THE ALKALOIDS

Edited by

GEOFFREY A. CORDELL

VOLUME 53

ACADEMIC PRESS

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Chemistry and Biology

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VOLUME 53

Academic Press
San Diego London Boston New York
Sydney Tokyo Toronto

This book is printed on acid-free paper. ♾

Academic Press

A Harcourt Science and Technology Company
525 B Street, Suite 1900, San Diego, California 92101-4495, U.S.A.
http://www.apnet.com

Academic Press
24-28 Oval Road, London NW1 7DX, UK
http://www.hbuk.co.uk/ap/

International Standard Book Number: 0-12-469553-1

PRINTED IN THE UNITED STATES OF AMERICA
99 00 01 02 03 04 BB 9 8 7 6 5 4 3 2 1

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PREFACE

Several transformative changes occur with this volume of *The Alkaloids: Chemistry and Biology.* The first is that the authors have prepared their respective chapters in camera-ready format in order to accelerate the publication process. Thanks to the fine cooperation of these authors, I believe that the excellent quality of the presentation of the series has been maintained.

The second transformation is an attempt to continue to broaden the coverage of the series. This has been done in this volume through several chapters. The first is a chapter by Song on the use of alkaloids as chiral transmitters in stereoselective synthesis. This area has developed very rapidly in recent years and some of the achievements have been spectacular. The second chapter reviews the alkaloids from marine organisms by compound class, reflecting this broadening of scope. This is now necessary as the number of interesting metabolites from marine sources has grown and the associated efforts of synthesis and biological investigation have burgeoned. Thus, Ozturk provides a detailed discussion of the isoquinolinequinone alkaloids. The chapter by Gerwick and Sutachitta on the alkaloids from marine bacteria also reflects the increasing attention being paid to this area of the marine biome. Finally, Le Hello reviews in-depth the diverse clinical aspects of colchicine, an alkaloid that is finding many new therapeutic uses. Chapters on the clinical aspects of alkaloids are rare in this series, and it is hoped that in the next few years the principal alkaloids used therapeutically can be covered.

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—— CHAPTER 1 ——

ALKALOIDS AS CHIRALITY TRANSMITTERS IN ASYMMETRIC CATALYSIS

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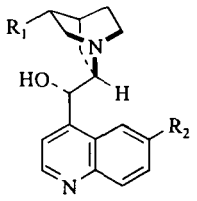
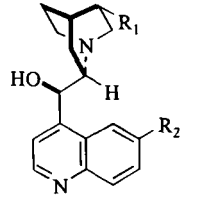
THE ALKALOIDS, VOL. 53
0099-9598/00 $30.00

I. Introduction

During the last decade a number of powerful catalytic asymmetric reactions have emerged as a result of the growing need to develop more efficient and practical synthetic methods for biologically active compounds. A wide variety of chiral ligands and chiral catalysts have been designed and their catalytic efficiency has been investigated. Especially, since the pioneering work (*1*) of Wynberg, naturally occurring alkaloids have been extensively utilized in asymmetric catalysis as chiral ligands or as chiral catalysts themselves. The parent alkaliods (Figure 1) possess nitrogen surrounded by highly asymmetric environment. They are also inexpensive and readily available in both enantiomeric forms in most cases, and can easily be modified to a variety of different derivatives. A number of processes have gained wide acceptance, and some are even used on an industrial scale, e.g. heterogeneous hydrogenation of α-ketoesters catalyzed by cinchona alkaloid-modified Pt (Ciba-Geigy) (section II.A.*1*.), Sharpless asymmetric dihydroxylation of olefins (Chirex Ltd.) (section III.A.*1*.), asymmetric alkylation of indanones using cinchona alkaloid-derived chiral phase-transfer catalysts (Merck) (section IV.A.), and cinchona alkaloid-catalyzed 2,2-cycloaddition of ketene and chloral or trichloroacetone (Lonza) (section IV.I.), etc. This research area has been very fast growing, nevertheless there has been no review, since 1986 when the first review (*1*) by Wynberg was published. This review is an overview of recent activities in this area.



R_1: CH=CH_2; Quinidine (R_2= OMe)
Cinchonine (R_2= H)
R_1: Et; Dihydroquinidine (R_2= OMe)
Dihydrocinchonine (R_2= H)



R_1: CH=CH_2; Quinine (R_2= OMe)
Cinchonidine (R_2= H)
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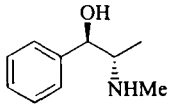
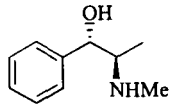
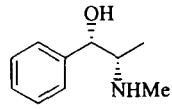


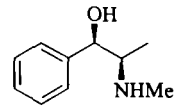
(1*R*,2*S*)-Ephedrine



(1*S*,2*R*)-Ephedrine



(1*S*,2*S*)-Pseudoephedrine



(1*R*,2*R*)-Pseudoephedrine

Sparteine

Isosparteine

FIGURE 1. Alkaloids frequently used as chirality transmitters in asymmetric catalysis

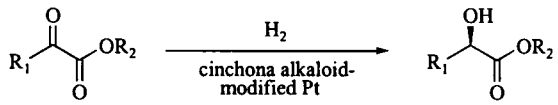
II. Enantioselective Carbon-Hydrogen Bond Formation

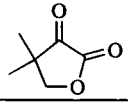
A. ASYMMETRIC CATALYTIC HYDROGENATION

1. Heterogeneous Asymmetric Carbonyl Hydrogenation

a. Asymmetric hydrogenation of α-ketoesters using cinchona alkaloid-modified Pt/Al_2O_3 (or Pt/C) catalysts. Asymmetric hydrogenation of α-ketoesters using cinchona alkaloid-modified Pt/Al_2O_3 (or Pt/C) catalysts (*2,3*), which was initially developed by Orito (*4-7*), is one of the most intensively studied areas in asymmetric catalysis, in which alkaloids are used as chirality transmitters. Over the last few years, Blaser (*8-15*) has made extensive studies of this reaction. Enantioselectivities up to 95% (*14*) were obtained by optimizing the catalyst, modifier, solvent, and reaction conditions (*4-15*) (Table I). Pretreatment of platinum on Al_2O_3 with hydrogen at high temperature (ca. 400 °C) is essential for high optical yields. Increasing the size of platinum particles by increasing the temperature with hydrogen led to more active and enantioselective catalysts (*8*). Hydrogenation of ethyl 2-oxo-2-phenylethanoate with unpretreated Pt/Al_2O_3 gave ethyl (-)-2-hydroxy-2-phenylethanoate in only 34% ee. After thermal treatment of the catalyst with hydrogen the ee increased to 84% ee.

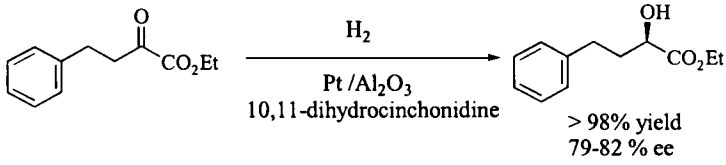
TABLE I
ASYMMETRIC HYDROGENATION OF α-KETOESTERS USING CINCHONA ALKALOID-MODIFIED Pt-CATALYSTS [from 2]



Substrate		Catalyst	Modifier	% ee
R_1	R_2			
Ph	Et	Pt / Al_2O_3	cinchonidine	89
Me	Et	Pt / Al_2O_3	dihydrocinchonidine	89 (95)[a]
n-Pr	Et	Pt / Al_2O_3	dihydrocinchonidine	57
Me	Me	Pt / Al_2O_3	dihydrocinchonidine	90
$PhCH_2CH_2$	Me	Pt / Al_2O_3	dihydrocinchonidine	85
$PhCH_2CH_2$	Et	Pt / Al_2O_3	dihydrocinchonidine	83 (91)[a]
$PhCH_2CH_2$	*n*-Bu	Pt / Al_2O_3	dihydrocinchonidine	82
		Pt / C	cinchonidine	82

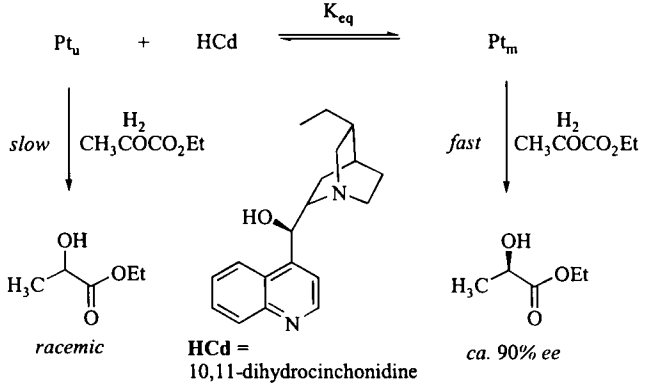
[a] solvent: toluene (acetic acid)

The practical utility was demonstrated in the synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutanoate, an ACE-inhibitor intermediate (*16*). The reaction with Pt/Al_2O_3 modified by dihydrocinchonidine can be carried out on 10-200 kg scale in greater than 98% chemical yield and in 79-82% optical yield (*2*) (Scheme 1).



SCHEME 1. Synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutanoate, an ACE-inhibitor intermediate

In the hydrogenation of α-ketoesters in the presence of cinchona alkaloid-modified Pt, alkaloid adsorption leads to a marked increase in reaction rate (*15*). Accordingly, this reaction can be classified as "ligand-accelerated catalysis (LAC)" (*17*). The initial rate for the enantioselective hydrogenation of α-ketoesters over chirally modified Pt catalysts is usually 5-20 times higher than that of the unmodified (racemic) reaction. The actual hydrogenation involves two kinds of reaction sites, chirally modified $Pt(Pt_m)$ and unmodified $Pt(Pt_u)$. Accordingly, the reaction is analyzed in term of a general two-cycle mechanism (Scheme 2). The first cycle is ligand-accelerated catalysis, which exhibits excellent enantioselectivity; the other cycle is a slow, chirally unmodified cycle to produce the racemic product. Both rate and product ee reach a maximum at extremely low concentration of alkaloid, corresponding to an alkaliod/Pt_{surf} ratio of 0.5 in toluene and 1 in ethanol (*15*). These data suggest that adsorption of the alkaloid on the metal surface is reasonably strong and/or only a small fraction of the surface is modifiable. A modified ensemble consists of one adsorbed alkaloid and 10-20 Pt atoms.

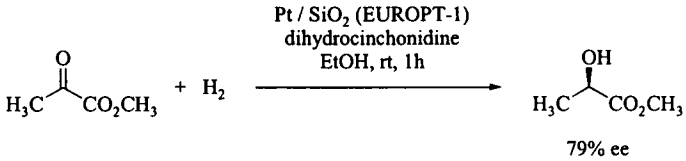


SCHEME 2. Two-cycle mechanism

However, unfortunately, the highly selective reaction is restricted to α-keto esters

as substrates. Despite many efforts to broaden the application range of this reaction, the results have been disappointing. Only 20% ee or below, was obtained in the enantioselective hydrogenation of β-diketones, β-ketoesters, aryl alkyl ketones and α-methoxy ketones (*18,19*). Better enantioselectivities were achieved in the hydrogenation of α-diketones (33-38% ee) (*20*), 2,2,2-trifluoroacetophenone (56% ee) (*21*), cyclic ketoamide (47% ee) (*22*) and α-ketoamides (up to 60% ee) (*23*).

b. Asymmetric hydrogenation of α-ketoesters using cinchona alkaloid-modified Pt/silica (EUROPT-1) catalysts. The well characterized 6.3% Pt/silica (EUROPT-1) (*24-27*) catalyst was also developed to be an enantioselective catalyst (*28,29*) by modifying with alkaloids. The rate for the hydrogenation of α-ketoesters over chirally modified EUROPT-1 catalyst is 25 times higher than that of the unmodified reaction. Modification with alkaloid can be best carried out in ethanol solution in the presence of air using a pre-reduced Pt/silica. Before modification, Pt/silica should be pre-reduced with H_2 (1bar) at 100 °C for 1h. This exposure to air confers on the catalysts a high hydrogenation rate and enantioselectivity. In the hydrogenation of α-ketoesters, the highest optical yields exceeded 80% ee when dihydrocinchonidine was used as a chiral modifier. Cinchonidine gave lower enantioselectivity. The superiority of the dihydrocinchonidine modifier, compared to cinchonidine, was ascribed to a different adsorption behavior. Whereas cinchonidine might bond to the surface either with the quinoline ring system or the vinylic double bond, the adsorption of dihydrocinchonidine is only possible by the quinoline ring system (*28,29*).



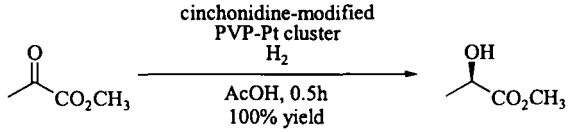
SCHEME 3. Enantioselective hydrogenation of methyl pyruvate using an alkaloid-modified EUROPT-1 catalyst

Other alkaloids such as codeine, 7,8-dihydrocodeine, brucine and strychnine, adsorbed on Pt/silica (EUROPT-1) also enhanced the rate of hydrogenation of methyl pyruvate and butane-2,3-dione, however, exhibited very low enantioselectivities (1-21% ee) (*30*).

c. Asymmetric hydrogenation of α-ketoesters using cinchona alkaloid-modified finely dispersed polyvinylpyrrolidine(PVP)-stabilized platinum clusters. Very recently, Liu and coworkers reported that finely dispersed polyvinylpyrrolidine(PVP)-stabilized platinum clusters modified with cinchonidine catalyzed the asymmetric hydrogenation of α-ketoesters, giving enantiomeric excesses in favor of (*R*)-(+)-methyl lactate up to 97.6% (*31*). The cinchonidine-modified PVP-Pt cluster immobilized onto alumina and a cross-linked polystyrene (PS) support also showed high enantioselectivities (91.3% ee for PVP-Pt/Al_2O_3 and 88.9% ee for PVP-Pt/PS, respectively) for the hydrogenation of methyl pyruvate

(*31*). The reaction runs best over a tiny cluster with a mean size of 1.4 nm (Scheme 4), which is quite different from Pt catalysts supported on aluminium oxide or carbon. As mentioned in section A.*1.a.*, the activity and enantioselectivity of cinchona alkaloid-modified Pt/Al_2O_3 (or Pt/C) increased by increasing particle size (*8*). When the particle size of cinchona alkaloid-modified Pt/Al_2O_3 (or Pt/C) is below 3.0 nm, both the activity and enantioselectivity decreased significantly.

It is also noteworthy that the protonated dihydrocinchonidine also functioned as the stabilizer of the Pt colloid (*32*). With excessive dihydrocinchonidine added into the reaction mixture to prevent agglomeration of the colloid, the hydrogenation of ethyl pyruvate was conducted at atmospheric pressure, and yielded products with an enantioselectivity of up to 78%. The activity of dihydrocinchonidine-stabilized platinum colloids decreases with increasing particle sizes as a result of decreasing dispersion.



97.6% ee with catalyst with particle diameter of 1.4 nm
93.6% ee with catalyst with particle diameter of 1.5 nm
95.9% ee with catalyst with particle diameter of 1.8 nm

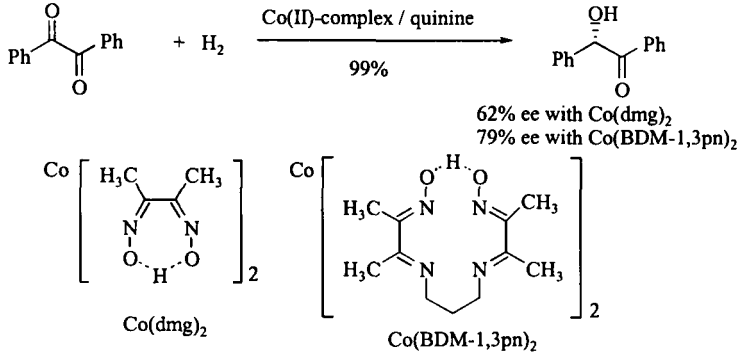
SCHEME 4. Enantioselective hydrogenation of methyl pyruvate using cinchonidine-modified PVP-Pt clusters

2. Co-Catalyzed Hydrogenation of 1,2-Diketones

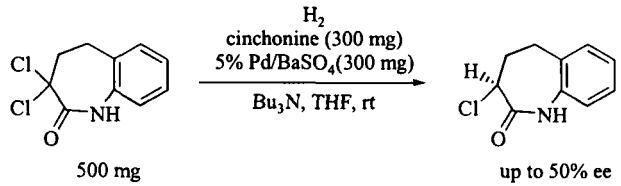
Cinchona alkaloids were also used as chirality transmitters in the Co-catalyzed hydrogenation of 1,2-diketones (Scheme 5) (*33-37*). The enantioselective hydrogenation of 1,2-diphenylethanedione with the catalytic system, $Co(dmg)_2$/quinine gave (*S*)-2-hydroxy-1,2-diphenylethanone in 99% yield and 62% ee (*33-36*). The enantioselectivity can be increased up to 78% by addition of one equivalent of benzylamine. With the catalyst, in which the mononegative 4,8-diaza-3,9-dimethyl-3,8-undecadiene-2,10-dionedioximato (BDM-1,3pn$^-$) was used as a ligand, 1,2-diphenylethanedione was hydrogenated to (*S*)-2-hydroxy-1,2-diphenylethanone in 99% yield and 79% ee (*37*).

3. Other Hydrogenations

The enantioselective hydrodehalogenation of the α,α'-dichlorobenzazepin-2-one with cinchona alkaloid-modified Pd- and Pt-catalysts was investigated by Blaser and coworkers (*38*). The best optical yields (up to 50% ee) were obtained with a 5% $Pd/BaSO_4$ catalyst modified with cinchonine in THF with NBu_3 as HCl acceptor. However, as shown in Scheme 6, very high modifier and catalyst concentrations were necessary to obtain good optical yields and reasonable reaction rates because the modifier decreases the catalyst activity.



SCHEME 5. Co-catalyzed hydrogenation of 1,2-diketones



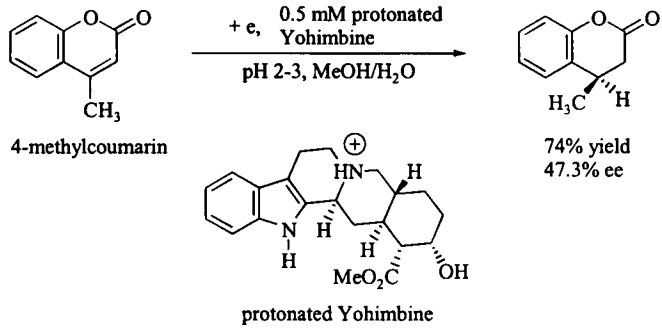
SCHEME 6. Enantioselective hydrodehalogenation of the α,α'-dichlorobenzazepin-2-one

Heterogeneous asymmetric hydrogenation of C=C bonds catalyzed by cinchona alkaloid-modified Pt has also been reported (*39*). However, the results are very disappointing because the most selective catalysts gave optical yields of <30%.

B. ALKALOID-INDUCED ENANTIOSELECTIVE ELECTROREDUCTION

Prochiral ketones, oximes and olefins can be electrochemically reduced to optically active alcohols, amines and alkanes in the presence of very small amounts of alkaloids, which are strongly adsorbed at the electrode (*40*). Pioneering work in the field of alkaloid-catalyzed enantioselective electroreduction has been done by Grimshaw and coworkers, who reduced 4-methylcoumarin in the presence of different alkaloids. With sparteine or yohimbine (+)-(*R*)-4-methyl-3,4-dihydrocoumarin was obtained with 17% or 12% ee, respectively (*41,42*). Recently, the optical yield of this reaction was increased up to 47.4% with yohimbine by systematic variation of the electrolysis conditions (Scheme 7) (*43*).

Electroreduction (*40*) using chiral quaternary ammonium salts as the supporting electrolytes has also been reported. Interestingly, for ketone reduction, the major enantiomer formed in the electroreduction was generally of opposite absolute configuration to that formed in the chemical reduction (*44*).

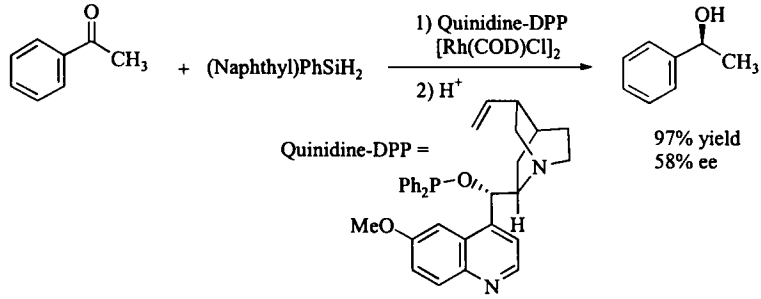


SCHEME 7. Catalytic enantioselective cathodic reduction of 4-methylcoumarin

C. OTHER KETONE AND IMINE REDUCTIONS

1. Enantioselective Hydrosilylation of Ketones

The use of phosphinite derivatives of cinchona alkaloids as chiral ligands in Rh(I)-catalyzed enantioselective hydrosilylation of aryl and alkyl ketones gave excellent chemical yield (88-98%), and, however, only low to moderate enantiomeric excess (5-58% ee) (*45*). For example, with quinidine-DPP (diphenyl phosphinite), (*S*)-1-phenylethanol was obtained in 97% yield and 58% ee by using α-naphthylphenylsilane as the reducing agent (Scheme 8). Chiral phase transfer catalysts **1** derived from alkaloids have also been used for the asymmetric hydrosilylation of 3-acetylpyridine (Scheme 9) (*46*).

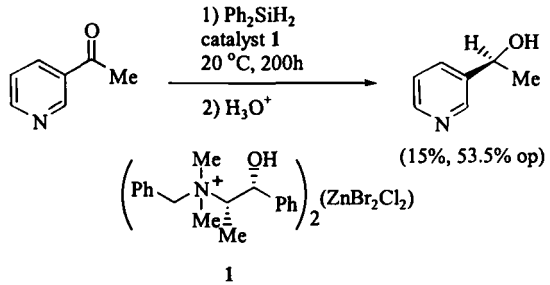


SCHEME 8. Rh-catalyzed asymmetric hydrosilylation of $Ph(CO)CH_3$ using quinidine-DPP as a chiral ligand

2. Additional Ketone and Imine Reductions

Additional ketone reductions catalyzed by cinchona (*47-49*) and ephedra alkaloids

(*47,48,50-55*) have been reported. Other imine reductions promoted by the cinchona and ephedra catalyst systems have been studied (*56*).



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SCHEME 9. Asymmetric hydrosilylation of 3-acetylpyridine catalyzed by **1**

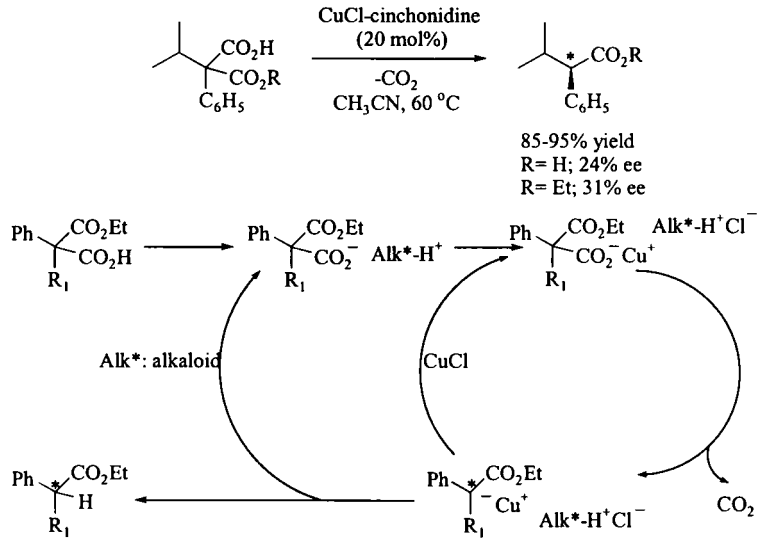
D. ENANTIOSELECTIVE PROTONATION

1. Catalytic Enantioselective Protonation of Prochiral Carbanions

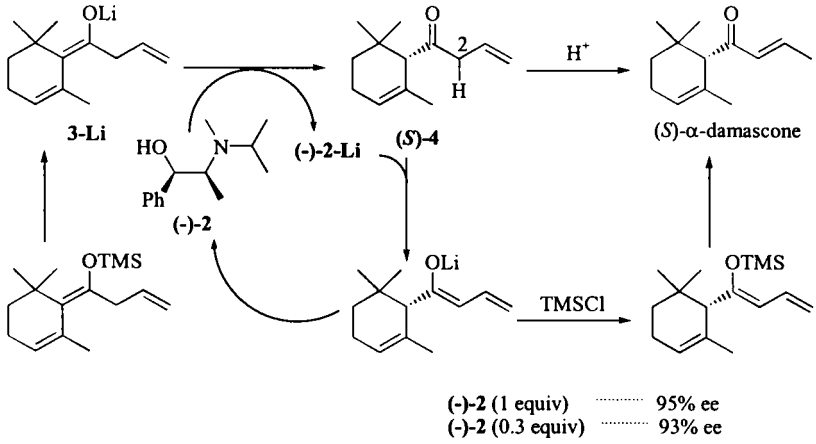
Only one reaction involving the asymmetric protonation of a prochiral carbanion catalyzed by alkaloids has been reported (*57-59*). Cu(I)-Catalyzed decarboxylation of racemic substituted malonic acids, followed by protonation of the intermediate carbanion by the HCl salt of alkaloid as a chiral proton donor gave the corresponding esters in up to 31% ee (Scheme 10) (*57*). Interestingly, inversion of the configuration in the product was observed when the alkaloids (quinine or cinchonidine) are acetylated (9-OAc). The substitution of copper (I) by silver (I) gave the same chemical yield for decarboxylation, but a lower optical yield. Other alkaloid-induced asymmetric decarboxylations of malonic acids have also been reported (*58,59*).

2. Catalytic Enantioselective Protonation of Prochiral Enols

Catalytic enantioselective protonation (*60-62*) of prochiral enol derivatives in the presence of catalytic amounts of cinchona and ephedra alkaloid derivatives as chiral proton donors is a very simple and attractive route for the preparation of optically active carbonyl compounds. During the last few years, this relatively new method has emerged as a powerful synthetic method for optically active carbonyl compounds, and has been successfully applied to the synthesis of some industrially important biologically active compounds, such as fragrances (*62*), antiinflammatory agents (*63-66*) and pheromones (*67,68*). For instance, Fehr (*69*) applied this method to the synthesis of α-damascone. (*S*)-α-Damascone, is an important perfume component. Protonation of enolate **3**-Li with 0.3 equivalents of *N*-isopropyl ephedrine (-)-**2** affords the α-damascone with 93% ee and 86% yield. This case is very particular, because the product ketone (*S*)-**4** is rapidly and exclusively deprotonated at C(2) (Scheme 11).

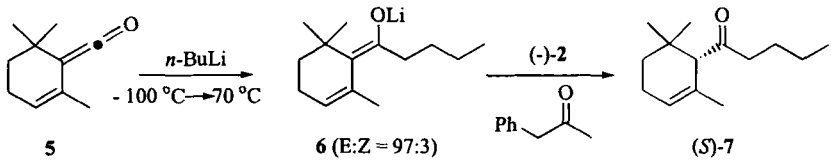


SCHEME 10. Asymmetric decarboxylation-protonation of racemic malonic acids



SCHEME 11. Autocatalytic enantioprotonation of 3-Li

An analogous autocatalytic reaction, as shown in Scheme 11, is not possible with enolate **6**, which can be readily obtained from ketene **5** and *n*-BuLi, because the acidity of the C(2) protons of (*S*)-**7** is substantially weaker. To circumvent this problem, phenyl-2-propanone has been successfully used as an external, achiral proton donor (Scheme 12) (*69*). The protonation of **6** with catalytic amounts of (-)-**2**, followed by the addition of phenyl-2-propanone affords the butyl ketone (*S*)-**7** with an enantiomeric excess of up to 98%.

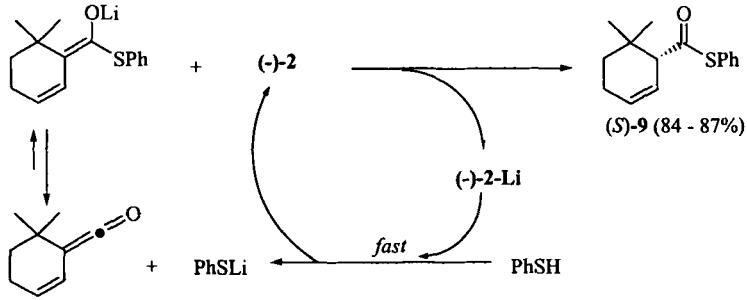


5 6 (E:Z = 97:3) (*S*)-7

(-)-2 (equiv)	Ph(C=O)CH$_3$ (equiv)	% ee
1.1	—	96
0.2	0.85	94
0.1	0.95	85
0.2	0.8, then TMSCl	98

SCHEME 12. Catalytic enantioselective protonation of **6**

Another impressive example is the synthesis of the industrially important (*S*)-α-cyclogeranium acid thioester **9** (*70*). Li-Salt of (-)-*N*-isopropyl ephedrine (-)-**2** catalyzes the addition of thiophenol to ketene **8** to give (*S*)-α-cyclogeranium acid thioester **9**. Thiophenol serves as both the nucleophile and the proton source. The rate of introduction of PhSH has to be kept low to prevent accumulation and to minimize the risk of enolate protonation of PhSH. As shown in Scheme 13, very high enantioselectivities (up to 89% ee) were achieved with as little as 2-5 mol% of (-)-**2**-Li. Of the other thiols tested, 4-chlorothiophenol, which is more acidic than PhSH, proved the most efficient (97% ee with 1 equiv (-)-**2**-Li; 90% ee with 0.05 equiv (-)-**2**-Li).



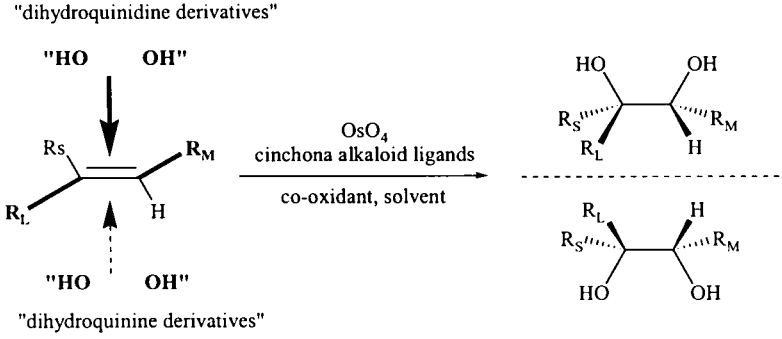
SCHEME 13. Catalytic enantioselective addition-protonation of thiophenol to a ketene

III. Asymmetric Oxidation

A. Asymmetric Dihydroxylation of Olefins

1. Sharpless Asymmetric Dihydroxylation of Olefins

The cinchona alkaloid-based catalytic asymmetric dihydroxylation of olefins (Scheme 14) is a very popular method that has been devised and continuously improved by Sharpless and coworkers (*71-75*). The method employs catalytic amounts of OsO_4 and dihydroquinidine or dihydroquinine derivatives (Figure 2) as chiral ligands together with *N*-methylmorpholine *N*-oxide (NMO) (*76,77*), potassium ferricyanide ($K_3Fe(CN)_6$) (*78*) or iodine (I_2) (*79,80*) as the co-oxidant. Dihydroquinidine- and dihydroquinine-derived auxiliaries have a pseudo-enantiomeric relationship that affords the antipodal diol products with similar enantioselectivities.



SCHEME 14. Asymmetric catalytic dihydroxylation of olefins (R_L, R_M, and R_S are respectively, the large, medium, and small substituents)

An asymmetric osmylation of olefins using stoichiometric amounts of dihydroquinidine acetate (DHQD-OAc) or dihydroquinine acetate (DHQ-OAc) was described in 1980 (*81*). Optical yields of up to 90 % were attained with *trans*-stilbene as the substrate. Although this reaction was a reliable synthetic method, the metal's cost and toxicity necessitated its use as a catalyst. In 1988, Sharpless discovered that the desired catalytic enantioselective reaction can be achieved selectively in aqueous acetone by using NMO as co-oxidant, catalytic amounts of OsO_4 and dihydroquinidine *p*-chlorobenzoate (DHQD-CLB) or dihydroquinine *p*-chlorobenzoate (DHQ-CLB) as a chiral ligand (*82*). Under this catalytic condition, *trans*-stilbene was converted to the corresponding 1,2-diol in 80% yield with 88% ee. However, the ees of diol products obtained under this catalytic condition were initially lower than those produced by the stoichiometric reaction. Sharpless explained this discrepancy with two catalytic cycles mechanism (Scheme 15) (*82,83*).

