 (1969).
188. T. Inaba and B. de B. Darwent, *J. Phys. Chem.*, **64**, 1431 (1960).
189. K. J. Rosengren, *Acta Chem. Scand.*, **16**, 1418 (1962).
190. P. Goldberg, *J. Phys. Chem.*, **40**, 427 (1964).
191. D. H. Volman, J. Wolstenholme and S. G. Hadley, *J. Phys. Chem.*, **71**, 1798 (1967).
192. P. S. H. Bolman, I. Safarik, D. A. Stiles, W. J. R. Tyerman and O. P. Strausz, *Canad. J. Chem.*, **48**, 3872 (1970).
193. W. A. Pryor and J. P. Stanley, *J. Amer. Chem. Soc.*, **93**, 1412 (1971).
194. A. B. Callear and D. R. Dickson, *Trans. Faraday Soc.*, **66**, 1987 (1970).
195. G. P. Sturm, Jr. and J. M. White, *J. Phys. Chem.*, **72**, 3679 (1968).
196. G. P. Sturm, Jr. and J. M. White, *J. Chem. Phys.*, **50**, 5035 (1969).
197. D. M. Graham and B. K. T. Sie, *Canad. J. Chem.*, **49**, 3895 (1971).

CHAPTER 18

The synthesis and uses of isotopically labelled thiols

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I. INTRODUCTION

Isotopic labelling of thiols has been used in research disciplines ranging from atomic physics to forestry in the study of practically every atomic, molecular and biological process that thiols are known to undergo. In this review we will consider the changes that substitution of deuterium for the

hydrogen of the thiol group introduces into the translational, rotational and vibrational processes of thiols both in the ground and transition states. These perturbations have helped to elucidate some of the most fundamental structural and chemical properties of thiols. The low energy β-rays emitted by the thiol group when it is substituted with tritium or sulphur-35 allow the thiol group and its constituent atoms to be located in complex reaction mixtures. In this review we will consider the tracer applications of radio-isotope labelling in mechanistic studies of thiol reactions. However, we will also consider the use of tritiated and sulphur-35 labelled thiols in the optimization of industrial processes, as well as to trace the path that thiols follow in the body. We extend this review into these two areas of research which are usually considered to be beyond the research interests of the organic chemists for two reasons. First, the physiochemical phenomena which underlie these processes are the same as those encountered in the reaction vessel by the organic chemist. The same radical transfer reactions of thiols take place in the photochemical reaction vessel, synthetic rubber polymerization chambers, and within the body of an animal exposed to ionizing radiation. The relative lipid- as compared to water-solubility of a thiol determines not only the best procedure for its extraction from a reaction mixture but also whether the thiol will penetrate the lipoidal blood–brain barrier. Second, we have included these industrial and biological studies for the sake of the chemist who may want to extend his research on thiols to more industrially or biological significant problems. In total, we will cover processes as delicate as the passage of a thiol over a transition state or as intractile as the wearing down of steel. We will trace the flow of a thiol down the axon of a neuron and through the ecosystem of a forest.

II. MOTIONAL PROCESSES

The most fundamental chemical questions concerning the molecular weight, atomic co-ordinates and bond strengths of thiols have been answered in the most precise way by careful physical measurements of the translational, rotational and vibrational motions of thiols. Since in any one measurement the number of physical variables usually exceeds the observable parameters, meaningful physical parameters could not have been obtained if measurements had not been made on a series of isotopically substituted molecules. It is now common practice in molecular spectrometry to site a motional process from several isotopically labelled positions in a molecule. In the following sections, we will briefly describe the physical origin of the isotope effect in mass spectrometry, microwave

and infrared spectroscopy and review how it has been used to answer fundamental chemical questions concerning thiols.

A. Translation

Mass spectrometry is a relatively accurate and convenient method for the determination of the molecular weight of a molecule. Moreover, in the course of the measurement, the molecule often fragments to smaller molecular ions, whose molecular weights are also measured. Later the pattern of molecular fragments can be pieced together in a way that will reveal the structure of the thiol. However, very often fragments originating from different parts of a molecule will have the same mass and will not be distinguishable from each other. As we will see, isotopic labelling readily overcomes this problem and precisely traces the origin of molecule ion fragments.

When thiols enter the mass spectrometer, they are first ionized and partially broken into fragments. Both the molecular parent ion and the fragment ion carry a charge, e, by virtue of which they can be accelerated through a potential, V. When the ions emerge from the accelerating chamber they all possess the same kinetic energy, Mv^2, and potential energy, eV (where M is the mass and V the velocity of the ion). When this process is applied to a mixture of normal and heavier isotopically labelled thiols, both the light and heavy ions will emerge with the same energy, but the light molecules will be travelling faster than the heavy molecules. The accelerated ions next enter the magnetic sector of the spectrometer, where the magnetic field, H, exerts a centripetal force, HeV, on the ions which is exactly balanced by a centrifugal force, Mv^2/r, i.e. $HeV = Mv^2/r$ (where r is the radius of the ions trajectory through the magnetic field). The lighter, normal ions travel with a greater velocity v and experience a greater centripetal force, and an even greater centrifugal force, than the heavier isotopically labelled thiols. Accordingly, the path of the lighter ions will have a smaller radius. The difference in paths of the light and heavy ions facilitates their separation and analysis[1].

The two most labile bonds in a thiol, R—CH_2—SH, are the S—H and C—H bonds. However, removal of a hydrogen from the CH_2 or the SH group yields fragments with the same mass. Amos and coworkers[2] have used isotopic labelling to show that CD_3SH fragments to $[CD_2{=}SH]^+$ and $[CD_3S]^+$ in the ratio 2 : 1, while the ratio in CH_3CD_2SH is approximately unity. Upon ionization, benzenethiol-S-d_1 has been shown by Lawesson, Madsen and Schroll[3] to lose equal amounts of mercapto-deuterium and ring hydrogen. In a later section, we will show how separation of ion fragments using isotope labelling has made possible a

number of mass spectrometric studies of the bond energies of thiols and the thermodynamics of their bond cleavages.

B. Rotation

Microwave spectroscopy has proved to be a powerful technique, providing data on the structure and bonding of gaseous molecules. The interaction of the dipole moment of the molecule with a microwave field induces transitions between the rotational energy levels of the gaseous molecule. The microwave frequencies, at which the transitions occur, depend entirely on the moments of inertia of the molecule about its principal rotational axes. The moment of inertia is determined by the atomic masses and bond lengths and angles of the molecule. Usually the determination of one set of moments of inertia is not sufficient to give a unique set of molecular parameters. To obtain such a unique set of molecular parameters, measurements must be made on a series of molecules, in which isotopic substitution has been used to create a series of changes in atomic mass along the molecule. As microwave measurements are quite sensitive, thiols containing ^{13}C, ^{33}S and ^{34}S at natural abundance can be observed and used to provide a series of naturally occurring isotopically substituted molecules[4].

In the first application of microwave spectroscopy to a thiol, Solimene and Dailey[5] measured the 0_{00}–1_{01} transition in several isotopically substituted methane thiols, including $^{12}CH_3{}^{32}SH$, $^{13}CH_3{}^{32}SH$, $^{12}CD_3{}^{32}SH$, $^{12}CH_3{}^{33}SH$, $^{12}CH_3{}^{34}SH$ and $^{12}CH_3{}^{32}SD$. From these data they derived the moments of inertia and corresponding structural parameters of methanethiol. Kadzar, Abbason and Imanev[6] determined the structure of ethanethiol using $CH_2CH_2{}^{32}SH$ and $CH_2CH_2{}^{34}SH$. A more comprehensive set of molecular parameters for ethanethiol has been obtained by Hayaishi and coworkers[7] from the spectra of the *trans* and *gauche* isomers of CH_3CH_2SH, CH_2DCH_2SH (syn and anti), CH_3CD_2SH, $CH_3CH_2{}^{34}SH$ and CH_3CH_2SD.

In addition to rotating with the molecule as a whole, the methyl group of methane thiol can rotate against the thiol group along the C—S bond. The resulting modes of hindered rotation (i.e. torsional vibration) create an additional series of spectral lines. Solimene and Dailey[5], by measuring the intensity of the lower-lying excited torsional states relative to the ground state in CH_3SH and CD_3SH, determined that the potential barrier for hindered rotation is sinusoidal with a height of 1·06 kcal/mole. Later Kojima[8], measuring the $\Delta J = \pm 1$, $\Delta K = \mp 1$ lines in the ground state and the $\Delta J = 0$ lines in the first excited state of CH_3SH and $CH_3{}^{34}SH$ determined the potential barrier of methanethiol to be $444 \pm 10\ cm^{-1}$.

In similar measurements of CH_2DSH and CHD_2SH, Knopp, Daniel and Quade[9] showed that the staggered conformation for the methyl and thiol group corresponds to a threefold minima in the potential energy function for hindered rotation. Reddington[10] has found that the height of the potential barrier of CF_3SH and CF_3SD is quite close to that of CH_3SH. The fact that substitution of CF_3 for CH_3 has little effect on the height of the barrier rules out repulsion between non-bonded atoms as the source of the potential barrier. Measurements like these can be expected to continue to provide insight into the nature of the interaction between two internally rotating groups.

It is interesting to note that one of the first measurements of the electric nuclear quadrapole moment of ^{33}S was made by Bird and Townes[11] who on close examination of Solimene and Dailey's microwave spectrum of methanethiol noticed a group of three very weak doublets. They ascribed the doublets to the interaction of the electric quadrupole moment of natural abundance ^{33}S with the electric field of the molecule as a whole.

C. Vibration

Infrared spectroscopy can be used not only in a qualitative way to identify functional groups in a molecule, but also to provide precise data on the bond strengths. Before such calculations can be made, however, every observed spectral band must be assigned to one of the vibrational modes of the molecule. Such assignments can often be ambiguous. Replacing an atom in a molecule with one of its isotopes does not, to a high order of approximation, change the electronic structure of the molecule, and therefore does not alter the potential functions governing the vibrations of the atoms. However, the frequency of the vibration will be affected and will reveal itself in a shift of the vibrational band. The shift will be small, when the isotopically substituted atoms moves very little in a particular vibrational mode; but when the atom has a large amplitude of vibration in a mode, the shift will be large[12]. Plant, Tarbell and Whiteman[13] reported the first isotope shift observed in the vibrational spectrum of a thiol. They found that in benzenethiol and *n*-hexanethiol deuteration of the thiol groups shifted the bands at 2600 cm^{-1} to 1839 and 1870 cm^{-1}, respectively. Since then isotope shifts have helped elucidate the infrared spectra of several thiols. For example, CF_3SH displays a band at 906 cm^{-1}, which shifts to 699 cm^{-1} in CF_3SD. This large spectral shift has allowed the band to be assigned to the CSH bending mode; whereas a series of bands near 500 cm^{-1} shift very little upon isotopic substitution, verifying their assignment to the CF_3 deformation modes[10].

Takeoka[14] has used the isotope shifts observed in the infrared spectrum of cyclohexanethiol-S-d_1 to assign the observed bands to the proper vibrational modes. In addition, bands belonging to the axial and equatorial conformations of cyclohexanethiol could be distinguished. Furthermore, the changes in the relative concentration of the two conformers on going from the liquid to the plastic to the hard crystalline phases could be followed.

Once the vibrational bands of a molecule have been assigned to their proper modes, calculations can be made of the interatomic forces that bind atoms together to form a molecule. The strength of these interatomic forces is measured in terms of a force constant for a particular vibrational mode. When the atomic co-ordinates and masses of a molecule are known, a complete set of force constants can be used in a normal co-ordinate analysis using the Wilson *FG* matrix method[15], to obtain a set of calculated vibrational bands. The set of force constants is then adjusted so as to obtain the best fit between observed and calculated frequencies. As occurs in other spectroscopic measurements, the number of force constants often exceeds the number of observed frequencies in any one spectrum. Since the force field is independent of isotopic substitutions, the spectra of isotopically substituted molecules can be used to provide additional frequencies. A particularly good check of a force field is its ability to predict the spectra of isotopically substituted molecules. May and Pace[16,17] have obtained a force field for methanethiol based on the frequencies of CH_3SH and CH_3SD and microwave structural parameters. Their force field accurately predicts all the observed frequencies of the normal and isotopically labelled molecules. Hayaishi and coworkers[18] have obtained a reliable set of force constants for ethanedithiol from the frequencies of $HSCH_2CH_2SH$ and $DSCH_2CH_2SD$. Furthermore, they have shown that when *trans, trans, trans* and *trans, trans, gauche* conformations are assumed, the force field satisfactorily predicts the observed frequencies of *n*-propanethiol, β-thiamethylethane thiol, β-halogenoethane thiol and 1,2-dithiamethyl ethane.

III. CLEAVAGE OF THE S—H BOND

A. The Primary Hydrogen Isotope Effect and the Nature of the Transition State

In the previous section we saw how isotope labelling has played an indispensable role in the elucidation of the motional processes and structure determinations of thiols. In this section we turn to the dynamics of the rupture of the S—H bond. The chemical phenomenon of the

S—H bond cleavage is indeed only another motional process, in which the thiol hydrogen moves independently of the rest of the thiol molecule in a sort of extended S—H stretching mode. As we have seen, substitution of deuterium for the thiol hydrogen has a pronounced effect on the motion of a thiol, particularly the S—H bond stretching vibration. We might expect that deuterium substitution will greatly affect the dynamics of the S—H bond cleavage. In this section, after having reviewed the theoretical basis for primary hydrogen isotope effects[19,20], we will construct several transition state models for S—H bond cleavage[20], predict the isotope effect for each model, and compare these to the measured values. Finally, we will turn to the use of isotopic labelling to trace the fate of the thiol hydrogen after it has been abstracted from a thiol.