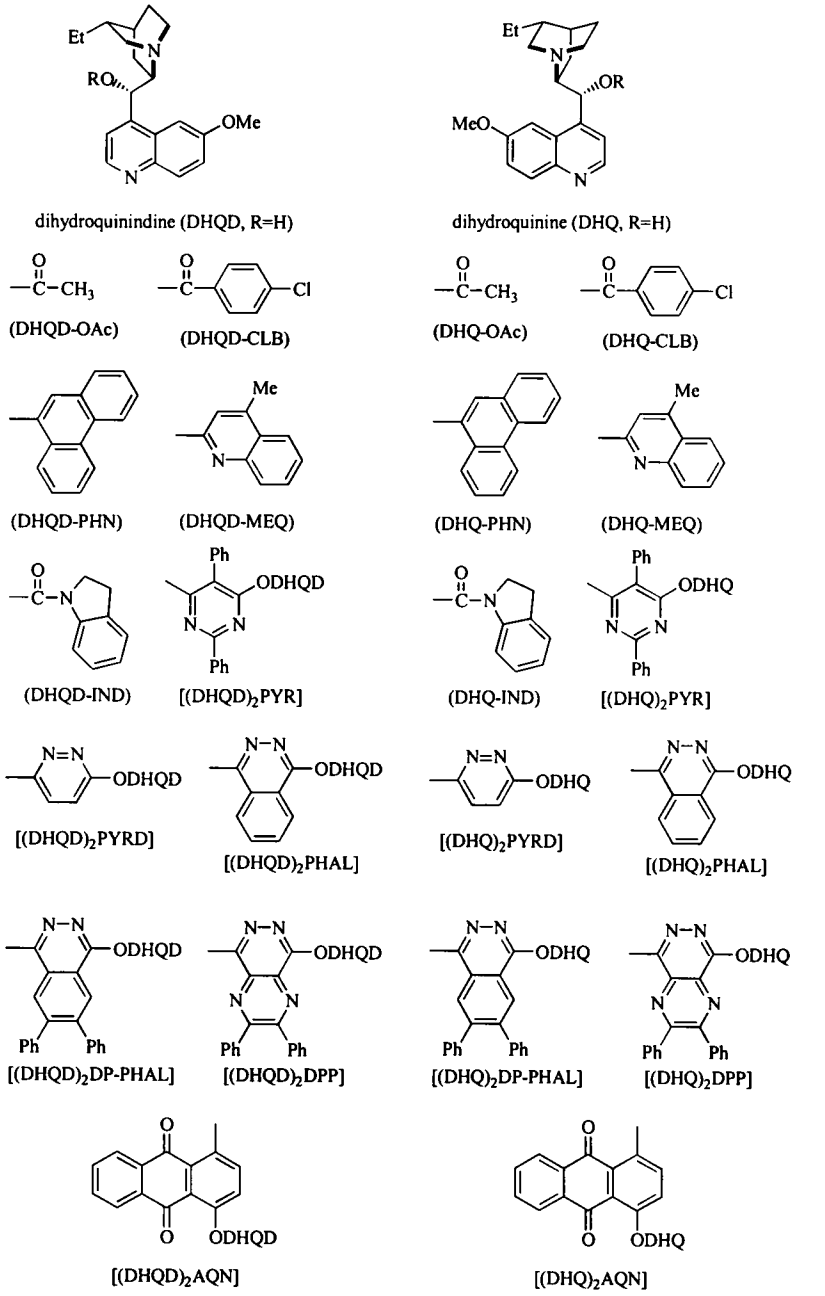
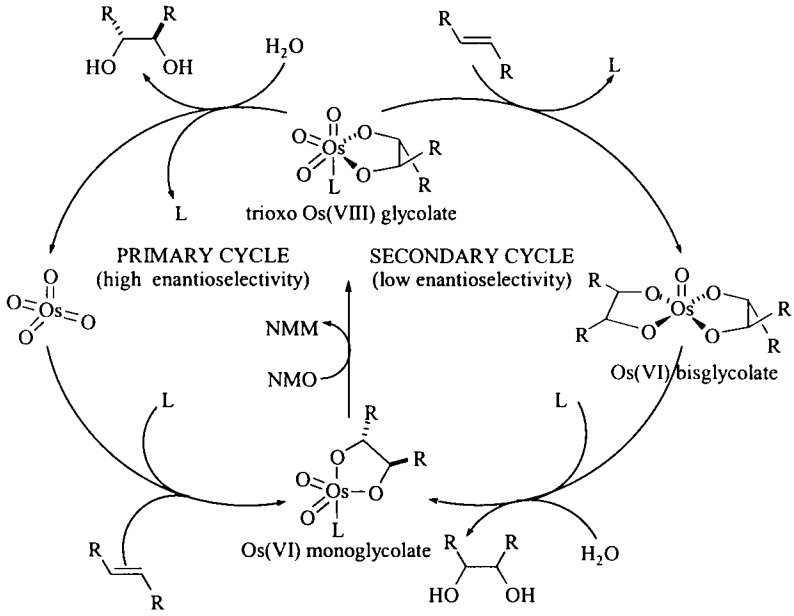


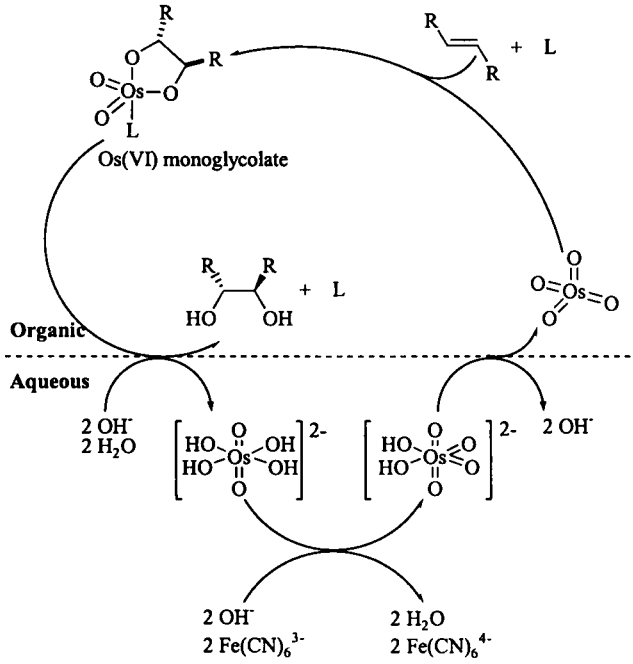
FIGURE 2. Structures of chiral ligands and their abbreviations

According to Sharpless, two competitive catalytic cycles operate, involving either direct hydrolysis of the reoxidized trioxo Os(VIII) glycolate ("first cycle") or its reaction with a second olefin to give an osmium(VI) bisglycolate ("second cycle") (*82,83*) (Scheme 15). Therefore, to minimize the low selectivity-generating second cycle the low concentration of substrates in the reaction mixture is essential, and thus, slow addition of the olefin is important to obtain high ees.



SCHEME 15. The two catalytic cycle in the AD reaction using NMO as cooxidant

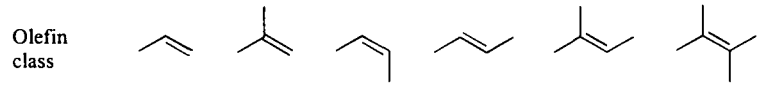
Subsequently, Sharpless and coworkers found that use of potassium ferricyanide ($K_3Fe(CN)_6$) as co-oxidant in a 1:1 *t*-BuOH-H_2O two-layer system can suppress the second cycle and lead to high enantioselectivity (*84*). In this case, there is no oxidant other than OsO_4 in the organic layer, in contrast to the homogeneous NMO conditions. Since the actual osmylation takes place in this layer, the resulting osmium(VI) monoglycolate undergoes hydrolysis, releasing chiral diol and ligand to the organic layer and $K_2OsO_2(OH)_4$ to the aqueous layer before its reoxidation to trioxo Os(VIII) glycolate can occur, and consequently entry of the Os(VIII) glycolate into the second cycle is prevented (Scheme 16). For the formation of the Os(VI) monoglycolate complex, two different mechanisms have been suggested: a [2+2]-addition of the olefin across an Os=O bond, followed by rearrangement of the resulting osmaoxetane intermediate to the glycolate complex (*85-89*) or a concerted [3+2]-cycloaddition pathway (*90,91*).



SCHEME 16. Catalytic cycle of AD reaction with $K_3Fe(CN)_6$ as the co-oxidant

A systematic ligand optimization (Figure 2) study undertaken by Sharpless group has resulted in considerable improvement of the reaction's efficiency. The cinchona alkaloid ligands first recommended were the acetate (Ac) (*81*) and *p*-chlorobenzoate (CLB) of dihydroquinidine and dihydroquinine (*82,83*). Soon, the 9-*O*-(9'-phenanthryl) (PHN) ethers and 9-*O*-(4'-methyl-2'-quinolyl) (MEQ) ethers were found to be more efficient (*92*). Currently, the best systems are the bis-cinchona alkaloids having a heterocyclic spacer, especially phthalazine (PHAL) (*93,94*), diphenypyrimidine (PYR) (*95*) and anthraquinone (AQN) (*96*). PHAL ligands are recommended for 1,1- and 1,2-*trans*-disubstituted, as well as trisubstituted, olefins, but are especially well suited to accommodate olefins with flat aromatic substituents. PYR ligands are the ligands of choice for monosubstituted terminal olefins, especially aliphatic olefins. However, a limitation of the method is the low enantioselectivity usually obtained in the asymmetric dihydroxylation of *cis*-olefins. This drawback was surmounted by Wang and Sharpless, who used IND ligands (*97*). Tetra-substituted olefins generate osmate esters whose *in situ* hydrolysis is difficult. However, when the reaction is run with a higher amount of catalyst in the presence of $MeSO_2NH_2$, useful selectivities can sometimes be obtained with PHAL and PYR ligands (*98*).This broad applicability of Sharpless asymmetric dihydroxylation for nearly every class of olefins affords enormous utility in organic synthesis (Figure 3). Thus, both unfunctionalized substrates and functionalized olefins with nearly all kind of substituents attached to olefins can be dihydroxylated to give products in

reasonable to very high selectivities. For examples, acrylic esters, unsaturated amides and ketones, dienes, enynes, vinyl silanes, acrolein acetals, and allylic halides, allylic phosphine oxides (*99,100*), allylic silanes (*101*) as well as allylic ethers and sulfur-containing olefins have been successfully dihydroxylated (*71,72*). Moreover, very recently, 1(*E*)-alkenylphosphonates were also successfully dihydroxylated to give a series of optically active threo-α,β-dihydroxyphosphonates (*102*).



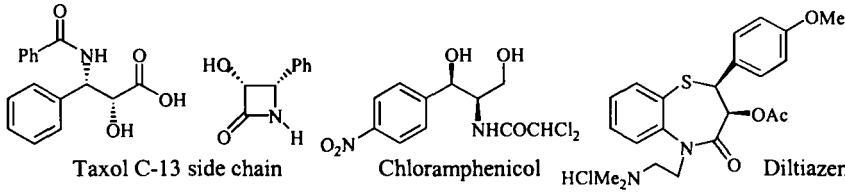
Olefin class						
Preferred ligand	PYR PHAL	PHAL	IND	PHAL	PHAL	PYR PHAL
ee range	30-97%	70-97%	20-80%	90-99%	90-99%	20-97%

FIGURE 3. Recommended ligand types for different olefin classes

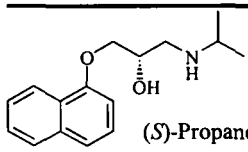
Moreover, the AD reaction is a good example of ligand accelerated asymmetric catalysis (LAC), in which the alkaloid ligands enhance the rate by one to two orders of magnitude (*17,72,103*). For example, rate improvements up to 14,771-fold were observed for the AD reaction of 2-vinylnaphthalene with $(DHQD)_2PHAL$ as a ligand. Interestingly, the rate acceleration is significantly greater with the alkaloid derivatives than with a simple quinuclidine. For example, the $(DHQD)_2PHAL$ promoted reaction occurs between about 240 (2-vinylnaphthalene) and about 14 (1-decene) times faster than the reaction in the presence of quinuclidine. That is, the rate accelerations are not directly related to the ground-state binding energy between the ligand and OsO_4, which is higher for quinuclidine (K_{eq} for quinuclidine: 2630 $Lmol^{-1}$ in *t*-BuOH at 25 °C and K_{eq} for $(DHQD)_2PHAL$ ligand of 27.7 $Lmol^{-1}$ in *t*-BuOH at 25 °C). Instead, "enzyme-like" noncovalent binding in a binding pocket of the ligand seems to be the cause of the substantial rate accelerations and the ability to deliver high enantioselectivity.

Asymmetric dihydroxylations have already been successfully applied to the commercial synthesis of intermediates for pharmacologically active compounds, such as the taxol C-13 side chain (*104-106*), propranolol (*107*) and CL-316243 (*108*), which is a anti-diabetic and anti-obesity agent (Chirex Ltd. U.K.). Other industrially interesting biologically active compounds, such as diltiazem (*109,110*), carnitine and 4-amino-3-hydroxybutyric acid (*111*), azole antifungals (*112*), chloramphenicol (*113*), reticuline intermediates (*114*), camptothecin analogs (*115-117*), khellactone derivatives (*118*), and antimalarial active cyclopenteno-1,2,4-trioxanes (*119*), etc., have been successfully synthesized using catalytic asymmetric dihydroxylation (Figure 4). More detailed information for the synthetic application of asymmetric dihydroxylation is available in several reviews (*71-75*).

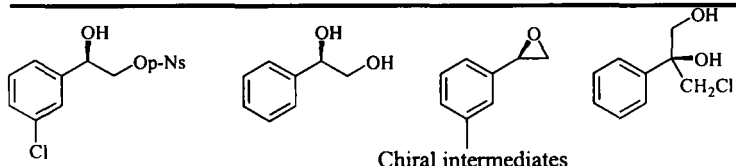
AD of *trans*-cinnamic acid derivatives



AD of allylaryl ethers



AD of Styrene Derivatives



Another Applications

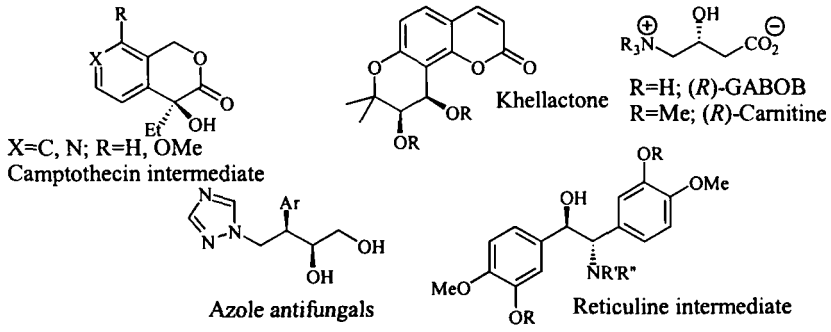


FIGURE 4. Synthetic applications of asymmetric dihydroxylations

2. Electrochemical Asymmetric Dihydroxylation of Olefins

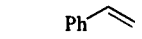
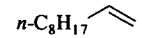
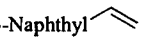
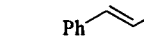
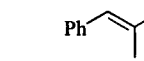
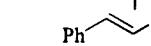
Gao and coworkers (*71*) at Sepracor developed an efficient electrocatalytic AD reaction using Pt-electrodes and PHAL-ligand in a glass H-type cell. A 5% aqueous solution of phosphoric acid is used in the cathode compartment, and the reaction in the anode compartment is stirred vigorously. Under a controlled anode potential of 0.4 V (vs. Ag/AgCl) and with $(DHQD)_2PHAL$ as chiral ligand, α-methylstyrene was converted to (*R*)-2-phenyl-1,2-propanediol in 15 hours with an electrical consumption of 2.1 F/mol. The product was isolated in 100% yield with 92% ee. More recently, Torii and coworkers (*79,80*) performed highly efficient electrochemical Os-catalyzed AD of olefins with PHAL ligands in an undivided cell equipped with two Pt-electrodes under a constant current of 2 mA/cm^2 (applied voltage: 1.0-3.0 V). After passage of 2.33 F/mol of electricity, the desired diols were obtained in very high values both on %ee and conversion yield. In addition, under these electrochemical conditions, they could minimize the use of the amount of $K_2OsO_2(OH)_4$ (0.0005 equiv) and $K_3Fe(CN)_6$ (0.1 equiv) (Table II).

TABLE II
ELECTROCHEMICAL OS-CATALYZED AD OF STYRENE

Conditions	Sharpless conditions	Electrolysis Run		
		A	B	C
Styrene	1	1	1	1
K_2CO_3	3	3	3	3
$(DHQD)_2PHAL$	0.01	0.01	0.01	0.01
$K_3Fe(CN)_6$	3	0.3	0.1	0.1
$K_2OsO_2(OH)_4$	0.002	0.002	0.002	0.0005
Electricity (F/mol)	-	2.33	2.33	2.33
Time (h)		31.2	31.2	31.2
Temp. (°C)	0	0	0	0
% Yield	88	95	95	72
% ee	97.2	97.2	97.3	99.0

Moreover, instead of $K_3Fe(CN)_6$, I_2 was also found to be an efficient co-oxidant (*80*). Iodine-assisted asymmetric electro-dihydroxylation of olefins in either a *t*-$BuOH/H_2O(1/1)$-K_2CO_3/$(DHQD)_2PHAL$-(Pt) or *t*-$BuOH/H_2O(1/1)$-K_3PO_4/K_2HPO_4 /$(DHQD)_2PHAL$-(Pt) afforded the diols in high yields and with excellent enantiomeric excesses (Table III). Iodine was also used as an efficient co-oxidant for the chemical dihydroxylation, and excellent results were obtained with nonconjugated olefins, in contrast to the case of $K_3Fe(CN)_6$.

TABLE III
ELECTROCHEMICAL Os-CATALYZED AD OF OLEFINS USING I_2 AS OXIDANT

Olefin	% Yield	% ee	Config.
	96.0	97.1	*R*
	87.6	>99	*R*
	87.6	>99	*R*
	92.9	97.3	*R,R*
	86.0	86.0	*R*
	88.2	88.2	*R,R*

Reaction conditions: $K_2OsO_2(OH)_4$ (0.0005 equiv), $(DHQD)_2PHAL$ (0.01 equiv), I_2 (0.5 equiv), K_3PO_4/K_2HPO_4 (1.2/1.8 equiv), electricity (2.33 F/mol), 31h, 0 °C.

3. Heterogeneous Asymmetric Dihydroxylation of Olefins

As mentioned in section III.A.*1.*, the Sharpless asymmetric dihydroxylation (AD) reaction has become an important process in organic chemistry for the synthesis of optically pure vicinal diols. However, there are limitations to performing the catalytic AD reaction on a large scale due to the toxicity of osmium tetroxide and the high cost of preparing the cinchona alkaloid ligands. Recently, in order to explore the possibility of the repetitive use of both components, several solid-supported cinchona alkaloid derivatives have been employed (*120-133*). However, most of polymers require complicated synthetic manipulations and, moreover, their catalytic efficiency remained far from satisfactory. Some of recent studies in this area led to an improved level of enantioselectivity. Salvadori and coworkers (*132*) used polymer-bound dihydroquinine derivatives as ligands (Figure 5) in AD reactions, which were obtained by radical copolymerisation of the cinchona alkaloid monomers **10** using EGDMA as the cross-linking agent. Optical yields of products ranged from 65% to 99%. Janda and coworker (*129,131*) have developed a soluble, polymeric form **11** (Figure 6) of $(DHQD)_2PHAL$ from polyethylene glycol monomethylether (MeO-PEG) for use in catalytic asymmetric dihydroxylation. The ligand **11** was prepared in five steps, and dihydroxylation was studied using four different substrates. In several cases excellent ees were obtained. We have also recently reported (*133*) a successful heterogeneous asymmetric dihydroxylation AD using silica gel-supported cinchona alkaloids containing 1,4-bis(9-*O*-quininyl)phthalazine [$(QN)_2PHAL$] (SGS-$(QN)_2PHAL$, **12**) (Figure 7).

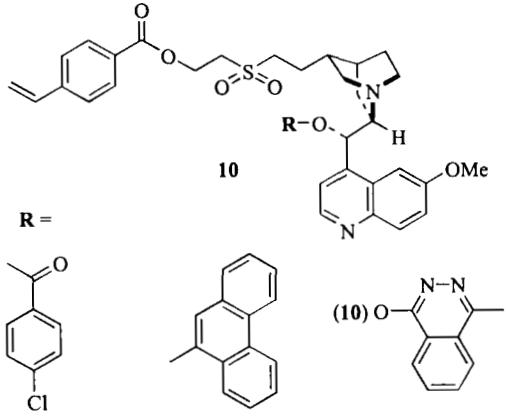


FIGURE 5. Salvadori's polymer-bound dihydroquinine derivatives

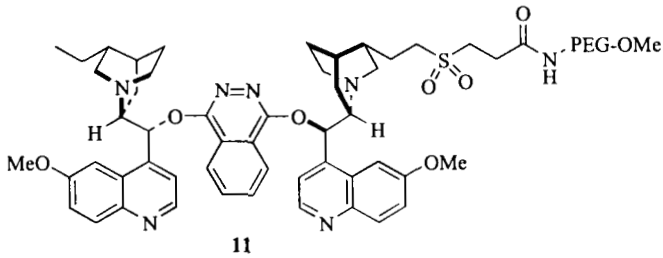
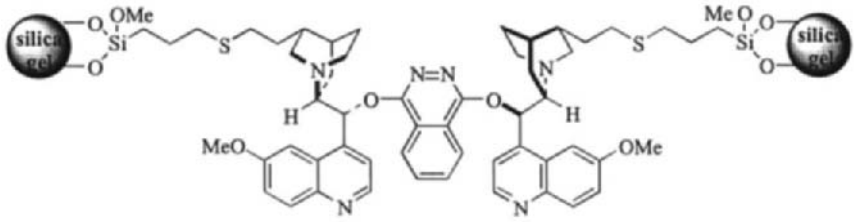


FIGURE 6. Janda's soluble polymer of $(DHQD)_2PHAL$



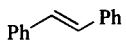
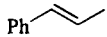
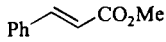
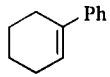
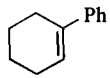
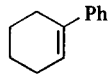
SGS-$(QN)_2$PHAL (**12**)

FIGURE 7. Song's SGS-$(QN)_2$PHAL

Substrate studies (Table IV) included *trans*-stilbene, β-methyl-*trans*-styrene, methyl *trans*-cinnamate, and 1-phenyl cyclohexene. Yields of between 88% and 95%, and ees of greater than 92% in all cases, represent a marked improvement on other reported investigations. Moreover, SGS-$(QN)_2$PHAL, **12** revealed much greater binding ability for OsO_4 than its homogeneous analogue. Thus, the silica gel supported alkaloid-OsO_4 complex could be reused without any significant loss of

enantioselectivity after simple filtration (entry 5). In the homogeneous solution, the complex formation of alkaloid and OsO_4 is in equilibrium. The binding constant K_{eq} for $(DHQD)_2PHAL$ is 27.7 (*103*). Thus, the enantiomeric excess in the homogeneous reaction approaches the maximum value with increasing ligand concentration. For the best results the reaction usually requires excess of expensive alkaloid ligands to osmium. However, in our system, excellent ee has been achieved with only an equimolar amount of ligand to osmium (entry 6).

TABLE IV
HETEROGENEOUS AD USING SGS-$(QN)_2$PHAL

Entry	Olefin	Time	% Yield [b]	% ee [c]	Config [c]
1		25h	88	>99	*S,S*
2		15h	92	97	*S,S*
3		20h	93	95	*2R,3S*
4		15h	96	96	*S,S*
5[d]		21h	92	92	*S,S*
6[e]		16h	95	95	*S,S*

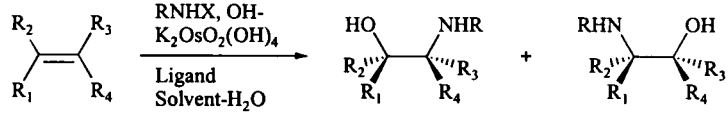
[a] The reaction conditions were not optimized: molar ratio of olefin/OsO_4/SGS- $(QN)_2$PHAL = 1/0.01/0.02; reaction temperature (10°C). [b] Isolated yields by column chromatography. [c] % ee and absolute configurations were determined by comparison of $[\alpha]_D$ with literature value. [d] Reaction was carried out with SGS-$(QN)_2$PHAL which had been used in entry 4 without further addition of OsO_4. [e] Molar ratio of olefin/OsO_4/SGS-$(QN)_2$PHAL = 1/0.01/0.01.

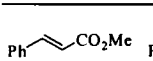
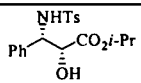
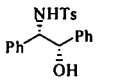
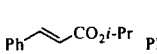
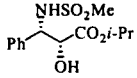
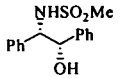
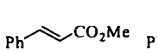
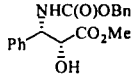
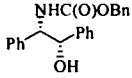
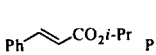
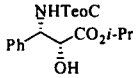
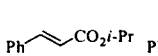
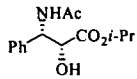
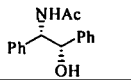
B. ASYMMETRIC AMINOHYDROXYLATION OF OLEFINS

The osmium-catalyzed (*134,135*) or palladium-mediated (*136*) aminohydroxylation of olefins has been known for over 20 years. The resulting β-aminoalcohols are one of the most abundant structural elements in biologically active compounds, as well as the starting points in the design of many chiral ligands. However, attempts to develop this reaction into a catalytic, asymmetric process were not successful, until Sharpless and coworkers discovered efficient methods for this reaction in 1996 (*137-141*). This reaction first emerged as a process in which TsNClNa (Chloramine-T) (*137,138*) was used as the nitrogen source/oxidant. Subsequently, with the

development of procedures which utilize carbamates- (*140*) and amide-derived oxidants (*141*) the substrate scope and selectivity was greatly improved (Table V). The products were obtained with moderate to high enantioselectivities, and with complimentary discrimination of the enantiotopic faces of the alkene by the two ligands, DHQ- and DHQD-derivatives. The same sense of the asymmetric induction as in the AD is observed, indicating that the transfer of chirality occurs by a similar pathway.

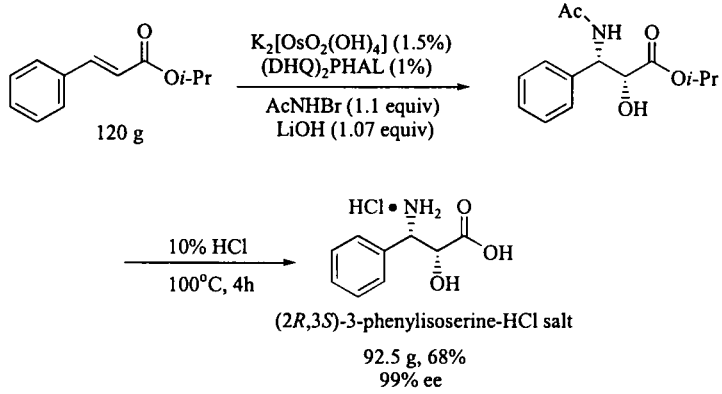
TABLE V
OS-CATALYZED ASYMMETRIC AMINOHYDROXYLATION OF OLEFINS



Substrate	Product	Nitrogen source and oxidant	Solvent	% ee $(DHQ)_2PHAL$	% ee $(DHQD)_2PHAL$	Yield (%)	Ref.
		$TsNClNa.3H_2O$	CH_3CN-H_2O	81	-71	64	*137*
		$TsNClNa.3H_2O$	CH_3CN-H_2O	62	-50	52	*137*
		CH_3SO_2NClNa	*n*-PrOH-H_2O	94	-95	65	*139*
		CH_3SO_2NClNa	*n*-PrOH-H_2O	75	-82	71	*139*
		BnOC(O)NClNa	*n*-PrOH-H_2O	94	-97	65	*140*
		BnOC(O)NClNa	*n*-PrOH-H_2O	91	-88	92	*140*
		TeoCNNaCl	*n*-PrOH-H_2O	99	-99	70	*143*
		CH_3NHBr, LiOH	*t*-BuOH-H_2O	99	-99	81	*141*
		CH_3NHBr, LiOH	*t*-BuOH-H_2O	94	-93	50	*141*

The AA reaction was applied to the large-scale synthesis of (2*R*,3*S*)-3-phenylisoserine (*141*), a precursor for the side chain of the anticancer drugs taxol and taxotere. The AA of isopropyl *trans*-cinnamate was carried out in the presence

of 1.5 mol% osmate salt and 1 mol% $(DHQ)_2PHAL$ using *N*-bromoacetamide as nitrogen source. The AA product was isolated by crystallization of the crude reaction mixture. Subsequent hydrolysis furnished the enantiomerically pure (2*R*,3*S*)-3-phenylisoserine as its HCl salt in 68% yield over two steps (Scheme 17). This amide-based AA protocol for the synthesis of the taxol C-13 side chain is superior to Sharpless's earlier AA- (*138*) or AD (*104-106*) approaches.

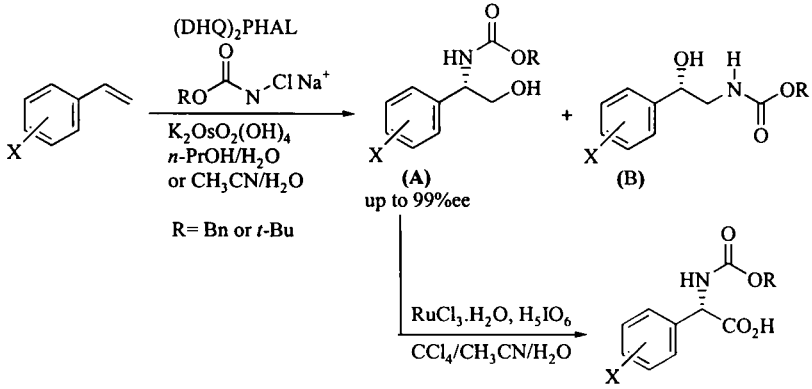


(2*R*,3*S*)-3-phenylisoserine-HCl salt

92.5 g, 68%
99% ee

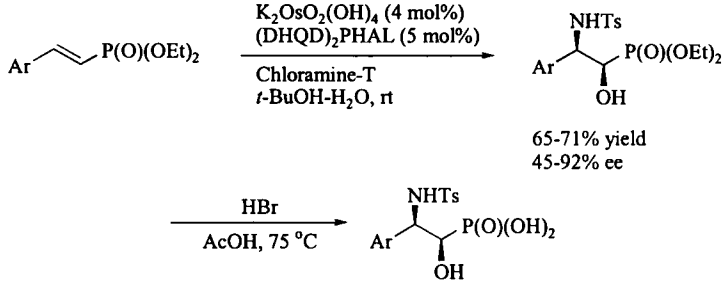
SCHEME 17. The synthesis of (2*R*,3*S*)-3-phenylisoserine as the taxol C13-side chain precursor

Another useful application of the AA reaction is the synthesis of optically pure α-arylglycinols. The catalytic asymmetric aminohydroxylation reaction of commercially available styrenes provides either (*R*)- or (*S*)-*N*-Cbz- or *N*-Boc-protected α-arylglycinols (Scheme 18) (*142-144*). The enantioselectivities are generally excellent and a subsequent oxidation step yields the corresponding α-arylglycines. The regioselectivity seems to be highly dependent on the nature of the styrenes as well as the choice of ligand, solvent and ligand-solvent combination. Phthalazine ligands such as $(DHQ)_2PHAL$ or $(DHQD)_2PHAL$ in *n*-PrOH favor the benzylic amine (A) over the benzylic alcohol regioisomer (B). In acetonitrile, the ratio of benzylic amines to benzylic alcohols (A/B) decreases significantly (*142*).



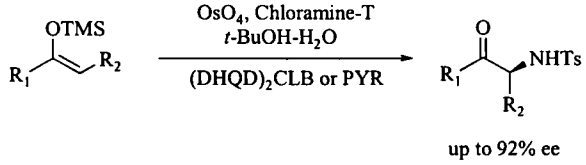
SCHEME 18. The synthesis of α-arylglycines via AA reaction

Recently, optically active β-amino-α-hydroxyphosphonic acids have been prepared by the asymmetric aminohydroxylation reaction of β-substituted vinylphosphonates followed by hydrolysis (Scheme 19) (*145*). The AA reaction of styrylphosphonate using $(DHQD)_2PHAL$ as chiral ligand afforded the corresponding β-amino-α-hydroxyphosphonate in 65% yield and 60% ee. After simple recrystallization and hydrolysis, β-amino-α-hydroxyphosphonic acid with high optical purity (>95% ee) was obtained.



SCHEME 19. Catalytic asymmetric aminohydroxylation of β-substituted vinylphosphonates

OsO_4-catalyzed aminohydroxylation of silyl enol ethers using cinchona alkaloids as chiral ligands and chloramine-T as the nitrogen source affords chiral α-amino ketones with an enantiomeric excess of up to 92% (Scheme 20) (*146*).

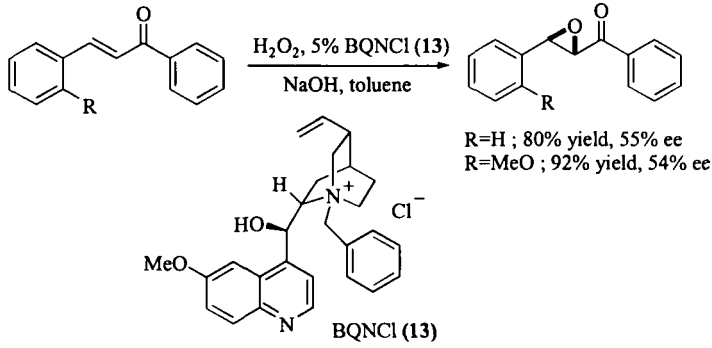


SCHEME 20. OsO_4-catalyzed asymmetric aminohydroxylation of silyl enol ethers

C. ASYMMETRIC EPOXIDATION OF ELECTRON-POOR OLEFINS

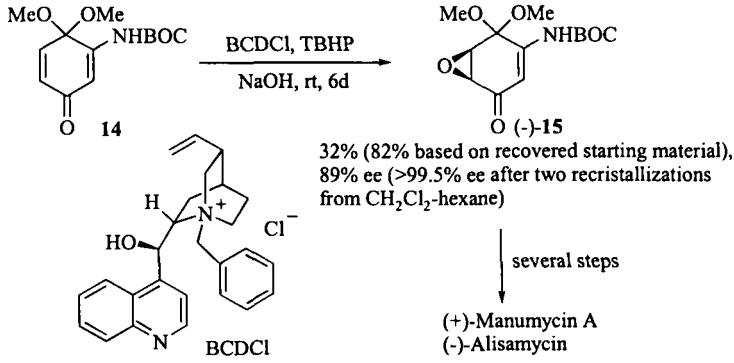
Cinchona alkaloid-derived quaternary ammonium salts as chiral phase transfer agents catalyze the asymmetric epoxidation of cyclic and acyclic electron-deficient alkenes, such as quinones and α,β-unsaturated carbonyl systems, with hydrogen peroxide, an alkyl hydroperoxide or sodium hypochlorite as the stoichiometric oxidant under basic conditions (*147-156*). Pioneering work of this reaction was done by Wynberg. However, the results were disappointing (ees generally <55%). Enantioselectivities as high as 55% ee were obtained in the epoxidation of *trans*-chalcone derivatives with basic hydrogen peroxide using *N*-benzylquininium chloride (BQNCl, **13**) (Scheme 21) (*147,149*). Epoxidation of *trans*-chalcone derivatives with sodium hypochlorite in the presence of **13** afforded a product with

about 25% ee, comparable to that obtained by employing the H_2O_2/NaOH system. However, interestingly, the absolute configuration of the major enantiomer obtained with bleach was opposite to that obtained by using H_2O_2 as oxidant (*148*).



SCHEME 21. Asymmetric epoxidation of *trans*-chalcones using phase-transfer catalyst **13**

Wynberg's epoxidation procedure using chiral phase transfer catalysts was recently applied by Taylor and coworkers to the total synthesis of manumycin A (*157*) and alisamycin (*158*) (Scheme 22). The epoxidation of the enone **14** with *tert*-butyl hydroperoxide using *N*-benzylcinchonidinium chloride (BCDCl) as the catalyst gave the epoxide (-)-**15** in 32% yield (82% based on recovered **14**) and 89% ee. Two recrystallizations of the reaction product from dichloromethane-hexane gave enantiomerically pure (-)-**15** (>99.5% ee). However, surprisingly, the use of the pseudoenantiomeric *N*-benzylcinchonium chloride (BCNCl) which would produce (+)-**15**, also gave (-)-**15**, albeit in only 10% ee.

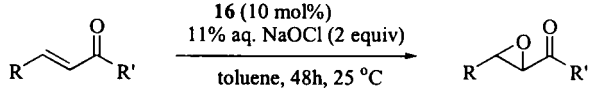


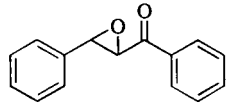
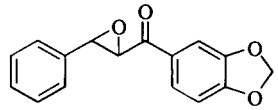
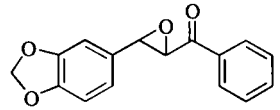
SCHEME 22. Synthesis of (+)-manumycin A and (-)-alisamycin

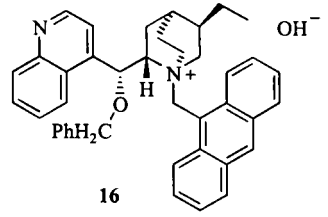
Very recently, much higher enantioselectivities (69-89% ee) have been achieved utilizing cinchona alkaloid-derived quaternary ammonium phase-transfer catalyst **16**

bearing an *N*-anthracenylmethyl function, in conjunction with sodium hypochlorite as the oxidant (Table VI) (*159*).

TABLE VI
ENANTIOSELECTIVE EPOXIDATION OF *trans*-CHALCONE DERIVATIVES USING **16**



Product	% ee (sign of rotation)	% yield
	86 (+)	90
	89 (+)	95
	83 (-)	97



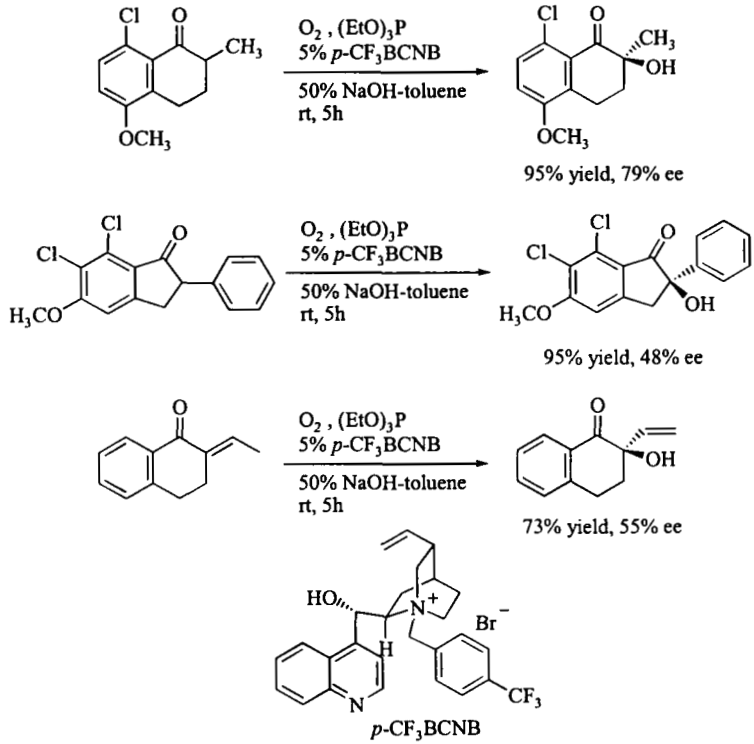
16

In another approach for the synthesis of non-racemic epoxides, direct epoxidation of benzaldehyde with dimethyl sulfide in the presence of chiral phase-transfer catalysts derived from ephedrine to give 2-phenyloxirane was also studied, however with little success (*160*).

D. ASYMMETRIC α-HYDROXYLATION OF KETONES

Ketones were converted by chiral PTC to optically active α-hydroxyketones with moderate asymmetric induction using molecular oxygen (Scheme 23) (*161*). Of the several different classes of quaternary ammonium salts examined, the best catalyst

was *N*-(*p*-trifluoromethylbenzyl)cinchonium bromide (*p*-CF_3BCNBr). α,β-Unsaturated ketones are also effective as starting materials in this reaction. For instance, (*E*)-2-ethylidene-1-tetralone was oxidized to the α-hydroxy ketone under the same reaction conditions in 73% yield and 55% ee.



SCHEME 23. Asymmetric α-hydroxylation of ketones

A hydrogen-bonded ion-pair formation depicted in Figure 8 between the catalyst and the enolate accounts for the enantioselectivity.

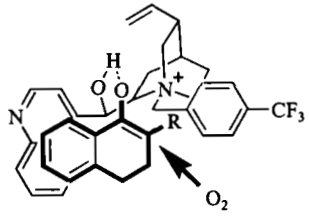
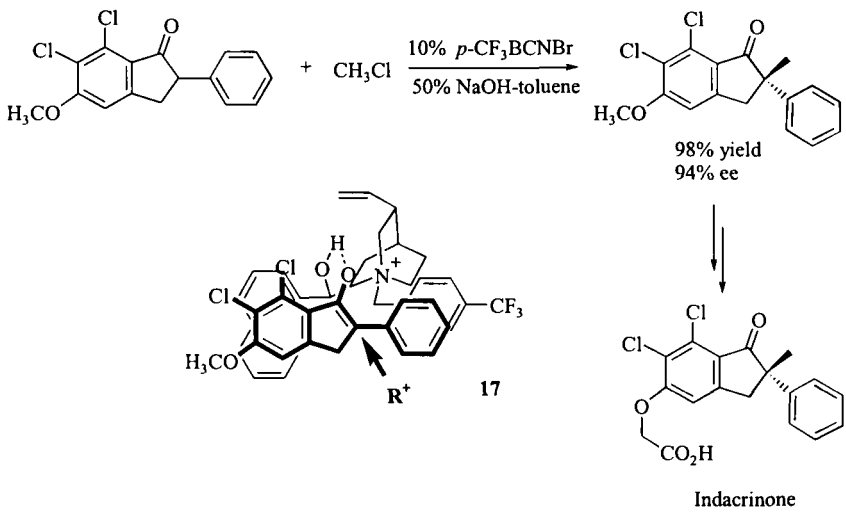


FIGURE 8. A hydrogen-bonded ion-pair formation

IV. Enantioselective Carbon-Carbon Bond Formation

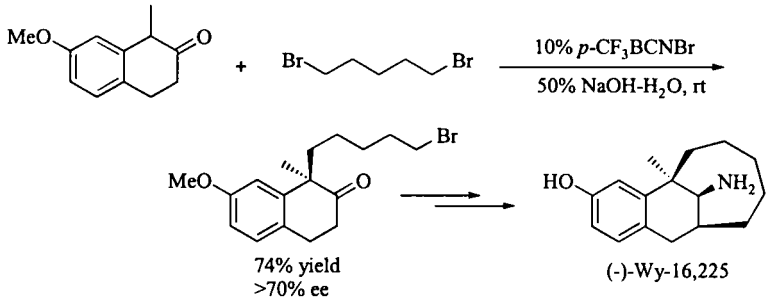
A. Asymmetric Alkylation using Phase-Transfer Catalysts

Asymmetric alkylations can be performed in the presence of catalytic amounts of alkaloid-derived chiral phase transfer catalysts. The first remarkable success in this area was achieved by Dolling and coworkers, who performed the enantioselective methylation of indanone derivatives under PTC conditions (*162-164*). The numerous reaction variables were optimized and the kinetics and mechanism of the reaction were studied in detail. The phase transfer reaction of indanone derivative and methyl chloride in 50% NaOH-toluene using *N*-(*p*-trifluoromethylbenzyl)cinchonium bromide (*p*-CF_3BCNBr) gives the methylated product in 98% chemical yield and 94% ee (Scheme 24). Alkylation takes place through a hydrogen-bonded enolate/catalyst ion pair **17**, from the least hindered side. The product is a precursor of the antihypertensive drug, (*S*)-indacrinone.

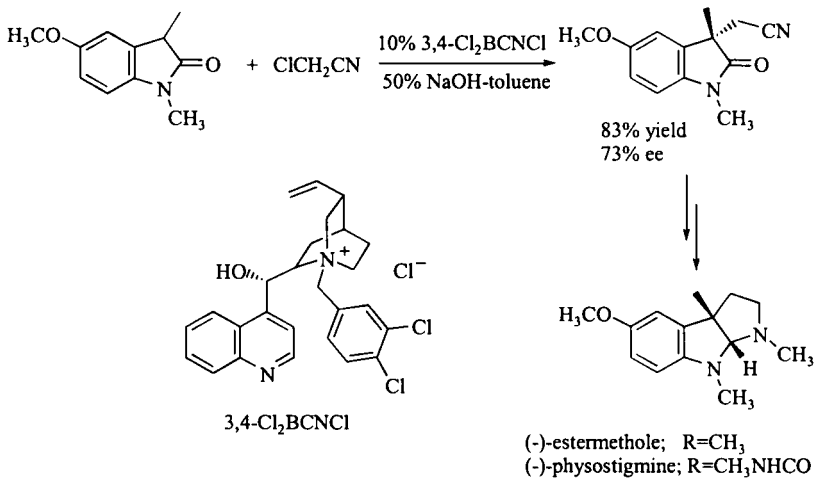


SCHEME 24. Asymmetric methylation of indanone derivative

Asymmetric induction in the phase-transfer alkylation of α-aryl substituted ketones, esters and lactones has also been evaluated (*165*). The potential of this method was demonstrated by the asymmetric synthesis of (-)-Wy-16,225, a bridged aminotetralin with potent analgesic properties (Scheme 25) (*165*). Similarly, asymmetric synthesis of (-)-estermethole and (-)-physostigmine, a clinically useful anticholinesterase agent, is accomplished by using phase-transfer alkylation of oxindoles (scheme 26) (*166*).



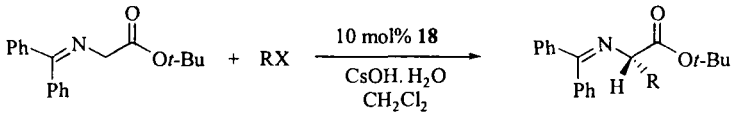
SCHEME 25. Asymmetric synthesis of (-)-Wy-16,225

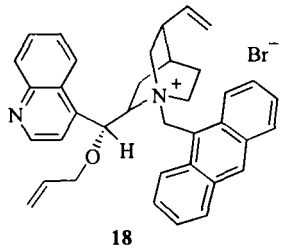


SCHEME 26. Asymmetric alkylation of oxindoles

α-Amino acids can be prepared by alkylation of the *tert*-butyl glycinate-benzophenone Schiff base ester using chiral PTC derived from cinchona alkaloids (*167-172*). This reaction had been thoroughly studied by the pioneering work of O'Donnell and coworkers. However, the enantioselectivity of this reaction was not very high (66-75% ee) (*167-170*). The asymmetric synthesis of chiral α,α-dialkylamino acids by a related method gave less satisfactory results (up to 50% ee) (*169*). However, very recently, Corey and coworkers (*171,172*) reported highly efficient enantioselective enolate alkylation using a well-designed cinchonidine-derived chiral phase-transfer catalyst **18** (Table VII). In this reaction, they used solid cesium hydroxide monohydrate as the basic phase in order to minimize the possibility of water in the organic phase (CH_2Cl_2) and to allow the use of lower reaction temperatures (-60 °C to -78 °C) than are possible with 50% aqueous KOH or NaOH.

TABLE VII
ENANTIOSELECTIVE CATALYTIC PHASE-TRANSFER ALKYLATION



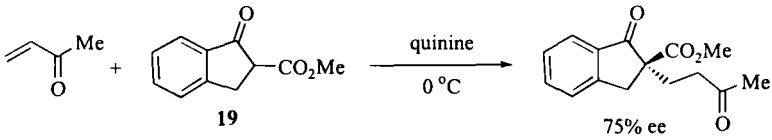


18

RX	% yield	% ee
CH_3I	71	97
EtI	82	98
$CH_3(CH_2)_4CH_2I$	79	99.5
	75	99
	89	97
$PhCH_2Br$	87	94
Ph_2CHBr	73	99.5

B. ASYMMETRIC MICHAEL ADDITION

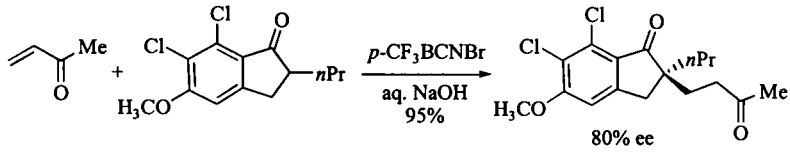
When the Michael donors have a sufficiently low pKa, the asymmetric Michael addition can be catalyzed by chiral bases. The most interesting results are those of Wynberg and coworkers (*173-176*). They performed the reaction of **19** with methyl vinylketone in the presence of quinine or quinidine and obtained either enantiomer of the Michael adduct (Scheme 27). The reactions of other ketoesters or of nitromethane are less enantioselective.



SCHEME 27. Asymmetric Michael addition catalyzed by cinchona alkaloid bases

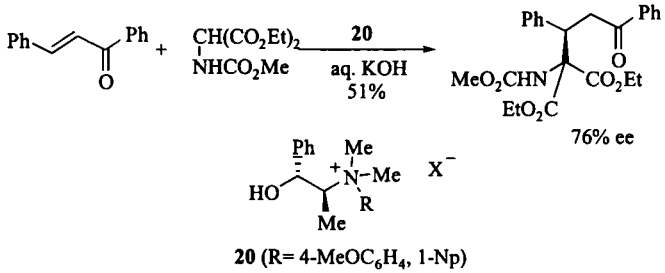
Michael additions can also be performed under phase transfer conditions with an achiral base in the presence of a chiral quaternary ammonium salt as a phase transfer agent. Weinstock and coworkers (*177*) conducted the Michael addition of 2-propyl-1-indanone to methyl vinylketone in a two-phase 50% aqueous NaOH-toluene system containing *N*-(*p*-trifluoromethylbenzyl)cinchonium bromide (*p*-CF_3BCNBr) as the catalyst (Scheme 28). The presence of a free hydroxyl group on the cinchona derivatives is necessary to observe good asymmetric induction. As in the case of alkylation, the interpretation of these results relies on the formation of a hydrogen

bond with the carbonyl of the α,β-unsaturated ketone, promoting its activation.



SCHEME 28

Ephedra alkaloid-derived quaternary ammonium salts **20** have also been employed in the Michael addition of protected glycinate to chalcone to yield optically active α-amino acid derivative (Scheme 29) (*178,179*). Michael adducts are obtained with good enantiomeric excess if the R substituent of **20** is 4-$MeOC_6H_4$ or 1-Np.



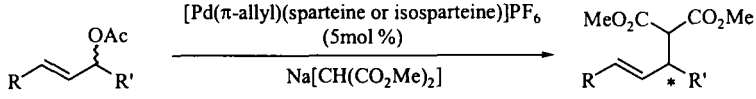
SCHEME 29. Asymmetric synthesis of α-amino acid derivatives by Michael addition

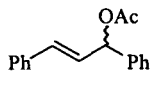
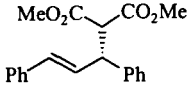
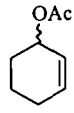
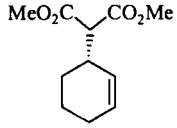
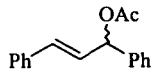
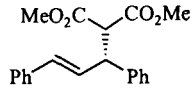
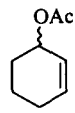
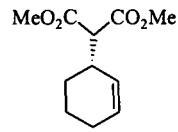
Conjugate addition of dialkylzincs to α-enones catalyzed by Ni(II)-complexes, based on norephedrine are also highly enantioselective (*180-183*).

C. Pd-CATALYZED ASYMMETRIC ALLYLIC ALKYLATION

Pd-catalyzed asymmetric alkylation reactions are useful synthetic methods for asymmetric C-C bond formation by allowing the Pd-catalyzed substitution of a nucleophile for a suitable leaving group in an allylic position (*184*). A number of chiral ligands have been developed for this kind of reaction. The readily available, naturally occurring alkaloid, (-)-sparteine has also been described as an effective chiral ligand for palladium-catalyzed asymmetric allylic alkylations (*185*). In some cases, high degrees of enantioselection were obtained (up to 85% ee). C_2-Symmetric (-)-α-isosparteine, which can be synthesized from (-)-sparteine in 2 steps (*186-188*), was also used by Kang and coworkers as a chiral ligand for asymmetric allylic alkylation (Table VIII) (*189*).

TABLE VIII
Pd-CATALYZED ASYMMETRIC ALLYLIC ALKYLATIONS [from 189]



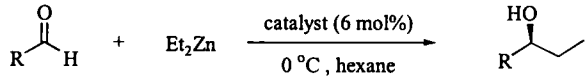
Ligand	Substrate	Product	% yield	% ee	Absolute config.
Sparteine			81	95	*R*
Sparteine			82[a)]	50[a)]	*S*
Isosparteine			86	88	*R*
Isosparteine			63	62	*S*

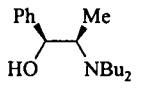
[a)] Results reported in ref. 185

D. ENANTIOSELECTIVE ADDITION OF DIALKYLZINCS TO ALDEHYDES

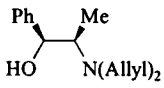
Catalytic enantioselective alkylation of aldehydes by chirally modified organometallics to give chiral secondary alcohols is one of the most important reactions in asymmetric synthesis (*190,191*). Whereas most of the attempts with organolithium or organomagnesium reagents have given disappointing results, very high enantioselectivities have been achieved by using dialkylzincs in combination with catalytic amounts of chiral β-aminoalcohols derived from norephedrines such as *N,N*-dibutylnorephedrine (DBNE) and *N,N*-diallylnorephedrine (DANE) (Table IX) (*190-193*). Dialkylzinc compounds do not react with aldehydes at room temperature, however, reactions do occur in the presence of catalytic amounts of aminoalcohols. A remarkable feature of these reactions is asymmetric amplification. Even when an aminoalcohol of low enantiopurity is used as a catalyst, the resulting secondary alcohol still often displays a high enantiomeric excess. This amplification mechanism has been studied in detail by Noyori (*191,194*).

TABLE IX
ENANTIOSELECTIVE ADDITION OF DIETHYLZINC TO ALDEHYDES CATALYZED BY NOREPHEDRINE-DERIVED AMINO ALCOHOLS





(1*S*,2*R*)-DBNE

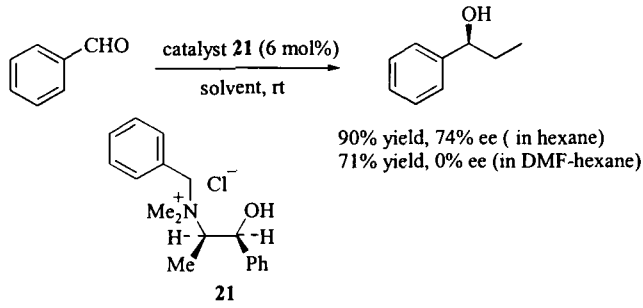


(1*S*,2*R*)-DANE

R	catalyst	yield (%)	% ee
Ph	(1*S*,2*R*)-DBNE	100	90
2-$MeOC_6H_4$	(1*S*,2*R*)-DBNE	100	94
4-$CF_3C_6H_4$	(1*S*,2*R*)-DBNE	92	91
4-FC_6H_5	(1*S*,2*R*)-DBNE	83	93
$PhCH_2CH_2$	(1*S*,2*R*)-DBNE	94	95
$(CH_3)_2CHCH_2$	(1*S*,2*R*)-DBNE	92	93
n-C_6H_{13}	(1*S*,2*R*)-DBNE	95	88
cyclo-C_6H_{11}	(1*S*,2*R*)-DBNE	94	78
n-C_8H_{17}	(1*S*,2*R*)-DBNE	95	87
n-C_8H_{17}	(1*S*,2*R*)-DANE	61	88

Diethylzinc has been added to benzaldehyde using an ephedra alkaloid-derived chiral quaternary ammonium salts, (1*S*,2*R*)-*N*-benzyl-*N*-methylephedrinium chloride (**21**) to give optically active secondary alcohols (Scheme 30) (*195*). This is an example in which the chiral catalyst affords a much higher enantioselectivity in the solid state than in solution. When benzaldehyde was treated with diethylzinc in hexane using **21** as a chiral solid state catalyst, (*S*)-1-phenylpropanol was obtained in 90% yield and 74% ee. In contrast, the reaction in DMF-hexane using the catalyst in solution **21** gave only racemic product in 71% yield. The degree of solvation of the ammonium cation of the catalyst is different between hexane and DMF. Very little solvation of the ammonium cation in hexane may be essential for the asymmetric induction. On the other hand, the oxygen or nitrogen atom of DMF strongly solvates the ammonium cation and may subsequently destroy the chiral complex of the ammonium catalyst and diethylzinc.

In another approach (*196-199*), polystyrene-bound *N*-butyl norephedrine **22** possessing a six-methylene spacer (Figure 9) has been used as a heterogeneous recyclable catalyst for the enantioselective addition of diethylzinc to aldehydes, producing secondary alcohols in high yields and with ees up to 82% (*198*).



SCHEME 30. Addition of Et_2Zn to benzaldehyde catalyzed by the ephedrinium chloride **21**

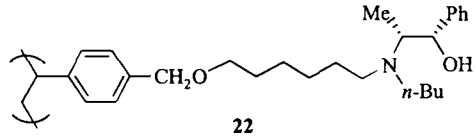


FIGURE 9. Polystyrene-bound *N*-butyl norephedrine **22**

Tridentate lithium salts derived from ephedrine (Figure 10) can also catalyze the reaction of diethyl zinc with benzaldehyde to exhibit high enantioselectivity (up to 95% ee) (*200,201*).

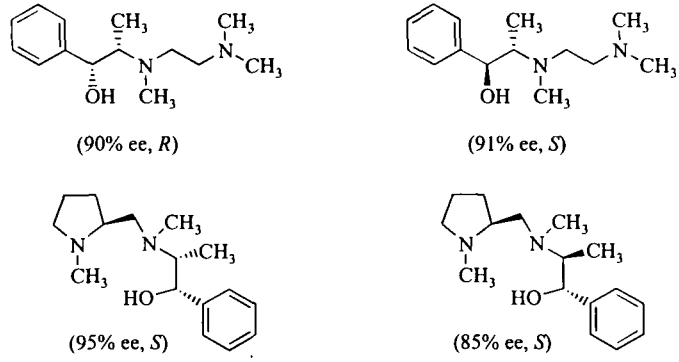
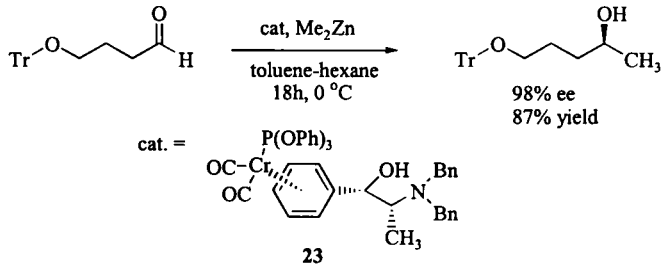


FIGURE 10. Ephedrine-derived tridentate ligands

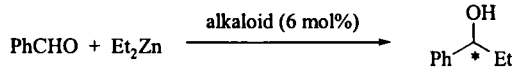
Other related sulfur derivatives of ephedra alkaloids (*202-205*) have also been used as catalysts in these reactions. Tricarbonyl chromium complexation of the aryl ring of ephedrine derivatives can lead to improved enantioselectivities (*206-208*). For example, the chromium complexed (1*S*,2*R*)-dibutylnorephedrine **23** can mediate the addition of dialkyl zincs to aldehydes giving *S*-carbinols in up to 99% ee (Scheme 31) (*208*).



SCHEME 31. 1,2-Addition of Me_2Zn to aldehyde catalyzed chromium-complexed DBNE **23**

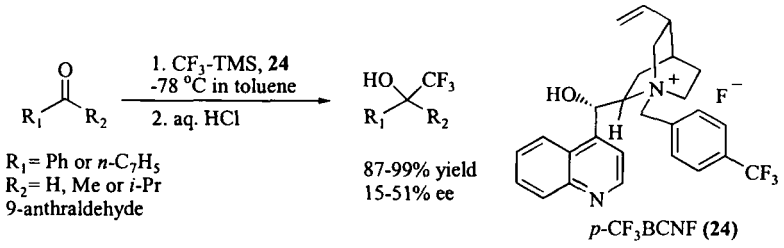
Cinchona alkaloids are also effective as chiral ligands for the 1,2-addition of diethylzinc to benzaldehyde (*209,210*). When diethylzinc is added to a solution of *o*-methoxybenzaldehyde in toluene in the presence of catalytic amounts of quinine, (+)-*o*-methoxyphenylethylcarbinol is formed in an ee of 92% (*209*). However, Buono and coworkers observed a very surprising effect of reaction temperature on reactivity and enantioselectivity in cinchona alkaloid-catalyzed 1,2-addition (Table X) (*210*). The best ee (73%) was obtained at elevated temperature (100 °C) and not, as could be expected, at a lower temperature. Through the appropriate choice of cinchona alkaloid, it is possible to obtain either enantiomers.

TABLE X
INFLUENCE OF REACTION TEMPERATURE ON ENANTIOSELECTIVITY IN CINCHONA ALKALOID-CATALYZED 1,2-ADDITION



catalyst	reaction time (h)	reaction temp (°C)	% yield	% ee	config.
Quinine	38	-10	90	48	*R*
	16	rt	97	64	*R*
	0.25	100	95	73	*R*
Quinidine	38	-10	92	45	*S*
	16	rt	98	51	*S*
	0.25	100	96	69	*S*

Chiral quaternary ammonium fluorides, such as *N*-(*p*-trifluoromethylbenzyl) cinchonium fluoride (*p*-CF_3BCNF, **24**), also catalyze the asymmetric 1,2-addition of trifluoromethylzinc to aldehydes and ketones to give optically active 1-substituted-2,2,2-trifluoroethanols in nearly quantitative yields, but in only low to moderate ees (Scheme 32) (*211*).



SCHEME 32. Asymmetric trifluoromethylation of carbonyl compounds with CF_3-TMS catalyzed by **24**

Recently, a number of chiral β-amino alcohols such as **25, 26** (Figure 11) derived from Abrine readily available from the seeds of *Abrus precatorius* have been prepared and used as chiral ligands for the enantioselective addition of diethylzinc with aldehydes (*212,213*). The compounds **25, 26** gave excellent enantiomeric excess (94.2% and 97.6%, respectively) (*213*).

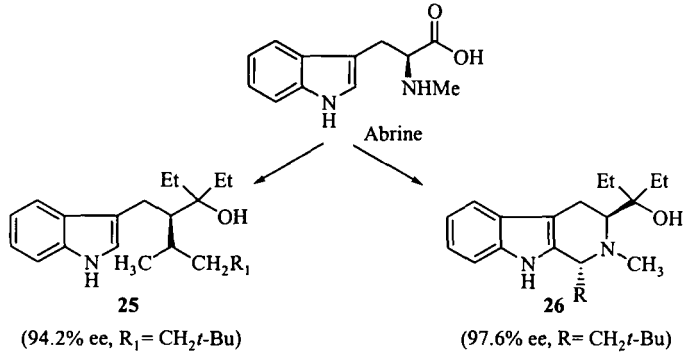
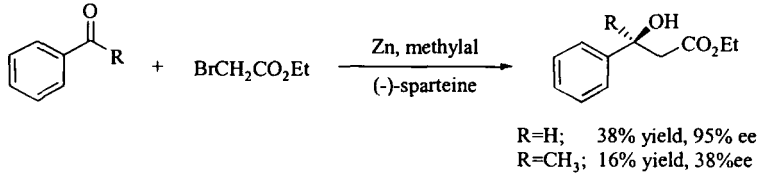


FIGURE 11. Chiral β-amino alcohols **25,26** derived from Abrine

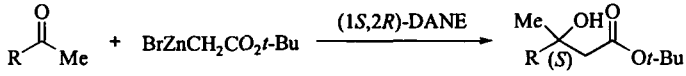
E. ASYMMETRIC REFORMATSKY REACTION

Sparteine was used as a zinc ligand as early as 1973 by Guette and coworkers in the Reformatsky reaction (*214*). A highly enantioselective reaction was observed with benzaldehyde. However, the Reformatsky reaction with ketones under the same conditions gave β-(*tert*-hydroxy)esters with only 7-39% ee (Scheme 33).



SCHEME 33. Asymmetric Reformatsky reaction using sparteine as a ligand

Higher ees and better yields were obtained using *N,N*-diallylnorephedrine (DANE) as a chiral ligand in the enantioselective Reformatsky reaction of *tert*-butyl bromoacetate and ketones (73-75% ee) (Scheme 34) (*215*).

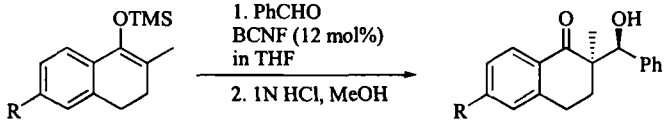


R= Ph; 65% yield, 74% ee
R= 2-Naphthyl; 38% yield, 75% ee
R= 4-FC_6H_4; 57% yield, 73% ee

SCHEME 34. Asymmetric Reformatsky reaction using DANE as a ligand

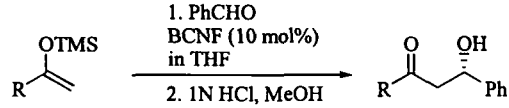
F. ASYMMETRIC ALDOL CONDENSATION

Asymmetric PTC aldol condensation of protected glycinate with aldehydes to give β-hydroxy-α-amino acid derivatives was reported (*216,217*). Unfortunately, the enantio- and diastereoselectivities in this reaction were very low (<12% ee). However, recently, moderate ees (up to 72% ee for the erythro isomer) were obtained in the catalytic enantioselective aldol reactions of benzaldehyde with silyl enol ethers by utilizing *N*-benzylcinchonium fluoride (BCNF) as a chiral catalyst (Scheme 35) (*218*).



R= H, MeO, Cl, Br

up to 72% ee



R= Ph, *t*-Bu

R= Ph; 65% yield, 39% ee
R= *t*-Bu; 62% yield, 62% ee

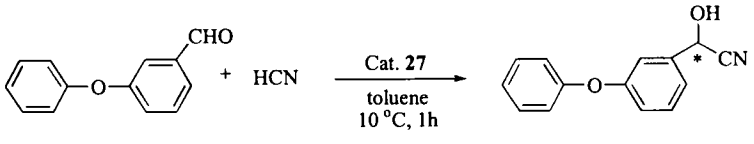
SCHEME 35. Enantioselective PTC aldol condensation

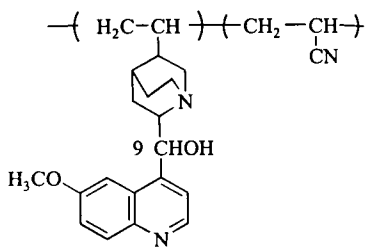
G. ASYMMETRIC CYANOHYDRIN SYNTHESIS

Optically pure cyanohydrins are versatile synthetic intermediates in the synthesis of a wide range of homochiral products such as α-hydroxy acids, β-hydroxy amines, and α-amino acid derivatives, etc., and have also been used as key intermediates in the synthesis of industrially important biologically active compounds (*219*). The usual synthetic route to cyanohydrins involves the addition of a cyanide source, such as HCN or TMSCN, to aldehydes or ketones in the presence of chiral catalysts (*219*). Cinchona alkaloids can also catalyze this type of reaction (*220-222*). In 1912, Bredig

and Fiske discovered the enantioselective addition of hydrogen cyanide to aldehydes catalyzed by cinchona alkaloids, which is probably one of the earliest studied asymmetric catalysis (*220*). However, the asymmetric induction by alkaloids was very low. Recently, Danda and coworkers have used the polymers **27**, containing quinidine or quinine, to catalyze the asymmetric addition of HCN to 3-phenoxybenzaldehyde (*221*). Very interestingly, polymeric alkaloids exhibited higher enantioselectivities than those obtained with the corresponding monomeric alkaloids, quinidine and quinine (Table XI). For example, a polymer containing quinidine gave the (*S*)-isomer of cyanohydrin in 98% yield and 46% ee, whilst quinidine gave the (*S*)-isomer of the cyanohydrin in 97% yield and 22% ee. The reaction mixture is gel-like in the case of polymeric alkaloids, whereas it is homogeneous in the case of the corresponding monomers. This gelation would be preferable both to increase the enantioselectivity and to decrease the racemization of the products.

TABLE XI
ASYMMETRIC HYDROCYANATION OF 3-PHENOXYBENZALDEHYDE CATALYZED BY CINCHONA ALKALOIDS





27

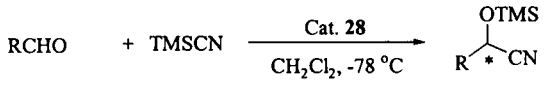
catalyst	% yield	% ee
quinidine	97	22 (*S*)
quinine	93	5 (*R*)
27a	98	46 (*S*)
27b	97	20 (*R*)

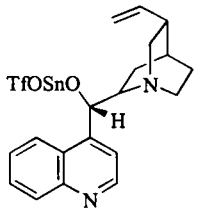
27a; poly(quinidine-co-acrylonitrile)
27b; poly(quinine-co-acrylonitrile)

Mukaiyama *et al.* have reported the use of the chiral tin (II) species **28**, derived from cinchonine, as a Lewis acid catalyst for the asymmetric addition of trimethylsilyl cyanide to aliphatic aldehydes (Table XII) (*222*). The enantiomeric excesses (72-96% ee) obtained by this method are much higher than those reported for cyanohydrins prepared using only alkaloids as catalyst or using polymeric derivatives of alkaloids. However, benzaldehyde did not react with TMSCN under the same conditions.

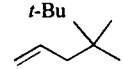
Cinchona and ephedra alkaloid-derived quaternary ammonium salts were also utilized as chiral catalysts for the synthesis of *O*-acetyl cyanohydrins under phase-transfer conditions. However, only low optical rotations in the products were observed (*223*).

TABLE XII
ASYMMETRIC ADDITION REACTION OF TMSCN WITH ALDEHYDES USING Ti(II)-LEWIS ACID DERIVED FROM CINCHONIDINE



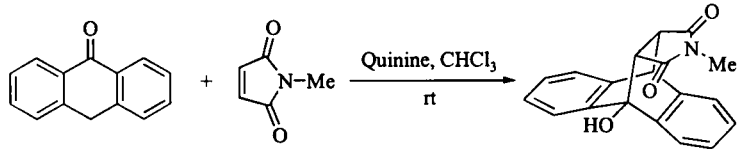


28

R	% yield	% ee
c-C_6H_{11}	89	72
n-C_8H_{17}	79	96
i-Pr	67	95
t-Bu	49	83
	27	93
Ph	0	—

H. ASYMMETRIC DIELS-ALDER REACTION

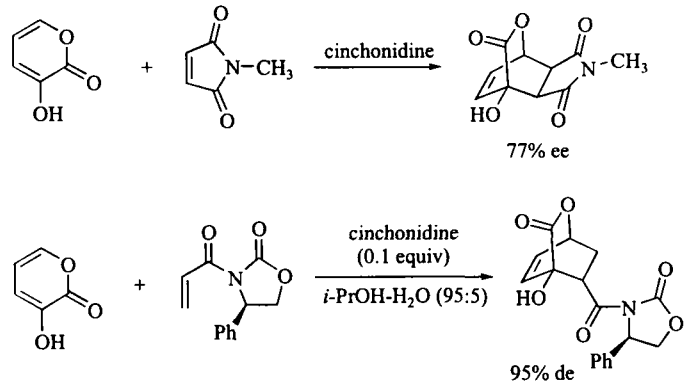
Diels-Alder reactions are usually catalyzed by Lewis acids, and several asymmetric acid-catalyzed reactions have been reported (*224*). However, Diels-Alder reactions catalyzed by bases are unusual and only a few asymmetric, base-catalyzed reactions are known. For example, the reaction of anthrone and *N*-methylmaleimide can be catalyzed by alkaloids (Scheme 36). However, only moderate ees were obtained (*225,226*).