For the purpose of theoretical discussion, we consider that the thiol lies on a surface of potential energy, whose co-ordinates are the bond lengths and angles of the thiol molecule in the horizontal direction and potential energy in the vertical direction. The exact topography of the surface is determined by the electronic structure of the molecule. During the processes of S—H bond cleavage, the thiol can be thought of as travelling across the surface along a pathway of lowest energy, which will correspond to the S—H stretching mode. The highest point along this pathway of lowest energy is called the transition state. The rate at which S—H bond cleavage will occur depends primarily on the probability of a thiol reaching the transition state, RSH^+. If we consider that ground state and transition state molecules are in equilibrium, then the process can be characterized by an equilibrium constant K^+ (eqns. 1 and 2).

$$RSH \rightleftharpoons RSH^+ \tag{1}$$

$$K^+ = \frac{[RSH^+]}{[RSH]} = \frac{\Pi Q^0_{\text{products}}}{\Pi Q^0_{\text{reactants}}} \exp(\Delta E/RT) \tag{2}$$

Equilibrium constants can be expressed in terms of the motional processes of a molecule, i.e. in terms of the partition function of the reactant and the product, which in this case is the transition state, as seen in equation (2). The partition function, Q or Q^0 (for unit volume of an ordinary molecule), denotes the probability of a molecule existing in any one particular motional state, summed over all the possible translational, rotational and vibrational states available to the molecule. The energies of the motional states are calculated taking the lowest classical state, as having zero energy. The exponential term in equation (2) corrects for the difference in energy between the reactant and transition states.

Having written K^+ in terms of motional states of the molecule, we are now prepared to ask how substitution of deuterium for the thiol hydrogen

will affect the probability of the thiol reaching the transition state $RSH^{\ddagger}$. Experimentally the question is posed in the ratio of the rate of the S—H bond cleavage over rate of S—D cleavage. These rates are largely determined by the equilibria in equations (3) and (4). As seen in equation (5) the hydrogen isotope effect can be written in terms of the partition functions for the light and heavy thiols.

A major advance in the theory of primary hydrogen isotope effects came when the approximation was made that substitution of deuterium for hydrogen does not greatly affect the classical properties of the molecule, such as the mass or moments of inertia and consequently neither the translational nor rotational partition functions[21]. This left only the quantum mechanical vibrational partition function as a source of the isotope effect. Writing the deuterium isotope effect in terms of the complete vibrational partition function, equation (6) is obtained, where $u_i = h\nu_i/kT$, ν_i is the frequency of the ith vibrational mode and N is the number of atoms in the molecule. The products and summations are

$$RSH \underset{}{\overset{K_H^{\ddagger}}{\rightleftharpoons}} RSH^{\ddagger} \tag{3}$$

$$RSD \underset{}{\overset{K_D^{\ddagger}}{\rightleftharpoons}} RSD^{\ddagger} \tag{4}$$

$$\frac{k_H}{k_D} = \frac{K_H^{\ddagger}}{K_D^{\ddagger}} = \frac{\prod Q_H^{0\ddagger}}{\prod Q_D^{0\ddagger}} \times \frac{\prod Q_D^{0}}{\prod Q_H^{0}} \tag{5}$$

$$\frac{k_H}{k_D} = \prod_i^{3N^{\ddagger}-7} \frac{1-\exp(-u_{i(D)}^{\ddagger})}{1-\exp(-u_{i(H)}^{\ddagger})} \times \prod_i^{3N-6} \frac{1-\exp(-u_{i(H)})}{1-\exp(-u_{i(D)})} \times \exp\left\{-\frac{1}{2}\left[\sum_i^{3N^{\ddagger}-7}(u_{i(H)}^{\ddagger}-u_{i(D)}^{\ddagger}) - \sum_i^{3N-6}(u_{i(H)}-u_{i(D)})\right]\right\} \tag{6}$$

taken over the $3N-6$ vibrational modes of the ground state and over $3N^{\ddagger}-7$ vibrational modes of the transition state, in which the vibrational mode corresponding to the reaction pathway (in our case the S—H stretch) is omitted. As seen in equation (6), an isotope effect will occur only when the deuterium participates in a vibrational mode, whose frequency changes on going from the ground to the transition state. We are now ready to characterize various transition states precisely in terms of what vibrational modes have changed, which is another way of locating the transition state on the potential surface.

The simplest model that can be chosen for the transition state is one in which the only vibrational mode that has changed is the S—H stretching mode. Since this vibrational mode is the reaction co-ordinate itself, it does not contribute to the isotope effect in the transition state. Molecular vibrations involving hydrogen generally have vibrational bands above 700 cm^{-1}, for which $\exp(-u)$ is 0·03 at 300 K and products involving this term will be close to unity. Equation (6) therefore reduces simply to

$$\frac{k_H}{k_D} = \exp[\tfrac{1}{2}(u_{RSH}-u_{RSD})] = \exp\left[\frac{hc}{2kT}(\tilde{\nu}_{RSH}-\tilde{\nu}_{RSD})\right] \tag{7}$$

where $\tilde{\nu}$ is the wave number of the thiol stretching mode in the ground state. Using the literature value[16] for the thiol stretching mode of methanethiol, 2605 cm^{-1} and 1893 cm^{-1} for CH_3SH and CH_3SD respectively, a value of 5·5 is obtained for k_H/k_D. Using equation (8), this corresponds to a value of

$$\frac{k_T}{k_H} = 1{\cdot}11\left(\frac{k_D}{k_H}\right) \times 1{\cdot}44 \tag{8}$$

or 11·29 for k_H/k_T, the primary tritium isotope effect.

Weakening the S—H bond in the transition state must certainly reduce the frequency of the C—S—H bending mode. If we consider the extreme case in which the frequency has gone to zero, the product term $[1-\exp(-u_{i(D)}^{\ddagger})]/[1-\exp(-u_{i(H)}^{\ddagger})]$ of equation (6) approaches $u_D^{\ddagger}/u_H^{\ddagger}$, which can be approximated by $(m_H/m_D)^{\frac{1}{2}}$, where m refers to mass. Equation (6) now reduces to

$$\frac{k_H}{k_D} = \left(\frac{m_H}{m_D}\right)^{\frac{1}{2}} \exp\left\{\frac{hc}{2kT}[(\tilde{\nu}_{SH\ stretch}-\tilde{\nu}_{SD\ stretch}) + (\tilde{\nu}_{CSH\ bend}-\tilde{\nu}_{CSD\ bend})]\right\} \tag{9}$$

Using values of 802 cm^{-1} and 623 cm^{-1} for the bending modes of CH_3SH and CH_3SD,[16] k_H/k_D increases to a value of 5·9 and k_H/k_T to 11·42. We may then expect that weakening the C—S—H bending mode will tend to increase slightly the isotope effect.

In addition to unimolecular dissociation of the S—H bond, thiol bonds are often ruptured when an acceptor molecule (usually a free radical) abstracts hydrogen from the thiol. In this case, the transition state will contain the three-centre linear system S—H—A, where A is the acceptor atom. The stretching and bending modes of the C—S—H group of the ground-state thiol will make the same contribution to the isotope effect as they did in the unimolecular dissociation, and the S—H stretch will

remain the reaction co-ordinate. However, in the transition state a new linear stretching mode associated with the S—H—A system will have to be introduced. If S—H—A is asymmetric, i.e. A does not resemble sulphur, then the stretching mode shown in Figure (1a) will tend to weaken the isotope effect for either of two reasons: (1) for large u and $u^{\ddagger}_{SHA} > u^{\ddagger}_{SDA}$, the transition state vibration will detract from the contribution made by the ground state molecules or (2) for small u, $[1-\exp(u^{\ddagger}_{i(D)})]/[1-\exp(u^{\ddagger}_{i(H)})]$ will introduce the term m_D/m_H. On the other hand, when S—H—A is symmetric, the linear vibration introduced, Figure (1b), in which H does not move, will not contribute to the isotope effect.

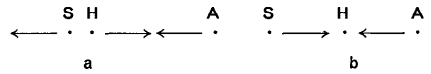


FIGURE 1. Stretching modes of the S—H—A system.

We might conceive of a reaction in which S—H bond cleavage occurs long before the thiol reaches the transition state, such as in the base-catalysed addition of RSH to an olefin, equations (10) and (11).

$$RSH + B \rightleftharpoons [RS^- HB^+] \tag{10}$$

$$[RS^- HB^+] + \text{>C=C<} \longrightarrow RS-\underset{|}{\overset{H}{\overset{|}{C}}}-\underset{|}{\overset{|}{C}}-+B \tag{11}$$

Here isotope substitution exerts its effect on the rate of the reaction, *via* the pre-reaction equilibrium, equation (10). Rather than calculating the kinetic isotope effect for the reaction, we will want to obtain an expression for the ratio of the equilibrium reactions.

$$RSH + B \overset{K_H}{\rightleftharpoons} [RS\cdots HB] \tag{12}$$

$$RSD + B \overset{K_D}{\rightleftharpoons} [RS\cdots DB] \tag{13}$$

The ratio of equilibrium constants, K_H/K_D, for equations (12) and (13) is equivalent to the equilibrium constant $K_{H/D}$ for the isotope exchange equilibrium.

$$RSH + [RS\cdots DB] \overset{K_{H/D}}{\rightleftharpoons} RSD + [RS\cdots HB] \tag{14}$$

Expressing $K_{H/D}$ in terms of the vibrational partition functions we obtain

$$K_{H/D} = \frac{Q_{RSH}\, Q_{RSDB}}{Q_{RSD}\, Q_{RSHB}} \tag{15}$$

Equation (15) is simply the individual partition function ratio of isotopic substituted RSH Q_{RSH}/Q_{RSD}, divided by Q_{RSHB}/Q_{RSDB},

$$\frac{Q_{RSH}}{Q_{RSD}} = \Pi \frac{u_{RSD}}{u_{RSH}} \times \exp\left(\Sigma \frac{u_{RSH} - u_{RSD}}{2}\right) \times \Pi \frac{1-\exp(-u_{RSH})}{1-\exp(-u_{RSD})} \tag{16}$$

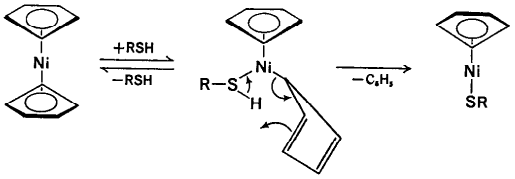
$$\frac{Q_{RSHB}}{Q_{RSDB}} = \Pi \frac{u_{RSDB}}{u_{RSHB}} \times \exp\left(\Sigma \frac{u_{RSHB} - u_{RSDB}}{2}\right) \times \Pi \frac{1-\exp(-u_{RSHB})}{1-\exp(-u_{RSDB})} \tag{17}$$

Just as in the case of the kinetic isotope effect, deuterium substitution is felt only in those vibrational modes that change on going from reactants to products.

The rate at which a particular reaction takes place is only partially accounted for by $K^{\ddagger}$. The rate of passage of a thiol over the potential barrier at the transition state is given by $\nu^{\ddagger}_L[RSH]$, in which $\nu^{\ddagger}_L$ is the frequency of the vibration that carries the thiol over the potential barrier and tears the S—H bond apart. The magnitude of $\nu^{\ddagger}_L$ is determined by the curvature of the potential surface near the transition state and since the curvature is concave downwards the frequency is imaginary, but has the same absolute value as if the surface were concave upwards, with a real vibrational frequency. The rate is influenced by two other parameters, which intimately depend on the topography of the potential surface. These are the transmission coefficient, i.e. the fraction of molecules passing over the barrier in the forward direction, and the percentage of tunnelling of the molecules under the potential barrier. These parameters are generally ignored or considered to introduce no isotope effect; however, in cases where large deviations from the predicted isotope effects are found, they have to be considered. The way in which these phenomena are affected by isotope substitution is an active field of theoretical study.

The observation of a large kinetic isotope effect indicates that isotopically substituted thiol hydrogen participates directly in a vibrational mode, whose frequency changes on going to the transition state, i.e. that S—H bond cleavage is an integral part of the transition state. The fact that a value for k_H/k_D of 2·80 was obtained for the addition of benzene-thiol-S-d_1 to nickelocene, led Ellgen and Gregory[22] to propose the mechanism below for the reaction. Although the authors did not comment

on the rather low value of k_H/k_D, it would seem to indicate that the zero point energy lost on cleavage of the S—H bond is partially offset by the formation of the cyclopentadienyl hydrogen bond. The abstraction of



thiol hydrogen by the triphenylmethyl radical proceeds with an anomalous large value for k_H/k_T of 14·9, which was attributed by Lewis and Butler[23] to tunnelling through the potential barrier, which occurs when a barrier is symmetrical.

Dmuchovsky, Vineyard and Zienty[24] observed a quite unusual inverse isotope effect for k_H/k_D of 0·65 for the base catalysed addition of *n*-butanethiol-S-d_1 to maleic anhydride. While inconsistent with any model of a transition state involving S—H bond cleavage, the inverse isotope effect could be accounted for by postulating a pre-reaction equilibrium between butanethiol and triethylamine, much like the one in equations (10) and (11). In fact, substitution into equations (16) and (17) of 2566 and 1850 cm^{-1} for the S—H and S—D stretching frequency, respectively, and 3253 and 2380 cm^{-1} for the N—H and N—D stretches of the amine–thiol complex, yields an equilibrium isotope effect of 0·68[23].

Isotope equilibrium exchange constants for a number of thiol–water systems have been measured and the value $K_{H/D}$ is usually referred to as the equilibrium isotope separation factor, α. Haul and Blenneman[25] have measured α for $HSCH_2CH_2SD$ as a function of temperature and obtained $\ln\alpha = 262/T - 0{\cdot}1162$, which corresponds to a ΔH of -520 cal/mole. Sakodynskii, Babkov and Zhavoronkov[26] found that changing the structure and composition of a thiol had very little effect on α, which indicates that, during hydrogen exchange with water, changes in vibrational frequencies are restricted to the C—S—H bonds.

The measurement of kinetic isotope effects have provided insight into economically important industrial processes. Early in the course of the synthetic rubber programme it was found that the molecular weight of a polymer such as G R—S, could be quantitatively regulated by the addition of thiols to the polymerization system. Normally the polymerization occurs as in equation (18); however, a growing polymer can abstract a hydrogen

atom from thiol, thereby transferring the radical to the thiol and inactivating the polymer chain, equation (19).

$$M_n^{\cdot} + M \xrightarrow{k_2} M_{n+1}^{\cdot} \quad (18)$$

$$M_n^{\cdot} + RSH \xrightarrow{k_3} M_nH + RS^{\cdot} \quad (19)$$

The chain length of the polymer formed is proportional to the transfer constant k_3/k_2, which is the ratio of the specific rate of radical transfer to the specific rate of chain propagation[27]. Wall and Brown[28] measured the isotope effect $k_{t(H)}/k_{t(D)}$ of the chain transfer step in the butanethiol-S-d_1 mediated polymerization of styrene. A value of 4, somewhat less than the predicted value of about 6, was obtained. The low kinetic isotope effect indicated that either the loss of zero point energy of the S—H bond had been compensated by the formation of unusually strong bonds or that the reaction was complicated by the abstraction of butyl hydrogens as well as thiol hydrogen. Data such as these can often aid in the search for more efficient transfer agents.

B. Tracers of Atoms and Free Radicals during S—H Bond Cleavage

In addition to its use in probing the nature of transition states, labelling with heavy hydrogen is an indispensable aid in following the fate of thiol hydrogen in the reaction mixture. It distinguishes thiol hydrogen not only from the hydrogens of the reaction mixture as a whole, but also from other hydrogen atoms of the thiol, which may have been dissociated under the reaction conditions that led to the dissociation of the S—H bond.

Greig and Thynne[29] have measured the relative rates at which methyl radicals abstract hydrogen and deuterium from CD_3SH. The hydrogen of the SH bond was abstracted 120 times faster than the methyl deuterium. Riesz and Burr[30] have measured the relative amounts of D_2 and HD produced by the reaction of deuterium atoms with cysteine-S-d_1 and n-butanethiol-S-d_1. The yields of D_2 were 80 and 83%, respectively, indicating that atom abstraction occurred primarily from the —SD group. Volman, Wolstenholme and Hadley[31] irradiated CH_3SD at 77 K with 2537 Å light and detected e.s.r. signals originating from D· but not from H. This indicated, that if $\cdot CH_2SD$ radicals were observed in the irradiated sample, they could only have been formed by a secondary radical abstraction reaction. Keyes and Harrison[32] were able to study the two major pathways of thiols that occur in the ion chamber of the mass spectrometer. Unlabelled CH_3SH yields fragments which cannot be separated, but CD_3SH, equations (20) and (21), yields $[CD_3S^+]$ and $[CD_2{=}SH^+]$ ions, whose heat of formation were found to be 214 and

219 kcal/mole, respectively. The difference in the heats of formation indicate the relative ease with which hydrogen can be abstracted from a mercapto group as opposed to a methyl group. Deuterium labelling has revealed that the fragmentation of benzenethiol is considerably more

$$CD_3SH \xrightarrow{9.54\ eV} e^- + [CD_3SH]^+ \xrightarrow{2.22\ eV} [CD_3S^+] + H \quad (20)$$

$$\searrow^{2.47\ eV} [CD_2{=}SH^+] + D \quad (21)$$

complex than that of methanethiol. Earnshaw, Cook and Dinneen[33] found that the fragment ions produced from benzenethiol-S-d_1 could be rationalized only by assuming that the parent ion $C_6H_5DS^+$ exists in two isomeric forms, an ionized benzenethiol (Figure 2a) and a cyclic seven-membered ion, in which the deuterium atom cannot be associated with any particular carbon atom (Figure 2b).

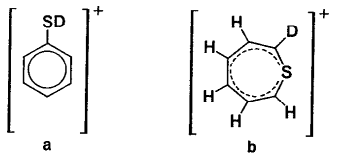


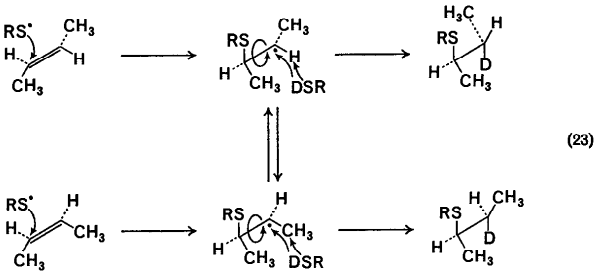
FIGURE 2. Isomeric forms of the $C_6H_5DS^+$ ion.

Labelling of the thiol group with heavy hydrogen can provide information concerning the nature of the hydrogen abstractor as well. The phenylethyl radical can exist as two isomers which can be interconverted by a 1,2 hydrogen migration (equation 22). Slaugh[34a] by

$$\begin{array}{ccc} PhCH_2\dot{C}H_2 & \rightleftharpoons & PhC\dot{H}CH_3 \\ \downarrow PhST & & \downarrow PhST \\ PhCH_2CH_2T & & PhCHTCH_3 \end{array} \quad (22)$$

allowing the radical to abstract hydrogen from benzenethiol-S-t_1 was able to mark the site of the radical with tritium. Methanethiol-S-d_1 adds across the double bonds of *cis*- and *trans*-2-butene to form identical mixtures of erythro- and threo-3-deuterio-2-(methylthio)butane. Skell and Allen[34b] found that the radical reaction takes place in two steps, the

addition of a methylthio radical to butene followed by the abstraction of deuterium from a molecule of CH_3SD by the 3-methylthio-2-butyl radical (equation 23). The fact that with deuterium labelling a mixture of threo



and erythro methylthiobutanes is obtained indicates that abstraction of thiol hydrogen is slower than the rate of rotation about the 2,3 carbon—carbon bond of the radical.

There are many exchange reactions that can be detected only with the use of isotopic labelling. One such reaction is hydrogen exchange between a thiol and a protic solvent. For example, Denisov, Kazakova and Ryl'tsev[35] studied mixtures of MeSH (or iso-BuSH) : MeOD and iso-BuSD : HOAc (or MeOH) to determine the relationship between the rate of hydrogen exchange and proton donor and acceptor properties. Sulphur-35 labelling was used by Dixon, Kornberg and Lund[36] in a study of the enzyme, malate synthetase, to determine whether the enzyme had a catalytic effect on exchange between coenzyme A-^{35}S and acetyl coenzyme A (equation 24)

$$CoA{-}^{35}SH + acetyl{-}S{-}Co \rightleftharpoons acetyl{-}^{35}S{-}CoA + CoA{-}S{-}H \quad (24)$$

In the photolysis of the S—H bond it is possible to introduce into the thiol more than enough energy for the cleavage of the S—H bond. The very subtle question of whether upon bond cleavage this excess energy is channelled into the vibrational modes of the radical or into the translational energy of the dissociated hydrogen atom has been answered by White and coworkers[37,38] by a clever use of isotope labelling. Translationally excited hydrogen atoms displace deuterium from D_2 to form HD (equation 25) to an extent that is proportional to the energy of the hydrogen atom. By photolysing CH_3SH in the presence of D_2 and measuring the amount of HD produced, they found that the excess energy resided chiefly in the

translational mode of the hydrogen atom. Furthermore, hydrogen atoms formed at 2282 Å appeared to have on the average significantly more energy than those produced at 2537 Å.

$$\left.\begin{array}{l} RSH \xrightarrow{h\nu} RS+H^{*} \\ H^{*}+D_2 \longrightarrow HD+D^{*} \end{array}\right\} \quad (25)$$

IV. TRACING ^{35}S-LABELLED THIOLS IN BIOLOGICAL SYSTEMS

In the previous section we have seen how isotopic labelling has been used to trace the fate of thiol sulphur and hydrogen atoms in the course of chemical reactions. However, by far the greatest application of isotopic labelling in tracer studies of thiols has been in biochemical, biological and clinical studies which have sought to map out the path followed by various thiols in the body from the time of their administration to their excretion. While many of these studies have been performed by scientists other than chemists, the phenomena they probe are essentially physiochemical in nature. For this reason we have taken the liberty to extend the scope of this review to the biological applications of isotopic labelling of thiols. We have done this in the hope that it will familiarize the chemist working in an interdisciplinary group with the nature of a biological system from the point of view of tracer studies, for which he may be asked to design a chemical probe.

A. Macromolecular Systems

Before turning to body tracer studies, we might consider the application of ^{35}S-tracing to a few isolated biochemical systems. The only place thiopurines and thiopyrimidines occur in nature are in the tRNA's (transfer ribonucleic acid). The question that was immediately posed after their discovery was whether whole thiopurines and thiopyrimides are incorporated in tRNA at the time of chain assembly or whether at some later time sulphur is exchanged for oxygen at particular sites in assembled tRNA chains that are deficient in sulphur. Sulphur-35 labelling has played an indispensable role in the discovery of the cysteine tRNA sulphurtransferase enzymes, that were found to substitute the sulphur-35 of labelled cysteine for the oxygen in the 4-position of uridine[39], in tRNA chains deficient in thiol sulphur. Sulphur-35 labelling also revealed that in some cases β-mercaptopyruvate could also serve as a donor of sulphur[40].

Sulphur-35 labelling of the cysteine residues in a protein has often been used as a convenient way of tagging a particular protein in the study of a

macromolecular phenomenon. For example, the macromolecular machinery used in the bacterial cell for the synthesis of proteins initially consists of (1) a chain of mRNA (messenger ribonucleic acid), (2) around which is clamped a 30S and a 50S ribosome particle, which together form an active 70S ribosome complex, (3) to which is bound a f-Met-tRNA$_F$ (N-formyl-L-methionyl transfer ribonucleic acid) molecule, that will supply the first amino acid to be incorporated. It was believed that upon completion of the synthesis of the polypeptide chain, the 70S ribosome is released in a form that cannot be immediately re-used and that it must first be dissociated back into 30S and 50S subunits. A protein known as initiation factor F_3 was later found to be essential for the formation of the initiation complex, and lately its function has been revealed in a study that has employed ^{35}S-labelled F_3[41]. ^{35}S-F_3 was shown to bind readily to 30S particles, but to neither 50S particles nor the 70S complex. When the ^{35}S-F_3 charged 30S subunit is induced to a 50S subunit by increasing the Mg^{2+} concentration of the media, ^{35}S-F_3 is released. This suggested that when an initiation complex is formed from 50S and F_3-30S subunits, F_3 is released and is free to dissociate other used inactive 70S complexes into subunits that can subsequently reform active 70S complexes.

^{35}S-Labelling has also been used in a quantitative fashion to obtain data on the number of binding sites available to a labelled molecule in a particular macromolecular complex. For example, the 30S particle was found to have one site available for ^{35}S-F_3[41]. Arabinosyl—6-mercapto purine-^{35}S (ara—MP-^{35}S), a non-toxic suppressor of the homograft response, was found to bind the surface red blood cells with a minimum of $6{\cdot}7 \times 10^5$ sites on B red blood cells and $1{\cdot}2 \times 10^5$ sites on tanned sheep blood cells[42].

Turning to a very simple biological system, ^{35}S-labelling has proved to be quite efficient in visualizing the behaviour of viruses. Virus particles usually consist of a strand of nucleic acid contained in a sheath of coat protein. Upon infection of a cell at 37°C, the nucleic acid enters the cell leaving its coat protein bound to the cell surface, whereas at 4°C the nucleic acid prefers to remain on the cell surface with its coat on. This phenomenon has been visualized with Sendai virus, whose coat proteins have been labelled with cysteine-^{35}S[43]. Ten minutes after infection of human amnion cell culture, faint uniformly distributed grains appear in the autoradiographs of the infected cell, reaching a maximum after 60 min. The uniform distribution of grains suggested that the labelled viral component was absorbed onto, but had not penetrated into, the cell. This was supported by the fact that identical grain counts were obtained at 37°C and 4°C. Mechanical shearing is often sufficient to

knock coat proteins off the cell surface. This technique together with ^{35}S-labelling can be used to distinguish between viral components injected into and absorbed onto cells[44]. MS-2 RNA coliphages contain two species of proteins, a coat protein and a maturation protein. The latter is required for both phage absorption to the F-pili of the host *Escherichia coli* cell and for the reconstitution of the infectious phage. ^{35}S-labelled MS-2 phage was used to determine whether the maturation protein enters the cell together with RNA. After infection at 37°C and shearing, 300 cpm/10^9 cells remained associated with the cell, whereas at 4°C only 20 cpm/10^9 cells were obtained. This implied that during infection the maturation protein had penetrated beyond the F-pili of the *E. coli* cell.

B. Whole Body Systems

In the remaining part of this section, we will consider the fascinating use of ^{35}S-labelling to follow the path taken by various thiols in an organism. After ingestion or intravenous or intraperitoneal injection, thiols rapidly cross the gastro-intestinal barrier and enter the vascular system of the organism, where they are swept by the blood flow past the membranes, lipoidal structures that insulate the organs and cells from the blood stream. At this point the thiol is evenly distributed in the vascular system of all the organs of the animal and its fate from here on will be determined largely by its physiochemical properties.

If the thiol is relatively soluble in lipids, it will be able to penetrate the lipoidal membranes, and will freely pass in and out of cellular structures. For example, thiopental, a rapidly acting anaesthetic, has a high solubility in lipids; and this allows it readily to penetrate the lipid membranes of the brain. A combination of ^{35}S-labelling and autoradiography has shown that the distribution of thiopental-^{35}S in the brain itself is not uniform[45]. Once inside the brain the distribution of the thiol depends not so much on its lipid solubility, but on the pattern of blood flow in the cortex, geniculates, colliculi and white matter of the cat brain. In fact, thiopental-^{35}S autoradiography has been used as a means of studying the physiological territory of supply of cerebral blood vessels[46]. While thiopental is freely passing in and out of the brain, its concentration in other organs is rapidly equilibrating in accord with the lipid solubility of the thiol. Ocular tissues, like the blood–brain barrier, behave as a lipid membrane and ^{35}S-thiopental, with its high lipid solubility, experiences no delay in penetrating the uveal tissue[47]. This is in contrast to more ionizable drugs, like phenobarbitone, which slowly penetrate the uveal tissue, but once inside bind to pigmented molecules. Thiopental-^{35}S forms no such complexes and is rapidly swept out of the tissue by the blood flow. In

vital organs, such as the brain, lung and liver, ^{35}S-activity reaches its maximum level within 15 s after injection and decreases to a plateau by 2 min. The liver then commences thiopental uptake again, obtaining a peak after 5 min, while depot fat takes up thiol at a constant rate. By the time the animal awakes, most of the thiol is concentrated in the liver and depot fat. It is interesting to observe that the lipid solubility of thiopental that allowed it to penetrate the brain so rapidly has led to the termination of its anaesthetic action[48]. With time thiopental will gradually accumulate in the kidneys and will be excreted[49].

A rough idea of the path that a thiol follows in the body can be obtained by measuring its rate of its excretion *via* urine, faeces and respiratory air. ^{35}S-Labelling has allowed the following kind of data to be obtained: 70% of glutathione-^{35}S subcutaneously injected in a mouse is excreted in the urine within 18 h[50]; the radioactivity of ^{35}S-thiobarbiturates are excreted 70–90% in the faeces and up to 1% by respiration[51]; SKF 525-A (2-diethylaminoethyl 2,2-diphenylvalerate) prolongs the thiopental induced sleeping time in mice by delaying the urinary excretion of injected ^{35}S-labelled thiopental[52].