98% yield
61% ee

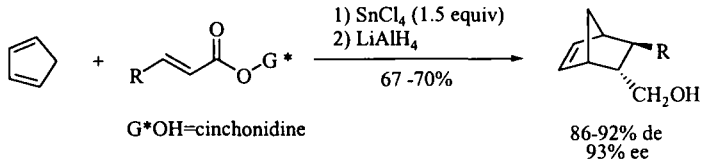
SCHEME 36. Quinine-catalyzed asymmetric Diels-Alder reaction

Recently, Nakatani and coworkers (*227-229*) reported that the reaction of 3-hydroxy-2-pyrone with an electron deficient dienophile can also be catalyzed by a base and afforded a Diels-Alder adduct in nearly quantitative yield (Scheme 37). In particular, when a cinchona alkaloid was used as an optically active base catalyst, up to 77% ee of the endo adducts was obtained in the reaction of 3-hydroxy-2-pyrone with *N*-methylmaleimide (*228*). In the presence of a cinchona alkaloid as a catalyst, the Diels-Alder reaction of 3-hydroxy-2-pyrone with chiral *N*-acryloyl oxazolidinone afforded a bicyclolactone adduct with high diastereoselectivities (up to 95% de), in almost quantitative yield (*229*).



SCHEME 37. Cinchonidine-catalyzed asymmetric Diels-Alder reaction

The use of cinchona alkaloids as chiral auxiliaries in the cycloadditions of cyclopentadiene with acrylic, crotonic or fumaric esters was also reported (*230*) (Scheme 38).

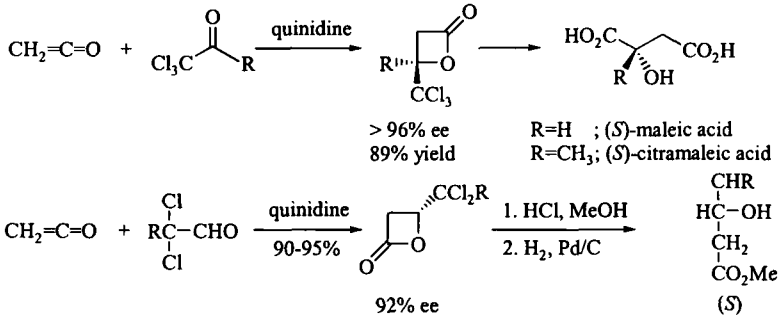


SCHEME 38. Asymmetric Diels-Alder reaction using cinchonidine as a chiral auxiliary

I. ASYMMETRIC 2,2-CYCLOADDITION OF KETENE AND ELECTRON-POOR ALDEHYDES OR KETONES

Acid chlorides (*231*) or ketenes (*232-237*) react with electron-poor aldehydes and ketones in the presence of optically active tertiary amines to give enantiomerically enriched β-lactones. The reaction of ketene with chloral or trichloroacetone has been studied in considerable detail by Wynberg. In the presence of catalytic amounts of cinchona alkaloid, the β-lactone is formed in virtually quantitative chemical and optical yield (up to 96% ee) (Scheme 39). This optical purity can be easily improved by a recrystallization of the β-lactones from methylcyclohexane. By the proper choice of the catalyst, either enantiomer of the β-lactone can be obtained. When quinidine is used as the catalyst the product has the (*R*) configuration, whereas the use of quinine gives access to the corresponding (*S*)-enantiomers. Thus, the reaction of 1,1,1-trichloroacetone with ketene in the presence of quinidine gives the β-lactone (*R*)-4-methyl-4-(trichloromethyl)oxetan-2-one from which (*S*)-citramalic acid, a useful chiral synthon of natural products (*238-240*), can be obtained by alkaline hydrolysis. Substitution of chloral for trichloroacetone in the cycloaddition reaction leads to the β-lactone, an intermediate in the production of (*S*)-malic acid. Similarly,

the quinidine-catalyzed cycloaddition of ketene to 2,2-dichloroaldehydes gives β-lactones of high optical purities which are easily converted to the corresponding methyl (3*S*)-hydroxyalkanoates (*235*).



SCHEME 39. Enantioselective cycloaddition of ketene and electron-poor aldehydes or ketones

Wynberg proposed the transition state picture **29** for the ketene-chloral addition in the presence of quinidine (Figure 12) (*241*).

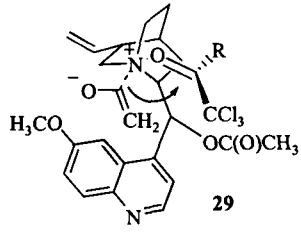
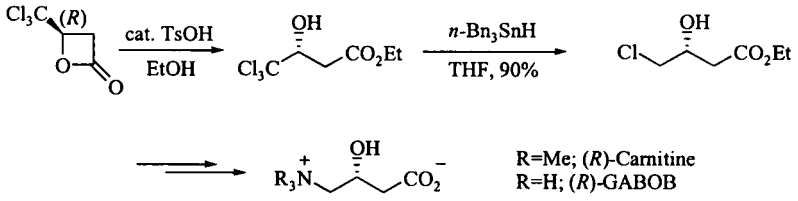


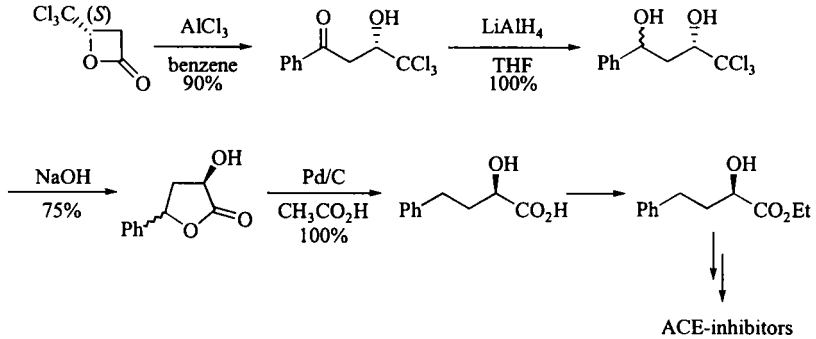
FIGURE 12. Ketene-chloral-quinidine acetate transition state

Song *et al.* reported that poly(cinchona alkaloid acrylate)s gave similar catalytic activity and enantioselectivity (up to 94% ee) compared to those of their monomeric alkaloids, quinidine and quinine (*242*).

(*R*)-4-(Trichloromethyl)oxetan-2-one can be easily converted to ethyl (*R*)-3-hydroxy-4-chlorobutyrate, a (*R*)-carnitine intermediate, by ethanolysis followed by selective bis-dechlorination of ethyl (*R*)-3-hydroxy-4,4,4-trichlorobutyrate (Scheme 40) (*243,244*). The optically pure β-lactone can serve an excellent chiral acylating agent under Friedel-Crafts-type conditions. The acylation of (*S*)-4-(trichloromethyl)oxetan-2-one with aromatic compounds provided an acylated product with a chiral trichloromethyl carbinol moiety, which can be used for the synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutanoate, an important precursor for the synthesis of angiotensin converting enzyme (ACE) inhibitors, e.g., enalapril (Scheme 41) (*245*).

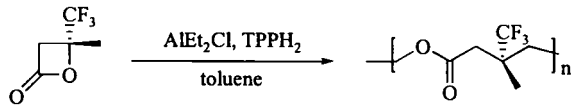


SCHEME 40. Synthesis of (*R*)-carnitine and (*R*)-GABOB from (*R*)-4-(trichloromethyl)oxetan-2-one



SCHEME 41. Synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutanoate from (*S*)-4-(trichloromethyl)oxetan-2-one

In the presence of catalysts such as $ZnEt_2/H_2O$ or $AlEt_3/H_2O$, optically active 2-substituted β-propiolactones readily polymerize to give optically active stereoregular polyesters exhibiting quite unique properties compared with the corresponding racemic polymers (Scheme 42) (*246-248*).

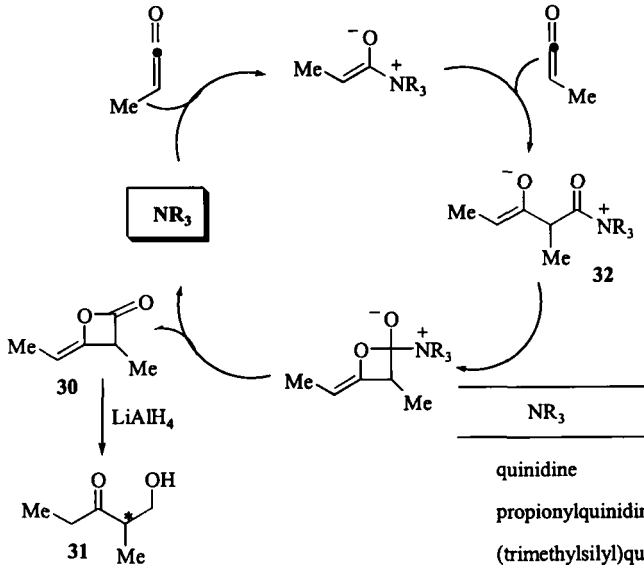


SCHEME 42. Synthesis of an optically active polyester from chiral β-propiolactone

J. ASYMMETRIC DIMERIZATION OF KETENES

Very recently, Calter has reported that cinchona alkaloids and their derivatives catalyze the dimerization of methylketene with high enantioselectivity (up to 98% ee), yielding β-lactone **30** via a formal Claisen condensation (Scheme 43) (*249*). This product could easily be transformed into a useful synthon for polypropionate synthesis. The dimerization of methylketene was carried out in the presence of 1 mol% of the alkaloid catalysts in THF at -78 °C. Due to the volatility and instability of the dimer **30**, the product was reduced with $LiAlH_4$ to produce the primary alcohol **31**. Possible conformations of ammonium enolate **32** derived from quinidine

and methylketene was proposed.

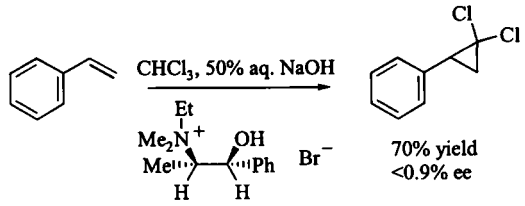


NR_3	% ee
quinidine	98 (*R*)
propionylquinidine	97 (*R*)
(trimethylsilyl)quinine	98 (*R*)
quinine	70 (*S*)
propionylquinine	54 (*S*)
(trimethylsilyl)quinine	93 (*S*)

SCHEME 43. Catalytic asymmetric dimerization of methylketene

K. ASYMMETRIC CYCLOPROPANATION

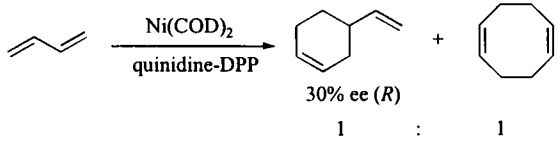
Although the asymmetric cyclopropanation of alkenes using an ephedrine-derived quaternary ammonium salt as a phase-transfer catalyst was reported early, the results were very disappointing (Scheme 44) (*250*). Another report of cyclopropanation has yielded, at best, low asymmetric inductions (*251*).



SCHEME 44. Catalytic asymmetric cyclopropanation of styrene

L. ASYMMETRIC DIMERIZATION OF BUTADIENE

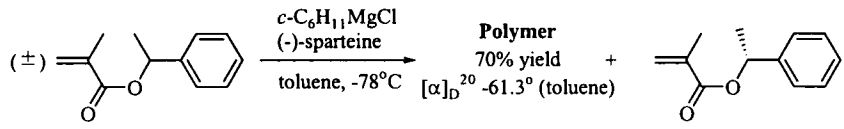
The dimerization of butadiene catalyzed by $Ni(COD)_2$ and quinidine-DPP as a chiral ligand gave rise to (*R*)-(+)-vinylcyclohexene with an ee of approximately 30%, accompanied with a selectivity for vinylcyclohexene versus cyclooctadiene of approximately 1 (Scheme 45) (*46,252*).



SCHEME 45. Catalytic asymmetric dimerization of butadiene

M. GRIGNARD-INDUCED ANIONIC POLYMERIZATION

Several sparteines, such as (-)-sparteine, (-)-α-isosparteine, (+)-6-benzylsparteine, etc., when combined with Grignard reagents catalyze the asymmetric polymerization of racemic α-methylbenzyl methacrylate (MBMA) in toluene at -78 °C (*253-257*). For instance, racemic 1-phenylethyl methacrylate is resolved efficiently by a cyclohexyl magnesium chloride-(-)-sparteine complex to give, at 70% conversion, optically active polymer and the unreacted monomer in greater than 90% ee (Scheme 46) (*253*). Similarly, reaction of racemic phenyl-2-pyridyl-*o*-tolylmethyl methacrylate in the presence of 4-fluorenyllithium and (+)- or (-)-2,3-dimethoxy-1,4-bis(dimethylamino)butane proceeds with a high degree of kinetic resolution (*258*).



SCHEME 46. Catalytic asymmetric Grignard induced anionic polymerization

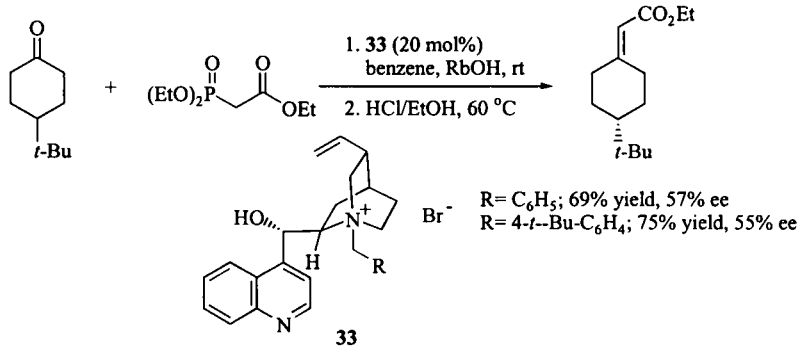
N. ASYMMETRIC HORNER-WADSWORTH-EMMONS REACTION

Very recently, Arai and coworkers (*259*) reported the first example of an asymmetric Horner-Wadsworth-Emmons reaction promoted by cinchonine-derived quaternary ammonium salts **33** as phase transfer catalysts (Scheme 47). Although catalytic activity and enantioselectivity are not high enough (up to 57% ee), these results will lead to further progress.

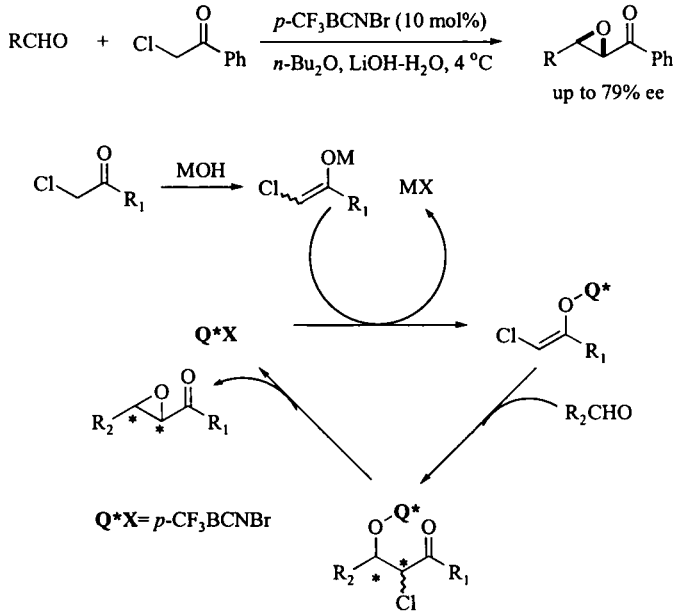
O. CATALYTIC ASYMMETRIC DARZENS CONDENSATION

A chiral quaternary ammonium salt derived from cinchonine catalyzes the asymmetric Darzens condensation under PTC conditions. The reaction of phenacyl chlorides with various aldehydes in dibutyl ether with LiOH monohydrate base in

the presence of 10 mol% of *p*-CF_3BCNBr affords α,β-epoxyketones with moderate to good enantiomeric excess (up to 79% ee) (*260*). Scheme 48 shows the proposed catalytic cycle for the asymmetric Darzens condensation under PTC conditions.



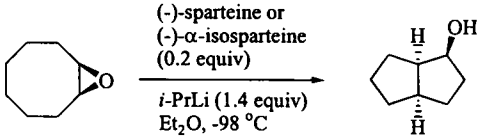
SCHEME 47. The catalytic asymmetric Horner-Wadsworth-Emmons reaction with PTCs



SCHEME 48. Catalytic asymmetric Darzens condensation under phase-transfer conditions and the proposed catalytic cycle

P. Enantioselective α-Deprotonation-Rearrangement of Achiral Epoxides

The enantioselective α-deprotonation-rearrangement of medium sized (8-, 9- and 10-membered) cycloalkene-derived achiral epoxides, using organolithiums in the presence of catalytic amounts of sparteine (*261*) or isosparteine (*262*) to give bicyclic alcohols in good yields and ees (up to 84% ee), has been recently reported (Scheme 49).



Using (-)-sparteine: 62% yield, 73% ee
Using (-)-α-isosparteine: 86% yield, 84% ee

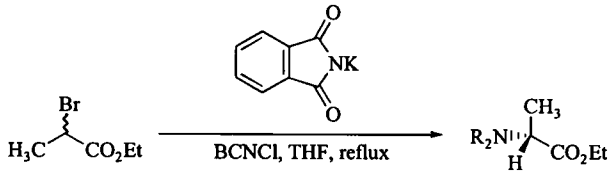
SCHEME 49. Asymmetric α-deprotonation-rearrangement of achiral epoxide

V. Enantioselective Carbon-Heteroatom Bond Formation

A. Carbon-Nitrogen Bond Formation

1. Synthesis of Optically Active α-Amino acids from α-Haloesters

Several groups have studied the synthesis of optically active α-amino acids from the inexpensive and readily available α-halo esters by displacement of the halides with phthalimide in the presence of cinchona alkaloid-derived quaternary ammonium salts as catalysts (Scheme 50) (*263-266*). Early studies, using chiral, nonracemic starting material, showed that this reaction occurred with partial inversion of configuration and likely involved a kinetic resolution (*265*).



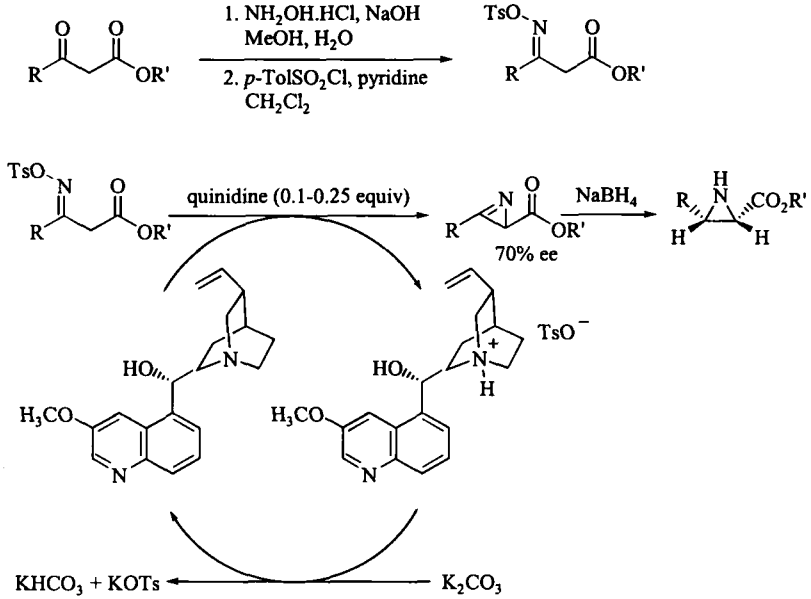
28% yield, 18.6% op

SCHEME 50. Asymmetric synthesis of optically active α-amino esters from a racemic α-halo ester

2. Catalytic Asymmetric Neber Reaction

Recently, Zwanenburg and coworkers (*267*) reported a novel catalytic asymmetric synthesis of aziridine carboxylic esters via the Neber reaction of ketoxime tosylates

derived from 3-oxocarboxylic esters (Scheme 51). Quinidine catalyzes the Neber reaction of ketoxime tosylates in the presence of K_2CO_3 to give optically active aziridines (up to ~70% ee). However, with sparteine, brucine, and strychnine no optically active aziridine was formed. It was suggested that in the mechanisim for asymmetric induction the alkaloid bases form a tightly bound complex with the ketoxime tosylate. Since the presence of an alcohol function in the alkaloid base seems to be a prerequisite, it was suggested that hydrogen bonding of the base and the substrate through this hydroxyl group is a governing factor in the enantiodifferentiation during the abstraction of the methylene protons. This proposal is supported by the observation that in a hydroxylic solvent no asymmetric induction takes place and that additives such as LiCl, and especially H_2O, lower the optical yield considerably.



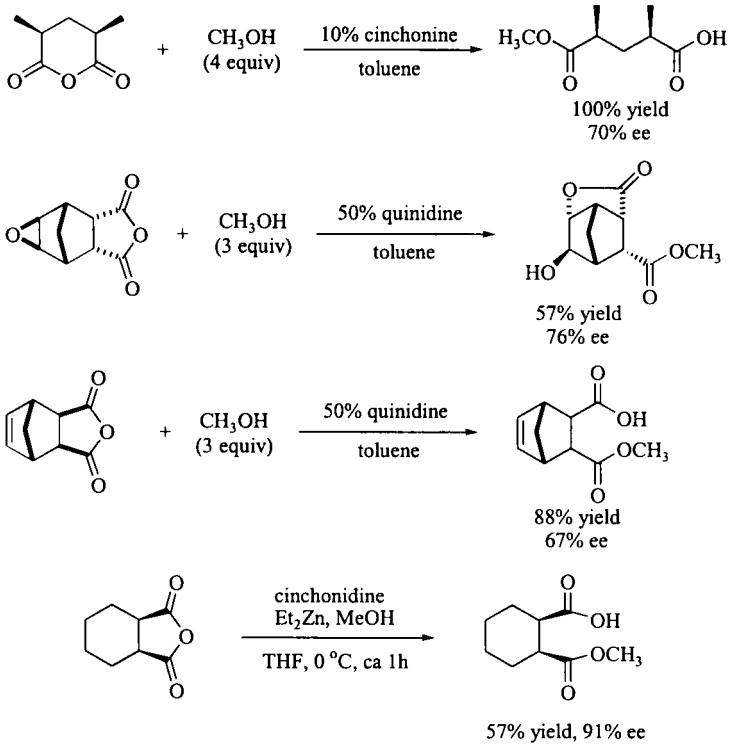
SCHEME 51. Catalytic asymmetric Neber reaction

B. CARBON-OXYGEN AND CARBON-SULFUR BOND FORMATION

1. Enantiotopic Differentiation Reaction of Meso-Cyclic Anhydrides (268-273)

In the presence of a cinchona alkaloid, certain cyclic carboxylic anhydrides with *meso* structures are converted to the chiral diacid monoesters in up to 76% ee (*268*). Quinine or cinchonidine and quinidine or cinchonine show opposite asymmetric induction. Asymmetric esterification of a prochiral cyclic anhydride can be also catalyzed by the complex formed from β-aminoalcohols, such as ephedrine or cinchona alkaloids, diethylzinc, and methanol. The use of (-)-ephedrine did not show

a satisfactory selectivity. However, cinchona alkaloids effected a good to excellent diastereofacial discrimination (up to 91% ee) (Scheme 52) (*273*).



SCHEME 52. Asymmetric methanolysis of *meso*-cyclic anhydrides

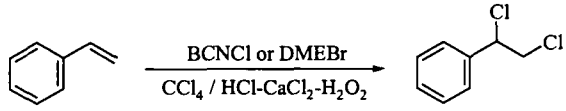
Polymer-supported quinine was also utilized for the asymmetric methanolysis of prochiral *cis*-2,4-dimethylglutaric anhydride. However, only low ees (up to 32% ee) were obtained (*270*).

2. O- and S-Alkylations

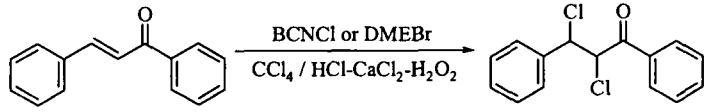
O-Alkylations with cinchona- and ephedra alkaloid-derived catalysts have been studied (*274*). The addition of thiophenol to cyclohexenone in the presence of cinchona alkaloid-derived quaternary ammonium salts gave the Michael adduct with 36% op in 85% yield (*275*). Other C-S bond formations with cinchona (*274,276-278*) and ephedra alkaloid (*274,275,279*) catalysts have been reported.

C. CARBON-HALOGEN BOND FORMATION

Very low asymmetric induction was observed in the addition of chlorine to alkenes under phase-transfer conditions using *N*-benzylcinchonium chloride (BCNCl) and *N*-dodecyl-*N*-methylephedrinium bromide (DMEBr) as catalysts (*280*) (Scheme 53). Alkaloid-catalyzed bromination of alkenes, which occurs with a maximum 5.5% optical yield was also reported (*281*).



with BCNCl; 30%, $[\alpha]_{546}$ +0.480°
with DMEBr; 25%, $[\alpha]_{546}$ -0.019°

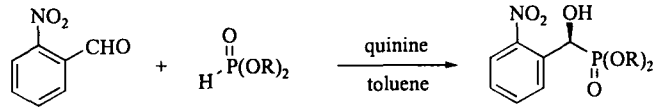


with BCNCl; 50%, $[\alpha]_{546}$ -0.401°
with DMEBr; 64.5%, $[\alpha]_{546}$ -0.170°

SCHEME 53. Catalytic asymmetric addition of chlorine to olefins

D. CARBON-PHOSPHOROUS BOND FORMATION

Cinchona alkaloids (e.g. quinine) catalyze the reaction between an aldehyde and a phosphite to produce an optically active α-hydroxyphosphonate ester (*1,282,283*). When the aldehyde has an *ortho* substituent such as nitro group, preferably one that aids in restricting the rotation of the aldehyde group, asymmetric induction takes place (*1*). The ees also increase (to nearly 100% for R= *t*-Bu) with an increasing bulk of the phosphonate ester, while the reaction rate decreases (Scheme 54) (*282*).



~100% ee for R= *t*-Bu

SCHEME 54. Cinchona alkaloid-catalyzed asymmetric addition of phosphite to an aldehyde

VI. Conclusion

This review has presented the current stage of asymmetric catalysis, in which alkaloids are utilized as chirality transmitters. As shown in many of the examples cited above, alkaloid-induced asymmetric catalysis has become a research field of great potential. A number of processes have gained wide acceptance, and some of them are even used on an industrial scale. However, in many cases a detailed knowledge of the mechanism is often unavailable. More careful and systematic studies to understand the details of the asymmetric induction step should be performed in the future. With such in-depth studies, it will be possible to design more efficient chiral ligands and catalysts that will lead to both high chemical yields and high levels of asymmetric induction.

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CHAPTER 2

BIOLOGICAL ASPECTS OF APORPHINOID ALKALOIDS

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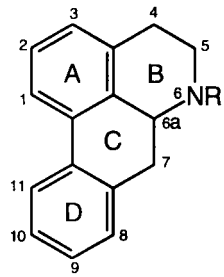
THE ALKALOIDS, VOL. 53
0099-9598/00 $30.00

I. Introduction

A. APORPHINES AND RELATED ALKALOIDS

The aporphinoids, aporphines and related alkaloids, constitute one of the largest and most widely distributed subgroup of benzylisoquinolines. Since 1975 a large number of reviews on the chemical structure and occurrence of aporphinoids have been published (*1-21*).

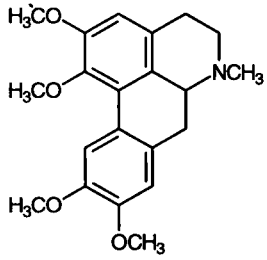
Chemically speaking, the aporphines are tetracyclic bases formed by direct union of the aromatic rings (A, D) of the typical benzylisoquinoline nucleus. They possess the simplest structure **1** with the numbering system most commonly used. The nitrogen at position 6 may be secondary, and in this case the compound is called noraporphine; tertiary, usually attached to a methyl group; or quaternary, with two methyl groups forming the aporphine salts, or less frequently with *N*-acetyl, *N*-formyl or *N*-methyl-*N*-oxide. In natural aporphines, positions 1 and 2 are always substituted by hydroxyl, methoxyl or methylenedioxy groups. Other positions, such as 9, 10 and 11, and less often 3 and 8, may also be frequently substituted. In a few cases, position 7 or 4 is oxygenated. The aporphinoids are optically active, possessing either the *R*-(−)- or *S*-(+)- absolute configuration, depending on the stereochemistry of C-6a.



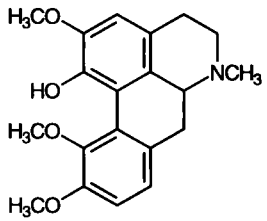
Basic skeleton of aporphines (**1**)

Considerable progress has been made in the field of this broad class of isoquinoline alkaloids called aporphinoids since the first naturally occurring aporphines, glaucine (**2**), corydine (**3**) and bulbocapnine (**4**), were isolated and identified.

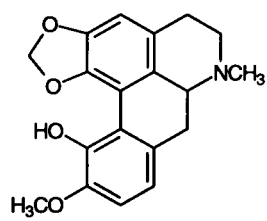
Apart from the aporphines, other biogenetically related alkaloids, like proaporphines, and catabolic derivatives, such as oxoaporphines, 4,5-dioxoaporphines, phenanthrenes, aristolochic acids and aristolactams, are also included in this group, in addition to the dimeric aporphinoids.



Glaucine (**2**)



Corydine (**3**)



Bulbocapnine (**4**)

B. OCCURRENCE

Aporphinoids are mainly distributed among the families Annonaceae, Berberidaceae, Hernandiaceae, Lauraceae, Magnoliaceae, Menispermaceae, Monimiaceae, Nymphaeaceae, Papaveraceae, and Ranunculaceae. They are also present in other families such as the Araceae, Aristolochiaceae, Canellaceae, Euphorbiaceae, Eupomatiaceae, Fumariaceae, Leguminosae, Liliaceae, Nelumbonaceae, Piperaceae, Rhamnaceae, Rutaceae and Saururaceae (*1-21*).

Proaporphines are found in the Annonaceae, Aristolochiaceae, Berberidaceae, Euphorbiaceae, Fumariaceae, Lauraceae, Menispermaceae, Papaveraceae, Ranunculaceae and Siparunaceae.

As far as the botanical occurrence of dimeric aporphinoids is concerned, they have been found in eleven plant genera: *Oxandra, Piptostigma, Polyalthia, Popowia, Trivalvaria* and *Unonopsis* of the Annonaceae, *Berberis* (Berberidaceae), *Dactylocapnos* (Fumariaceae), *Hernandia* (Hernandiaceae), *Thalictrum* (Ranunculaceae) and *Roemeria* (Papaveraceae). Proaporphine-tryptamine dimers have only been located in the genus *Roemeria*.

Phenanthrenes have been isolated from species belonging to the Annonaceae, Eupomatiaceae, Fumariaceae, Hernandiaceae, Lauraceae, Menispermaceae and Ranunculaceae. The last six families also contain oxoaporphines, as do the Aristolochiaceae, Magnoliaceae, Monimiaceae, Papaveraceae and Rhamnaceae families, while dioxoaporphines are confined to seven families (Annonaceae, Aristolochiaceae, Fumariaceae, Menispermaceae, Papaveraceae, Piperaceae and Saururaceae). The Menispermaceae is the only source of oxoisoaporphines.

Other catabolic derivatives of aporphines, such as the aristolochic acids and aristolactams, are present in the Annonaceae, Aristolochiaceae, Menispermaceae, Monimiaceae, Piperaceae and Saururaceae.

C. PLANT PHYSIOLOGY

Although alkaloids are the most widespread nitrogen-containing substances arising from secondary metabolism, their contribution to chemical ecology has been the subject of controversy. Their defensive role against mammalian

herbivores is well documented, but the protection that these compounds provide plants against insect attack has not been so extensively investigated. In this context, the principal groups of plant alkaloids acquired from dietary sources by insects are simple pyrrolidines, piperidines and indolizidines. However, nitrophenanthrenes can be added to the list of toxins that are sequestered by members of Lepidoptera, since certain *Aristolochia*-feeding butterflies accumulate large quantities of this type of aporphinoid-like compound (*22*). This protection appears to occur at larval stages of the papilionid butterfly *Battus archidamas*, which feeds on *Aristolochia chilensis* (Aristolochiaceae). In this case, both the plant and the insect contain different aristolochic acid toxins due to the existence of a selective uptake and/or metabolic transformation (*23*).

Alkaloids can also modify the feeding preferences of polyphagous herbivores. The high alkaloid content of its leaves constitutes the chemical defence of the tulip tree *Liriodendron tulipifera* (Magnoliaceae). The leaves contain fourteen alkaloids, including **2**, which is able to reduce the survival and upset the growth characteristics of the gypsy moth *Lymantria dispar* when the insect feeds on them (*22*). Aristolochic acids possess strong antifeedant activity, comparable to that of the potent limonoid azadirachtin, and it has been demonstrated that the free carboxylic acid group close to a nitro group is the most important structural feature (*24*).

In spite of the non-volatility of most plant alkaloids, certain types of these compounds have been characterized in plant-insect interactions as oviposition stimulants. They are perceived by the female insect and specifically stimulate her to lay her eggs on the chosen host plant. Among other examples are reported the swallowtail butterfly *Atrophaneura alcinous* feeding on the leaves of *Aristolochia debilis*, and the female pipevine swallowtail *Battus philenor* on *A. macrophylla*, where a mixture of aristolochic acids and one inositol have been assumed to be the elicitors. These chemicals are used as a defence by the butterfly against predators, such as tree sparrows, which obtain them through larval feeding (*22,25*).

II. Chemistry

A. Biosynthesis

In this section we only consider some general aspects of the biogenetic pathways of the aporphinoids. For further reading, Shamma and Guinaudeau (*26*) and Cavé *et al.* (*27*) are recommended.

Aporphinoids have the same amino acid precursor, tyrosine, as the rest of the isoquinoline classes. They derive from the benzylisoquinoline skeleton formed from two units of tyrosine, one of them via dopamine, derived by hydroxylation of tyramine, and the other via *p*-hydroxy-phenylacetaldehyde. The product of the condensation of these two units, catalyzed by (*S*)-norcoclaurine synthase, is *S*-(+)-norcoclaurine. It is transformed to an intermediate by *O*-methylation at position 6; subsequent *N*-methylation yields *N*-methylcoclaurine, and then a sequence of 3'-

hydroxylation, followed by 4'-*O*-methylation, leads to the tetrahydrobenzylisoquinoline *S*-(+)-reticuline, the key intermediate and the main building block for the aporphine alkaloids.

Direct, intramolecular phenolic oxidative coupling of *S*-(+)-reticuline in the bisdienone radical form is a key reaction in the formation of aporphines. The substitution pattern of the tetrahydrobenzylisoquinoline precursor gives rise to the corresponding aporphines, although certain positions of *O*-substitution, such as at C-3 or C-7, arise by oxidation of the aporphinoid nucleus. Methylation at C-7 can be induced by the action of *S*-adenosyl methionine.

Aporphines can also originate from a proaporphine intermediate by the cyclization of an *ortho-para* diradical tetrahydroisoquinoline form, direct protonation and subsequent dienone-phenol rearrangement. An alternative is a dienol-benzene rearrangement via a proaporphinol.

Oxoaporphines can arise from the oxidation of aporphines via a dehydroaporphine and subsequent 4,5,6a,7-didehydroaporphine, which is susceptible to oxidation at C-7. This leads to the unstable 7-oxo-*N*-methyl quaternary aporphinium ion, which is easily *N*-demethylated.

B. CHEMICAL CLASSIFICATION OF APORPHINOIDS

In the present review we have followed the classification of aporphinoids adopted by Bentley (*19-21*).

1. Proaporphines

This structural group has been considered the biogenetic precursor of some other aporphinoid types, and is sometimes included in, and at other times excluded from, the aporphinoids. The majority of these compounds possess a cyclohexadienone system incorporated into the tetrahydroisoquinoline nucleus, which may be partially or fully saturated and yield dihydro- or hexahydroproaporphines (*28*).

2. Aporphines

The aporphines are the most relevant group, and possess the basic tetracyclic skeleton with the tetrahydroisoquinoline nucleus. They can be divided into four subgroups (*27*):

a. Aporphines sensu stricto. The known aporphines *sensu stricto* are derivatives possessing *O*-substitution on ring A, or on rings A and D, and include: noraporphines, aporphines, aporphine *N*-oxides, *N,N*-dimethyl and *N*-methyl-*N*-oxide quaternary salts of aporphines, and *N*-carbonyl (*N*-formyl, *N*-acetyl and *N*-carbamoyl) aporphines.

b. Dehydroaporphines. The dehydroaporphines are characterized by an additional unsaturation at C-6a(7). They are sometimes found as 7,7'-dimeric dehydroaporphines and as dehydroaporphine-benzylisoquinoline dimers.

c. 7-Alkyl-aporphinoids. The 7-alkyl-aporphinoids are derivatives exhibiting a methyl substituent, such as 7,7-dimethyl-aporphines, 7-hydroxy-7-methyl-aporphines, 7-hydroxy-*N*-methyl-aporphines or 7,7-dimethyl-*N*-methyl-aporphines, and they have additional unsaturations as dehydroaporphines, 6,6a-dehydroaporphines, or are alkaloids with a completely aromatized B ring, like the 7-hydroxy-7-methyl-, 7-formyl- and *N*-formyl-7-methyl-dehydroaporphines.

d. 7-Oxygenated and/or 4-oxygenated aporphines. The 7-oxygenated and/or 4-oxygenated aporphines, include the 7-hydroxy-aporphines, 7-methoxy-aporphines, 7-hydroxy-*N*-methyl-*N*-oxide-aporphines, 4-hydroxy-noraporphines and 4-hydroxy-*N*-methyl-aporphines.

3. Dimeric aporphinoids

Aporphinoids are also found as dimers that can be classified into subgroups depending on the type (ether or carbon-carbon linkage) and location of the bridge formed between the monomers. They are (*2,5,11*):

a. Oxygen-bonded aporphine-benzylisoquinolines. They include reticuline-reticuline dimers (thalicarpine type and foetidine type), reticuline-coclaurine dimers (istanbulamine type and thalifaberine type) and coclaurine-coclaurine dimers (pakistanine type and kalashine type).

b. Oxygen-bonded proaporphine-benzylisoquinolines. This subgroup comprises pakistanamine and epivaldiberine types.

c. Oxygen-bonded and oxidized aporphine-benzylisoquinolines, and proaporphine-benzylisoquinolines. The first subgroup includes the hernandaline type and the second one the coyhaiquine type.

d. Carbon-bonded dimers. They include aporphine-benzylisoquinoline dimers (uskudaramine type) and bisaporphines.

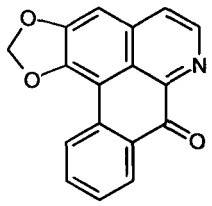
4. Phenanthrenes

The most common degradative route of aporphines is the one that yields phenanthrenes (*27,29*). This group of alkaloids, frequently called secoaporphines, is based on a phenanthrene nucleus with an ethylamine side chain, where the nitrogen carries one or two methyl substituents.

5. Oxoaporphines and dioxoaporphines

Oxoaporphines are a small group of metabolites formed by the natural oxidation of aporphines (*27,29*). They exhibit an oxo substituent group at C-7 and a complete aromatic aporphine skeleton. Liriodenine (**5**) is considered the prototype of this group. A limited number of derivatives present a 4-oxo (4-oxoaporphines)

or a 4,5-dioxo (4,5-dioxoaporphines) substitution in ring B. An unusual 11-oxo-ring-C-reduced-dehydroaporphine has also been isolated (*13*).



Liriodenine (**5**)

6. Aristolochic acids and aristolactams

Oxidation of ring B of an aporphine, probably through a 4,5-dioxoaporphine intermediate, yields aristolochic acid, which may be reduced and dehydrated to an aristolactam. In spite of the fact that neither aristolochic acids nor aristolactams possess the isoquinoline nucleus and are non-basic compounds, they are considered aporphinoids resulting from the degradative metabolism of aporphines (*28*).

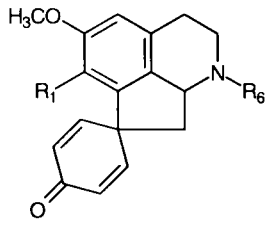
7. Miscellaneous

Among the miscellaneous aporphinoids are included other catabolic derivatives of aporphines. Azaoxoaporphines (*30*) derive from oxoaporphines by an extra-diol cleavage leading to an intermediate that, by subsequent decarboxylation and addition of ammonia, gives rise to the second pyridine nucleus characteristic of these alkaloids. Azaphenanthrenes are formed by oxidative cleavage of both rings A and B of a 4,5-dioxoaporphine.

Tropolonoisoquinoline alkaloids (*29,31*), also called homoaporphines, are formed via a cyclopropanone intermediate by incorporation of the carbon monoxide unit of an oxoaporphine into the lower ring, which becomes a seven-membered ring. Oxoisoaporphines present ring D attached to carbons 7 and 7a of ring C and the oxo group in a *para* relationship with respect to the nitrogen atom.

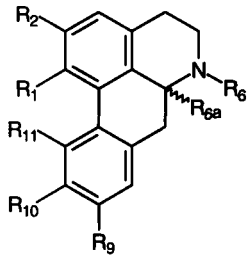
The chemical structures of the most relevant pharmacologically active aporphinoids are listed in Tables I-VIII.

TABLE I
ACTIVE PROAPORPHINES



No.	Name	Structure	
		R_1	R_6
6	Glaziovine	OH	CH_3
7	Stepharine	OCH_3	H

TABLE II
ACTIVE APORPHINES

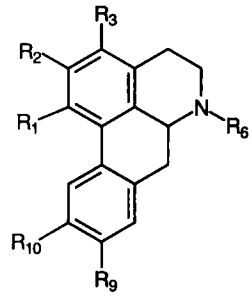


No.	Name	Structure						
		R_1	**R_2**	**R_6**	**R_{6a}**	**R_9**	**R_{10}**	**R_{11}**
8	Actinodaphnine	O-CH_2-O		H	αH	OH	OCH_3	H
9	Anolobine	O-CH_2-O		H		OH	H	H
10	Anonaine	O-CH_2-O		H	βH	H	H	H
11	Asimilobine	OCH_3	OH	H		H	H	H
12	Asimilobine-2-*O*-β-D-glucoside	OCH_3	OGlc	H	βH	H	H	H
13	Boldine	OCH_3	OH	CH_3	αH	OH	OCH_3	H
14	Bracteoline	OH	OCH_3	CH_3		OCH_3	OH	H
15	Corytuberine	OH	OCH_3	CH_3		H	OCH_3	OH
16	Dicentrine	O-CH_2-O		CH_3		OCH_3	OCH_3	H
17	Domesticine	OH	OCH_3	CH_3		O-CH_2-O		H
18	Hernangerine (nandigerine)	O-CH_2-O		H	αH	H	OH	OCH_3
19	Hernovine	OCH_3	OH	H	αH	H	OH	OCH_3
20	Isoboldine	OH	OCH_3	CH_3	αH	OH	OCH_3	H
21	Isocorydine (artabotrine)	OCH_3	OCH_3	CH_3	αH	H	OCH_3	OH
22	Isolaureline	O-CH_2-O		CH_3	βH	OCH_3	H	H
23	Isothebaine	OH	OCH_3	CH_3	αH	H	H	OCH_3
24	Launobine	O-CH_2-O		H	αH	H	OCH_3	OH
25	Laurelliptine	OH	OCH_3	H		OH	OCH_3	H
26	Laurifoline (laurofoline)	OH	OCH_3	$(CH_3)_2$		OH	OCH_3	H
27	Laurotetanine	OCH_3	OCH_3	H	αH	OH	OCH_3	H
28	Laurolitsine	OCH_3	OH	H	αH	OH	OCH_3	H
29	Lirinidine	OH	OCH_3	CH_3		H	H	H
30	Lirioferine	OCH_3	OCH_3	CH_3		OCH_3	OH	H
31	Liriotulipiferine	OCH_3	OH	CH_3		OCH_3	OH	H

TABLE II (*Continued*)

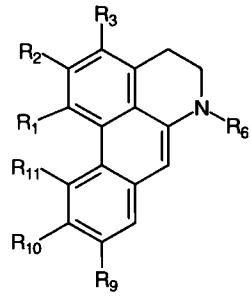
No.	Name	Structure						
		R_1	**R_2**	**R_6**	**R_{6a}**	**R_9**	**R_{10}**	**R_{11}**
32	Magnoflorine	OH	OCH_3	$(CH_3)_2$	αH	H	OCH_3	OH
33	*N*-Acetylanonaine	O-CH_2-O		$COCH_3$	βH	H	H	H
34	*N*-Hydroxy-ovigerine	O-CH_2-O		OH	αH	H	O-CH_2-O	
35	Nantenine	OCH_3	OCH_3	CH_3		O-CH_2-O		H
36	*N*-Methylactino-daphnine (cassythicine)	O-CH_2-O		CH_3		OH	OCH_3	H
37	*N*-Methylactino-daphnine *N*-oxide	O-CH_2-O		(→O)CH_3	αH	OH	OCH_3	H
38	*N*-Methylglaucine	OCH_3	OCH_3	$(CH_3)_2$	αH	OCH_3	OCH_3	H
39	*N*-Methyl-hernangerine (*N*-methyl-nandigerine)	O-CH_2-O		CH_3	αH	H	OH	OCH_3
40	*N*-Methyl-hernovine	OCH_3	OH	CH_3	αH	H	OH	OCH_3
41	*N*-Methyl-laurotetanine	OCH_3	OCH_3	CH_3		OH	OCH_3	H
42	*N*-Methylputerine	O-CH_2-O		CH_3		H	H	OCH_3
43	Nordicentrine	O-CH_2-O		H	βH	OCH_3	OCH_3	H
44	Nornantenine	OCH_3	OCH_3	H		O-CH_2-O		H
45	Nornuciferine	OCH_3	OCH_3	H		H	H	H
46	Nuciferine	OCH_3	OCH_3	CH_3		H	H	H
47	*O*-Methyl-bulbocapnine	O-CH_2-O		CH_3	αH	H	OCH_3	OCH_3
48	Ovigerine	O-CH_2-O		H	αH	H	O-CH_2-O	
49	Phanostenine	O-CH_2-O		CH_3	βH	OCH_3	OH	H
50	Predicentrine	OCH_3	OH	CH_3	αH	OCH_3	OCH_3	H
51	Puterine	O-CH_2-O		H		H	H	OCH_3
52	Roemerine	O-CH_2-O		CH_3	βH	H	H	H
53	Roemeroline	O-CH_2-O		CH_3	βH	OH	H	H
54	Thaliporphine	OH	OCH_3	CH_3	αH	OCH_3	OCH_3	H
55	Xylopine	O-CH_2-O		H	βH	OCH_3	H	H

TABLE III
ACTIVE 3-HYDROXY-APORPHINES



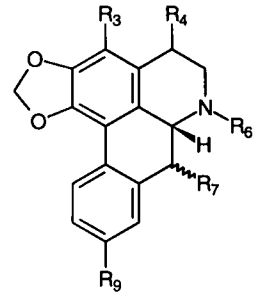
No.	Name	Structure					
		R_1	R_2	R_3	R_6	R_9	R_{10}
56	3-Hydroxyglaucine	OCH_3	OCH_3	OH	CH_3	OCH_3	OCH_3
57	3-Hydroxynornuciferine	OCH_3	OCH_3	OH	CH_3	H	H
58	Isopiline	OH	OCH_3	OCH_3	H	H	H
59	Norstephalagine	$O\text{-}CH_2\text{-}O$		OCH_3	H	H	H
60	*N*-Methylisopiline	OH	OCH_3	OCH_3	CH_3	H	H
61	Ocoteine	$O\text{-}CH_2\text{-}O$		OCH_3	CH_3	OCH_3	OCH_3

TABLE IV
ACTIVE DEHYDROAPORPHINES



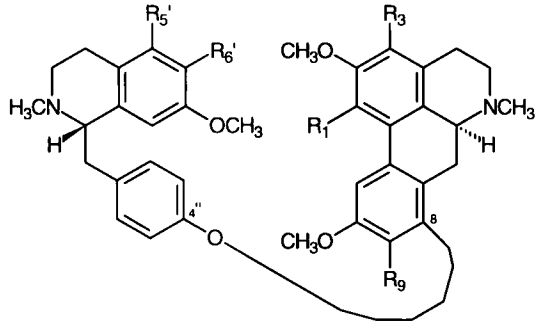
No.	Name	Structure						
		R_1	**R_2**	**R_3**	**R_6**	**R_9**	**R_{10}**	**R_{11}**
62	Cabudine	O-CH_2-O		CH_2OH	CH_3	OCH_3	H	H
63	Dehydrodicentrine	O-CH_2-O		H	CH_3	OCH_3	OCH_3	H
64	Dehydroglaucine	OCH_3	OCH_3	H	CH_3	OCH_3	OCH_3	H
65	Dehydroroemerine	O-CH_2-O		H	CH_3	H	H	H
66	*N*-Formyldehydroovigerine	O-CH_2-O		H	CHO	H	O-CH_2-O	

TABLE V
ACTIVE 7-OXYGENATED OR 4,7-DIOXYGENATED-APORPHINES



No.	Name	Structure				
		R_3	**R_4**	**R_6**	**R_7**	**R_9**
67	Guatterine	OCH_3	H	CH_3	βOH	H
68	Norushinsunine	H	H	H	αOH	H
69	Oliveridine	H	H	CH_3	βOH	OCH_3
70	Pachypodanthine	H	H	H	βOCH_3	H
71	Pachystaudine	H	βOH	CH_3	βOCH_3	H

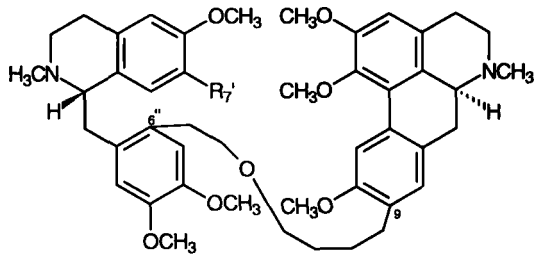
TABLE VI
ACTIVE DIMERIC APORPHINES
8,4'' Aporphine-benzyltetrahydroisoquinolines



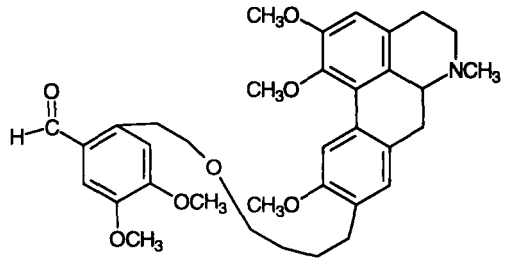
No.	Name	Structure				
		R_1	**R_3**	**R_9**	**$R_{5'}$**	**$R_{6'}$**
72	Thalifaberidine	OCH_3	OCH_3	OH	H	OH
73	Thalifaberine	OCH_3	OCH_3	OCH_3	H	OCH_3
74	Thalifalandine	OH	OCH_3	OCH_3	H	OCH_3
75	Thalifasine	OCH_3	OH	OCH_3	OH	OCH_3

TABLE VI (*Continued*)

9,6'' Aporphine-benzyltetrahydroisoquinolines

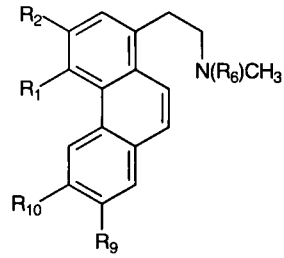


No.	Name	Structure
		$R_{7'}$
76	*O*-Ethylthalmelatine	OCH_2CH_3
77	Thalicarpine (thaliblastine)	OCH_3
78	Thalmelatine	OH
	Aporphine-benzaldehyde	



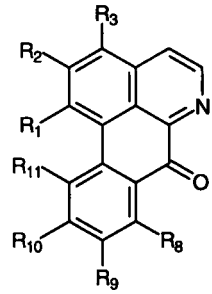
79 Hernandaline

TABLE VII
ACTIVE PHENANTHRENES



No.	Name	Structure				
		R_1	R_2	R_6	R_9	R_{10}
80	Argentinine	OCH_3	OH	CH_3	H	H
81	Atherosperminine	OCH_3	OCH_3	CH_3	H	H
82	Atherosperminine *N*-oxide	OCH_3	OCH_3	$(\rightarrow O)CH_3$	H	H
83	Atherosperminium I	OCH_3	OCH_3	$(CH_3)_2$	H	H
84	Dicentrine methine	O-CH_2-O		CH_3	OCH_3	OCH_3
85	Glaucine methine *N*-oxide	OCH_3	OCH_3	$(\rightarrow O)CH_3$	OCH_3	OCH_3
86	*N*-Allylsecoboldine	OCH_3	OH	$CH_2CH{=}CH_2$	OH	OCH_3
87	*N*-Allylsecoglaucine	OCH_3	OCH_3	$CH_2CH{=}CH_2$	OCH_3	OCH_3
88	*N*-Cyanosecoboldine	OCH_3	OH	CN	OH	OCH_3
89	*N*-Methylsecoboldine	OCH_3	OH	CH_3	OH	OCH_3
90	*N*-Methylsecoglaucine	OCH_3	OCH_3	CH_3	OCH_3	OCH_3
91	*N*-Methylsecopredicentrine	OCH_3	OH	CH_3	OCH_3	OCH_3
92	*O*-Acetylargentinine	OCH_3	OAc	CH_3	H	H
93	Secoboldine	OCH_3	OH	H	OH	OCH_3
94	Xylopine hydroxylamine	O-CH_2-O		OH	OCH_3	H

TABLE VIII
ACTIVE OXOAPORPHINES



No.	Name	Structure						
		R_1	**R_2**	**R_3**	**R_8**	**R_9**	**R_{10}**	**R_{11}**
95	Atherospermidine	O-CH_2-O		OCH_3	H	H	H	H
96	Dicentrinone (oxodicentrine)	O-CH_2-O		H	H	OCH_3	OCH_3	H
97	Hernandonine	O-CH_2-O		H	H	H	O-CH_2-O	
98	10-Hydroxyliriodenine	O-CH_2-O		H	H	H	OH	H
99	Lauterine (10-methoxyliriodenine)	O-CH_2-O		H	H	H	OCH_3	H
100	Liridine (*O*-methylmoschatoline)	OCH_3	OCH_3	OCH_3	H	H	H	H
101	Lysicamine	OCH_3	OCH_3	H	H	H	H	H
102	Oxoglaucine	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H
103	Oxophoebine	OCH_3	OCH_3	OCH_3	H	O-CH_2-O		H
104	Oxopurpureine	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	H
105	Oxoputerine	O-CH_2-O		H	H	H	H	OCH_3
106	Oxostephanine	O-CH_2-O		H	OCH_3	H	H	H
107	Oxoxylopine (lanuginosine)	O-CH_2-O		H	H	OCH_3	H	H
108	Thalicminine	O-CH_2-O		OCH_3	H	OCH_3	OCH_3	H

III. Pharmacology

A. Introduction

In recent years, the pharmacological activity of aporphinoids has been the subject of a great deal of research in the field of natural products (*32-34*). After the first studies of the relationship between aporphine chemical structure and dopaminergic activity, many other reports on the interactions with neurotransmitter receptors have been published. Many of the known pharmacological effects of aporphinoids on smooth muscle or the cardiovascular system can be explained today on the basis of this research and by the studies on ion channel effects.

Other activities to consider are the antimicrobial and cytotoxic ones. Since the cytotoxicity of some oxoaporphines was first reported, many new alkaloids have been included in the potential list of active compounds in this field. In addition, some interesting studies on the relationship between chemical structure and antimicrobial or cytotoxic activity have been published.

Aporphinoids have also been observed to have other effects. The anti-oxidative properties of phenolic and non-phenolic aporphines have been reported so frequently that the therapeutic use of some alkaloids like boldine (**13**) seems well justified.

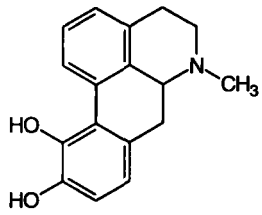
We have focused this review on the main pharmacological effects of natural aporphinoids reported between 1989 and 1998. However, some studies on synthetic or semisynthetic aporphines are included in order to improve our knowledge of the pharmacodynamics of these compounds. It should be pointed out that although aristolochic acid and derivatives are considered aporphinoids by some authors because of their biosynthetic origin, we do not deal with them in the pharmacological section because in some chemical and biological aspects they differ strikingly from the aporphinoids. Moreover, the research on aristolochic acid can constitute *per se* a separate theme for further evaluation.

B. Interactions with Neurotransmitter Receptors

1. Dopamine

a. Aporphines as a tool for studying dopamine receptors. Five types of dopaminergic receptors have been identified. The dopamine (DA) receptors D1 and D5 are associated with the G_s protein and increase in the cAMP level after activation, whereas receptors D2, D3 and D4 are associated with the $G_{i/o}$ protein. The D2 receptors are coupled to multiple effector systems, such as adenylate cyclase inhibition, K^+ current activation and calcium current inhibition, but the effector systems of the D3 and D4 receptors have not been established. In this chapter we use the nomenclature and classification established in the latest review of receptor nomenclature (*35*).

Bulbocapnine (**4**), apomorphine (**109**) and its derivatives have served as excellent tools for the investigation of DA and its receptors. The structural relationships between different aporphinoids have been studied to find new potential agonists and antagonists of DA receptors and to identify their different types and characteristics. On the other hand, the role of DA in human schizophrenia, Parkinson's disease and Huntington's chorea has been clearly established, and the implication of D2 receptors in the pathophysiology of these diseases explained. For this reason, D2-antagonists are used as antipsychotic drugs, and D2-agonists are used in Parkinson's disease (*36,37*).



Apomorphine (**109**)

Relevant advances in the field of DA receptors have been made with natural alkaloids (e.g., **4**) and semisynthetic drugs (**109** and its derivatives). Using these compounds, different research groups have established the relationship between the structure of aporphinoids and dopaminergic activity, and on the basis of these findings, Neumeyer (*38*) proposed a DA receptor model.

In light of the studies of Neumeyer, Baldessarini, Booth and Gao, certain structural features of aporphinoids in relationship to their dopaminergic activity can be considered. The substitution at the nitrogen atom of aporphines increases the affinity and selectivity for the D2 over the D1 receptor sites, with the *N-n*-propyl derivative being the most potent compound, followed by ethyl, allyl, methyl, isopropyl and isobutyl congeners (*39*).

A key structural feature of the dopaminergic activity of aporphines is the absolute configuration at C-6a. The *R*-(−) configuration is essentially what determines the efficacy and potency of the agonists, and the *R*-(−)-aporphines are full agonists. 10-Hydroxy substitution is not essential, but increases potency (*40*).

Studies with synthetic *R*-(−)- and *S*-(+)-isomers of different aporphines have demonstrated that the *R*-(−)-aporphines have greater affinity than the *S*-(+)-aporphines for both D1 and D2 receptors, but only the 10,11-dihydroxy-aporphines are D1 agonists, whereas the 11-hydroxy derivatives are antagonists at D1 receptors (*41*). In fact, the interaction of the C-11 hydroxyl group of the aporphine with the binding site on the receptor is essential to impart affinity for the D1 receptor, since all of the 11-hydroxy-derivatives assayed possess D1 antagonist properties (*41,42*). Although *R*-(−)-enantiomers are generally agonists and *S*-(+)-enantiomers are antagonists, there are compounds, for example *R*-(−)-11-

monohydroxy derivatives, that maintain the agonist properties against D2, but act clearly as D1 antagonists. In addition, some authors hypothesize that the absolute configuration at C-6a establishes the orientation of the lone pair on the nitrogen in space, and that only the *R*-(−)-form is suitable for binding to the DA receptor (*41*).

In a complementary study, Booth *et al.* (*43*) evaluated the effect of both enantiomers of mono- and di-hydroxy aporphines on tyrosine hydroxylase activity using minced striatal tissue from rat brain. Their findings demonstrated that the catechol derivative *N*-*n*-propylnorapomorphine (NPA) is a full agonist that inhibits striatal DA synthesis via a presynaptic autoreceptor of the D2 type, with slight stereoselectivity, whereas its mono-hydroxy derivative 11-hydroxy-*N*-*n*-propylnoraporphine (11-OH-NPa) acts as a partial D2 agonist.

Baldessarini *et al.* (*44*) screened the *R*-(−)- and *S*-(+)-enantiomers of aporphines for affinity at 40 representative places in rat brain tissue, and obtained higher selectivity for the *R*-(−)- than *S*-(+)-aporphines, as well as higher selectivity for the D2 than the D1 receptor.

Different patterns of substitution can modify the dopaminergic activity of aporphinoids. For example, a hydroxy at C-2 in ring A diminishes the activity in aporphines (*45*). In the case of *R*-(−)-NPA there is an increase in the affinity for the D2 receptor (*46*). Among the 2-substituted aporphines the 2-fluoro-derivative was the most potent of all the compounds assayed, with a high D2 affinity (K_i 12 pM) and D2 selectivity (about 57,500 *vs* D1) (*47*).

Aporphinoids with hydroxy substitution at C-8 or C-10 in ring D are less active as DA agonists than the C-11 hydroxy derivatives, and the latter are less active than C-10,C-11-dihydroxy aporphines (*48*). In fact, a catechol group at C-10,C-11 gives affinity and efficacy to this kind of alkaloid, but the absence of hydroxy, or substitution with other radicals at C-10, gives no active compounds. The C-10,C-11 *ortho*-dihydroxy aporphines had higher affinity than the C-11 hydroxy aporphines additionally substituted at the C-8 position, whereas the C-11 and C-9 hydroxy substituted aporphines have the lowest affinity of all the assayed compounds. Blockage of catechol to give the corresponding methoxy- or methylenedioxy-derivative dramatically decreases both the affinity and antagonist activity of the aporphinoids (*41,48*).

Transformation of aporphines into 7-oxoaporphines gave no active alkaloids (*49*). The oxidation of C-7 to give 7-oxoaporphines transforms active compounds, for example, anonaine (**10**), into inactive alkaloids (e.g., **5**), as was demonstrated by Protais *et al.* (*50*) in a screening of different structural types of aporphinoids.

After studying different aporphinoids, Schaus *et al.* (*41*) proposed a model for aporphine binding to the D1 receptor, in which the basic nitrogen and the C-11 hydroxy group of the aporphine were needed for high-affinity binding to the receptor, and the C-10 hydroxyl binding site was required for agonist activity. There is a hydrophobic binding site that could explain certain abnormal features, such as the affinity and antagonist potency of *S*-(+)-bulbocapnine (**4**) at the D1 receptor. Neumeyer *et al.* (*51*) proposed the possible use of aporphines as precursors for the preparation of *N*-*n*-propyl congeners as ligands for further characterization of the DA receptor.

When Kula *et al.* (*52*) assayed six aporphines, they demonstrated that of the derivatives tested, especially *R*-(−)-NPA, showed substantial D3 affinity, but none

of the *S*-(+)-derivatives showed D3/D2 selectivity. The authors suggest that *S*-(+)-aporphines may have a preferential affinity for another limbic-selective D2-like receptor, type D4. In fact, Seeman and Van Tol (*53*) assayed three pairs of aporphine stereoisomers and demonstrated that the *S*-(+)-aporphine derivatives were 4 to 20 times more selective at the D4 than the D2 receptors, and that *S*-(+)-NPA was the most selective compound. Although the *R*-(−)-aporphines tested also bound to D4 receptors with reasonable selectivity (three to ten times higher for D4 than for D2), they caused the classic signs and symptoms of DA-mimetic drugs (stereotypy, hallucinations, etc.). The authors concluded that the *S*-(+)-aporphines might be a good source for atypical neuroleptic drugs.

Similar results were obtained by Baldessarini *et al.* (*54*) when they assayed the effects of *S*-(+)-NPA and its 11-hydroxy-derivative as partial agonists at D2 receptors. They demonstrated that unlike the typical neuroleptic drugs, *S*-(+)-aporphines had no effect on rat prolactin, and concluded that the lack of induction of hyperprolactinemia by the *S*-(+)-aporphines thus adds to properties that are attractive in atypical antipsychotic drugs.

b. Natural aporphinoids as dopaminergic agents. Only a small number of natural aporphinoids are reported to be dopaminergic agents. Stepharine (**7**) and nuciferine (**46**) were cited as D2 agonists, and bulbocapnine (**4**) as a D1 antagonist. Some of these alkaloids, isoboldine (**20**), 1,2-dihydroxyaporphine, **2**, **4** and **46**, produce inhibition of dopamine-sensitive adenylate cyclase and increased levels of DA in brain (*34*). Some alkaloids have been reported to have contradictory effects on the same system. Nuciferine (**46**), for example, has been described as a dopaminergic agonist and antagonist (*34*).

Protais *et al.* (*50*) assayed 23 aporphinoids for their ability to inhibit the *in vitro* [^{3}H]-DA uptake by rat striatal synaptosomes and compared their activity at striatal D1 and D2 binding sites. Of the three noraporphines, isopiline (**58**), norstephalagine (**59**) and **10**, that had an effect, **10** was the most active on [^{3}H]-DA uptake, in comparison with their affinities for the D1 and D2 receptors. Among the tested aporphinoids, only non-substituted ring-D derivatives were active on DA uptake, but *N*-methylisopiline (**60**) had no selectivity, whereas **58** and **59**, two structurally analogous compounds, did. Other structural types, such as oxoaporphines, 7-alkyl-aporphines, bis-aporphines, cularines, and seco-cularines had no selectivity. However, the same authors (*55*) had previously described some of these derivatives as having high affinity for receptors D1 and D2.

Table IX indicates some of the aporphines active on the DA system, including the most relevant semisynthetic and synthetic derivatives.

TABLE IX
SEMISYNTHETIC APORPHINOIDS ACTIVE AS DA AGONISTS OR ANTAGONISTS

DA-agonists	References
R-(−)-Apomorphine (**109**)	*42*
R-(−)-*N*-*n*-Propylnorapomorphine (NPA)	*42*
R-(−)-11-Hydroxy-*N*-*n*-propylnoraporphine (11-OH-NPa)	*42,51,56*
R-(−)-11-Methoxy-*N*-*n*-propylnoraporphine	*56*
R-(−)-11-Hydroxy-*N*-allylnoraporphine	*51*
R-(−)-10,11-Dihydroxy-*N*-allylnoraporphine	*51*
D2-agonists (specific)	**References**
2,10,11-Trihydroxy-*N*-*n*-propylnoraporphine	*46*
N-*n*-Propylnorapomorphine	*57*
11-Hydroxy-*N*-*n*-propylnoraporphine	*57*
Partial D2-agonists	**References**
S-(+)-*N*-*n*-Propylnorapomorphine	*43,54,58*
S-(+)-11-Hydroxy-*N*-*n*-propylnoraporphine	*43,58*
DA-antagonists	**References**
S-(+)-10,11-Methylenedioxy-*N*-*n*-propylnoraporphine	*59*
S-(+)-*N*-*n*-Propylnorapomorphine	*42,59*
S-(+)-11-Hydroxy-*N*-*n*-propylnoraporphine	*42,59*
R-(+)-11-Hydroxyaporphine	*41*
S-(+)-Bulbocapnine (**4**)	*42*
S-(+)-Apomorphine (**109**)	*41*

c. Effects of aporphinoids on central nervous system. Más *et al.* (*60*) have studied whether the inhibition of sexual behavior that follows unrestricted mating could be caused by a blockade of dopaminergic transmission. Male rats, after verification that they were sexually inactive, were injected **109** at different doses and their behavior with receptive females was observed. The dose of 200 μg/kg had the maximal stimulatory effects on mating, but treatment with **109** was unable to fully restore the copulatory pattern shown by sexually rested animals. This observation could be due to additional factors such as other transmitter systems involved in the mechanisms of sexual satiety.

Atypical antipsychotic drugs are characterized by highly selective anti-dopaminergic action in limbic *vs* extrapyramidal regions of rat brain and a lack of

induction of dopaminergic supersensitivity. Haloperidol is a typical D2 antagonist which elevates the circulating prolactin in male rats, while *R*-(−)-11-OH-NPa lowers it. However, *S*-(+)-NPA and its 11-monohydroxy analogues, all of which are partial agonists at D2 dopamine receptors, had little or no effect (*54*). The impression that *S*-(+)-NPA had some of the properties of atypical antipsychotic agents was also reported by Nemeroff *et al.* (*61*). They studied the effects of the isomers of NPA on regional concentrations of neurotensin in rat brain and compared the results with those of haloperidol. Haloperidol at the lower dose did not produce significant changes, but at the higher dose it increased neurotensin in accumbens, caudate, and substantia nigra. *S*-(+)-NPA, which has some of the properties of a limbic-selective DA antagonist, yielded small increases in neurotensin in accumbens and piriform cortex, and even smaller ones in caudate, substantia nigra, and mesoprefrontal cortex. All of these effects persisted for 5 days after treatment. The *R*-(−)-enantiomer increased neurotensin only in substantia nigra (*61*).

The effects of low doses of **109** on rat motor activity induced by the psychostimulant drugs amphetamine, fencamfamine and cocaine have been evaluated. Thirty minutes after treatment with **109**, rats received one of the stimulants. The parameters of motor activity, including locomotion, rearing and sniffing, were quantified in the animals' home cages for 60 min at 15 min intervals. Apomorphine (**109**) modified the changes induced by cocaine, decreasing its stereotyped effect, but did not change the effects of fencamfamine or amphetamine (*62*).

Corytuberine (**15**), **2**, **4** and **13** were studied in mice for their neuroleptic-like, anticonvulsant and antinociceptive effects. The results were compared with some relevant centrally effective drugs such as haloperidol, phenobarbital and morphine (*63*). The pharmacological effects were more intense when the alkaloids were administered after the agonists than before them. All of the aporphinoids inhibited, in a dose-dependent fashion, the exploratory rearing activity and elicited palpebral ptosis, catalepsy, hypothermia and prolonged anesthesia with thiopental. Corytuberine (**15**) showed central stimulatory, proconvulsive and prostereotypic activities that differentiate it from the other aporphines.

Compounds **2**, **4** and **13** seem to have a neuroleptic quality, as does haloperidol, but there were differences in the mechanism of action. The mechanisms of **4** and haloperidol are similar, and differ substantially from those of **2** and **13**. The antignawing and anticlimbing effects of **2**, **4** and **13** could be explained by its anti-DA activity. D2 receptors may be involved in the antistereotypic action of aporphinoids. However, the biochemical results from rat striatum indicate that **4** blocks D1 receptors more than D2 receptors. This compound and haloperidol were less potent against methylphenidate than **109**, whereas the opposite was true of **2** and **13**. In this respect, **4** was haloperidol-like, but the other aporphines were not.