Often in the course of a thiol's travels through the body, it will encounter a compound with which it will form a complex. In contrast to thiopental, penicillamine-^{35}S rapidly enters the plasma after oral administration where it is bound to the serum albumin[53]. In this bound state, penicillamine is no longer able to pass through the semi-permeable membrane of the kidneys, which retards its excretion in the urine. Penicillamine-^{35}S subsequently becomes evenly distributed in the body fluids, affording the drug an opportunity to scavenge copper efficiently from the body fluids. The resulting widespread and long-lasting action of the thiol makes it the drug of choice in the treatment of Wilson's Disease.

Inside a cell, a thiol might form a stable complex with a particular cellular constituent. Cystamine-^{35}S does not seem to form any particularly marked complexes with the cell nuclei, mitochondria and microsomes of liver and spleen[54], while cysteamine-^{35}S forms a very tight complex with the dinucleoprotein, which cannot be disrupted by repeated water shock and extraction[55].

In addition to forming a complex with a particular cellular substance, the thiol may encounter an enzyme that will alter its chemical composition. A change in the structure of the thiol can profoundly alter its distribution within the body. One of the most striking examples of this phenomenon is the accumulation of 6-methyl-thiopurine ribonucleotide-^{35}S (6-MMPR) by erythrocytes. The ratio of radioactivity in the erythrocyte as compared to plasma is 40 : 1, whereas in the case of 6-mercapto-purine-^{35}S the ratio is 1 : 100, representing a 4000-fold difference between

the two compounds. The selective accumulation of 6-MMPR-^{35}S in erythrocytes has been attributed to its intracellular phosphorylation to the more ionizable and hence less diffusible ribonucleotide[56]. The fact that the behaviour of a thiol within an organism is largely determined by physical properties such as lipid as opposed to water solubility suggested that more efficient drugs might be designed on the basis of their solubility properties. An interesting experiment along this line was the conversion of the water-soluble, carcinostatic drug 9-(β-D-xylofuranosyl)—9H—purine 6-thiol (xyl—6-MP) to its triacetyl derivative (xyl—6-MP—TAC). It was hoped that the derivative, which is relatively insoluble in water, would be retained in the body longer than xyl—6-MP. Surprisingly, xyl—6-MP—TAC-^{35}S was excreted in the form of xyl—6-MP-^{35}S and sulphate-^{35}S even more rapidly than xyl—6-MP-^{35}S itself[57].

If the thiol does not bind tightly to a cellular constituent or encounter an enzyme into whose binding site it can fit, it will eventually be excreted in an unaltered form. In one of the earliest applications of ^{35}S-labelling of thiols in a biological tracer experiment, mercaptohistidine-^{35}S was administered to rats and boars to test whether a metabolic pathway exists for the conversion of mercaptohistidine to its betaine derivative, the naturally occurring ergothioneine. Ergothioneine did not take up radioactivity and 90% of the administered 2-mercaptohistidine-^{35}S was excreted in the urine by the twenty-first day[58, 59].

Tracer studies such as those just described have found a particularly important application in the design of drugs that retard the growth of tumours and increase the survival times of afflicted animals, including man. One of the basic strategies that underlie the search for effective carcinostatic drugs is the design of a drug that has a high toxicity for tumour cells, while relatively non-toxic for the host animal. The fast turnover rate of tumour cells, and the demands that this places on the synthesis of purines and pyrimidines and their incorporation into DNA have proved to be the Achilles heel of the tumour cell.

One group of compounds that have proved to be particularly effective in interfering with DNA synthesis of tumour cells are the mercaptopurines and pyrimidines and their alkyl derivatives: 6-mercaptopurine (6-MP) blocks the *de novo* synthesis of purines[60]; 9-(β-D-arabinofuranosyl)—9H—purine—6-thiol (ara—6-MP) inhibits the incorporation of L-aspartic acid and orotic acid into DNA cystosine[61, 62]; 9-(β-D-xylofuranosyl)—9H—purine—6-thiol (xyl—6-MP) inhibits the utilization of exogenously administered guanine[57]; the periodic acid oxidation product of 9-(β-D-ribosyl)—6-methyl—thio purine (MMPR—OP) blocks the incorporation of thymidine into DNA[63]. The effective clinical use of thiols

such as these depends on two phenomena: whether the thiol will selectively accumulate in tumour cells, while the remainder of the drug is rapidly flushed out of the body and whether the thiol is selectively metabolized by the tumour cell to a more toxic substance.

The correlation of therapeutic action with the distribution of a drug had already been found in one of the earliest tracer studies of a labelled thiol. The powerful antithyroid drug, 4-methyl-2-thiouracil-^{35}S, was distributed more or less evenly in the different organs of the cockerel, with only the thyroid gland, the pituitary gland and the fast-growing base of the feathershafts showing distinctly above normal concentrations[64]. ^{35}S-Labelling has continued to be an indispensable tool in studying both of these phenomena during the testing of thiol drugs.

Both 6-mercaptopurine and buthiopurine (δ-(purinyl-6)mercaptovaleric acid) are carcinostatic drugs. However, buthiopurine is 8 times less active, but 30 times less toxic on chronic administration than 6-mercaptopurine. The origin of this effect was thought to lie in the relative tissue distributions of the drugs, which were studied using ^{35}S-labelling[65]. Mercaptopurine-^{35}S passed rapidly through the gastro-intestinal barrier and flooded many tissues, especially the liver, lungs, spleen and heart, as compared to the more gradual accumulation of buthiopurine in these organs. This was thought to account for the higher toxicity of 6-mercaptopurine. In the tumour itself, 6-mercaptopurine achieved a high level of accumulation, which then fell off as a function of time; whereas, buthiopurine persisted at a lower level for a longer time. The lower level of buthiopurine in the tumour as compared to that of mercaptopurine is in correlation with the effectiveness of the two drugs.

The oxidation of the ribosyl moiety of MMPR to MMPR—OP completely changes the mode of action of the drug as well as its stability. MMPR—OP-^{35}S is no longer selectively concentrated in tissues, but is rapidly excreted in the urine, most of it unchanged. The rapid passage of the drug through the body spares the host animal. However, a small portion of the drug is bound to the ascite tumour membrane and is responsible for the drug's therapeutic effect. Although the drug is cleaved in part to methylthiopurine, intact MMPR—OP was assumed to be the active agent[63]. Ara—6-MP-^{35}S rapidly appears in the blood, after intraperitoneal injection, where it is evenly distributed between plasma and red blood cells. At 3 min, the tumour cells already contained the largest percentage of the drug. By 30 min the drug is found in all tissues, except those beyond the blood–brain barrier. The concentration of the drug in the kidneys steadily increases with time, as the drug is cleared from the blood. The rapid clearance of the drug from the vital organs is thought

to account for its low toxicity. After 6 h 76% of the injected dose had been excreted, of which 87% could be accounted for as unchanged drug. The tumour cells themselves did not cleave ara—6-MP-^{36}S to 6-MP-^{35}S, nor appreciably converted it to the nucleotide, nor incorporated it into nucleic acids[61, 62].

6-Mercaptopurine-^{35}S is converted in the tumour cell to 6-methylthiopurine ribonucleotide. The ribonucleotide was shown to be much more efficient than the nucleotide of the parent compound, 6-MP, in inhibiting the enzyme, phosphoribosyl pyrophosphate amidotransferase, and subsequently bringing to a halt *de novo* purine synthesis in the tumour cell. The conversion of 6-MP follows the pathway 6-MP → MP nucleotide → 6-Me—MP nucleotide. Tumour cells lacking the enzyme hypoxanthine phosphoribosyl transferase, which is needed for the conversion to nucleotide, are spared the action of 6-MP. Compounds that would be active against 6-MP-resistant tumours have been actively sought, and those found include: 6-MeMP, MMPR—OP, ara—6-MP, 9-Me—6-MP and 9-Et—6-MP. ^{35}S-Labelling studies showed that these thiols are rapidly excreted unaltered[60, 61, 63, 66].

Till now we have considered the behaviour of thiols that are essentially foreign to the metabolism of the animal. However, perhaps the most sophisticated tracer techniques yet applied to the study of labelled thiols have been developed in the course of investigations of the utilization of a pulse-labelled cysteine in the on-going process of the synthesis of body proteins. After administration, ^{35}S-cysteine quickly enters the various amino acid pools of the body and is incorporated along with naturally occurring cysteine into the polypeptides synthesized in various tissues.

When amino acid sequencing techniques were first applied to proteins, the sequence Cys–Gly–Gly was found to occur with greater than chance frequency. This suggested that perhaps this sequence originated from glutathione, rather than from free amino acids. To check this, oviduct mince was incubated with glutathione labelled with ^{35}S in the cysteine residue and ^{14}C in the carboxyl group of the glycyl residue. The ovalbumin produced was hydrolysed and the specific activity of cysteic acid and glycine originating from the sequence Cys–Gly was compared to the activity of those amino acids from other positions in the polypeptide chain. The results indicated that glutathione played no specific role in the biosynthesis of the Cys–Gly sequence[67].

The rate of uptake of labelled cysteine into proteins has been extensively used as an indicator of the metabolic activity of tissues. ^{35}S-L-cysteine administered to mice was found to be preferentially incorporated into growing hair follicles and claws. In other forms of epithelia the rate of

incorporation was found to be related to the cell turnover rate end and in glandular cells to the rate of protein synthesis[68]. Bleeding caused an arrest or delay in the incorporation of cysteine-^{35}S into organ proteins, followed by a period of enhanced incorporation[69]. Zinc deficiency in rats impairs the incorporation of L-cystine-^{35}S in skin protein while enhancing the rate of incorporation of L-cystine-^{35}S into pancreas protein. This suggested that zinc is essential to the synthesis of skin keratin and collagen[70].

Many hormones are rich in cysteine and the tissues in which they accumulate can be easily recognized by a marked uptake of ^{35}S-L-cysteine. For instance, mature virgin mice, mature mice of both sexes and castrated males display a ^{35}S-labelled juxtamedullary X-zone in the brain, whereas normal adult male mice do not[71]. The neurosecretory system of the earthworm markedly accumulates cysteine-^{35}S[72]. The neurosecretory cells of rapidly developing female locusts and females in the second gonotropic cycle take up cysteine-^{35}S at a greater rate than either newly emerged or slowly developing females[73].

The neurosecretory system that has been studied in greatest detail is the brain's hypothalamo-hypophysial tract, that is concerned with the synthesis of the octapeptide hormones, oxytocin and vassopressin, and their secretion into the blood stream. Bargmann[74] and Schrarrer[75] have proposed that the neurophysial octapeptides are synthesized in the perikaryon of specialized nerve cells. They are subsequently bound to carrier proteins, the neurophysins, which are then organized into granules. These granules of neurosecretory material are then transported down the axon of the neuron and stored in the terminals of the nerve fibres. The release of the hormones into the blood vessels is accompanied by the dissociation of the hormone from the carrier protein. Morphologically[74, 75, 76], the system consists of two paired nuclei, the supraoptic and the paraventricular nuclei, which lie in the hypothalamus of the brain. The axons that extend from these parikaryons run through the hypothalamo-hypophysial tract and reach the neurohypophysis, where they terminate next to the basement membrane of the blood capillaries.

The neurosecretory material is rich in cysteine and can be spotted with histochemical reagents specific for S—H and S—S bonds. Histochemical staining has located neurosecretory material in the Golgi bodies of the parikaryon and stored in vesicles in the nerve terminals[74]. However, such staining techniques cannot detect the flow of hormones through the neurosecretory system, while the use of single pulses of ^{35}S-cysteine offers the possibility of observing the fascinating process of the flow of neurosecretory material through the cells of the secretory system.

In 1959 Sloper[77] first performed the now much repeated experiment of administering ^{35}S-labelled cysteine and methionine to rats and observing the appearance of radioactivity in various parts of the neurosecretory system. Labelled cysteine and methionine rapidly appeared in the supraoptic nuclei, and only later labelled cysteine, but not methione, appeared in the infundibular process of the neurophysis. This suggested that the supraoptic nuclei were actively engaged in protein synthesis, and one of these polypeptides, particularly rich in cysteine, had migrated to the neurophysis. Ficq and Flament-Durand[78] similarly observed that cystine-^{35}S appeared in the supraoptic and paraventricular nuclei within 5 min after administration of labelled cystine, and only 10 h later did labelled material appear in the neurohypophysis. Talanti and coworkers[79,80] have monitored as function of time elapsed after the administration of labelled cysteine the radioactivity that appears in the supraoptic and paraventricular nuclei, as well as in three sites along the hypothalamo-hypophysial tract and in the neurohypophysis. When one has such a set of data, stating as a function of time the amount of label present in an anatomical structure, a kinetic model of the system can be set up that consists of a number of discrete pools of compounds whose flow from compartment to compartment obeys simple mathematics. When Talanti and coworkers[79,80] analysed their data in terms of such a kinetic model, they could detect a component that first appeared in the supraoptic and paraventricular nuclei and slowly flowed through the hypothalamo-hypophysial tract to the neurohypophysis. Superimposed on the slow component was a rapidly abating pulse of radioactivity that moved through the hypothalamo-hypophysial tract at a constant speed of 0·6 mm/h without experiencing any delays. The fast component was thought to represent neurosecretory material, while the slow component represents structural proteins.

The identity of the labelled material that was seen to flow through the neurosecretory system was established only when the system was taken apart chemically. Sachs[81], by directly infusing highly labelled cysteine-^{35}S into the third ventricle of the brain of a dog, succeeded in isolating minute quantities of vasopressin-^{35}S. Vasopressin-^{35}S associated with the neurosecretory particle always had the lowest specific activity, whereas vasopressin-^{35}S found in the cell nuclei and in large granules had the highest specific activity. Norström and Sjöstrand[82] later showed in a very elegant experiment that following the injection of cysteine-^{35}S in the area of the supraoptic nuclei, radioactivity appeared in a group of proteins that migrated through the hypothalamo-neurohypophysial tract, at a speed of 2–3 mm/h. Approximately 90% of the radioactivity of these

soluble proteins was recovered in a single protein component. Norström, Hansson and Sjöstrand[83] later showed that when the microtubuli of the axons are depolymerized with colchinine, the amount of labelled material that reaches the hypothalamo-neurohypophysial tract and the neurohypophysis is considerably reduced.