According to the results, other neurotransmission systems are involved. Opioid as well as adrenergic mechanisms contribute to the antijumping action of **13**, whereas the mechanism of **4** is predominantly adrenergic, and that of **15** is opioid.

Corydalis cava (Papaveraceae) forms part of a phytotherapeutic drug usually employed to treat nervousness-induced insomnia, agitation and anxiety. This plant

contains a variety of different alkaloids, the main one of which is **4**. In addition, domesticine (**17**), predicentrine (**50**), **2**, **15** and **20** are present (*64*).

Bulbocapnine (**4**) has shown in animal models, especially in cats, effects ranging from sleep-inducing and narcotic action to induction of catalepsia and cramps. Moreover, it decreases reflex excitability, slows down peristaltic movement of the intestine, lowers blood pressure and shows vasodilatatory effects. The action of this alkaloid is due to interactions with the dopaminergic neuronal transmitter system. Bulbocapnine (**4**) has a selective action on DA receptors, and influences other systems such as the serotonergic and cholinergic sytems in experiments with rats and mice (*65*).

2. Noradrenaline

Several authors have reported that some aporphines increase or decrease adrenergic activity, and some of these have been compiled by Ríos *et al.* (*34*). Isocorydine (**21**), cabudine (**62**), 8-hydroxyaporphine, 8-OH-NPa, **2**, **3**, **4** and **46** were cited as active compounds.

De las Heras *et al.* (*66*) described the adrenergic properties of **2**, **13** and **109** on rat vas deferens, and hypothesized that the observed effects could relate to the inhibition of adenylate cyclase, previously described in the case of **20** in the central nervous system, but not to a direct effect on α-adrenoceptors. However, Ivorra *et al.* (*67*) demonstrated that **2** had affinity for [^{3}H]-prazosin (an α_1-antagonist) binding sites, and probably acts as an α_1-adrenoceptor antagonist. In a later study, Ivorra *et al.* (*68*) clearly demonstrated the antagonism of another aporphine, *S*-(+)-boldine (**13**), in a dose-non competitive response curve of noradrenaline (NA) in rat aorta, whereas *R*-(−)-apomorphine (**109**) acted by a complex mechanism with a non-reversible effect, eliciting contraction at lower doses and relaxation at higher doses. After analysis of results, the authors proposed an apomorphine mechanism as partial agonist at the α_1-adrenoceptor. Both alkaloids interacted with [^{3}H]-prazosin binding to rat cerebral cortex, and **13** had higher affinity than **109** (K_i 0.16 *vs* 2.16). In the dose-response curves for NA in the rat aorta, **13** gave an IC_{50} of 12.4 μM, with a slight increase in the E_{max}, and **109** gave an IC_{50} of 14.4 μM with no modification of the E_{max}. The conformational features of the two aporphines explain their different behavior at α_1-adrenoceptors. The difference is similar to that demonstrated for aporphines *vs* dopaminergic receptor sites. Both alkaloids can bind to adrenergic receptors, but only **109** activates them, whereas **13** acts as an adrenoceptor antagonist.

Using guinea-pig aorta, Chuliá *et al.* (*69*) established the effect of **13** as a competitive α_1-adrenoceptor antagonist with a pA_2 of 5.6, whereas in the case of prazosin the pA_2 was 8.6. The fact that this effect was present in the guinea-pig aorta without endothelium indicates that the smooth muscle relaxant properties are not mediated by any endothelium-derived relaxing factor (EDRF), nor by prostacyclin (PGI_2). Since **13** did not inhibit the contraction produced by caffeine, the effects of **13** on NA-induced contraction is not attributable to direct smooth muscle contractile inhibition or calcium movement, but to direct antagonism of α_1-adrenoceptors or inhibition of receptor-mediated signal transduction. Analysis of the action of **13** on the formation of [^{3}H]-inositol phosphates induced by NA

showed that this aporphine, at a concentration of 30 μM, which almost completely relaxes the NA-induced contraction of the guinea-pig aorta, is able to block the phosphatidylinositol formation pathway linked to α_1-adrenoceptor activation.

In the same way, Madrero *et al.* (*70*) studied the selectivity of **2**, **13** and **50** on α_{1A}-adrenoceptor subtypes by examining [^{3}H]-prazosin competition binding in rat cerebral cortex. Boldine (**13**) and **50** had greater selectivity than **2** (70, 56 and 17 fold α_{1A}-selective, respectively). The three alkaloids have the same absolute configuration, *S*-(+)-, and pattern of substitution, but there is a substantial difference in the degree of methylation. Boldine (**13**) is the 2,9-dihydroxy-1,10-dimethoxy-; **50** is the 2-hydroxy-1,9,10-trimethoxy-, and **2** is the 1,2,9,10-tetramethoxy-derivative. Experimental data showed that the increase in methylation produces a decrease in binding affinity and selectivity for α_1-adrenoceptor subtypes. Predicentrine (**50**), maintains boldine's ability to discriminate between α_{1A} and α_{1B} subtypes with similar potency and selectivity, which suggests that the free hydroxy at C-2 increases the α_1–subtype selectivity shown by *S*-(+)-aporphines.

Yu *et al.* (*71*) studied semisynthetic derivatives from **13** as vasorelaxant agents on the rat aorta, and demonstrated that *N*-allylsecoboldine (**86**) was the most active, inhibiting the contractions induced by phenylephrine in a concentration-dependent manner with a pA_2 of 6.45. This effect persisted in endothelium-denuded aorta (pA_2 of 6.45). In cultured A 10 vascular smooth muscle cells, **86** displaced the binding of [^{3}H]-prazosin with a K_i of 0.4 μM and slightly inhibited the level of inositol monophosphate (IMP) increased by phenylephrine, but did not affect the levels of cGMP and cAMP. Other seco-derivatives such as secoboldine (**93**) and *N*-allylsecoglaucine (**87**) had no activity, but the original compounds, **2** and **13**, had a similar potency as α_1-antagonists (Table X).

S-(+)-Glaucine (**2**) inhibited contractions induced by NA (pA_2 5.88) and methoxamine (pA_2 6.14) in a dose-dependent manner, shifting curves to the right by increasing the maximal response of NA. However, **2** did not inhibit the uptake of NA. In a study with electrically stimulated vas deferens, the same authors demonstrated that **2** enhances the contractions produced by electrical stimulation and antagonizes the inhibition produced by clonidine in a competitive manner. These results suggest that **2** can act on pre- (α_2) and post-synaptic (α_1) adrenoceptors (*74*).

In isolated intact rat aorta, **2** competitively inhibited the contractions induced by NA (pA_2 7.14) and was active in a calcium-free medium. The aporphine did not modify the basal uptake of calcium, but inhibited the influx of calcium induced by NA through α_1-adrenoceptors. This effect confirms the postsynaptic antagonism of **2** on α_1-adrenoceptors (*75*).

TABLE X
INHIBITORY POTENCIES OF ALKALOIDS ON THE CONTRACTIONS INDUCED BY NA ON RAT AORTA

Alkaloid	IC_{50}	Reference
Anonaine (**10**)	$(1.1 \pm 0.1) \times 10^{-5}$	*72*
Apomorphine (**109**)	$(1.4 \pm 0.4) \times 10^{-7}$	*68*
Boldine (**13**)	$(1.2 \pm 0.1) \times 10^{-7}$	*68*
Dehydroroemerine (**65**)	$(2.3 \pm 0.1) \times 10^{-5}$	*72*
Glaucine (**2**)	$(8.0 \pm 0.1) \times 10^{-7}$	*67*
Liriodenine(**5**)	$(2.3 \pm 0.4) \times 10^{-5}$	*73*
Norushinsunine (**68**)	$(5.0 \pm 0.3) \times 10^{-5}$	*73*
Roemerine (**52**)	$(5.8 \pm 0.7) \times 10^{-6}$	*72*
Prazosin (reference)	$(5.1 \pm 0.4) \times 10^{-10}$	*67*

Chen *et al.* (*76*) isolated *S*-(+)-dicentrine (**16**), and identified it as the vasorelaxant principle, from *Lindera megaphylla* (Lauraceae), and Teng *et al.* (*77*) studied its ability to react with adrenergic receptors. Dicentrine's pattern of substitution is analogous to that of **13**, and its relaxant effect is mediated by the adrenergic system. In fact, **16** acted as a competitive α_1-adrenoceptor antagonist in rat thoracic aorta, and its pA_2 was 8.19 when assayed against the dose-response curve of NA, and 9.01 in the case of phenylephrine. In the same experiment, the pA_2 obtained for prazosin was 10.6, and for phentolamine 7.5. Analysis of the contraction-response curves of **16** showed that competitive antagonism against NA in rat aorta was independent of the presence of endothelium in the biological preparation. Therefore neither EDRF nor PGI_2 mediate the vasorelaxation caused by **16**.

These results were corroborated by Yu *et al.* (*78,79*), who studied the effects of **16** on spontaneously hypertensive and normotensive rats and on human hyperplastic prostates. In the former work (*78*), the authors demonstrated that **16** induced a dose-related reduction in the mean arterial pressure in anaesthetized normotensive rats without any significant changes in heart rate, cardiac output or stroke volume, but with increased tail blood flow. The hypotensive activity was abolished by α_1-adrenoceptor blockade. This was ratified in a study using radioligand binding and *in vitro* isometric tension experiments on human hyperplastic prostates in which Yu *et al.* (*79*) demonstrated the α_1-adrenoceptor antagonist effect of **16**. The pA_2 values obtained for **16** against NA and phentolamine were 8.04 and 8.33, respectively. When prazosin, phentolamine and

rauwolscine were assayed against phenylephrine-induced response, the pA_2 values were 8.75, 6.11 and 5.55, respectively. Dicentrine (**16**) was less potent than prazosin as an α_1-antagonist, but stronger than the other known antagonists assayed.

Mustafa *et al.* (*80*) studied the effect of **16** on different types of α_1-adrenoceptors that are involved in the contractions of the rat-aorta (α_{1D}) and spleen (α_{1B}), and obtained a competitive antagonism. The dose-response curves shifted to the right, and pA_2 values of 8.3 in the rat aorta and 6.1 in the spleen were obtained. On the basis of these results, the authors hypothesized than **16** is more selective on α_{1D} than α_{1B}-adrenoceptors.

S-(+)-*N*-Methylactinodaphnine (**36**) (structurally related to **16**) was isolated from *Illigera luzonensis* (Hernandiaceae), and it was assayed as an α-adrenoceptor antagonist in functional and binding experiments with rat thoracic aorta (*81*). The pA_2 value was calculated for **36** and compared with the values obtained with prazosin in rat aorta, vas deferens and spleen, known to contain distinct α_1-adrenoceptor subtypes. The pA_2 values obtained were 7.11 *vs* phenylephrine in rat aorta and 5.01 *vs* clonidine in rat vas deferens. When prazosin, phentolamine and yohimbine were assayed, the results were 10.35 and 6.89, 7.43 and 7.47, and 6.54 and 8.02 in rat aorta and rat vas deferens, respectively, for each drug. The ratio order of α_1/α_2-antagonist selectivity for the tested compounds was 2,884 for prazosin, 126 for **36**, 0.91 for phentolamine and 0.03 for yohimbine. In addition, the effects of **36** in rat aorta denuded of endothelium were similar, with a pA_2 value of 7.18 that demonstrated that the endothelium did not modify the antagonistic effect of **36**. In a complementary assay, this aporphine inhibited the increase in [^{3}H]-IMP produced by NA, but had no effect on the contents of cAMP and cGMP elevated by forskolin and sodium nitroprusside, respectively. These results demonstrate that the relaxant effects of **36** are not mediated by an increase in the cellular cyclic nucleotide concentration, or by EDRF. Because **36** showed a higher potency in rat spleen (pA_2 7.38) than in rat vas deferens (pA_2 6.58), the authors concluded that this aporphine is an α-adrenoceptor antagonist, with preferential selectivity for the α_{1B}-adrenoceptor subtype *vs* the α_{1A}-adrenoceptor subtype.

Chuliá *et al.* (*73*) isolated **5** and norushinsunine (**68**) from *Annona cherimolia* (Annonaceae) and studied their effects on the contraction elicited by NA in rat aorta. Both alkaloids relaxed the NA-contraction in a concentration dependent manner, but **5** is more potent than **68**. Although the relaxing effects are mediated by an inhibition of calcium entry via L-type calcium channels, when extracellular calcium is removed, the contractile response of rat aorta to NA is attributable to calcium release from intracellular stores via α_1-adrenoceptor activation. In these conditions **5** and **68** inhibited NA-induced contractions in calcium-free medium. This study suggests that these aporphines show a relaxant action in rat aorta, which is mediated by an interaction with α_1-adrenoceptors, together with an alteration of the calcium entry via voltage operated channels (VOC). From the roots of the same species, Chuliá *et al.* (*72*) isolated three aporphinoids and studied their relaxant activity on isolated rat aorta. Their findings demonstrated antagonism against NA curves, with IC_{50} values ranging between 23 and 5.8 μM (Table X). Roemerine

(**52**) was the most potent compound, but it was clearly weaker than other alkaloids, like **2** and **13**, tested in similar experimental conditions.

S-(+)-Laurotetanine (**27**), an aporphine isolated from *Litsea cubeba* (Lauraceae), inhibited the phenylephrine-induced phasic and tonic contractions in rat aorta in a dose-dependent manner, with an IC_{50} of 36.8 μM (*82*). The effect was greater on the tonic contraction than on the phasic one, and no differences were observed in the experiments with denuded aorta. The authors concluded that **27** acts as an α_1-adrenoceptor antagonist.

Studies on principles isolated from *Aristolochia papillaris* showed that **20** relaxed isolated guinea pig trachea with an IC_{50} of 0.7 mM. This effect was antagonized by the β-antagonist propranolol, but it was not clearly identified as an adrenergic antagonism (*83*).

Some apomorphine derivatives have affinity for α-adrenoceptors. Baldessarini *et al.* (*44*) assayed the affinity of six aporphinoids and demonstrated that the *R*-(−)-isomers preferred α_2-adrenoceptors, while the *S*-(+)-forms were α_1-selective. The derivatives assayed were **109**, NPA and 11-OH-NPa, in their *R*-(−)- and *S*-(+)-forms.

Sotníková *et al.* (*84*) studied the activity of isothebaine (**23**), magnoflorine (**32**), **15** and **21** against different contractile agents, and observed the relaxation of the NA-rat aorta induced contractions in a standard and in a calcium-free medium when **21** and **23** were assayed. On the other hand, both alkaloids shifted the phenylephrine dose-response curves to the right in a non-competitive manner. The authors confirmed that these alkaloids act as antagonists of α_1-adrenoceptors.

3. Serotonin

Some aporphinoids have been described as serotonergic agents. Stepharine (**7**), apocodeine and **52** were described as agonists, and nantenine (**35**) and **46** as antagonists (*34*). Asimilobine (**11**) and liriniding (**29**), two aporphines isolated from the leaves of *Nelumbo nucifera* (Nymphaeaceae), inhibited the contractions of isolated rabbit aorta induced by serotonin (5-HT). The pA_2 values were 5.78 and 7.36, respectively (*85*).

Cannon *et al.* (*86*) synthesized two enantiomers of 11-hydroxy-10-methylaporphine, and both bound strongly to $5\text{-}HT_{1A}$ receptors from rat forebrain membrane tissue. The *R*-(−)-enantiomer was an agonist and the *S*-(+)-enantiomer an antagonist in the guinea pig ileum preparation, but neither of them showed significant activity as DA-receptors. However, in previous work, Gao *et al.* (*42*) had demonstrated the activity of *R*-(−)-11-hydroxyaporphine as a DA agonist and the *S*-(+)-enantiomer as an antagonist. For this reason, Cannon *et al.* (*87*) synthesized and studied the activity of the enantiomers of 10-methylaporphine. They found that the *R*-(−)-10-methylaporphine was the most active compound for inhibiting the guinea pig ileum contraction induced by electrical stimulation, an assay procedure for $5\text{-}HT_{1A}$ receptor studies, with an ID_{50} of 1.2 μM. Its *S*-(+)-enantiomer, and *R*-(−)-, and *S*-(+)-aporphine, had similar potency in the same experiment, with ID_{50} values between 6.7 and 5.4 μM. None of them modified the cardiovascular effects of epinephrine, acetylcholine or histamine, nor did they

modify arterial pressure or heart rate when administered intravenously. These results demonstrated that aporphines need the 11-hydroxy group to exert a high effect on 5-HT_{1A} receptors. In the former experiment (*86*), *R*-(−)-11-hydroxy-10-methylaporphine had an ED_{50} of 0.05 μM for the inhibition of contractions produced by a transmural single shock stimulation on guinea pig ileum. The *S*-(+)-11-hydroxy-10-methylaporphine was inactive in this experiment, but it had an ED_{50} of 0.03 μM for antagonism of inhibition of contractions produced by 8-hydroxy-2-(di-*n*-propyl-amino)tetralin, a 5-HT_{1A} agonist. The *R*-(−)-enantiomer was inactive in this test. Both stereoisomers had a high affinity for the 5-HT_{1A} binding sites, expressed as an ability to displace [^{3}H]-8-hydroxy-2-(di-*n*-propyl-amino)tetralin from the membranes of the rat forebrain (*86*).

In a later study, Cannon *et al.* (*88*) amplified the experiment to include other structural analogues. The *R*-(−)- and *S*-(+)-forms of 10-hydroxy-9-methyl-, 10-methoxy-9-methyl-, 9-hydroxy-10-methyl-, and 9-methoxy-10-methyl-aporphine were tested on guinea pig ileum contractions produced by electrical stimulation. The derivatives 10-hydroxy-9-methyl- and 9-hydroxy-10-methyl-aporphines were inactive, whereas the methoxy-derivatives were active, with an ED_{50} range of 2.6 to 5.9 μM. *R*-(−)-9-Methyl-10-methoxy-aporphine (ED_{50} 2.6 μM) was the most active of the analogues, but clearly weaker than *R*-(−)-11-hydroxy-10-methylaporphine, the most active compound of the series, with an ED_{50} of 0.05 μM in this experiment. In view of these results, the authors concluded that 11-hydroxy-10-methylaporphines are highly selective agonists (*R*-isomer) or antagonists (*S*-isomer) at 5-HT_{1A} receptors.

Similar results were obtained by Hedberg *et al.* (*89*), who demonstrated that *R*-(−)-11-hydroxyaporphine was a low-potency partial 5-HT_{1A} agonist with affinity for D1 and D2A receptors and that *R*-(−)-11-hydroxy-10-methyl-aporphine was a potently selective and efficacious 5-HT_{1A} receptor agonist. They hypothesized that a lipophilic pocket in the 5-HT_{1A} receptor accommodates a methyl group at C-10, and that the binding site of the D2 receptor (*89*) does not accommodate it. The elimination of the methyl group on the nitrogen in *R*-(−)-11-hydroxyaporphine, increases the selectivity for 5-HT_{1A} receptors (100-fold), and substitution by *N*-propyl increases the selectivity of *R*-(−)-11-hydroxy-10-methyl-aporphine for D2A receptors (3-fold) (*90*). This feature was verified in a complementary study with different C-10 and *N*-substituted aporphines, and it was observed that the *N*-alkyl group produced a marked decrease in the affinity for the 5-HT_{1A} receptor (*91*).

Extracts from the fruit, seeds and leaves of *Annona muricata* were tested to see if they inhibited the binding of [^{3}H]-rauwolscine at the 5-HT_{1A}-receptor in the calf hippocampus. A strong inhibition was obtained for the fruit extract (*92*). Three aporphines were then isolated from this source, and the serotonergic activity was studied. Nornuciferine (**45**), **10** and **11** inhibited binding of [^{3}H]-rauwolscine to 5-HT_{1A}-receptors with IC_{50} values of 3, 9 and 5 μM, respectively. In a later study (*93*), the same authors reported the inhibition of forskolin-stimulated cAMP accumulation in NIH-3T3 cells stably transfected with human 5-HT_{1A}-receptors. The results indicated that these compounds act as 5-HT_{1A} agonists. The alkaloids **10** and **45** (EC_{50} < 10 μM) were more potent than **11** (EC_{50} 20 μM) in this assay.

4. Acetylcholine

Only a few reports on the anti-cholinergic activity of aporphinoids have been published. Speisky *et al.* (*94*) explained the effects of *Peumus boldus* (Monimiaceae) extract as a result of the activity of the major alkaloid **13**. In this study they demonstrated the competitive antagonism of **13** on the contraction of the rat ileum induced by acetylcholine (ACh), with a pA_2 of 4.78. In the same experiment, atropine inhibited the contraction with a pA_2 of 7.78. Hue *et al.* (*95*) ratified the effects of **13** on cholinergic receptors, using in this case an insect central nervous system preparation. The electrophysiological results confirm that **13** acts as a nicotinic cholinergic receptor antagonist, without affecting muscarinic and gabergic receptors.

Liriodenine (**5**), isolated from *Fissistigma glaucescens* (Annonaceae), showed anti-muscarinic properties. Using different isolated tissues, Lin *et al.* (*96*) demonstrated the competitive antagonism on contractions induced by carbachol on guinea-pig trachea (pA_2 6.22), ileum (pA_2 6.36), left atria (pA_2 5.24) and right atria (pA_2 5.35 for inotropic and 5.28 for chronotropic effects). The functional antimuscarinic studies were complemented by other biochemical and receptor-binding experiments. Competitive inhibition by **5** of the binding of ^{3}H-*N*-methyl scopolamine to canine cultured tracheal smooth muscle cells followed a two-site model and revealed features analogous to those of 4-diphenylacetoxy-*N*-methylpiperidine (DAMP), an M_3 antagonist. Both **5** and DAMP reduced more strongly the accumulation of inositol triphosphate (IP_3) (an M_3 signal-transducer) than of cAMP (an M_2 signal-transducer), which confirms the relative selectivity of **5** for smooth muscle tissues instead of cardiac muscle (*97*).

C. Effects on Muscular Contraction

1. Smooth muscle

It has been stated in section III.B that aporphines share important structural features with physiological catecholamines, interact with their receptors, and thereby produce notorious effects on smooth muscle. The present chapter focuses on the interactions with processes such as those associated with VOC that also affect smooth muscle, and considers certain related aspects of cardiovascular pharmacology. Since various cellular events link receptor activation to ion channel activation, sections III.B and III.C.*1* should be taken together.

a. Effects on uterus. Norstephalagine (**59**) and atherospermidine (**95**), which is its corresponding oxoaporphine (a less basic, fully planar aromatic compound), were both isolated from *Artabotrys maingayi* (Annonaceae), and inhibited the contractions of rat uterus induced by 56.3 mM KCl, with EC_{50} values of 3.54 and 3.22 μM, respectively. The rhythmic contractions induced by oxytocin were also reduced, or even completely abolished, by 33 μM **59** and 100 μM **95**. In a calcium-free medium, **59** did not reduce the oxytocin contraction plateau, and even increased it at higher doses, whereas **95** reduced it dose-dependently (EC_{50} 3.38

μM). The vanadate (sodium orthovanadate)-induced plateau was also reduced dose-dependently by **95**, while **59** reduced it at low doses and enhanced it at high doses. Since it is known that high $[K^+]$ increases extracellular calcium influx through VOC, it was concluded that both alkaloids inhibited extracellular calcium entry, and that **95** also inhibited intracellular calcium release. Moreover, **59** seems to be readily oxidized *in vivo* to give **95** and therefore the effects of both alkaloids appear mixed (*98*).

S-(+)-Glaucine (**2**) proved to have relaxant properties on KCl-induced and oxytocin-induced contractions that were quantitatively similar to those of papaverine. However, in a calcium-free medium, **2** did not inhibit either oxytocin- or vanadate-induced contractile responses. Therefore, it probably can not affect calcium intracellular sites, but merely blocks extracellular influx (*99*). Glaucine (**2**) and **13** were compared with diltiazem for their inhibition of the effects of some agonists. The relaxant potency of **2** on KCl-induced contractions was nearly eight times greater than that of **13**, and on ACh-induced contractions, fourteen times greater. After washing, a second contraction induced by KCl or ACh was smaller in amplitude as a result of the pre-treatment with **2** and, less markedly, with **13**. In a calcium-free medium, **13**, like **2**, did not modify the oxytocin-induced plateau. In conclusion, it was assumed that the blocking of free hydroxyl groups by methylation and the consequent increase in lipophilicity do not modify the mechanism of action, but increase the relaxant effect of these alkaloids (*100*).

b. Effects on aorta. The relaxant properties of **2** and **13** on arterial vessels in connection with their underlying mechanisms were examined in detail by Ivorra *et al.* (*67*). The contractile response to NA of rat aorta strips incubated in a calcium-free medium was completely abolished by **2**, but under the same conditions this alkaloid failed to inhibit the caffeine-induced response. An interaction with cellular contractile machinery or with intracellular calcium release can therefore be ruled out. Glaucine (**2**) competed with [^{3}H]-(+)-*cis*-diltiazem for the benzothiazepine-binding site of rat cerebral cortex homogenates, with an IC_{50} of 10 μM, but did not affect the binding of [^{3}H]-nitrendipine to the dihydropyridine binding site. Additionally, **2** inhibited the type IV (rolipram-sensitive)-cAMP-phosphodiesterase (PDE) from bovine aorta with a K_i of 3.3 μM, which represents half the potency of papaverine (K_i 1.68 μM). The high selectivity that **2** showed towards this form of PDE ruled out the possibility that this mechanism was important in determining glaucine's vascular effect, because the type III-PDE, which is not affected by the alkaloid, is in fact the major isozyme involved in vasodilation and cardiac output increase. It is therefore assumed that **2** acts through its α_1 adrenoceptor antagonism (see section III.B) and calcium channel blocking properties. It was reported later that **13** also binds to the benzothiazepine-binding site of rat cerebral cortex, with an IC_{50} of 33.28 μM, which is a third of the potency of **2**, and does not affect PDE activity (*68*).

Loza *et al.* (*101*) reported that **2** inhibited the two phases, transient and sustained, of the aorta functional response to NA in a calcium-free medium. The phases depend on the IP_3-mediated release of intracellular calcium stores and on diacylglycerol-mediated activation of protein kinase C (PKC), respectively. Moreover, as **2** did not affect the aortic cell basal calcium intake, measured as ^{45}Ca,

but strongly reduced the calcium intake induced by K^+ and NA, it was suggested that a blockade of receptor-operated calcium channels (ROC) also occurred. Glaucine (**2**) also reduced *in vitro* the contractions induced by 5-HT in a non-competitive manner, consistent with a decrease in the calcium influx induced by the same agent. *In vivo*, however, it affected the cardiovascular effects of nicotine and did not influence those of 5-HT or NA (*75*).

Similar pharmacological profiles were studied in relationship with the alkaloid **27**, isolated from the Chinese herbal remedy *Litsea cubeba*. This compound inhibited the response of rat thoracic aorta to $CaCl_2$ (0.3-3 mM) in a 60 mM K^+-calcium-free medium, with an IC_{50} of 19.8 μM, for a $[Ca^{2+}]$ of 1 nM, and the contractions induced by KCl in normal Krebs medium. When **27** was added 10 min after KCl, it managed to reduce the magnitude of the effect to about 65% of the initial one, whereas under these conditions nifedipine completely relaxed the preparation. Neither the contractions induced by caffeine, nor the levels of cAMP or cGMP, were affected. Given that **27** also relaxed the tonic (sustained) contractions induced by the α-agonist phenylephrine, in both intact and denuded aorta, it was suggested that it must act both as a VOC and ROC blocker (*82*). Isocorydine (**21**), a naturally occurring aporphine in some Malaysian medicinal plants, was also reported to inhibit the responses induced by KCl and phenylephrine (*102*).

From the roots of *Annona cherimolia*, two aporphines, **10** and **52**, and one dehydroaporphine, dehydroroemerine (**65**), were isolated and tested for their aorta relaxant properties. The experiments to study the effect against KCl-induced contractions were performed in the presence of 10 μM phentolamine, to avoid any interference of NA released by depolarization. Under these conditions, the three alkaloids showed similar potencies, with IC_{50} values in the 10^{-5} range, whereas nifedipine had an IC_{50} of 1.1 nM. Anonaine (**10**) and **52** at 100 μM abolished the NA-induced contractions in a calcium-free medium, and caffeine contractions were not affected. When the contact with the alkaloids was allowed to continue during the calcium storage refilling, **10** was the most effective alkaloid against caffeine challenge (*72*). Recently, the relaxant activity of the aporphines from *Mahonia aquifolium* (Berberidaceae) was reported. Isocorydine (**21**), as mentioned above, and **23** (but not **3** or **32**) behaved as anti-adrenergic and calcium channel blockers in a fashion similar to that observed for **2**. One hypothesis compares the structural features of aporphines and 8-chlorodiltiazem (*84*).

In a study of five, closely-related phenanthrene derivatives obtained from **13**, **86** was found to be nearly five times more potent than **27** as a relaxant of the calcium-induced contractions in 60 mM K^+-calcium-free medium (IC_{50} 4 μM). In a $[Ca^{2+}]$ = 1.9 mM medium, both **86** and verapamil relaxed the plateau caused by 15 and 60 mM KCl, with greater potency and efficacy at the higher $[K^+]$, whereas nifedipine exerted its relaxant effect independently of $[K^+]$. This fact supports the idea that **86** may act as a VOC blocker in a potential-dependent manner. Moreover, its effect was not associated with endothelial factor release, interaction with ATP-sensitive K^+-channels or cyclic nucleotide synthesis (*79*).

Other alkaloids, like *O*-methylbulbocapnine (**47**), xylopine (**55**), atherosperminine (**81**), **16**, and certain derivatives, reported to have antiplatelet activity (see section III.F), were also effective as relaxants of aortic smooth muscle

contracted by 80 mM KCl or 3μM NA. The most active compounds were the phenanthrene derivatives with a methoxyl function at C-2 or a methylenedioxy bridge at C-3–C-4. Formation of quaternary or hydroxylamine derivatives and double methoxyl substitution at C-6 and C-7 reduced the effect (*103*).

A four-week dietary treatment with **16** made it possible to establish the long-term *in vivo* effects of this substance on vascular muscle reactivity. At first, it was demonstrated that the aorta excised from hyperlipidemic (fat diet-fed) rats was significantly more reactive to phenylephrine (EC_{50} 36 nM) than that of normal rats (EC_{50} 200 nM). Addition of **16** (10 mg/kg) to normal chow diet resulted in a decrease in the phenylephrine potency (EC_{50} 1.25 μM). Relaxation induced by ACh or sodium nitroprusside was not affected. The effects observed in spontaneously hypertensive rats fed the high fat-high cholesterol diet were even slightly more pronounced: Treatment with **16**, at 10 mg/kg, caused a reduction in the phenylephrine potency to one fiftieth. Apart from the effects on smooth muscle contraction, **16** also lowered some quite important cardiovascular parameters such as the mean arterial pressure and plasmatic lipid levels (*104*).

It was later demonstrated that a single intravenous dose of **16** (0.5 mg/kg) improved head and body arterial circulation in dogs. Impedance of ascending aorta, wave reflection force and total peripheral vascular resistance were reduced, whereas total peripheral load compliance was increased (*105*). In close connection with blood flow homeostasis, it should be noted that **16**, like other vasodilators, caused dose-dependent inhibition of the serum-induced proliferation of rat glomerular mesangial cells (*106*)

c. Effects on other smooth muscles. Boldine (**13**) possessed a relaxant activity *per se* on rat ileum (pD_2 3.77) though its relative potency as a competitive antagonist of ACh was higher. This alkaloid also exerted a non-competitive antagonism on $BaCl_2$-induced contractions (*94*).

The oxoaporphine thalicminine (**108**), from *Thalictrum isopyroides* (Ranunculaceae) relaxed guinea pig ileum, trachea and aorta, and pulmonary arteries. Its effect on pulmonary artery and ileum was not influenced by the β-adrenergic antagonist propranolol, and was increased by quinacrine. This indicates the participation of an AA-mediated mechanism. In anesthetized animals, **108** transiently reduced arterial pressure and heart rate (*107*).

A great deal of research has been done to elucidate the mode of action of **5** and **81** as tracheal relaxants. Atherosperminine (**81**) reduced the contractions induced by carbachol, high K^+ and some eicosanoid agonists, with pIC_{50} values between 4.2 and 4.5. Neither the removal of endothelium, nor the presence of propranolol, modified the relaxant effect of **81**. Further, it augmented cAMP levels, but not those of cGMP, potentiated the effect of forskolin (an adenylate cyclase stimulator) and inhibited PDE activity (IC_{50} 57 μM for cAMP-PDE) in homogenates of guinea pig tracheal rings. Consequently, it seemed that its relaxant effect lies mainly in the interaction with this enzyme (*108*).

Liriodenine (**5**) at high concentrations (300 μM) partially reduced the response of the trachea to other spasmogens such as neurokinin A, high K^+ and some eicosanoid agonists, lowering the maximum effect. When 1 μM nifedipine was present or calcium was not, 300 μM **5** did not affect the reactivity to neurokinin A

and U-46619, a thromboxane B_2 (TXB_2) agonist. The levels of cAMP and cGMP also remained unchanged. It was therefore concluded that **5** blocks extracellular calcium entry (*96*).

In addition to its α-adrenoceptor antagonism (see section III.B), **2** showed relaxant activity in rat vas deferens on calcium-induced contractions in a 50 mM K^+-calcium-free medium. Higher doses produced a reduction in the maximum effect (pD'_2 3.65 μM). Glaucine (**2**) did not modify the calcium basal uptake, but inhibited the K^+-induced uptake (*74*).

2. *Skeletal muscle*

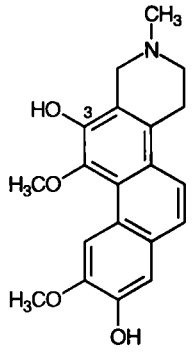
Boldine (**13**) showed neuromuscular blocking ability on a mouse phrenic nerve-diaphragm preparation. At 100 μM it produced, first, an immediate twitch, and then muscular paralysis, which was eliminated on addition of the anti-cholinesterase agents neostigmine or physostigmine. This led researchers to suspect a competitive antagonism on motor plate nicotinic receptors, while the twitch increase of tension might be purely of muscular origin because it also appeared in muscle (direct)-evoked contractions. The blockade of the nicotinic receptors was confirmed by the inhibition of ACh-induced contractions in a denervated diaphragm (*109*). The effect of **13** on calcium movements inside the muscular cell was studied in the presence of ryanodine, an activator of intracellular calcium channels. At doses between 10 and 200 μM, **13** potentiated, in a dose-dependent manner, the contraction-enhancing effect of ryanodine on an isolated mouse diaphragm subjected to electrical stimulation. At a dose of 300 μM, **13** produced a biphasic response: a fast tonic contraction (1.4 g), dependent on extracellular calcium entry, followed by a slow phasic one (4 g), that depends on sarcoplasmic reticulum (SR) calcium release. By using SR vesicles obtained from rat and rabbit back muscles, it was demonstrated that **13** is an activator of the ryanodine-sensitive channels, and this effect is directly related to an increase in the ryanodine binding to its receptor (*110*).

D. Effects on Enzyme Activity

1. *Acetylcholinesterase*

Litebamine (**110**) is a natural phenanthrene aporphinoid that can also be readily obtained by alkaline solvolysis from **13**, through a series of secoboldine-type intermediates. Following a colorimetric method, based on the use of 5,5'-dithio-(bis-2-nitrobenzoic acid) as a coupler and ACh iodide as substrate, the inhibition of type V-acetylcholinesterase activity by several different *N*-substituted litebamine homologues and related secoaporphines was evaluated. The most powerful inhibitors were the tertiary *n*-propyl, *n*-butyl, *i*-propyl and *i*-butyl-*N*-derivatives of litebamine, with IC_{50} values ranging from 6.5 to 8.0 μM, and especially the quaternary *N*-methyl-*N*-propyl homolog, in the form of its iodide salt, with an IC_{50} of 2.79 μM. The similarity in the distances between the nitrogen and oxygen (at C-

3) atoms of litebamines (ca. 4.288 Å) and ACh (ca. 3.815 Å) is believed to explain their effect (*111*).



Litebamine (**110**)

2. Pro-inflammatory enzymes

Corytuberine (**15**), one of the alkaloids from *Mahonia aquifolium*, showed 30 % inhibition of the activity of sunflower seed lipoxygenase (LOX) at 100 μM. This effect may relate to the moderate lipid peroxidation (LP) inhibition also caused by this alkaloid. It is postulated that high concentrations of **15** and other benzylisoquinolines might account for the anti-psoriatic activity of *Mahonia* extracts (*112*).

Thaliporphine (**54**) is a vasoconstrictor alkaloid isolated from *Neolitsea konishii* (Lauraceae) and endowed with protective activity against endotoxin shock. Its mechanism of action seems to be based on its influence on inducible NO synthase (iNOS) activity. At concentrations between 0.1 and 100 μM **54** inhibited nitrite accumulation in murine macrophages induced by lipopolysaccharide (LPS) or interleukin-1β (IL-1β), but did not affect that induced by tumor necrosis factor α (TNFα). In bovine artery endothelial cells, **54** did not affect bradikinin/Ca^{2+}-induced NO formation (measured as nitrite), unlike the NOS inhibitor N^G-methyl-L-arginine. As the alkaloid failed to inhibit transformation of NO into nitrite, and also the conversion of [^{3}H]-arginine to [^{3}H]-citrulline, it was established that it possesses an inhibitory activity on iNOS expression, associated with an inhibition of IL-1β synthesis or release (*113*).

3. Protein kinases

Some aporphines exert *in vitro* inhibitory activity on certain kinases, such as myosin light chain kinase (MLCK) or the catalytic subunit of cAMP-dependent protein kinase (CAK). Boldine (**13**) inhibited MLCK and CAK with IC_{50} values of

12 μM and 82 μM, respectively, whereas **4** moderately inhibited MLCK (IC_{50} 30 μM). Apomorphine (**109**) was also active against these enzymes, but was notably more potent against CAK (IC_{50} 1 μM) and against PKC (IC_{50} 8 μM) (*114*).

4. Protein phosphatases

Among the aporphines isolated from *Rollinia ulei* (Annonaceae), **10** (IC_{50} 17 μM) and **45** (IC_{50} 5.3 μM) exhibited the highest potency as *in vitro* inhibitors of recombinant CD45 protein tyrosine phosphatase, an enzyme with recognized importance in T and B lymphocyte receptor signaling, and therefore in autoimmune disorders. Glaucine (**2**), **5** and **52** were less potent (IC_{50} values ranging from 80 to 90 μM). None of the alkaloids inhibited other, less specific, phosphatases, like human acid and alkaline phosphatase, or bovine calcineurin phosphatase (*115*).

5. Tyrosine hydroxylase

Bulbocapnine (**4**) moderately inhibited the tyrosine hydroxylase obtained from bovine adrenal medulla. The inhibition (K_i 0.2 mM) was non-competitive with respect to L-tyrosine. This may be the reason for the bulbocapnine-induced decreased dopamine biosynthesis in PC12 cells (*116*).

E. Antioxidant and Free-Radical Scavenger Activity

During the last few years, research on the influence of aporphine alkaloids, particularly **13**, on the biological systems that regulate the oxidative metabolism of lipids has received enormous attention. Around this central theme, different studies on free radical generation and scavenging have been performed in order to establish the mechanism of action of these alkaloids.

S-(+)-Boldine (**13**) is the major alkaloid of *Peumus boldus*, a South American tree believed to have many curative properties, and the leaves of which are used around the world to treat hepato-biliary disorders. This alkaloid (10-100 μM) was effective as an inhibitor of natural LP in rat brain, and this auto-oxidation is independent of superoxide and free hydroxyl radicals. It reduced oxygen consumption, chemoluminescence and accumulation of thiobarbituric acid-reacting substances (TBARS) with IC_{50} values of 28.7, 25 and 19 μM, respectively. The IC_{50} obtained for propyl gallate and butylated hydroxyanisole, two standard antioxidants, were 0.5 and 1.6 μM in the chemoluminescence test. In contrast with the behavior of these standard drugs, **13** showed a time-dependent increase in its effect, possibly due to its transformation into a more hydrophobic, though phenolic, derivative. In an erythrocyte cytoplasmatic membrane preparation, **13** reduced the 2,2'-azobis(2-amidinopropane) (AAP)-induced LP in a dose-dependent, time-independent fashion, thus suggesting a mechanism based on alkylperoxyl radical scavenging (*117*). In rat liver microsomal fractions, **13** inhibited LP induced by either $Fe(NH_4)_2(SO_4)_2$, Fe^{3+}-ATP/NADPH (or NADH), *tert*-butyl hydroperoxide (TBH) or CCl_4, at concentrations ranging from 5 to 30 μM (IC_{50} close to 15 μM). Boldine (**13**) did not affect xanthine oxidase (XOD)-induced reduction of

cytochrome C, only slightly inhibited the oxidation of ethanol to acetaldehyde carried out by catalase/H_2O_2, and reduced the production of ethylene from 2-keto-4-thiomethylbutyric acid. These findings indicated that this aporphine reacts with the hydroxyl radical, but not with superoxide or H_2O_2. Additionally, **13** protected some microsomal enzymes from the inactivation caused by LP (*118*). In a model of LP/cellular damage produced by TBH, **13** (200 μM) suppressed the cytotoxicity in hepatocytes, provided that TBH concentrations were not higher than 0.87 mM, drastically reduced TBARS accumulation, and did not prevent glutathione (GSH) depletion. When **13** was added to isolated hepatocytes after TBH, no inhibition was observed, and sometimes cytolysis was even exacerbated, although TBARS levels were reduced. This phenomenon must imply that LP is not responsible for the TBH toxic effects, which could arise from excessive oxidation of thiol groups and pyridine nucleotides, or from an increase in cytosolic calcium (*119*).

The effects of **13** were assessed in human liver microsomal preparations, in which CCl_4 and Fe^{3+}/ATP/NADPH (or NADH) were used separately as LP stimulators. Boldine (**13**) dose-dependently inhibited the production of TBARS in both models. However, it was only able to reduce the LP-mediated inactivating effect on cytochrome P4502E1, when LP was induced by Fe^{3+}. Therefore, it seems unquestionable that CCl_4 drives a non-peroxidative mechanism to suppress the activity of cytochrome P4502E1, possibly through the direct generation of the $^{\bullet}CCl_3$ radical at the catalytic site (*120*).

In the course of a study covering 19 benzylisoquinoline alkaloids, several aporphines at 100 μM strongly reduced $FeSO_4$/cysteine-induced LP in hepatic microsomes. Isoboldine (**20**) (IC_{50} 11 μM), **4** (IC_{50} 12.5 μM) and **13** (IC_{50} 20 μM) were the most potent natural alkaloids, although **109** surpassed all three (IC_{50} 3.3 μM) (*121*). Other agents such as $FeSO_4$/ascorbate, CCl_4/NADPH and Fe^{3+}-ADP/NADPH were also employed as inducers of microsomal LP, in order to study the influence of the same aporphines (except **20**). The maximum potencies of the natural alkaloids coincided in the Fe^{3+} (100 μM)-ADP/NADPH-induced LP, and **4** and **13** were the ones with the lowest IC_{50} values (5.6 and 9.5 μM, respectively), although again, **109** was more potent, and was the only aporphine that efficiently scavenged superoxide radicals. Given that this compound was also the only one with two free *ortho* hydroxyl groups, it was supposed that this functionality affords the aporphines their reactivity towards free radicals, as occurs with other aromatic molecules. Nevertheless, other facts like the formation of phenoxy radicals must be involved, because other, non-free, hydroxyl aporphines like **2** showed an inhibitory effect on LP (*122*). When the aporphines (100 μM) were tested for their microsomal antiperoxidative effect against 200 μM Fe^{3+}, their effects were weaker than those observed against other iron-induced models. The alkaloids **2**, **4** and **13** scavenged hydroxyl radicals in the Fe^{3+}-EDTA/H_2O_2-induced deoxyribose degradation system, while **10** and **109** increased hydroxyl radical production. Anonaine (**10**) changed its stimulation into inhibition when Fe^{3+} was stabilized by the presence of ascorbate (*123*).

The effect of **2** was further studied, and compared with that of other related compounds, by applying rat brain lipid auto-oxidation and lysozyme inactivation by AAP. Glaucine (**2**) and **13** were equipotent in inhibiting auto-oxidation, measured as luminescence, and both were nearly six times less potent than **109**. On

the other hand, the presence of 20 μM **2** caused a 3-fold increase in the time required for a 25% reduction in lysozyme activity, whereas **13** showed a 6-fold increase, and *N*-methylglaucine (**38**) (a quaternary alkaloid) had no lasting effect, and ultimately caused a two-thirds loss in the protective effect of **2** on lysozyme activity. These facts support the idea that aporphines lacking free hydroxyl groups, such as **2**, possess antioxidant activity when they have a non-shared electronic pair on the nitrogen atom, which could stabilize a radical on the neighboring C-6a (*124*).

It is well-established that oxidative stress is one of the multiple cellular events produced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and participates in the translocation of PKC and thereafter in the tumor-promoting activity of that compound. Accordingly, TPA-induced gap junctional intercellular communication (GJIC) down-regulation, one of the crucial steps in tumor promotion, is potentiated when antioxidant defences are reduced by GSH depletion. Considering these conditions and the antioxidant properties of some aporphines, **2** and **13** were studied for their activity in TPA-treated WB-F344 rat liver epithelial cells. Both alkaloids inhibited PKC translocation from the cytosol to the particulate fraction, hyperphosphorylation and internalization of gap junctional connexin 43 (a protein forming the intercellular bridge connexons), and the accumulation of oxidant species, such as oxygen radicals and peroxides. Neither of the two aporphines affected either phorbol-dibutyrate binding or XOD activity, which reinforces the hypothesis that antioxidants play an important role as anti-tumor promoting agents (*125*).

F. Effects on Platelet Aggregation

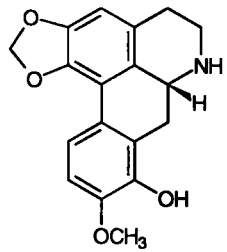
S-(+)-Dicentrine (**16**), which is present in the roots of *Lindera megaphylla*, was an efficient inhibitor of rabbit platelet aggregation. At a 300 μM concentration, it almost completely abolished the aggregation induced by ADP, collagen, AA, and platelet-activating factor (PAF) (*126*). In order to study its mechanism of action, the effect of **16** was evaluated in both washed and lysed platelet from rabbit plasma. Between 30 and 150 μM it managed to suppress AA-induced aggregation with an IC_{50} of 70 μM, and, at higher doses (225-300 μM), also suppressed aggregation induced by ADP, collagen, thrombin and U46619. This effect was paralleled by an inhibition of TXB_2, a natural pro-aggregant metabolite of AA, and ATP release and did not depend on the drug incubation time, since the same values were obtained for 3 and 30 min pretreatment. The levels of IMP suffered only an insignificant reduction at 300 μM, whereas those of cAMP were significantly increased at the same dose. For this reason an influence on the adenylate cyclase-mediated signal transduction pathway should be considered.

As **16** was also a good inhibitor of PGD_2 formation, it was thought that the inhibition of TXB_2 release could be due to an inhibition of the shared biosynthetic enzyme COX. This hypothesis, however, is hardly admissible because the potency of **16** in inhibiting AA-induced aggregation is 70 times lower than that of indomethacin, a rather specific COX inhibitor (*127*).

In addition to **16**, other aporphine alkaloids were also isolated from the flower buds and peduncles of *L. megaphylla*, one of which, **47**, was also effective against AA- and collagen-induced aggregation, whereas dicentrinone (**96**) and dehydrodicentrine (**63**) were pro-aggregant (*128*). The effectiveness of *N*-acetylanonaine (**33**) from *Zanthoxylum simulans* (Rutaceae) (*129*) and hernangerine (**18**), *N*-methylhernangerine (**39**) and ovigerine (**48**), from *Hernandia sonora* (Hernandiaceae) was similar to that of **16** (*130*).

Two phenanthrene aporphinoids, **81** and atherosperminium I (**83**), isolated from *Fissistigma glaucescens*, and four, closely related, semisynthetic phenanthrenes, atherosperminine *N*-oxide (**82**), dicentrine methine (**84**), glaucine methine *N*-oxide (**85**) and xylopine hydroxylamine (**94**) were studied. The natural compounds **81** and **83**, at 100 μg/mL, completely abolished the platelet aggregation induced by AA, ADP, collagen and PAF. Among the other four alkaloids, **82** was the most effective because it inhibited aggregation induced by both AA and collagen. A comparison of the results on the assayed compounds showed that the presence of an *N*-oxide function is a positive feature for the activity, and that 3,4-dioxygenated, or quaternary phenanthrene derivatives with hydrogen at C-6 and C-7, reach the highest potency. When some methoxyl groups substitute for these positions, activity decreases (*103*).

An extensive study covering twenty-five aporphines, most of them of direct natural origin (from *Fissistigma*, *Illigera*, *Lindera* and *Litsea* species), while a few were semisynthetic, was run using the same four above-mentioned inducers. The most active inhibitors were actinodaphnine (**8**), *N*-methylnandigerine (**39**), *N*-methyllaurotetanine (**41**), norannuradhapurine (**111**), **36**, **47** and **55**. *N*-Methylactinodaphnine *N*-oxide (**37**) was active only in the ADP-induced aggregation test. It should be mentioned that **55** and **111**, both belonging to the 6a*R* series, displayed strong effects against every inducer, even PAF, at a concentration lower than 25 μg/mL. Apart from the common 1,2-oxygenated substitution on ring A of tertiary or quaternary aporphines, a variety of mono- and di-oxygenated substitutions at 8,9 and 10 on ring D, particularly a hydroxyl or methoxyl group at C-9, favored the anti-platelet effect (*131*).



Norannuradhapurine (**111**)

The mechanism of action of **13**, laurolitsine (**28**) from *Phoebe formosana* (Lauraceae), the phenanthrene **110** and four semisynthetic seco-aporphinoids, *N*-

cyanosecoboldine (**88**), *N*-methylsecoglaucine (**90**), *N*-methylsecopredicentrine (**91**) and **93,** was investigated in rabbit and human platelets. All the alkaloids inhibited aggregation induced by AA and collagen, but the highest potency was attributed to **88**, which, at 15 μM, inhibited the AA-induced aggregation by 80%. The production of TXB_2 was also reduced by all of the assayed alkaloids, especially by **110** (77% at 15 μM) and **88** (97% at 30 μM). The seco-derivatives, particularly **91** and **90**, also inhibited aggregation caused by PAF, thrombin and U46619. As seen in many other cases, aggregation induced by PAF was difficult to inhibit. In this model, the seco-derivatives showed notable activity at around 150-300 μM, although at lower concentrations the effect fell dramatically. *N*-Methylsecoboldine (**89**) and **110** inhibited the release of ATP by platelets in a dose-dependent manner, and, at 300 μM, **89** markedly increased the production of cAMP, one of the most important markers of platelet inactivation. Human platelet-rich plasma was employed solely to determinate the influence on the biphasic aggregation induced by adrenaline and ADP. *N*-Methylsecoboldine (**89**) suppressed both phases, whereas **110** suppressed only primary aggregation (*132*).

Finally, a survey of the active principles of *Illigera luzonensis* led to the identification of certain aporphines and oxoaporphines, some of which had previously been characterized as anti-aggregant drugs (e.g. **16**). *S*-(+)-*N*-methylactinodaphnine (**36**) was the most potent alkaloid: at 20 μg/mL it completely abolished the effect of AA, and at 10 μg/mL showed 84% inhibition, whereas other alkaloids such as hernovine (**19**), launobine (**24**) and **4** were less active (*133*).

G. Hypotensive and Anti-arrhythmic Effects

The Mapuche Amerindians use the leaves of *Laurelia sempervirens* (Monimiaceae) to treat headaches, and the plant has been recognized as a diuretic agent. In 1992, Schmeda-Hirschmann *et al.* (*134*) demonstrated that the extract had strong hypotensive properties on normotensive rats. Bioassay-guided isolation of the extract yielded the alkaloid **27** as the main principle with hypotensive activity. At 1 mg/kg it elicited a hypotensive response of –29% in the mean blood pressure for 2 min (*135*).

Fernández-Alzueta *et al.* (*136*) observed that **2** produced a characteristic hypotension in the cardiovascular system in anaesthetized rats, accompanied by a significant decrease in heart rate. This alkaloid directly relaxes vascular and non-vascular smooth muscle, as papaverine does, but with a different mechanism of action, as the authors demonstrated later and is reported above (section III.C.*1*) (*75*).

A study of **16** as an anti-arrhythmic drug was conducted and the results were compared with those of quinidine, procainamide and *N*-acetylprocainamide. They were examined in the Langendorff perfused rabbit heart and rabbit isolated cardiac cells (*137*). The His-Purkinje conduction time, the ventricular repolarization time and its effective refractory period were significantly increased by the higher dose of **16**, quinidine and procainamide. Dicentrine (**16**) inhibited the action potential upstroke and prolonged action potential duration. The voltage clamp study proved that the prolongation of rabbit atrial action potential duration by **16** is due to its

inhibition of the transient outward current. In ventricular cells from the rabbit, **16** also suppressed the K^+ outward current. Dicentrine (**16**) inhibits both Na^+ and K^+ channels like procainamide, quinidine and amiodarone, which are used in treating supraventricular, as well as ventricular, arrhythmia. Similar results were found by Su *et al.* (*138*), who studied the electrophysiological properties of **16** in guinea pig isolated heart cells and rat ventricular cells. Again, **16** exerted anti-arrhythmic action by inhibiting excitability through the suppression of the sodium inward current (anti-arrhythmic class I) and prolongation of the action potential duration (anti-arrhythmic class III). In conclusion, **16** is potentially a useful type I and III anti-arrhythmic agent (*137*), the anti-arrhythmic effect of which may be associated with a smaller suppression of cardiac contractility than that produced by quinidine (*138*).

Liriodenine (**5**) showed anti-arrhythmic potential and positive inotropic effects. The anti-arrhythmic action seems to be mediated by the blockade mainly of the Na^+ and transient outward current (I_{to}) channels. The inhibition of I_{to} could participate in the prolongation of the action potential duration that **5** produces in the cardiomyocytes, and account for the positive inotropy of this agent. The positive inotropic effect was not antagonized by prazosin, propranolol or carbachol, which suggests that the inotropic effect is not due to activation of α- or β-adrenoceptors or to muscarinic receptor activation. The fact that verapamil did not attenuate the positive inotropic effect of **5** rules out activation of the L-type calcium channel. Aminopyridine markedly attenuated the inotropic property, which indicates that its mechanism is similar to that of **5**, *i.e.* blockade of K^+ channel and hence prolongation of the action potential duration. The similarity between the concentrations of **5** needed for positive inotropy and K^+ outward current blockade suggests that inhibition of both currents contributes to the positive inotropy. Liriodenine (**5**), like quinidine, has a higher affinity for the inactivated Na^+ channels, but, unlike this therapeutic agent, **5** did not retard the recovery time course of Na^+ channels from the inactivated state (*139*).

H. ANTIMICROBIAL AND ANTIVIRAL ACTIVITY

Villar *et al.* (*140-141*) established the antimicrobial structure activity relationships of aporphinoids. They evaluated twenty-six benzylisoquinolines, including aporphinoids such as nornantenine (**44**), puterine (**51**), 3-hydroxynornuciferine (**57**), **9** and **55** (noraporphines), *O*-methylmoschatoline (**100**), lysicamine (**101**), oxoputerine (**105**), lanuginosine (**107**) and **5** (oxoaporphines), and *N*-methylputerine (**42**), **10**, **11**, and **46** (aporphines), against Gram positive bacteria, Gram negative bacteria and one yeast. Comparison of the minimal inhibitory concentration (MIC) of the alkaloids tested showed that the noraporphines are the most active substances against Gram positive bacteria and yeast. In the oxoaporphine group, **105** was the most active alkaloid against all the microorganisms tested, and the only alkaloid that inhibited *Candida albicans* at 6 μg/mL. The results suggest that the basic structural requirement for antimicrobial activity is a 1,2-methylenedioxy-noraporphine. Introduction of a methoxyl group and/or blockage of nitrogen with a methyl group decreases the activity or

eliminates the antimicrobial properties. For example, **10** had a MIC in a range of 3-50 μg/mL, whereas its methoxylated derivatives **51** (11-methoxyanonaine) and **55** (9-methoxyanonaine) showed a MIC range of 25 to 100 μg/mL. *N*-Methylation of **51** eliminated the antimicrobial activity. In a complementary study (*142*), **9**, **100** and **101** were assayed against 87 clinical isolates. The activity of **101** against Gram positive bacteria was variable. About 90% of *Streptococcus pneumoniae* isolates were inhibited at 25 μg/mL, but other Gram positive bacteria were susceptible to 50 μg/mL. Similar results were found for the other two alkaloids.

The bactericidal or bacteriostatic effects of the alkaloids **5**, **9** and **101,** at different concentrations, against *Staphylococcus aureus* and *Escherichia coli* was established by growth curves analysis. The results obtained led to the conclusion that the effect of **9** against *S. aureus* and *E. coli* was bactericidal at 32 and 64 mg/L, respectively. Lysicamine (**101**) also had a bactericidal action similar to **9**. The cidal effect of **5** against *S. aureus* was faster than that of **101** (*142*).

In order to discover other structural requirements for the antimicrobial activity, and to establish the potency of the alkaloids isolated from the stem bark of *Annona cherimolia*, fourteen benzylisoquinoline alkaloids were evaluated *in vitro* for their antimicrobial activity (*143*). The results obtained with aporphines confirmed that the basic active skeleton against Gram positive bacteria is the 1,2-methylenedioxy noraporphine. Anonaine (**10**) inhibited the growth of Gram positive bacteria, *Mycobacterium phlei*, *Klebsiella pneumoniae* and *C. albicans*. Anolobine (**9**) (inactive against *C. albicans*), inhibited *E. coli* and *Salmonella typhimurium*, while **68** was the only alkaloid that inhibited the growth of *Pseudomonas aeruginosa*. The oxoderivatives of aporphines were inactive against Gram negative bacteria, and showed little activity against the yeast, but they were more active against Gram positive bacteria than the corresponding reduced forms (*143*).

Glaucine (**2**), the major constituent of the nonphenolic alkaloid fraction of *Liriodendron tulipifera*, was a more effective inhibitor of the growth of the fungi assayed than lirioferine (**30**), liriotulipiferine (**31**) and **41**, the major constituents of the phenolic fraction. The extraordinarily high concentration of **2** in the discolored sapwood formed in response to injury in the living yellow poplar tree, and its high antifungal activity, suggest that this injury stimulates the biosynthesis of **2** in order to protect the tree against attack by microorganisms that invade the tree stem after wounding (*144*).

At 1 mg/mL, the aporphinoids laurelliptine (**25**), **10** and **20,** isolated from *Annona salzmanii*, showed antifungal activity. Anonaine (**10**) was the only alkaloid that inhibited the Gram positive bacteria assayed, and this effect may be responsible for the antidysenteric effect of the drug (*145*).

Research on antiviral activity is quite difficult due to the complexity of the different techniques and parameters that can condition the final results. The number of aporphinoids that have been examined for antiviral activity is very small. Montanha *et al.* (*146*) assayed nineteen aporphine alkaloids against *Herpes simplex* type 1 (HSV-1) by determining the 50% cytotoxic concentration (CC_{50}) and the concentration that had no effect on the cell number at the maximum tolerated concentration. The antiviral activity was determined as the reduction factor (log) of the viral titre by comparison with untreated controls. The CC_{50} values varied from 7.5 μM for oliveridine (**69**) (the most toxic alkaloid) to 500 μM for **101** (the least

modify arterial pressure or heart rate when administered intravenously. These results demonstrated that aporphines need the 11-hydroxy group to exert a high effect on 5-HT_{1A} receptors. In the former experiment (*86*), *R*-(−)-11-hydroxy-10-methylaporphine had an ED_{50} of 0.05 μM for the inhibition of contractions produced by a transmural single shock stimulation on guinea pig ileum. The *S*-(+)-11-hydroxy-10-methylaporphine was inactive in this experiment, but it had an ED_{50} of 0.03 μM for antagonism of inhibition of contractions produced by 8-hydroxy-2-(di-*n*-propyl-amino)tetralin, a 5-HT_{1A} agonist. The *R*-(−)-enantiomer was inactive in this test. Both stereoisomers had a high affinity for the 5-HT_{1A} binding sites, expressed as an ability to displace [^{3}H]-8-hydroxy-2-(di-*n*-propyl-amino)tetralin from the membranes of the rat forebrain (*86*).

In a later study, Cannon *et al.* (*88*) amplified the experiment to include other structural analogues. The *R*-(−)- and *S*-(+)-forms of 10-hydroxy-9-methyl-, 10-methoxy-9-methyl-, 9-hydroxy-10-methyl-, and 9-methoxy-10-methyl-aporphine were tested on guinea pig ileum contractions produced by electrical stimulation. The derivatives 10-hydroxy-9-methyl- and 9-hydroxy-10-methyl-aporphines were inactive, whereas the methoxy-derivatives were active, with an ED_{50} range of 2.6 to 5.9 μM. *R*-(−)-9-Methyl-10-methoxy-aporphine (ED_{50} 2.6 μM) was the most active of the analogues, but clearly weaker than *R*-(−)-11-hydroxy-10-methylaporphine, the most active compound of the series, with an ED_{50} of 0.05 μM in this experiment. In view of these results, the authors concluded that 11-hydroxy-10-methylaporphines are highly selective agonists (*R*-isomer) or antagonists (*S*-isomer) at 5-HT_{1A} receptors.

Similar results were obtained by Hedberg *et al.* (*89*), who demonstrated that *R*-(−)-11-hydroxyaporphine was a low-potency partial 5-HT_{1A} agonist with affinity for D1 and D2A receptors and that *R*-(−)-11-hydroxy-10-methyl-aporphine was a potently selective and efficacious 5-HT_{1A} receptor agonist. They hypothesized that a lipophilic pocket in the 5-HT_{1A} receptor accommodates a methyl group at C-10, and that the binding site of the D2 receptor (*89*) does not accommodate it. The elimination of the methyl group on the nitrogen in *R*-(−)-11-hydroxyaporphine, increases the selectivity for 5-HT_{1A} receptors (100-fold), and substitution by *N*-propyl increases the selectivity of *R*-(−)-11-hydroxy-10-methyl-aporphine for D2A receptors (3-fold) (*90*). This feature was verified in a complementary study with different C-10 and *N*-substituted aporphines, and it was observed that the *N*-alkyl group produced a marked decrease in the affinity for the 5-HT_{1A} receptor (*91*).

Extracts from the fruit, seeds and leaves of *Annona muricata* were tested to see if they inhibited the binding of [^{3}H]-rauwolscine at the 5-HT_{1A}-receptor in the calf hippocampus. A strong inhibition was obtained for the fruit extract (*92*). Three aporphines were then isolated from this source, and the serotonergic activity was studied. Nornuciferine (**45**), **10** and **11** inhibited binding of [^{3}H]-rauwolscine to 5-HT_{1A}-receptors with IC_{50} values of 3, 9 and 5 μM, respectively. In a later study (*93*), the same authors reported the inhibition of forskolin-stimulated cAMP accumulation in NIH-3T3 cells stably transfected with human 5-HT_{1A}-receptors. The results indicated that these compounds act as 5-HT_{1A} agonists. The alkaloids **10** and **45** (EC_{50} < 10 μM) were more potent than **11** (EC_{50} 20 μM) in this assay.

toxic one). The best results on the viral titre reduction were obtained with *S*-(+)-cassythicine (**36**), pachystaudine (**71**), oxostephanine (**106**), **5**, **8** and **69**. The rest of the alkaloids assayed were inactive. The alkaloid **106** appeared to be the most active against HSV-1, with a high selectivity. The results suggest that the active alkaloids interfere with a stage subsequent to virus attachment of the replicative cycle. To determine the mechanism of antiviral action, alkaloids were added after inoculation and HSV-1 DNA synthesis was evaluated. According to the results, it seems that **69** and **106** interfere with an earlier event of the HSV-1 replication, but they may also inhibit a later stage, whereas **71** interferes only with earlier events of HSV-1 replication. In relation to the effect of alkaloids on HSV-1 DNA synthesis, the results indicated that the best inhibitory effect was reached with **71**. For **69** and **106**, the decrease in DNA synthesis alone could not explain the reduction in the virus titre. Other events of the replicative cycle are probably inhibited by these alkaloids.

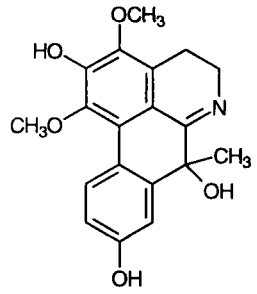
Some requirements of the antiviral structure activity relationships are similar to those found previously in the antimicrobial activity research: The presence of a methylenedioxy group at C-1–C-2 is positive for the activity (**5** and **106**), but some products like guatterine (**67**) or pachypodanthine (**70**) were inactive, which means that this function is not the only requirement for activity. This could be explained by the negative role of the 7-hydroxyl, because substitution on C-7 of methoxyl by hydroxyl eliminated the antiviral activity (*146*).

Natural aporphine alkaloids isolated from plants have also been tested for their antiparasitic activity. Thus, thalifasine (**75**) was isolated from *Thalictrum faberi* and was assayed for its antimalarial activity. It was able to inhibit the chloroquine-sensitive D-6 and the chloroquine-resistant W-2 clones of *Plasmodium falciparum* with a selectivity index of 19 and 93, respectively (*147*).

The secondary and tertiary bases of *Stephania pierrei* (Menispermaceae) tubers, such as (–)-asimilobine-2-*O*-β-D-glucoside (**12**), demonstrated appreciable antimalarial activity with ED_{50} values of 950 and 470 ng/mL in the D-6 and W-2 strains, respectively (*148*).

The alkaloids **2**, **13**, **50** and **109** were tested against the epimastigotes of different strains of *Trypanosoma cruzi*, the causative factor of the Chagas's disease. The trypanocidal effects appear to correlate with their anti-oxidative properties (*121*). Apomorphine (**109**), together with **2** and **13**, showed the strongest inhibition of culture growth. The mechanism of action of these products could be the blockage of mitochondrial electron transport in the parasite (*149*).

The alkaloids present in the extract of *Guatteria foliosa* (Annonaceae) are responsible for the antiprotozoal activity that induces complete lysis of the promastigote forms of three species of *Leishmania* and the epimastigote form of three strains of *T. cruzi*. Isoguattouregidine (**112**) was the only alkaloid that showed significant activity against *L. donovani* and *L. amazonensis*. The alkaloids were evaluated in the bloodstream form (trypomastigote) isolated from a blood sample of *T. cruzi* infected mice. 3-Hydroxynornuciferine (**57**), **112** and argentinine (**80**) were the most active alkaloids, with lysis percentages of over 60% (*150*).



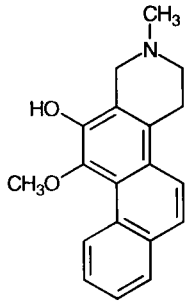
Isoguattouregidine (**112**)

I. CYTOTOXIC AND ANTITUMORAL ACTIVITY

Within the isolated plant aporphinoids, oxoaporphines are the most frequently reported as cytotoxic principles, although other active compounds have been also described. Glaziovine (**6**) (proaporphine), ocoteine (**61**), **2**, **3**, **8**, **10**, **16**, **36** (aporphines); oxoglaucine (**102**), oxopurpureine (**104**), **5**, **95**, **100**, **101**, **107** (oxoaporphines); *O*-ethylthalmelatine (**76**), thalicarpine (**77**), thalmelatine (**78**), thalifalandine (**74**) and hernandaline (**79**) (dimeric forms) have been described as cytotoxic, antitumoral and antileukemic principles (*34*).

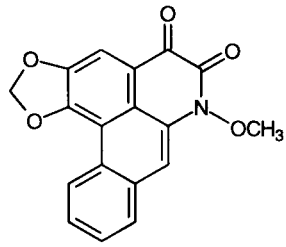
Bioassay-directed fractionation of the methanol extract of stem parts of *Polyalthia longifolia* (Annonaceae) led to the isolation and identification of **5**, which inhibited the growth of several cell lines (*151*). This oxoaporphine was also the main cytotoxic alkaloid found in *Paramichelia baillonii* (Magnoliaceae), *Artabotrys uncinatus*, *Thalictrum sessile* (*152,153*), and *Annona montana* (*154*). Moreover, from this species, annoretine (**113**) and **80** were isolated. The former showed significant activity against different cell lines, with ED_{50} values between 2.51 and 14.47 μg/mL, whereas **80** inhibited the growth of the same cell lines with ED_{50} values ranging from 2.93 to 9.89 μg/mL. *O*-Acetylargentinine (**92**) was cytotoxic with similar potency to the other alkaloids tested (*154*).

Six aporphines, five oxoaporphines, nine aporphine *N*-oxides, seven phenanthrenes and four phenanthrene *N*-oxides were tested for their cytotoxicity against five cell lines in order to find out more about the relationships between chemical structure and cytotoxic activity (*155*). The most active alkaloids were oxoaporphines and one of the phenanthrenes assayed. Liriodenine (**5**) showed potent, wide-spectrum activity against all the cell lines tested. These results are in agreement with previous studies in which activity *in vitro* and *in vivo* was demonstrated (*156,157*). Other active oxoaporphines were oxodicentrine (**96**) and oxoxylopine (**107**). When the oxo function was removed, the activity was strongly reduced, as reflected by the comparison between **107** and **55**, as well as the fact that most of the aporphines tested were inactive. The oxo function is essential for the cytotoxic activity.



Annoretine (**113**)

From *Artabotrys zeylanicus* a new *N*-methoxyl derivative was isolated and identified as *N*-methoxynorcepharadione A (**114**) (*158*). The known oxoaporphine **95**, previously isolated from the stem bark of *A. uncinatus* (*152*), was also isolated. The bioactivity profile of these substances was established through mechanism-based yeast mutant bioassays, in which both alkaloids showed significant, selective activities with the wild-type strain. In addition, **114** was active against two cell lines, P388 (wild-type, ED_{50} 1.59 μg/mL) and P388 (camptothecin-resistant, ED_{50} 1.12 μg/mL).