Quite early in the course of these tracer studies it was noted that marked changes in the uptake of cysteine-^{35}S occur following periods of water deprivation. Wells[84] found that in rats thirst causes a marked increase in the uptake of radioactivity in the supraoptic nucleus and to a lesser extent in the paraventricular nucleus. Talanti[85] later observed that thirst accelerates the rate of disappearance of radioactivity from the supraoptic and paraventricular nuclei, as well as the disappearance of radioactivity from the neurohypophysis. These results indicated that thirst activates both the synthesis and release of neurosecretory hormones that regulate the function of the kidneys.

V. APPLICATION OF ^{35}S-TRACER STUDIES TO AGRICULTURAL SCIENCE AND INDUSTRY

Perhaps the largest system in which ^{35}S-labelling has been used to follow the distribution of a thiol was a 20 acre forested area that was aerially sprayed with Malathion-^{35}S during a study of the ecological transport of the insecticide[86]. Samples were taken in a number of ingenious ways. Air samples were taken on frosted glass discs suspended from helium balloons to measure the above canopy drift of the insecticide off the area. Samples of bark were taken to measure the settling out of the insecticide at different layers within the canopy. Soil samples were measured to determine the subsurface distribution. Samples collected on spotting enamel paper placed throughout the forest monitored the horizontal distribution of the insecticide. Samples from streams, insects, mammals, reptiles and birds indicated the initial and subsequent transport of the insecticide and its metabolities in the ecosystem.

The cream of cows which have consumed the weed, landcress, becomes tainted upon heat treatment with α-toluenethiol. In order to determine the efficiency of steam distillation for the removal of the taint, ^{35}S-labelled α-toluenethiol was added to cream. The measurement of radioactivity proved to be a convenient analytical method to determine the amount of thiol that remained in the cream[87].

The SH : SS ratio in gluten has been conveniently measured by assaying the relative ^{35}S-activity of NEMI-cysteine and cystine in gluten prepared

from dough that had been made from the flour of wheat that was grown on soil supplemented with sulphate-^{35}S[88].

The friction produced by a chrome-steel ball-bearing moving against discs and steel and brass creates a layer of FeS on the disc when it is lubricated with a mixture of cetane- and dodecane-thiol. The rate of formation of FeS and its subsequent wear were quantitatively measured by taking autoradiographs of the tracks of $Fe^{35}S$ left by the ball-bearing on the steel discs when dodecane thiol-^{35}S was added to the lubricant[89].

VI. ISOTOPE LABELLING AND COUNTING IN PRACTICE

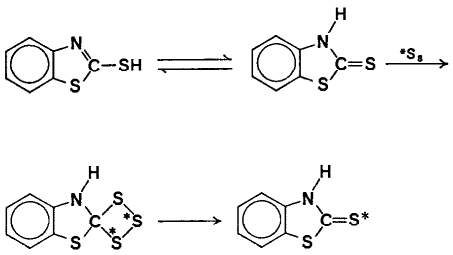
Having reviewed the phenomena that can be probed with isotopically labelled thiols, we now turn to the technical problems associated with the execution of an experiment using isotope labelling. While many of the isotopically labelled thiols discussed in this review are now commercially available, we will review the synthetic procedures that have been used in the past to incorporate deuterium, tritium and sulphur-35 into these thiols, in the hope that it will allow the researcher with a less common thiol to choose the best synthetic route to its preparation. Having prepared a ^{35}S-labelled thiol, various methods are available for the assay of its sulphur-35 activity. The method, best suited to a particular study, will depend on the accuracy desired, the level of sulphur-35 activity in the sample, and the nature of the medium in which the ^{35}S-labelled thiol is dispersed. These and the various auxiliary techniques used to prepare the sample for counting will be discussed. Finally, we will turn to various methodological and phenomenological considerations which have rendered past ^{35}S-labelling studies, especially in endocrinology, subject to criticism.

A. Synthetic Methods

Perhaps the simplest and most elegant method of labelling a thiol with ^{35}S would be to add a neutron to the nucleus of natural abundance ^{34}S by the nuclear reaction $^{34}S(n,\gamma)^{35}S$. To date, this method has not been used, probably because there is no effective way to prevent the heat generated by the nuclear reaction from decomposing the molecule.

If the sulphur in a thiol cannot be rendered radioactive itself, it might be exchanged for thermally activated radioactive ^{35}S atoms. For instance, the sulphur atoms of mercaptobenzothiazole exchange with ^{35}S recoil atoms generated *in situ* by the nuclear reactions, $Cl(n,p)^{35}S$ (where $C_6H_3Cl_3$ is used as the Cl source) or $^{34}S(n,\gamma)^{35}S$ where elemental sulphur

is the source of natural abundance ^{34}S[90]. The yield of ^{35}S-labelled mercaptobenzothiazole is ~2–5% for ^{35}S generated from ^{35}Cl and ~30% for ^{35}S from ^{34}S. It is not necessary to use ^{35}S recoil atoms to accomplish the exchange. It has long been known that during the heating of a solution of 2-mercaptobenzothiazole with sulphur-^{35}S, the sulphur of the mercapto group is exchanged for radio-sulphur[91]. Since the thiol group of mercaptobenzothiazole is in tautomeric equilibrium with the thion form, exchange is thought to occur by the addition of elemental sulphur to the C=S bond of the thio tautomer (equation 26). Morávek and Kopecky[92,93] have



(26)

found the exchange to be generally synthetically useful for the labelling of thiols that can exist in a tautomeric form. Table 1 lists the thiols that have been labelled in this way.

The exchange of labelled sulphur can be promoted by enzyme catalysts, instead of heating. Bird egg yolk[94] and the cysteine desulphydrase[95,96,97] that it contains catalyse the exchange of sulphur-35 from $Na_2{}^{35}S$ to L-cysteine, L-cystine and L-cysteic acid. In a typical experiment, 150 ml of a buffer solution containing 2 millimoles of cysteine–HCl, 2 millimoles of $Na_2{}^{35}S$ and 500 mg of cysteine desulphydrase preparation is incubated at 38°C for 15 h. A mixture of 74·4% cystine-^{35}S and 25·3% cysteine-^{35}S is obtained. L-Cystine-^{35}S is subsequently reduced electrolytically to cysteine-^{35}S. The total yield of L-cysteine-^{35}S obtained by isotope exchange is 70%.

Although isotope exchange by virtue of its simplicity and ability to form compounds of high specific activity is the method of choice for the labelling of tautomeric thiols, a synthetic method is often better suited to other thiols. For example, heating α-toluenethiol with sulphur-^{35}S in benzene at 135–140°C for 6–12 h, yields α-toluenethiol-^{35}S with a specific activity of only 2–9%. However, the synthesis of the compound from benzyl-magnesium chloride and sulphur-^{35}S yields α-toluenethiol-^{35}S

TABLE 1. Isotopically labelled thiols

Compound	Source of the isotope	Method of synthesis	Reference
Methanethiol-S-d_1	D_2O	Isotope exchange	16
Methanethiol-C-d_3	CD_3I	$+SC(NH_2)_2$	5
Methanethiol-^{35}S	Thiourea-^{35}S	$+(CH_3)_2SO_4$	110, 115
		$+CH_3I$	111
Ethanethiol-C_1-d_2	$LiAlD_4$	$+CH_3C{=}S{-}OEt$	2
Ethanethiol-^{35}S	Hydrogen-sulphide-^{35}S	$+C_2H_4$	100
Ethanedithiol-S-d_2	D_2O	Isotope exchange	18
2-Mercaptoethanol	Hydrogen sulphide-^{35}S	$+ClCH_2CH_2OH$	112
β-Mercaptoacetic acid (thioglycollic acid)	Hydrogen sulphide-^{35}S	$+CH_2CH_2O$	112
	Thiourea-^{35}S	$+ClCH_2COOH$	117
	Dithioglycollic acid-$^{35}S_2$	H_2 reduction	124
β-Dimethylaminoethane-thiol-^{35}S	Thiourea-^{35}S	$+(CH_3)_2NCH_2CH_2Cl$	118
β-Diethylaminoethanethiol-^{35}S	Thiourea-^{35}S	$(CH_3CH_2)_2NCH_2CH_2Cl$	119
n-Butanethiol-S-d_1	DCl, D_2O	Isotope exchange	28, 24
n-Butanethiol-^{35}S	Thiourea-^{35}S	$+CH_3CH_2CH_2CH_2Br$	108
	Sodium hydrogen sulphide-^{35}S	$+CH_3CH_2CH_2CH_2Br$	116
iso-Butanethiol-^{35}S	Sulphur-^{35}S	$+(CH_3)_2CHCH_2MgBr$	104
2,3-Dimercaptosuccinic acid-^{35}S	$(^{35}SCH_2COOH)_2$	H_2 reduction	127
n-Hexanethiol-S-d_1	D_2O	$+CH_3(CH_2)_4CH_2SNa$	13
Cyclohexanethiol-S-d_1	D_2O	Isotope exchange	14
Benzenethiol-S-d_1	D_2O	$+C_6H_5SNa$	13
Benzenethiol-S-t_1	HTO	Isotope exchange	23
p-Halogen-benzene thiol-S-d_1	Methanol-O-d_1	$+XC_6H_4S{-}Si(CH_3)_3$	134
Benzenethiol-^{35}S	Sulphur-^{35}S	$+C_6H_5MgBr$	105
α-Toluenethiol-S-t_1	HTO	Isotope exchange	34

TABLE 1 (*cont.*)

Compound	Source of the isotope	Method of synthesis	Reference
α-Toluenethiol-^{35}S	Sulphur-^{35}S	$+C_6H_5CH_2MgCl$	106
	Sulphur-^{35}S	Isotope exchange	98
	Sodium hydrogen sulphide-^{35}S	$+C_6H_5CH_2Br$	108
p-Toluenethiol-^{35}S	Sulphur-^{35}S	$+CH_3C_6H_4SMgBr$	108
2-Phenylethanethiol-^{35}S	Sulphur-^{35}S	$+C_6H_5CH_2CH_2MgBr$	109
p-Methoxybenzenethiol-^{35}S	Carbon disulphide-^{35}S	$+p$-$CH_3OC_6H_4N_2Cl$	108
p-Phenylbenzenethiol-^{35}S	Carbon disulphide-^{35}S	$+p$-$C_6H_5C_6H_4N_2Cl$	108
α-Naphthalenethiol-^{35}S	Sulphur-^{35}S	$+\alpha$-$C_{10}H_7MgBr$	110
2-Mercaptobenzothiazole-$^{35}S_{2,3}$	Sulphur-^{35}S	$+C_6H_5NCS$	162
2-Mercaptobenzothiazole-^{35}S	Sodium hydrogen sulphide-^{35}S	2-Chloromercapto-benzothiazole	122
	Sulphur-^{35}S	Isotope exchange	91
D,L-Cysteine-^{35}S	$C_6H_5COSCH_2CH(NHCOC_6H_5)CO_2CH_3$-$^{35}S$	Hydrolysis	
L-Cysteine	Sulphur-^{35}S or	Enzymatic isotope exchange	94, 95
	Sodium sulphide-^{35}S		96, 97
	SO_4^{2-}-^{35}S	Biosynthesis	127
α-Amino-β-mercaptobutyric acid	4-Carboxy-5-methyl-2-phenyl thiozoline-^{35}S	Acid hydrolysis	126
Thiopental-^{35}S (5-Ethyl-5(-1-methylbutyl)-2-thiobarbituric acid)	Sulphur-^{35}S	Isotope exchange	163, 164 165
Thiopyrimidines	Sulphur-^{35}S	Isotope exchange	166
2,4-Dithiouracil	Sulphur-^{35}S	Isotope exchange	93
4-Thiouracil	Sulphur-^{35}S	Isotope exchange	93
6-Methyl-2-thiouracil	Sulphur-^{35}S	Isotope exchange	167, 168, 93

TABLE 1 (*cont.*)

Compound	Source of the isotope	Method of synthesis	Reference
2-Thiouracil	Sulphur-^{35}S	Isotope exchange	166, 168, 93
4-Amino-2-thiouracil	Sulphur-^{35}S	Isotope exchange	93
6-Phenyl-2-thiouracil	Sulphur-^{35}S	Isotope exchange	93
2-Thio-6-azouracil	Sulphur-^{35}S	Isotope exchange	93
6-Amino-2-thiouracil	Sulphur-^{35}S	Isotope exchange	93
2-Thiobarbituric acid	Sulphur-^{35}S	Isotope exchange	93
6-Mercaptopurine	Sulphur-^{35}S	Isotope exchange	168, 93
	Barium sulphate-^{35}S	6-Chloropurine	123
2-Methyl-6-mercaptopurine	Sulphur-^{35}S	Isotope exchange	93
6-Thioguanine	Sulphur-^{35}S	Isotope exchange	168
6-Thioguanosine	Sulphur-^{35}S	Isotope exchange	93
6-Mercaptopurine riboside	Sulphur-^{35}S	Isotope exchange	93
2-Hydroxyl-6-mercaptopurine	Sulphur-^{35}S	Isotope exchange	93
6-Hydroxy-2-mercaptopurine	Sulphur-^{35}S	Isotope exchange	93
8-Mercaptoguanosine	Sulphur-^{35}S	Isotope exchange	93
2-Thioxanthine	Sulphur-^{35}S	Isotope exchange	168
Coenzyme-A-^{35}S	$^{35}SO_4^{2-}$	Biosynthesis	131
Glutathione-^{35}S	$^{35}SO_4^{2-}$	Biosynthesis	130
γ-Globulin-^{35}S	Cysteine-^{35}S	Biosynthesis	132
Insulin-^{35}S	Cysteine-^{35}S	Biosynthesis	129
Dysentery bacteria-^{35}S	Cysteine-^{35}S	Biosynthesis	133

with a specific activity of 27%[98]. There are a number of synthetic routes available for the synthesis of labelled thiols, ranging in specificity from high temperature and hot atom reactions to the biosynthesis of complex thiols, such as coenzyme A.

Thermolysis and recoil atom reactions yield quite complex mixtures of thiols. For example, when an equimolar mixture of $C_2H_2-N_2-H_2{}^{35}S$ was passed through an empty quartz tube C_3H_8, 0·00001%; C_4H_{10}, 0·001%; cyclobutane, 0·001%; ethanethiol, 0·1%; butanethiol, 0·2%; isobutanethiol, 0·2%; and many other unidentified products was obtained[99]. When a 1 : 1·3 mixture of $^{14}C_2H_4$ and $H_2{}^{35}S$ was heated for 10 h at 310°C at 20 atmospheres, $^{14}CH_3{}^{14}CH_2{}^{35}SH$ and $(^{14}CH_3{}^{14}CH_2)_2{}^{35}S$ were obtained in mole fractions of $3{\cdot}3\times10^{-4}$ and $3{\cdot}9\times10^{-4}$, respectively[100]. ^{35}S-Recoil atoms produced in a mixture of methane–HCl by the atomic reaction $^{35}Cl(n,p)^{35}S$, yield a mixture containing $H_2{}^{35}S$ and $CH_3{}^{35}SH$ as the major constituents[101]. The relative amounts of the products can be controlled by adjusting the concentration of Ar and NO, which serve as moderator and radical scavenger. Hot ^{35}S atoms formed by the neutron bombardment of CCl_4 react with a cyclopentane : cyclohexane mixture to give a mixture of ^{35}S-labelled thiophene, tetrahydrothiopyran, cyclopentanethiol, cyclohexanethiol, ethanethiol, proparethiol, butanethiol, dicyclopentyl sulphide and polymeric mercaptans and sulphides[102]. Neutron bombardment of a 1 : 1 mixture of CCl_4 and cyclohexane yields a reaction containing $C_6H_{11}SH$ and $C_5H_{10}S$ at levels of 3·5 and 8% of the total radioactivity, respectively; however, the majority of the activity is found in non-volatile products[103]. In practice, the more conventional synthetic methods used for the preparation of thiols in general are better suited to the preparation of labelled thiol, especially when the ^{35}S-labelled precursor is commercially available.

Thiomagnesium halides formed by the reaction of sulphur with a Grignard reagent can be decomposed to the corresponding thiols (equation 27). While the reaction has not been extensively used for the preparation of non-labelled arenethiols, it is particularly well suited to the

$$RX+Mg \longrightarrow RMgX \xrightarrow{^{35}S} R^{35}SMgX \xrightarrow{HX} R^{35}SH+MgX_2 \quad (27)$$

synthesis of ^{35}S-labelled thiols, since the ^{35}S-labelled reactant, sulphur-^{35}S, is readily available. Among the ^{35}S-labelled thiols that have been prepared by this method are iso-butanethiol[104], benzenethiol[105], α-toluenethiol[98, 106, 107], *p*-toluenethiol[108], 2-phenylethanethiol[109] and α-naphthalenethiol[110]. Yields vary from 44 to 90%.

In recent years the method of choice for the preparation of thiols in the laboratory has become the addition of an alkyl-halide to thiourea to form

an S-alkylisothiouronium halide which is subsequently decomposed with alkali to the alkanethiol (equation 28). The reaction is easy to control and

$$RI + SC(NH_2)_2 \longrightarrow RSC(:NH)NH_2HI \longrightarrow RSH + H_2NCN \quad (28)$$

the isothiouronium salts are stable and can be stored. The decomposition of S-methylisothiouronium sulphate, prepared from thiourea and dimethylsulphate, has been used as a convenient source of methanethiol-^{35}S in the course of a number of syntheses. The quaternization of thiourea with methyl iodide[111] has been reported to give higher yields than with dimethyl-sulphate. The ^{35}S-labelled precursor, thiourea-^{35}S, is prepared from H_2^*S by reaction with H_2O, NH_2CN, NH_4OH[112] or from $Ba^{35}S$ by treatment with H_2O, NH_4HCO_3 and a trace of powdered sulphur[113, 114]. A number of ^{35}S-labelled thiols have been prepared in this way, including methane thiol-^{35}S[111, 114, 115], *n*-butanethiol-^{35}S[116], β-mercaptoacetic acid-^{35}S[117], dimethylaminoethanethiol-^{35}S[118], diethylaminoethanethiol-^{35}S[119] and 2-thiouracil-^{35}S[120]. Yields up to 90·5% have been reported.

In 1840 Regnault passed ethyl chloride into potassium hydrogen sulphide in a retort and obtained ethanethiol[121] (equation 29). This classical synthetic method has been used to prepare labelled thiols from $Na^{35}SH$ and organic halides. The thiols prepared by this method include

$$RCl + NaSH \longrightarrow RSH + NaCl \quad (29)$$

n-butane-thiol-^{35}S[108], α-toluenethiol-^{35}S[108] and 2-mercaptobenzothiazole-^{35}S[122]. In variations on the method, 2-mercaptoethanol-^{35}S has been prepared from $H_2{}^{35}S$ and 2-chloroethanol[112] and 6-mercaptopurine was obtained by heating 6-chloropurine with $Ba^{35}SO_4$[123].

A standard method for making aromatic thiols from relatively unreactive aromatic halides is to convert them to the aromatic diazonium salt,

$$\left.\begin{aligned} RN_2Cl + KSCSOEt &\longrightarrow RSCSOEt + N_2 + KCl \\ RSCSOEt + H_2O &\longrightarrow RSH + COS + EtOH \end{aligned}\right\} \quad (30)$$

which readily reacts with a xanthate, such as $EtOCS_2K$ (equation 30). ^{35}S-Labelled $EtOCS_2K$ has been prepared by treating Na_2S and sulphur-^{35}S with CS_2 to form uniformly labelled $NaCS_3$, which is then decomposed with HCl and the resulting CS_2-^{35}S passed through a EtOH/EtOK solution. Both ^{35}S-labelled *p*-methoxy- and *p*-phenyl-benzenethiol have been prepared from $EtOCS_2K$-^{35}S and the corresponding diazonium chloride[108].

When ^{35}S-labelled disulphides are available, the corresponding ^{35}S-thiol can be readily prepared by electrolytic or H_2 reduction (equation 31). β-Mercaptoacetic acid[124] and cysteine have been obtained in this way[125].

$$RSSR + H_2 \longrightarrow 2\,RSH \quad (31)$$

Although the addition of H_2S to unsaturated bonds proceeds in quantitative yields, e.g. the addition of hydrogen sulphide to ethylene gives ethyl mercaptan with no by-products, the reaction has been used only once to prepare ethanethiol-^{35}S from H_2S-^{35}S and ethylene (equation 32)[100]. The addition of H_2S-^{35}S across strained heteroatomic bonds in

$$H_2S + \underset{\diagdown X \diagup}{CH_2CH_2} \longrightarrow HSCH_2CH_2XH \quad (32)$$

small ring compounds has been used to prepare 2-mercaptoethanol[112] from ethylene oxide and 2-mercaptoethylamine from ethyleneimine[112].

In addition to these standard methods, a number of specialized reactions of limited scope have been used to prepare some biologically important ^{35}S-labelled thiols. For instance, 2-thiouracil-^{35}S has been prepared by the condensation of thiourea-^{35}S with $NaOCH{=}CHCO_2Et$[120]. α-Amino-β-mercaptobutyric acid-^{35}S was prepared by the acid hydrolysis of 4-carboxy-5-methyl-2-phenylthiazoline-^{35}S[126]. 2,3-Dimercaptosuccinic acid-$^{35}S_2$ was obtained by the hydrolysis of 2,3-bis(acetylthio)succinic acid-^{35}S[127]. D,L-Cysteine-^{35}S was obtained by the acid hydrolysis of $PhCOS{-}CH_2CH(HNCOC_6H_5)CO_2Me$[128].

However, as the biologically interesting thiols become more complex, biosynthetic routes would appear to be the method of choice for their synthesis, in spite of the inherent loss of ^{35}S isotope in the biological system and need for chromatographic separation of the isotopically labelled molecule from a complex biological mixture. L-Cysteine-^{35}S[129], glutathione-^{35}S[130] and coenzyme-A-^{35}S[131] have been obtained from labelled sulphate by biosynthetic routes, while complex polypeptides, such as γ-globulin[132] and insulin[129] have been obtained from organisms grown on cysteine-^{35}S. Even highly labelled whole organisms such as dysentery bacteria[133] have been grown on cysteine-^{35}S.

Deuterium and tritium labelling of the SH group can be carried out most conveniently by isotope exchange with D_2O or T_2O by simply dissolving the thiol in the labelled solvent, followed by evaporation. The thiols labelled by isotope exchange are, CH_3SD[5, 16], $DSCH_2CH_2SD$[18], $CH_3(CH_2)_3SD$[24, 28], $C_6H_{11}SD$[14], C_6H_5ST[23], $C_6H_5CH_2ST$[34]. Thiols have also been deuterated by the D_2O solvolysis of Na mercaptides, such as

$CH_3(CH_2)_5SNa$[13] and C_6H_5SNa[13] and by the reaction of MeOD with $XC_6H_4S{-}SiMe_3$ (X = halide)[134].

As in any synthesis employing radioisotopes, special care must be taken not to contaminate the laboratory. Special glassware which minimize the escape of the isotope are usually designed to meet the needs of a specific synthetic route. The preparation of thiols from radioactive sulphur and a Grignard reagent is a good illustrative example of the design of such vessels for an organic reaction and the subsequent extraction of the labelled compound with organic solvents[110].

An apparatus for the reaction of ^{35}S with a Grignard reagent is shown in Figure 3. The Grignard reagent is pipetted in tube A, ether is added and

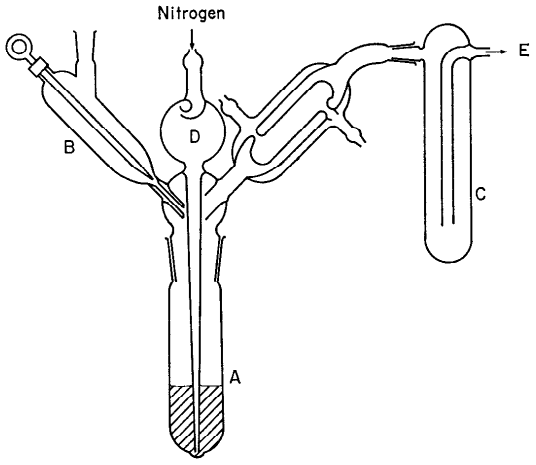


FIGURE 3. Reaction vessel used for Grignard reaction.

the apparatus is flushed with nitrogen. Sulphur-35 dissolved in xylene is added to the mixture from B and the reaction mixture is stirred under nitrogen at 0°C. The liquid air trap C protects the mixture from moisture, while tube D acts as a liquid trap in a case of a pressure backflow due to a pressure build-up in a series of aqueous sodium hydroxide traps connected at E. Upon completion of reaction the Grignard reagent is decomposed by addition of HCl.

The labelled thiol is extracted from the reaction mixture by rapidly transferring the reaction flask A to the apparatus shown in Figure 4. The extraction is carried out under a nitrogen atmosphere. By properly adjusting the traps, the reaction mixture is transferred from A to the separatory funnel G, to which ether is added through H. The two phases are agitated by the magnetic stirrer I, and the aqueous layer is returned to A and the ether layer to F. The aqueous layer is extracted with more

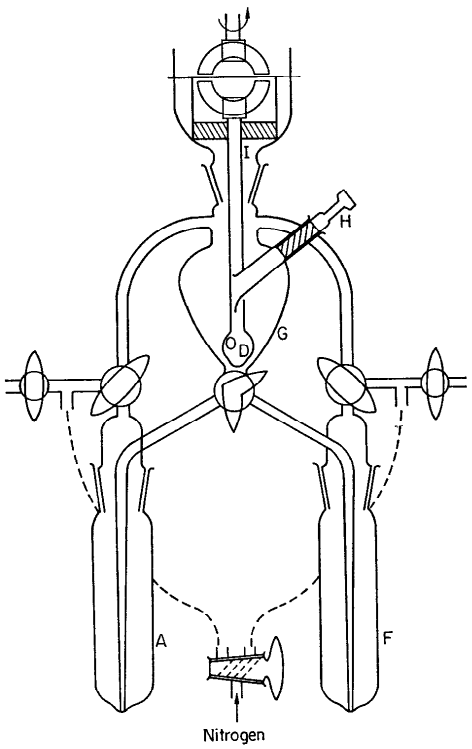


FIGURE 4. Vessel for the extraction of ^{35}S-labelled thiols.

portions of ether added through H. The thiol can be precipitated from the ether layer as the Na salt by simply extracting the aqueous layer with the 10% aqueous sodium hydroxide.

B. Counting Methods

The low energy β-rays emitted by ^{35}S can be counted in a number of different ways, including gas flow counting, liquid scintillation counting and autoradiography on photographic emulsions. The particular method chosen depends on the nature of the sample.

Perhaps the simplest counting procedure is to place the same on a planchet and assay its radioactivity under either a windowless gas flow counter or a mica end window counter. This method of counting has most often applied to $BaSO_4$-^{35}S[58,59,64] or benzidine sulphate-^{35}S[65], which is layered on the planchet. In addition, films of polymers[110], TCA precipitated proteins, whole blood[42], and red blood cell ghosts[42] labelled with ^{35}S have been counted in this way. Often the counting of a layer of material is complicated by the self-absorption of the radiation from the bottom of the sample. The self-absorption of radiation is generally standardized by preparing layers that are 'infinitely thick', e.g. 15–16 mg sulphate per cm^2. This ensures that radiation from the bottom of the sample is completely adsorbed. When ^{35}S to be counted is in the gas phase, as in SO_2 or H_2S, it can be introduced together with methane directly into a Geiger–Müller tube and counted at efficiencies of 95–96%[135]. Sulphur dioxide-^{35}S can be introduced up to 7·5 torr, whereas hydrogen sulphide-^{35}S can be counted at much higher partial pressures. A novel application of this type of counting was the measurement of β-activity of alkanethiols, as they emerge from a gas–liquid chromatograph, in which methane was used as the carrier gas[136].

A convenient and fast method of locating ^{35}S-labelled spots on thin-layer plates or on paper chromatograms is to pass the chromatogram under a windowless gas flow counter[89,92]. Radiochromatogram scanners of this type are commercially available and their design have been described[137,138]. However, for accurate determination of radioactivity the spot must be counted in a liquid scintillation counter.

Liquid scintillation counting has been used to assay the radioactivity of numerous ^{35}S-labelled compounds after they have been separated on TLC plates. However, the ^{35}S-labelled compound must first be located by radiochromatogram scanning, scraped off the plate and eluted off the absorbent into the counting solution. Alternatively, the absorbent together with the ^{35}S-labelled compound can be suspended in the counting solution by Cab-O-Sil. Paper chromatograms, on the other hand, are usually cut into 1 cm strips which are eluted and counted in a toluene scintillator. Polyacrylamide gels have been embedded in 2% agar gel, mounted on a rubber plate and cut into 1 mm thick slices in a mechanical chopper. The strips are then placed in a liquid scintillator phial, extracted in 1 ml of toluene and subsequently counted[82]. Alternatively, gels were thickened with 10% glycerol and sliced in a dry-ice acetone–hexane bath[139]. The radioactivity of ^{35}S-labelled compounds, emerging from a liquid chromatograph, has been measured as they flow through a plastic scintillator spiral[140]. A number of liquid scintillator fluors particularly suited for the low energy β-rays of ^{35}S have been developed[141,142,143].

Autoradiography has been extensively used to locate radioactive areas on chromatograms. Usually the chromatogram is pressed against a no-screen X-ray film and allowed to develop[131]. The development time can extend over a period of weeks or months, which allows radioactive areas of very low activity to be detected[46].

Autoradiography is particularly well suited for determining the distribution of radioactivity in tissue. In principle, the distribution of radioactivity in a tissue could be assessed by gas flow counting, if the tissue was dissected, its parts weighed and uniformly spread as a dry film on a degassed planchet. However, very often it is difficult to identify exactly the part of the tissue that has been dissected. Furthermore, the fluids which surround the tissue in the body may often be very highly labelled and will contaminate the dissected specimen. The use of autoradiography readily overcomes these difficulties[77].

The methods of preparing the autoradiographs most commonly used in ^{35}S tracer studies are those of Doniach and Pelc[144] and Ullberg[145]. The choice of exposure time and counting methods has been discussed by Pelc[146]. The activity recorded on the photographic film can be determined either by directly counting silver grains[73] or by mounting the autoradiograph on a microscope slide and measuring the relative amount of light transmitted using a photocell at the ocular of a microscope[84]. The former is more accurate and the data are obtained in a form that can be treated by statistical methods, i.e. silver granules/μ^2 ($\pm$S.E.M.). The absolute sensitivity of electron microscope autoradiography, i.e. ratio of developed grains to radioactive decays in the specimen, were determined for ^{35}S with Ilford L4 and Kodak NTE emulsions and found to be 1/21 for ^{35}S in a monomolecular layer[147]. The resolution that can be obtained depends on the photographic emulsion. The observed radioactivity depends on several physical factors, including the thickness of the sample, the nature of the tissue, the exposure time and the modalities of the

developing procedures[78]. Autoradiography has been used to follow the whole body distribution of ^{35}S in plants and animals[68], as well as the movement of ^{35}S down the axon of a nerve cell[77-80].

C. Sample Preparation

1. Wet ashing

The wet ashing technique was originally designed to convert sulphur contained in organic material into a form, such as $BaSO_4$, which could be layered on a planchet for gas flow counting. This was achieved by decomposing the sample with a mixture of HNO_3 and $HClO_4$[148], or a mixture of HCl and HNO_3 together with a copper salt catalyst[58,59,65], followed by the precipitation of SO_4^{2-} by barium. The method also lends itself to liquid scintillation counting when the $BaSO_4$ is suspended in a liquid scintillator solution that has been gelled by Cab-O-Sil[148]. Alternatively, the sample can be reduced to H_2S, which is subsequently absorbed in a solution of NaOH, and assayed in a liquid scintillation counter[149].

2. Oxygen flask combustion

The oxygen flask method converts organic sulphur to a form suitable for liquid scintillation counting. In principle, the sample is combusted in an oxygen atmosphere. Sulphur is converted to SO_2, which is trapped in a liquid scintillator solution. In practice, a good deal of development has gone into increasing the speed, efficiency and safety of the technique. The sample can be held in a number of ways, such as in a Pt basket[150,151] or a paper cup held by a Pt–Ir wire[152], or impregnated on a cotton pellet, placed in a paper cup that is held in a glass ring or watch-glass-type combustion platform[153]. The reaction vessel, which can be either a 2 l glass flask[151] (accommodating 20–200 mg of matter), a liquid scintillation phial (holding 10–15 mg)[152], or a plastic bag[153], is flushed with oxygen. The sample is ignited most often by focusing a light beam on a dark spot which has been made on the paper sample holder or by heating electrically the Pt sample holder. The sample is combusted and SO_2 is collected in a trapping agent such as phenylethylamine[153,154] or ethanolamine[155,156] in nine parts of methanol. The trapping solution is subsequently mixed with the liquid scintillator and counted. Usually the trapping agent, which is a flammable organic mixture, is added to the reaction vessel prior to ignition, and therefore poses a hazard when the sample is ignited. To avoid explosions, the reaction vessel is either cooled in dry-ice acetone to lower the volatility of the trapping solution or

alternatively the vessel is fitted with a balloon attached to the side-arm[151]. A non-flammable trapping solution consisting of a 1 : 1 : 2 mixture of toluene, triton X-100 and water has also been used[151]. The efficiency of counting which takes into account the recovery of radioactivity and the quenching of the scintillant by the trapping agent is usually 90–95%. In human samples, in which a large amount of material with a very low activity is combusted, a compromise must be struck between the counting rate and the quenching level. The large amounts of trapping agent that are required, quench the counting mixture, while dilution of the trapping agent reduces the counting rate to the background level of the counter[160].

3. Specialized techniques

In addition to the wet ashing and oxygen flask methods, a number of rather specialized techniques have been used to convert a sample to a form which can be sufficiently counted. Methanethiol-^{35}S has been added to $Hg(CN)_2$ and precipitated as $(MeS)_2Hg$ and counted under a gas flow counter[157]. ^{35}S-Labelled scintillation counting was carried out by an in-phial degradation by heating in a xylene solution containing *t*-butyl hydrogen peroxide and OsO_4[158]. Labelled H_2S released into the atmosphere by micro-organisms has been trapped on paper strips impregnated with basic lead acetate, which are subsequently treated with glyoxal, H_3PO_4 and zinc powder and counted in a Tracerlab counter[159].

D. Methodological Considerations

A number of important methodological considerations enter into the design of body tracer studies. The number of labelled thiol molecules that will be incorporated into a particular macromolecule or tissue depends on (1) the dilution of the isotope in the added molecule, (2) the pre-existing concentration of the compound in different organs and cells, (3) the presence of different precursors of the compound, (4) the turnover rate of the compound and its precursors, and finally (5) the rate of synthesis of the complex polypeptide into which it will be incorporated. Furthermore, in endocrine research, polypeptide hormones may be quickly metabolized and lead to an unspecified labelling sometimes difficult to detect. Hormones are usually physiologically active at very low concentrations, which requires that they be very highly labelled if they are to be observed at all[78]. As the metabolic pathways of cells are often ramified, in addition to the hormone, labelled sulphur may also be incorporated into structural proteins, lipid sulphatides, sulphonated mucopolysaccharides and water-soluble substances, such as cystine, methionine, glutathione, taurine and

inorganic sulphates. Labelled methionine can be used to determine the rate of accumulation of labelled sulphur in structural proteins, and labelled sulphate can be used to check the localization of sulphur in other compounds[77]. If the specific activity of the labelled polypeptide is to be determined then a technique such as autoradiography must be used in conjunction with quantitative cytochemical methods.

The interpretation of autoradiographs can be ambiguous, especially if the anatomy of the tissue furnishes few points of reference and the area to be counted is far from the cell nucleus. Often the shape of the cell may impede the exact determination of its centre[80].

Kinetic measurements of the rate of transport of a labelled compound in a tissue depend on the specification of the time and the site of entry of the labelled compound into the system. Ideally, one would like to inject the labelled compound directly into the system under study. However, the local application of the labelled substances introduces a serious risk of disturbing both the timing of precursor adsorption into the system and the rate of incorporation. There may be no way of knowing whether the true physiological circumstances are preserved. Furthermore, the local application of the labelled compound does not enhance the specificity of its incorporation in the polypeptide, as opposed to other uptake mechanisms[79]. Since the measurement of isotope accumulation requires that the animal be sacrificed, it is not possible to take consecutive samples from the same animal as a function of time. The kinetic measurements must therefore represent a picture of the mean behaviour of the isotope in a population of animals[80].

VII. REFERENCES

1. K. Biemann, *Mass Spectrometry*, McGraw-Hill, New York, 1962.
2. D. Amos, R. G. Gillis, J. L. Occolowitz and J. F. Pisani, *Org. Mass.*, **2**, 209 (1969).
3. S.-O. Lawesson, J. Ø. Madsen and G. Schroll, *Acta Chem., Scand.*, **20**, 2325 (1966).
4. G. Herzberg, *Molecular Spectra and Molecular Structure*, Vol. I, Van Nostrand, New York, 1950.
5. N. Solimene and B. P. Dailey, *J. Chem. Phys.*, **23**, 124 (1955).
6. Ch. O. Kadzar, A. A. Abbasov and L. M. Imanev, *Opt. Spektrosk.*, **24**, 629 (1968).
7. M. Hayaishi, H. Imaishi, K. Ohno and H. Murata, *Bull. Chem. Soc. Japan*, **43**, 872 (1971).
8. T. Kojima, *J. Phys. Soc. Japan*, **15**, 1284 (1960).
9. J. V. Knopp, D. D. Daniel and C. R. Quade, *J. Chem. Phys.*, **53**, 4352 (1970).

10. R. L. Reddington, *J. Mol. Spectroscopy*, **9**, 469 (1962).
11. G. R. Bird and C. H. Townes, *Phys. Rev.*, **94**, 1203 (1954).
12. G. Herzberg, *Molecular Spectra and Molecular Structure*, Vol. II, Van Nostrand, New York, 1950.
13. D. Plant, D. S. Tarbell and C. Whiteman, *J. Amer. Chem. Soc.*, **77**, 1572 (1955).
14. Y. Takeoka, *J. Mol. Spec.*, **15**, 29 (1965).
15. E. B. Wilson, J. C. Decius and P. Cross, *Molecular Vibrations*, McGraw-Hill, New York, 1955.
16. I. W. May and E. L. Pace, *Spectrochimia Acta*, **24A**, 1605 (1968).
17. I. W. May and E. L. Pace, *Spectrochimia Acta*, **25A**, 1903 (1969).
18. M. Hayaishi, Y. Shiro, T. Oshima and H. Murata, *Bull. Chem. Soc. Japan*, **38**, 1734 (1965).
19. A. A. Frost and R. G. Pearson, *Kinetics and Mechanism*, Wiley, London, 1953, Chap. 5.
20. L. Melander, *Isotope Effects on Reaction Rates*, Ronald Press, New York, 1960, Chap. 2.
21. J. Bigeleisen and M. G. Mayer, *J. Chem. Phys.*, **15**, 261 (1947).
22. P. C. Ellgen and C. D. Gregory, *Inorg. Chem.*, **10**, 980 (1971).
23. E. S. Lewis and M. M. Butler, *J. Org. Chem.*, **36**, 2582 (1971).
24. B. Dmuchovsky, B. Vineyard and F. Zienty, *J. Amer. Chem. Soc.*, **86**, 2874 (1964).
25. R. A. W. Haul and D. Blenncmann, *Z. physik. Chem.*, **23**, 300 (1960).
26. K. I. Sakodynskii, S. I. Babkov and N. M. Zhavoronkov, *Doklady Akad. Nauk S.S.S.R.*, **121**, 681 (1958).
27. W. V. Smith, *J. Amer. Chem. Soc.*, **68**, 10; 2059 (1946).
28. L. A. Wall and D. W. Brown, *J. Polymer Sci.*, **14**, 513 (1954).
29. G. Greig and J. C. J. Thynne, *Trans. Faraday Soc.*, **62**, 379 (1966).
30. P. Riesz and B. E. Burr, *Rad. Res.*, **16**, 661 (1962).
31. D. H. Volman, J. Wolstenholme and S. G. Hadley, *J. Phys. Chem.*, **71**, 1798 (1967).
32. B. G. Keyes and A. G. Harrison, *J. Amer. Chem. Soc.*, **90**, 5671 (1968).
33. D. G. Earnshaw, G. L. Cook and G. N. Dinneen, *J. Phys. Chem.*, 296 (1964).
34. a. L. H. Slaugh, *J. Amer. Chem. Soc.*, **81**, 2262 (1959). b. P. S. Skell and R. G. Allen, *J. Amer. Chem. Soc.*, **82**, 1511 (1960).
35. G. S. Denisov, E. Kazakova and E. V. Ryl'tsev, *Zh. Prikl. Spektrosk.*, **8**, 690 (1968).
36. G. H. Dixon, H. L. Kornberg and P. Lund, *Biochim. Biophys. Acta*, **41**, 217 (1960).
37. J. M. White and G. P. Sturm, Jr., *Canadian J. Chem.*, **47**, 357 (1969).
38. J. M. White, R. L. Johnson, Jr. and D. Bacon, *J. Chem. Phys.*, **52**, 5212 (1970).
39. J. W. Abrell, E. E. Kaufman and M. N. Lipsett, *J. Biol. Chem.*, **246**, 294 (1971).
40. M. N. Lipsett, J. S. Norton and A. Peterkofsky, *Biochemistry*, **6**, 855 (1967).
41. S. Sabol and S. Ochoa, *Nature New Biol.*, **234**, 233 (1971).
42. A. P. Kimball, S. J. Herriot and G. A. LePage, *Proc. Soc. Exp. Biol. Med.*, **121**, 931 (1965).

43. V. M. Zhdanov, A. G. Bukrinskaya and G. P. Romenskaya, *Acta virol.*, **7**, 1 (1963).
44. M. Kozak and D. Nathans, *Nature New Biol.*, **234**, 209 (1971).
45. L. J. Roth and C. F. Barlow in *Isotopes in Experimental Pharmacology* (Ed. L. J. Roth), University of Chicago Press, Chicago, 1965, pp. 49–62.
46. M. D. Joy, *J. Physiol. (London)*, **215**, 49 (1971).
47. G. B. Cassano, S. E. Sjöstrand, G. F. Placidi and E. Hansson, *Exp. Eye Res.* **7**, 196, (1968).
48. N. Ebata, *Sapporo Med. J.*, **29**, 23 (1966).
49. J. D. Taylor, R. K. Richards and D. L. Takern, *Current Researches Anesthesia and Analgesia*, **29**, 101 (1950).
50. W. H. Chapman and J. W. Duckworth, *Glutathione*, Proc. Symp., Ridgefield, Conn., 1953, p. 292.
51. B. Block and I. Ebigt, *Arzneimittel-Forsch.*, **7**, 572 (1957).
52. L. B. Achor and E. M. K. Geiling, *Proc. Soc. Exp. Biol. Med.*, **87**, 261, (1954).
53. K. Gibbs and J. M. Walshe, *Quart. J. Med.*, **40**, 276 (1971).
54. V. G. Vladimirov, *Radiobiologiya*, **8**, 258 (1968).
55. I. D. Vinogrodova, Z. N. Fakeva and Ya. L. Shekhtman, *Radiobiologiya*, **8**, 34 (1968).
56. T. L. Loo, D. H. W. Ho, D. R. Blossom, B. J. Shepard, and E. Frei, *Biochemical Pharmacol.*, **18**, 1711 (1969).
57. K. Sato, G. A. LePage and A. P. Kimball, *Cancer Res.*, **26**, Part 1, 741 (1966).
58. H. Heath, C. Rimington, T. Glover, T. Mann and E. Leone, *Biochem. J.*, **54**, 606 (1953).
59. H. Heath, *Biochem. J.*, **54**, 689 (1953).
60. L. L. Bennett and P. W. Allan, *Cancer Res.*, **31**, 152 (1971).
61. A. P. Kimball, G. A. LePage and B. Bowman, *Can. J. Biochem.*, **42**, 1753 (1964).
62. G. A. LePage, J. P. Bell, and M. J. Wilson, *Proc. Soc. Exp. Biol. Med.*, **131**, 1038 (1969).
63. J. P. Bell and R. H. Gisler, *Biochem. Pharmacol.*, **18**, 2103 (1969).
64. J. J. Bezem, F. Brunnekreeft, M. J. E. Ernsting, J. Lever and W. Th. Nauta, *Acta Endocrinol.*, **3**, 151 (1949).
65. V. Francova, Z. Franc and J. Jelinek, *Neoplasma*, **10**, 193 (1963).
66. H. J. Hansen, W. G. Giles and S. B. Nadler, *Cancer Res.*, **22**, 761 (1962).
67. M. Flavin and C. B. Anfinsen, *J. Biol. Chem.*, **211**, 375 (1954).
68. B. Forslind, *Acta Derm. Venerol.*, **51**, 1 (1971).
69. N. R. Oster, *Sbornik Nauch. Trudov Tashkent Med. Inst.*, **No. 12**, 345 (1958).
70. J. M. Hsu and W. L. Anthony, *J. Nutrition*, **101**, 445 (1971).
71. G. Garweg, I. Kinsky and H. Brinkmann, *Z. Anat. Entwicki-Gesch.*, **134**, 186 (1971).
72. I. Tork, B. Aros, J. Kiss and B. Vigh, *Acta Biol. Acad. Sci. Hungary*, **17**, 185 (1966).
73. K. C. Higham and A. J. Mordue (Luntz), *Gen. and Comp. Endocrinology*, **15**, 31 (1970).
74. W. Bargman, *Internatl. Rev. Cytol.*, **19**, 183, (1967).

75. E. Scharrer and B. Scharrer, *Neuroendocrinology*, Columbia University Press, New York (1963).
76. J. C. Sloper, *British Medical Bull.*, **22**, 209, (1966).
77. J. C. Sloper, D. J. Arnott and B. C. King, *J. Endocrin.*, **20**, 9 (1960).
78. A. Ficq and J. Flament-Durand, Autoradiography, in *Techniques in Endocrine Research*, Academic Press, London–New York, 1963, p. 73.
79. S. Talanti, U. Attila and M. Kekki, *Progr. Brain Res.*, **34**, 115 (1971).
80. S. Talanti, U. Attila and M. Kekki, *Z. Zellforsch.*, **124**, 342 (1972).
81. H. Sachs, *J. Neurochem.*, **10**, 299 (1963).
82. A. Norström and J. Sjöstrand, *J. Neurochem.*, **18**, 29 (1971).
83. A. Norström, H.-A. Hansson and J. Sjöstrand, *Z. Zellforsch.*, **113**, 271 (1971).
84. J. Wells, *Experimental Neurobiology*, **8**, 470 (1963).
85. S. Talanti, *Z. Zellforsch.*, **115**, 110 (1971).
86. T. J. Peterle, *Pesticides in the Environment and Their Effects on Wildlife*, Proceedings of an Advanced Study Institute (Ed., N. W. Moore) Blackwell, London, 1965, p. 181.
87. N. J. Walker, *J. Dairy Res.*, **32**, 229 (1965).
88. C. C. Lee and E. R. Samuels, *Cereal Chem.*, **39**, 482 (1962).
89. S. Tochio, S. Ikeda and H. Okabe, *Amer. Soc. Lubrication Engrs., Trans.*, **5**, 67 (1962).
90. K. Taki, *Bull. Chem. Soc. Japan*, **43**, 2626 (1970).
91. G. A. Blokh, F. A. Golubkova and G. P. Miklwkin, *Doklady Akad. Nauk S.S.S.R.*, **90**, 201 (1953).
92. J. Morávek and Z. Nejedly, *Chem. Industry*, **42**, 1788 (1967).
93. J. Morávek and J. J. Kopecky, *Coll. Czech. Chem. Commun.*, **34**, 4013 (1969).
94. F. Chapeville, H. Maier-Huser and P. Fromageot, *Radioisotopes Phys. Sci. Ind., Proc. Conf. Copenhagen*, 1960, p. 139.
95. H. Teodoru, *Rum. Pat.* 51,247; *Chem. Abstr.*, **71**, 9746w, (1969).
96. E. Teodoru, *Fr. Pat.* 1,566,692; *Chem. Abstr.*, **73**, 35,760 g, (1970).
97. F. Chapeville and P. Fromageot, *Fr. Pat.* 1,244,192; *Chem. Abstr.*, **55**, 18,865 gh, (1961).
98. G. A. Anorova and V. P. Shishkov, *Metody Polncheniya Radioaktivn. Preparatov, Sb. Statei*, **5**, 1962; *Chem. Abstr.*, **59**, 1749a (1963).
99. K. Samochocka and M. Taube, *Nukleonika*, **13**, 313 (1968).
100. R. Kanski, M. Borkowski and H. Pluciennik, *Nukleonika*, **16**, 37 (1971).
101. K. Panek and K. Mudra, *Chem. Effects Nucl. Transformators*, Proc. Symp., Vienna, 1964, **1**, 195.
102. B. G. Dzantiev and I. M. Barkalov, *Radioisotopes. Phys. Sci. India*, Proc. Conf. Use, Copenhagen, 1960, p. 27.
103. B. G. Dzantiev and A. P. Shvedchikov, *Radiokhimiya*, **9**, 276 (1967).
104. G. Ayrey, *J. Labelled Compd.*, **2**, 51 (1966).
105. M. Fischer, G. Reinhard and H. Schmidt, *Isotopenpraxis* **7**, 30 (1971).
106. R. C. Thomas and L. J. Reed, *J. Amer. Chem. Soc.*, **77**, 5446 (1955).
107. P. T. Adams, *J. Amer. Chem. Soc.*, **77**, 5357 (1955).
108. V. N. Vasil'eva and E. N. Gur'yanova, *Zhur. Obschei Khim.*, **26**, 677 (1956); *Chem. Abstr.*, **50**, 14,615e.
109. R. Wolff, *Commun. Energie Atomique* (France), Rappt. No. 605 (1957).

110. G. Ayrey, C. G. Moore and W. F. Watson, *J. Polymer Sci.*, **19**, 1 (1956).
111. O. Oiták and J. Zikmund, *Coll. Czech. Chem. Communs.*, **24**, 4053 (1959).
112. H. Simon and G. Apel, *Z. Naturforsch.*, **116**, 693 (1956).
113. Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. E. Zhukova, N. A. Kosoloapova and M. N. Schukina, *Doklady Akad. Nauk S.S.S.R.*, **91**, 1129 (1953).
114. K. Heise and E. Mittag, *Kernenergie*, **8**, 181 (1965).
115. V. I. Maimind, M. N. Schukina and T. E. Zhukova, *Zhur. Obschei Khim.*, **22**, 1234 (1952).
116. G. N. Vinogradov, M. M. Kusakov, P. I. Sanin, Yu. S. Zaslavskii, E. A. Razumovskaya, A. V. Ul'yanova and D. V. *Ryabova. Khim. i Tekhnol. Topliva*, 1956, **No. 6**, 14; *Chem. Abstr.*, **50**, 16,089c (1956).
117. I. Kh. Fel'dman and A. A. Abremzon, *Mechenye Biol. Aktivn. Veshchestva, Sb. Statei*, 1962, 48; *Chem. Abstr.*, **59**, 6247e (1963).
118. I. Kh. Fel'dman, N. N. Bel'tsova and V. K. Grishkova, *Mechenye Biol. Aktivn. Sv. Statei*, 1962, 90; *Chem. Abstr.*, **59**, 7369h (1963).
119. S. G. Kuznetsov, Z. I. Bobyshera and E. M. Balonova, *Zhur. Obshchei*; *Khim* **28**, 635 (1958).
120. J. B. Ziegler, A. C. Shabica and M. Sonenberg, *J. Org. Chem.*, **23**, 1546 (1958).
121. V. Regnault, *Ann.* **34**, 24 (1840).
122. M. Bentov, *Commun. Energie atomique* (France), Rappt. No. 831 (1958).
123. G. B. Elron and G. H. Hitchings, *J. Amer. Chem. Soc.*, **76**, 4027 (1954).
124. C. Michou-Saucet and J. C. Merlin, *Bull. Soc. Chim. France*, 1962, 1184.
125. R. Emiliozzi, L. Pichat and M. Herbert, *Bull. Soc. Chim. France*, 1959, 1544.
126. H. R. V. Arnstein, *Biochem. J.*, **68**, 333 (1958).
127. H. Chang and C.-H. Yang, *Hua Hsueh Hsueh Pao*, **28**, 263 (1962); *Chem. Abstr.*, **59**, 12,634e (1963).
128. H. Emiliozzi, *Commun. Energie atomique* (France), Rappt., No. 128,617 (1959).
129. I. Voelker, E. Schuemann and C. V. Holt, *Biochem. Z.*, **335**, 382 (1962).
130. L. Laufer and S. Gutcho, *U.S. Pat.*, 2,711,989; *Chem. Abstr.*, **49**, 15,183f.
131. S. P. Sen and S. C. Leopold, *Biochem. et Biophys. Acta* **18**, 320 (1955).
132. E. Jermoljev, J. Pozdena and J. Baker, *Biol. Plant.*, **12**, 382 (1970).
133. T. S. Sedova and V. V. Grechko, *Zhur. Mikrobiol., Epidemiol. i Immunobiol.*, **31**, 31 (1960); *Chem. Abstr.*, **55**, 23,666 g (1961).
134. A. R. Bassindale, C. Eaborn and D. R. M. Walton, *J. Chem. Soc., C (London)*, 1577 (1970).
135. S. Mlinko, I. Gács, and T. Szarvas, *Intern. J. Appl. Rad. Isotopes*, **18**, 457 (1967).
136. K. Panek and K. Murda, *Radiokhimiya*, **7**, 246 (1965).
137. P. E. Schulze and M. Wenzel, *Angew. Chem.*, **74**, 777 (1962).
138. G. Boucke, *Atompraxis*, **11**, 263 (1965).
139. G. L. Eliceiri, *Biochim. Biophys. Acta*, **209**, 387 (1970).
140. I. H. W. Scharpenseel and K. H. Menke, *Z. anal. Chem.*, **180**, 81 (1961).
141. J. M. Sadler, J. R. Bethany and J. W. B. Stewart, *Can. J. Soil. Sci.*, **51**, 308 (1971).
142. E. Kragelund and M. Dyrbye, *Scand. J. Clin. Lab. Invest.*, **19**, 129 (1967).
143. E. Varrone and S. N. Albert, *J. Nucl. Med.*, **10**, 263 (1969).

144. J. Doniach and S. R. Pelc, *Brit. J. Radiol.*, **23**, 184 (1950).
145. S. Ullberg, *Acta Radiol. Suppl.*, **118** (1954).
146. S. R. Pelc, *Int. J. Appl. Radiat. Isotop.*, **1**, 172 (1956).
147. L. Bachmann, M. M. Salpeter and F. McHenry, *J. Cell Biol.*, **33**, 299 (1967).
148. C. P. Willis, D. G. Olson and C. W. Clande, *Anal. Chem.*, **42**, 124 (1970).
149. G. J. Blair and F. C. Crofts, *Soil Sci.*, **107**, 277 (1969).
150. K. R. Millar and T. F. Allsop, *N.Z. J. Sci.*, **13**, 149 (1970).
151. N. G. Grundon and C. G. Asher, *J. Agr. Food Chem.*, **20**, 794 (1972).
152. G. N. Gupta, *Proc. Int. Conf. Methods Prep. Stor. Label Compounds*, 2nd ed., 1966, p. 1165.
153. G. N. Gupta, *Microchem. J.*, **13**, 4 (1968).
154. H. E. Dobbs, *Anal. Chem.*, **35**, 783 (1963).
155. M. Takamatsu, *Radioisotopes*, **19**, 286 (1970).
156. E. N. Chirkova, *Vop. Med. Khim.*, **14**, 98 (1968); *Chem. Abstr.*, **68**, 84,905p (1968).
157. R. H. Rolfe, *Anal Chem.*, **34**, 340 (1962).
158. D. A. Chapman and C. R. Parks, *Anal. Chem.*, **43**, 1242 (1971).
159. J. Morre and L. Richou, *Bull. Acad. Vet. France*, **37**, 85 (1964).
160. R. R. Roncucci, G. Lambelin, M. J. Simon and W. Soudyn, *Anal. Biochem.*, **26**, 118 (1968).
162. E. N. Gur'yanova and M. Ya. Kaplunov, *Doklady Akad. Nauk S.S.S.R.*, **94**, 53 (1954).
163. G. A. Anorova and V. P. Shiskov, *U.S.S.R. Pat.*, 118,557; *Chem. Abstr.*, **46**, 22,026c (1952).
164. J. Kalina, *Radiochem. Conf., Abstr. Pap., Bratislavo*, 1966, 58.
165. G. A. Anorova, *Metody. Polncheniya i Izmeren. Radioktivn. Preparatov., Sb. Statei*, 1960, 177; *Chem. Abstr.*, **57**, 8572 (1962).
166. J. Kolina, J. Fejtek and F. Horak, *Radioisotopy*, **10**, 825 (1969).
167. I. Zamfir and C. N. Turcanu, *Rev. Roum. Chim.*, **14**, 339 (1969).
168. C. Chiotan, I. Zamfir, M. Szabo and C. N. Turcanu, *Nov. Metody. Poluch. Radioaktiv. Prep., Sb. Doklady Simp.*, 1969, 386; *Chem. Abstr.*, **74**, 386 (1971).

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