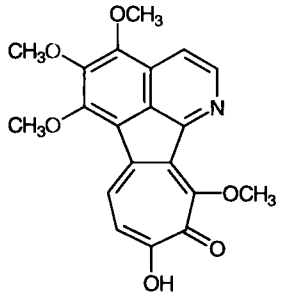
N-Methoxynorcepharadione A (**114**)

The bioactivity-directed separation of the ethanol extract of *Stephania pierrei* tubers resulted in the isolation of twenty-three isoquinoline alkaloids. Two of them were new aporphinoids: **12** and nordicentrine (**43**). Among the aporphine alkaloids, the secondary and tertiary bases with the 1,2-methylenedioxy group such as *R*-(−)-dicentrine (**16**), (−)-isolaureline (**22**), *R*-(−)-cassythicine (**36**), roemeroline (**53**), (−)-phanostenine (**49**), **10**, **43** and **55**, displayed general cytotoxicity in nearly all cancer cell lines tested, while those without the above mentioned group showed no inhibitory effect on the growth of the cancer cells. The enhanced activity of **10** relative to **12** (which was inactive) implies that the 1,2-methylenedioxy substituent is required for the expression of cytotoxicity (*148*).

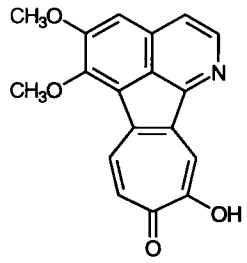
Thalictrum faberi is a perennial herb used in China as an antiphlogistic drug and in the treatment of stomach cancer. The phenolic alkaloid extract of the roots after fractionation yielded a new aporphine identified as thalifaberidine (**72**). Its cytotoxic activity was studied together with the major alkaloids from this plant, thalifaberine (**73**) and **75**. In order to obtain some information about structural modification and biological activity, **73** and **75** were subjected to biological evaluation for cytotoxic, antimalarial and HIV-reverse transcriptase inhibitory activities. The compounds showed cytotoxic activity against the different cell lines assayed, with ED_{50} ranging from 0.6 to 17.7 μg/mL (*147*). No remarkable differences in activity were evident in the exchange of phenolic and methoxyl functions, or in the presence of oxygenated functions at the C-3 and C-5' positions.

Hernandia nymphaeifolia (*159,160*) is a plant rich in lignans and alkaloids, including aporphines and oxoaporphines. From the methanol extract of this plant *N*-formyldehydroovigerine (**66**) and four known aporphines, *N*-methylhernovine (**40**), **19**, **27** and **32**, were isolated. Most of the compounds were less potent than the reference drug mithramycin, but (+)-*N*-hydroxyovigerine (**34**) (ED_{50} 92 ng/mL), **66** (ED_{50} 72 ng/mL) and hernandonine (**97**) (ED_{50} 92 ng/mL) exhibited cytotoxicities close to that of mithramycin (ED_{50} 61 ng/mL). Furthermore, **97** (*160*) (ED_{50} <1 ng/mL against P388) was 60 times more toxic than mithramycin. Magnoflorine (**32**) (ED_{50} 229 ng/mL) and **19** (ED_{50} 214 ng/mL) showed selective cytotoxicities against the P388 cell line *in vitro*. By comparison, the noraporphine **19** had higher cytotoxic activity than its *N*-methyl analogue **40** against different cell lines.

A novel tropoloisoquinoline alkaloid was isolated from *Cissampelos pareira* (Menispermaceae) and identified as pareirubrine A (**115**), which inhibited the growth of the P-388 cell line with an IC_{50} of 0.33 μg/mL (*161*). Further bioassay-directed purification of the extract, guided by cytotoxicity against P-388 cells, led to the isolation of pareirubrine B (**116**) (IC_{50} 0.17 μg/mL), together with other inactive tropoloisoquinolines (*160*).



Pareirubrine A (**115**)



Pareirubrine B (**116**)

From *Xylopia aethiopica* (Annonaceae) two oxoaporphines, oxophoebine (**103**) and **5**, were isolated and studied for their DNA topoisomerase II inhibition activity. Both compounds showed selective activity against *Saccharomyces cerevisiae rad 52* and *rad 6* mutants (*162*). Related compounds such as lauterine or 10-methoxyliriodenine (**99**) and the 10-hydroxyliriodenine (**98**) were also active, but they had greater toxicity against the *rad 52.top 1* mutant than against *rad* 52. All these substances were assayed for the inhibition of purified mammalian DNA topoisomerase II, and were active. Alkaloid **98** was twice as active as **99**, which is consistent with the difference between the two compounds observed previously against mutant yeast. It is interesting to note that the presence of a methylenedioxy function at C-1–C-2 (**5**, **98** and **99**) and of the C-10 substitution (**98** and **99**) reduce the toxicity against the *rad 52* mutant (*162*).

In conclusion, oxoaporphinoids, particularly those with methylenedioxy substituents may represent a novel class of DNA topoisomerase II inhibitors (*162*).

Liriodenine (5) was found to be a potent inhibitor of topoisomerase II in both *in vivo* and *in vitro* assays. These tests also indicated that **5** is a strong catalytic topoisomerase II inhibitor, but differs from other inhibitors in that it does not have the strong poisonous effect. Topoisomerase II could be the target of **5** that accounts for its biological activities. Inhibition of topoisomerase by topoisomerase II poisons causes chromosome damage, including illegitimate recombination, deletions, sister chromatid exchanges and translocations. These substances have also been reported to be mutagenic agents. Woo *et al.* only studied the inhibition of mammalian topoisomerase II by **5** (*163*).

The cytotoxicity of thaliblastine (**77**) and/or cisplatin (DDP) was comparatively determined in DDP-sensitive (0-342) and resistant (0-342/DDP) rat ovarian tumor cell lines. The ID_{50} of **77** and cisplatin were 39.3 μg/mL and 6.2 μM, respectively, in the sensitive line. Against the resistant line the ID_{50} values were 23.4 μM for cisplatin and 27.3 μg/mL for **77**. Furthermore, simultaneous exposure of cells to cisplatin and **77** showed a significant superiority over cisplatin alone (*164*).

Cisplatin induces a higher level of DNA interstrand crosslinks in the sensitive 0-342 cells, while **77** produces DNA single strand breaks in a dose-dependent manner in 0-342/DPP cells, but not in 0-342 cells. The combination of these two compounds shows a synergistic ability to produce DNA single strand breaks in resistant cells. Therefore, **77** and cisplatin affect the cellular DNA in different ways, and together produce synergistic cytotoxicity in the resistant cells. The mechanisms involved in this process remain unclear, but **77**, in combination with cisplatin, might be able to eliminate the resistance in clinical use (*165*).

Recently Seifert *et al.* (*166*) analyzed by flow cytometry the cell cycle changes that take place on exposure to **77**, using the rat ovarian tumor cell lines, mentioned above. The two cell lines, sensitive and resistant to cisplatin, differ in the number of chromosomes and, consequently, in the DNA content per cell. In both lines similar cell cycle effects are discernible. Thaliblastine (**77**) blocks the cells in the premitotic interval (G2), in the period of mitosis (M) and in the period between mitosis and the beginning of DNA synthesis (G1). However, this effect appears to occur later in resistant cells, although both types show a similar DNA distribution pattern. In other words, **77** produces both G1 and G2/M blockage in tumor cells

during growing and proliferation. This effect appears to be earlier in the cisplatin-sensitive cells than in cells that are resistant to the drug (*166*).

Roemerine (**52**), an aporphine alkaloid isolated from the ethyl acetate-soluble extract of *Annona senegalensis*, showed weak cytotoxic activity in many of the tumor cell lines tested. To investigate its effect on reversing multidrug resistance, KB-V1 cells were treated with different concentrations of the alkaloid in the presence or absence of 1 μg/mL vinblastine. This concentration did not affect the growth of drug-resistant KB-V1 cells, although it was lethal for sensitive KB-3 cells. Addition of **52** to the culture medium of KB-V1 cells did not modify the growth of these cells, but in the presence of vinblastine an ED_{50} value of 0.6 μg/mL was obtained. These results suggest a clear reversion of multidrug resistant KB-V1 cells to drug sensitivity. Further experiments were carried out in order to explore this activity by treating KB-3 and KB-V1 cells with various concentrations of vinblastine in the presence or absence of **52**. The cytotoxic response mediated by the reference drug was augmented in the presence of a fixed concentration of **52**. Inhibition of ATP-dependent vinblastine binding to vesicles isolated from multidrug-resistant KB-V1 cells was also observed in the presence of the alkaloid, and was dose-dependent (*167*).

J. IMMNUNOPHARMACOLOGICAL ACTIVITY

The immunopharmacological activity of eight series of isoquinoline alkaloids, including aporphinoids, was evaluated on complement activation, phagocytosis and antibody synthesis tests (*168*). The results showed that the alkaloids studied were able to influence these immune reactions *in vitro*. Oxoglaucine (**102**) was the only substance with complement inhibiting properties. It was also the only one that inhibited the classical pathway complement activity in guinea-pig serum much more than in normal human serum. The authors suggest that this alkaloid might act by a reaction of its carbonyl group directly with some of the complement proteins. Compounds with two phenolic functions inhibited the process of phagocytosis by peritoneal macrophages. The strongest alkaloid was bracteoline (**14**), which is characterized by the presence of hydroxyl groups at C-1 and C-10. All the substances tested possessed suppressive effects on antibody synthesis against sheep red blood cells (SRBC). The replacement of one of the two methoxyl groups with hydroxyl diminished the effect (*168*).

3-Hydroxyglaucine (**56**), dehydroglaucine (**64**), **2**, **14**, **20**, **54** and **102** showed similar behavior in the reactions tested. The most potent suppressor of antibody production by spleen lymphocytes in a dose-dependent manner was **102**. A concentration of 10 μM caused strong inhibition without changing the cell viability. Incubation of splenocytes with **102** for 24 h, and the addition of antigen after the substance had been removed, resulted in a strong suppression of antibody synthesis. This result suggests that the exposure of **102** before the antigen exposure causes a large number of inactivated splenocytes. However, when splenocytes were previously activated, the addition of **102** did not affect them. The data suggest that **102** acted on some T-lymphocyte clones on rather than, mature B-lymphocytes. This result was confirmed by measuring [^{3}H]-thymidine incorporation by splenic

lymphocytes in the presence of T- or B-cell mitogens. Oxoglaucine (**102**) suppressed T-cell responses to concanavalin A and phytohaemagglutinin, and augmented B-cell response (*168*).

In order to confirm the above-cited results, Ivanovska *et al.* (*169*) studied **102** to evaluate its immunomodulatory action *in vitro* after the *in vivo* application. The *in vitro* proliferative response to concanavalin A was inhibited more effectively when the alkaloid was added at the same time as the mitogen. Lipopolysaccharide (LPS)-induced proliferation decreased only when LPS and **102** were added together. The immunomodulatory effect of **102** of altering antibody response to SRBC and LPS antigens was tested in mice models employing the arthritis adjuvant and *K. pneumoniae* infection. The results obtained after application *in vivo* indicated that its effect on the proliferation of immune cells may result in inhibition of antibody response. After multiple treatment with 0.05 mg/kg of **102** (ineffective in a single injection), an inhibition of the serum levels of anti-SRBC and anti-LPS antibodies was detected. The application of **102** in the poplyteal node assay, in which the reaction involves T-cell activation, and in the repeated injection of high LPS which induces B-cell activation, resulted in augmentation of both reactions (*169*).

Daily application of **102** (1 mg/kg) in different phases of inflammation in adjuvant arthritic mice showed the complicated character of this alkaloid in its effect on the immune cells. Before or during the development phase the drug increased the paw swelling, while in the established phase, treatment caused a decrease in paw edema. This same duality was observed in *K. pneumoniae* infection in mice: **102** (1 mg/kg) administered from seven to five days before inoculation augmented host resistance. Single doses of 10 and 20 mg/kg impaired host immunity significantly. It is obvious that further experiments are needed to explain the mechanism of the immunological action of **102** (*169*).

Analogous results were obtained when **102** was studied on delayed-type hypersensitivity (DTH) reaction and *K. pneumoniae* infection in normal and immunosuppressed mice in order to analyze its immunomodulatory effects (*171*). When **102** was administered during the development phase, the DTH reaction was increased, abolishing the inhibitory action of cyclophosphamide. On the other hand, it showed a restorative effect in *K. pneumoniae* infection in immunosuppressed mice when it was administered together with indomethacin, prednisolone or artemisinin. These data seem to indicate that **102** may act on selected lymphocyte clones involved in the anti-infectious host resistance (*170*).

The effect of **13** on cellular immune functions was studied *in vitro* with regard to its immunomodulatory effect. Treatment with vitamin A has beneficial effects on patients displaying an impairment of cell-mediated immunity, while administration of vitamin E to hyperlipidemic rats showed a beneficial effect on blast transformation, TNF activity and IL-1. In general, the favorable changes in the immune system observed in humans and in animal models are attributable to the antioxidant properties, inhibition of LP and cellular membrane-stabilizing activity of the alkaloid (*117*).

González-Cabello *et al.* (*171*) studied the suppression of blast transformation in healthy donors and patients with breast tumours and chronic lymphocytic leukemia (CLL) by **13**. The mechanism for this effect may be related to the entry of calcium blockade or the inhibition of IL-2 expression and transferrin receptors. In this

study, the natural killer cells (NK) of CLL patients were lower than those of healthy controls. NK cells in CLL patients were normal, but they had deficient cytoplasmatic granulation. Boldine (**13**) increases the NK activity in patients with CLL, but this effect cannot be explained only in terms of antioxidant properties. *In vitro* and *in vivo* NK activity is enhanced by the effect of IL-2, as well as by interferon (IFN), and it is very likely that the higher activity of NK cells resulted from IL-2 and IFN production by **13** (*171*).

K. ANTI-INFLAMMATORY AND CYTOPROTECTIVE EFFECTS

Boldine (**13**) seems to be involved in the hepatoprotective activity of *Peumus boldus* extract, since 33 μg/mL strongly inhibited the LP (reduction of malondialdehyde production) and cell membrane disturbances (reduction of lactate-dehydrogenase leakage) induced in rat isolated hepatocytes. *In vivo*, **13** was active against CCl_4–induced hepatitis at 10 mg/kg (*172*).

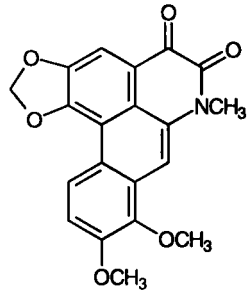
With respect to anti-inflammatory activity, **13** did not appear to be responsible for the pharmacological activity shown by the plant extract, since doses of 10 and 20 mg/kg did not inhibit the inflammation. According to Backhouse *et al.* (*173*), this lack of activity could be due to the small dose assayed. They have demonstrated that **13** has dose-dependent anti-inflammatory activity in the carrageenan-induced guinea pig paw edema assay with an oral ED_{50} of 34 mg/kg. In this inflammation model, the COX products of AA metabolism and the production of reactive oxygen species are involved. The same authors (*173*) also studied the antipyretic activity of **13** in rabbits treated with bacterial pyrogen. The dose found to elicit this antipyretic effect is in the same range as the anti-inflammatory oral ED_{50}. Boldine (**13**) inhibited prostaglandin synthesis *in vitro*, and this effect could explain both the antipyretic and anti-inflammatory effects. The ability of **13** to inhibit the biosynthesis of prostanoids may be due to the anti-oxidant activity of the alkaloid. This can be deduced from the reduction in 6-keto-PGF levels, which implies a decreased biosynthesis of PGI_2 that may be due to a direct inhibitory effect of **13** on COX or to a decreased availability of PGH_2. Gastric mucosal irritation or the appearance of duodenal ulcers often accompanies the therapeutic use of non-steroidal anti-inflammatory drugs (NSAIDs). The main difference between **13** and a NSAID is that **13** is present in galenical preparations to aid digestion and there are no reports of it having negative gastrointestinal effects (*173*).

It has been proposed that the oxidative modification of cell components mediated by reactive oxygen metabolites (ROMs) is a key event in the etiology of several inflammatory diseases. Enhanced oxygenated metabolite generation has been implicated in the tissue injury that is present in inflammatory bowel disease. The drugs used in the treatment of this disease, such as sulfasalazine or 5-aminosalycilic acid (5-ASA), act as ROM scavengers (*174*). Gotteland *et al.* (*175*) investigated the antioxidant and cytoprotective effects of **13** in an *in vivo* experimental model of colonic inflammation in rats treated with the intrarectal administration of acetic acid. This agent induces a marked inflammatory response in the colonic mucosa and submucosa after 24 h. Pretreatment of rats with a single

dose of **13** afforded significant protection against macroscopic injuries. The neutrophil recruitment in the rat colonic tissue, determined by measuring the myeloperoxidase (MPO) activity, was dramatically augmented by acetic acid, but it was clearly diminished by the effects of **13**. Administration of acetic acid causes colitis, and also induces LP, which was not reduced by **13**. Pretreatment of rats with **13** or 5-ASA did not alter the colonic fluid absorption in the presence of acetic acid. The results obtained demonstrate again that **13** possesses anti-inflammatory and cytoprotective activities against acetic acid-induced colonic inflammation. The decrease in MPO activity suggests a lower infiltration of neutrophils in the acid-exposed tissue. Despite the scavenging properties of **13**, the alkaloid failed to inhibit LP induced by acetic acid, probably because the cytoprotective effects of **13** focus primarily on the inhibition of the oxidative modification of certain proteins whose modification and loss of function are very important to the development of cell injury (*175*). On the other hand, the cytoprotective effect may also be due to the capacity of **13** to act as a calcium antagonist, like verapamil (*68*). This drug caused significant mucosal protection because it is able to reduce the mucosal leukotriene production, and to increase the PGE_2 synthesis (*176*). Based on these properties, a similar mechanism of action may be proposed for **13**. 5-ASA has a protective effect comparable to **13**, and like **13**, does not affect acetic acid-induced LP. Consequently, prevention of LP is not essential for cytoprotection. The effect of 5-ASA on MPO activity suggests that an inhibitory activity on inflammatory mediators is more relevant for cytoprotection (*174*).

L. Mutagenic, Genotoxic and Clastogenic Effects

Nineteen aporphinoids including **5** and **13** were investigated for their possible clastogenicity using a cultured Chinese hamster lung cell line (*177*). Chromosome preparation was made by a direct method (without metabolic activation) or by an S9 method (with metabolic activation). The number of cells with chromosome aberration was counted on 100 well-spread metaphases. The results demonstrated that eighteen alkaloids induced chromosomal aberration with the direct method or the S9 method. Laurofoline (**26**) was the only aporphine that did not induce chromosome aberrations even at the highest dose, 150 μg/mL. Anolobine (**9**), **5** and 4,5-dioxodehydrocrebanine (**117**) induced chromosomal aberrations at relatively low doses, and **9**, **16** and **117** induced chromosomal aberrations with high frequencies. The common chemical structure of these four alkaloids is the presence of a methylenedioxy group at the C-1–C-2 position in the aporphine nucleus (*177*).



4,5-Dioxodehydrocrebanine (**117**)

In a previous Ames test (*178*), the potent mutagenic alkaloids were C-10,11 unsubstituted, while compounds with substituents at positions C-9 and C-10 of the D ring (alkaloid **13**) were alkaloids with little or no mutagenicity. In this test, C-10,11 unsubstituted alkaloids, such as **5** and C-10- or C-10,11-substituted compounds (**16**, **17** and **109**), showed clastogenic effects.

Boldine (**13**) did not show genotoxic activity with or without metabolic activation on the SOS chromotest and different Ames tester strains. Moreover, it was not able to induce point and frameshift mutations in haploid *S. cerevisiae* cells. However, mitotic recombinational events, such as crossing-over and gene conversion, were weakly induced in diploid yeast cells by **13** (*179*). Tavares and Takahashi (*18.01*) studied the possible clastogenic effects of this drug in terms of induction of chromosome aberrations and sister-chromatid exchanges in human peripheral blood lymphocytes treated *in vitro*, and of chromosome aberrations in bone marrow cells of BALB/c mice treated *in vivo*. The *in vitro* experiments for the evaluation of frequency of chromosome aberrations showed that chromatid gaps were the most frequent alterations and that **13** has no statistically significant clastogenic effect. The results obtained for the *in vivo* assay on BALB/c mouse bone marrow cells also showed no clastogenic effect in the different treatments with **13**. Toxicity appears at concentrations higher than 900 mg/kg.

IV. Conclusions

Aporphinoids constitute a good chemical source of dopaminergic agents, and studying them could therefore lead to new neurotropic drugs. In addition, some alkaloids have activity on adrenergic and serotonergic transmission, and in some cases the alkaloid has different effects on the three neuronal systems.

Inhibition of extracellular calcium entry by many aporphinoids has been assessed, and some of them have shown additional tissue-specific influence on intracellular calcium movements. These alkaloids belong predominantly to the *S*-(+)-series, and their potencies sometimes approach those of clinically used drugs. Consequently, they possess a vasodilator effect, which, together with the anti-

adrenergic and anti-platelet activities, explains why aporphine-containing plants are used in ethnotherapeutics.

Aporphines have the appropriate chemical features to behave as antioxidant drugs, and have proved useful in treating hepatic diseases. The presence of free hydroxyl groups, substitution at the nitrogen atom and double bond resonance explains the radical scavenger and antiperoxidative activities of boldine and related alkaloids.

Noraporphines and oxoaporphines have antimicrobial and antitumoral properites. The presence of a methylenedioxy group is a structurally relevant feature in aporphinoids as antibacterial, antiviral and antitumoral agents. In this last case, the presence of this substituent in the oxoaporphine group gives rise to a novel class of DNA topoisomerase II inhibitors.

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CHAPTER 3

ALKALOIDS CONTAINING AN ISOQUINOLINEQUINONE UNIT

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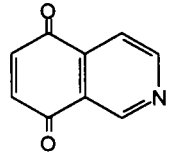
THE ALKALOIDS, VOL. 53
0099-9598/00 $30.00

I. Introduction

The first alkaloid containing the isoquinolinequinone unit **1**, bostrycoidin, appeared in the literature almost half a century ago, in 1954. Since then, nearly seventy alkaloids containing the same unit were reported. Although some are simple compounds, their challenging structures and interesting biological activities made synthetic chemists develop splendid synthetic methodologies for their total syntheses, as well as for their analogues and derivatives. During the course of these studies a series of articles appeared, including reviews *(1-6)*. These alkaloids have been classified in the literature as members of four groups, the aza-anthraquinone type, the saframycin type, the isoquinolinequinone type and the naphthyridinomycin type. Interestingly, and contrary to the alkaloids containing quinolinequinoneimine units *(7)*, to the best of our knowledge, there is no known alkaloid which possesses an isoquinolinequinoneimine unit.



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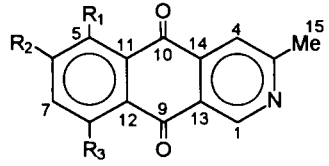
II. Aza-anthraquinone Type Alkaloids

A. BOSTRYCOIDIN

1. Isolation and Structure Elucidation.

In 1954, Cajori *et al.* reported the isolation of a pigment called bostrycoidin **2** from the culture filtrates of *Fusarium bostrycoides (8)*. The molecular formula of this first isolated alkaloid with an isoquinolinequinone unit was initially assigned as

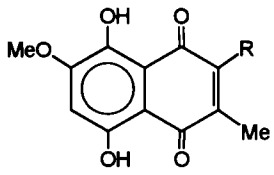


bostrycoidin	**2** R_1=R_3=OH, R_2=OMe
8-*O*-methylbostrycoidin	**3** R_1=OH, R_2=R_3=OMe
5-deoxybostrycoidin	**4** R_1=H, R_2=OMe, R_3=OH
6-*O*-demethyl-5-deoxybostrycoidin	**5** R_1=H, R_2=R_3=OH

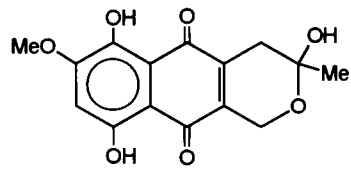
$C_{18}H_{14}O_7$. Later, in 1965, Arsenault isolated the same pigment from the culture filtrate of *Fusarium solani* D_2 together with javanicin (**6**), (+)-solaniol (**7**) and fusarubin (**8**) *(9,10)*.

In 1979, Steyn *et al.* reported the isolation of a derivative of bostrycoidin, 8-*O*-methylbostrycoidin (**3**), from the cultivation of *Fusarium moniliforme* on soil media *(11)*. Javanicin (**6**), solaniol (**7**) and fusarubin (**8**) were also isolated. 8-*O*-Methylbostrycoidin gave a color change from dark-red to light-pink when treated with Zn in AcOH, and on removal of the Zn by filtration, the material was re-oxidized to the starting material. It forms blue alkali-metal salts, and acts as strong chelating agent with cations such as $Ca^{2+}$and Mg^{2+}.

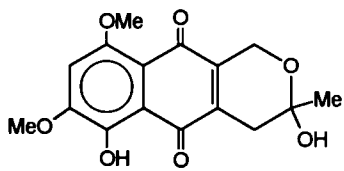
Tatum *et al.* reported in 1985 that 8-*O*-methylbostrycoidin was also produced by *Fusarium oxysporum* isolates obtained from the roots of diseased citrus trees, along with 9-*O*-methylfusarubin (**9**), 9-*O*-methylanhydrofusarubin (**10**), 5-*O*-methyljavanicin (**11**), 5-*O*-methylsolaniol (**12**) and 3,4-dihydroxy-5,7-dimethoxy-2-(2-oxopropyl)-1,4-naphthalenedione (**13**) *(12)*.



javanicin **6** $R=CH_2COCH_3$
(+)-solaniol **7** $R=CH_2CHOHCH_3$



fusarubin **8**



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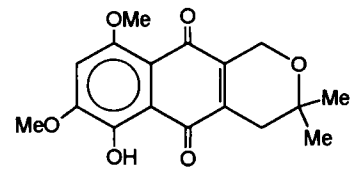

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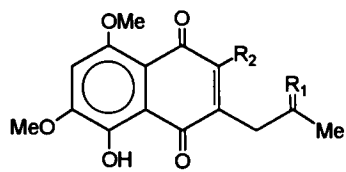


11 $R_1=O$, $R_2=Me$
12 $R_1=H$, OH, $R_2=Me$
13 $R=O$, $R_2=OH$

In 1989, Barbier *et al.* reported the isolation of another derivative of bostrycoidin called 5-deoxybostrycoidin (**4**) from cultures of the fungus *Nectria haematococco (13)*. More recently, 6-*O*-demethyl-5-deoxybostrycoidin (**5**), was isolated by Barbier *et al.*, from the same fungus *Nectria haematococco* in 1990 *(14)*.

For the determination of mycotoxins and other secondary metabolites, including bostrycoidin, some analytical methods using high-performance liquid chromatography have also been developed *(15, 16)*.

Bostrycoidin (**2**): $C_{15}H_{11}NO_5$; brown or lath-shaped crystals; sublimes when heated *in vacuo* as bright red crystals; insoluble in water, soluble in strong acids and alkalis, orange-red in acid solution and deep red in alkaline solution; moderately soluble in organic solvents (1,4-dioxane, benzene, ethanol, $CHCl_3$, CCl_4, acetone); mp 241-243°C; MS *m/z* 285.063 M^+; IR ν 3100, 1615 cm^{-1}; ^{1}H-NMR (60 MHz, $CDCl_3$) δ 9.47 (1H, s, H-1), 2.78 (3H, s, H-15), 7.91 (1H, s, H-4), 13.10 (1H, s, -OH), 4.00 (3H, s, H-6), 6.70 (1H, s, H-7), 13.38 (1H, s, -OH).

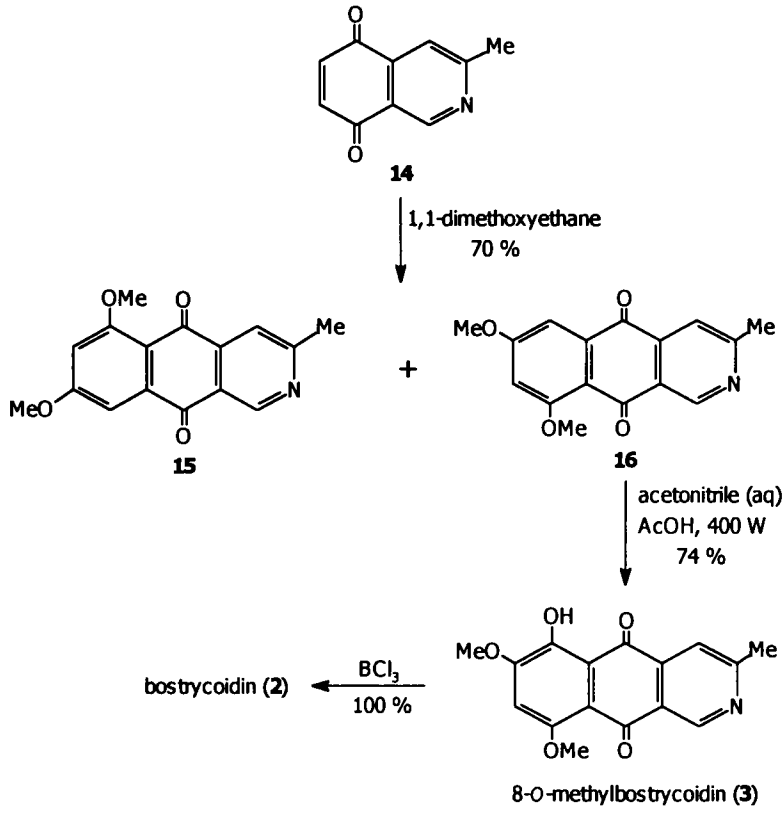
8-*O*-Methylbostrycoidin (**3**): $C_{16}H_{13}NO_5$; mp 215-216°C; MS *m/z* 299.08 M^+; UV (log ε) λ 247.5 (4.50), 318 (3.92), 480 (3.83 nm); (MeOH-acidic) λ 227 (4.32), 262 (4.20), 310 (3.88), 510 (3.73); (MeOH-alkaline) λ 259 (4.44), 306 (3.82), 546 (4.0) nm; IR ν 1641, 1591, 1311, 1265 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 9.44 (1H, s, H-1), 7.85 (1H, s, H-4) 6.86 (1H, s, H-7), 2.76 (3H, s, H-15), 4.05 (6H, s, 2xOCH_3), 13.19 (1H, s, -OH); ^{13}C-NMR ($CDCl_3$) δ 149.6 (C-1), 163.9 (C-3), 116.9 (C-4), 148.9 (C-5), 155.1 (C-6), 104.2 (C-7), 156.0 (C-8), 179.0 (C-9), 188.7 (C-10), 115.4 (C-11), 110.9 (C-12), 125.5 (C-13), 137.4 (C-14), 25.1 (C-15), 56.4 (6-OCH_3), 57.0 (8-OCH_3).

5-Deoxybostrycoidin (**4**): $C_{15}H_{11}NO_4$; yellow needles; mp 195-196°C (CH_2Cl_2-hexane) MS *m/z* 269 M^+; IR (KBr) ν 1680, 1635, 1590 cm^{-1}; UV (MeOH) λ 207, 237, 270, 322 (sh), 414 nm; ^{1}H-NMR (220 MHz, $CDCl_3$) δ 9.40 (1H, s, H-1), 7.86 (1H, s, H-4), 7.32 (1H, d, *J*=2.5 Hz, H-7), 6.74 (1H, d, *J*=2.5 Hz, H-7), 2.78 (3H, s, -CH_3), 3.94 (3H, s, -OCH_3), 12.76 (1H, s, -OH); ^{13}C-NMR (220 MHz, $CDCl_3$) δ 149.1 (C-1), 165.6 (C-3), 118.5 (C-4), 134.6 (C-14), 186 (C-10), 138.7 (C-11), 107.4 (C-5), 165.9 (C-6), 108.1 (C-7), 166.54 (C-8), 110.5 (C-12), 182 (C-9), 124 (C-13), 149.1 (C-1), 25.3 (CH_3), 56.1 (OCH_3).

6-*O*-Demethyl-5-deoxybostrycoidin (**5**): $C_{14}H_9NO_4$; yellow platelets; mp 300-305°C (dec); MS *m/z* 255 M^+; IR (KBr) ν 3200-2400, 1682, 1637, 1600, 1450, 1417, 1342, 1298, 1218, 1200, 1166 cm^{-1}; UV (MeOH, log ε) λ 205 (4.40), 239 (4.42), 280 (4.12), 320 sh. (3.83), 421 (3.75); ^{1}H-NMR (DMSO-d_6) δ 9.20 (1H, s, H-1), 7.80 (1H, s, H-4), 7.10 (1H, d, *J*=2 Hz, H-5), 6.60 (1H, d, *J*=2 Hz, H-7), 2.70 (3H, s, -CH_3), 12.57 (1H, s, -OH-8); ^{13}C-NMR (DMSO-d_6) δ 147.7 (C-1), 164.4 (C-3), 117.8 (C-4), 108.3 (C-5), 165.2 (C-6), 108.1 (C-7), 165.2 (C-8), 185.4 (C-9), 181.6 (C-10), 138.1 (C-11), 109.1 (C-12), 123.8 (C-13), 134.6 (C-14), 24.5 (-CH_3).

2. Synthesis

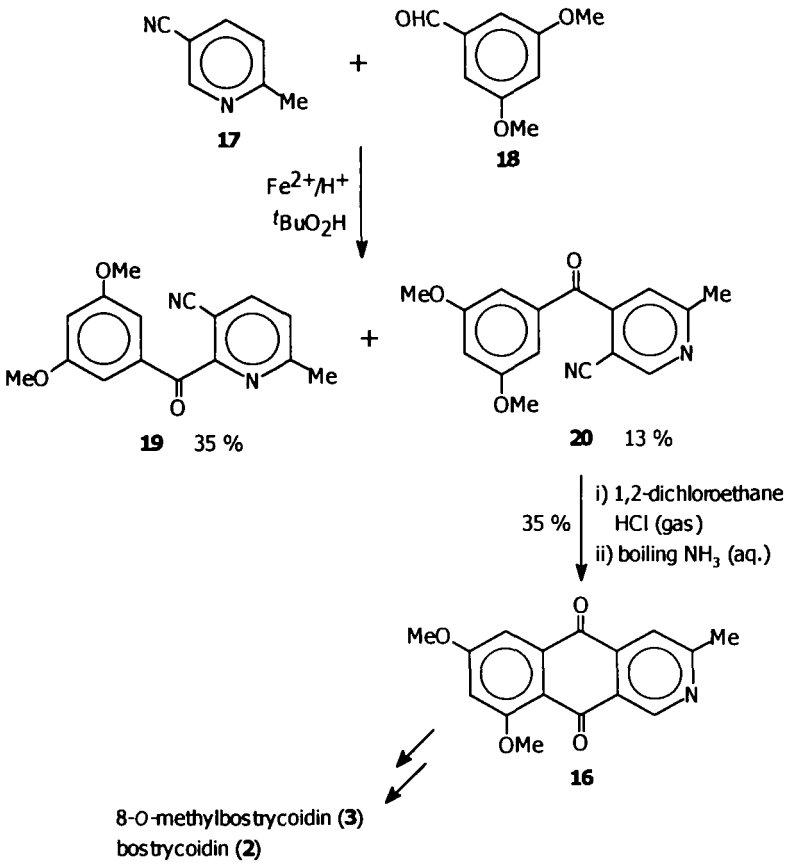
The first total synthesis of bostrycoidin (**2**) and its derivative 8-*O*-methylbostrycoidin (**3**) was reported by Cameron *et al.* in 1980 (Scheme 1) *(17)*. Their synthesis started with the annulation of isoquinolinequinone (**14**) with 1,1-



SCHEME 1. Total synthesis of 8-*O*-methylbostrycoidin (3) and bostrycoidin (2) *(17)*.

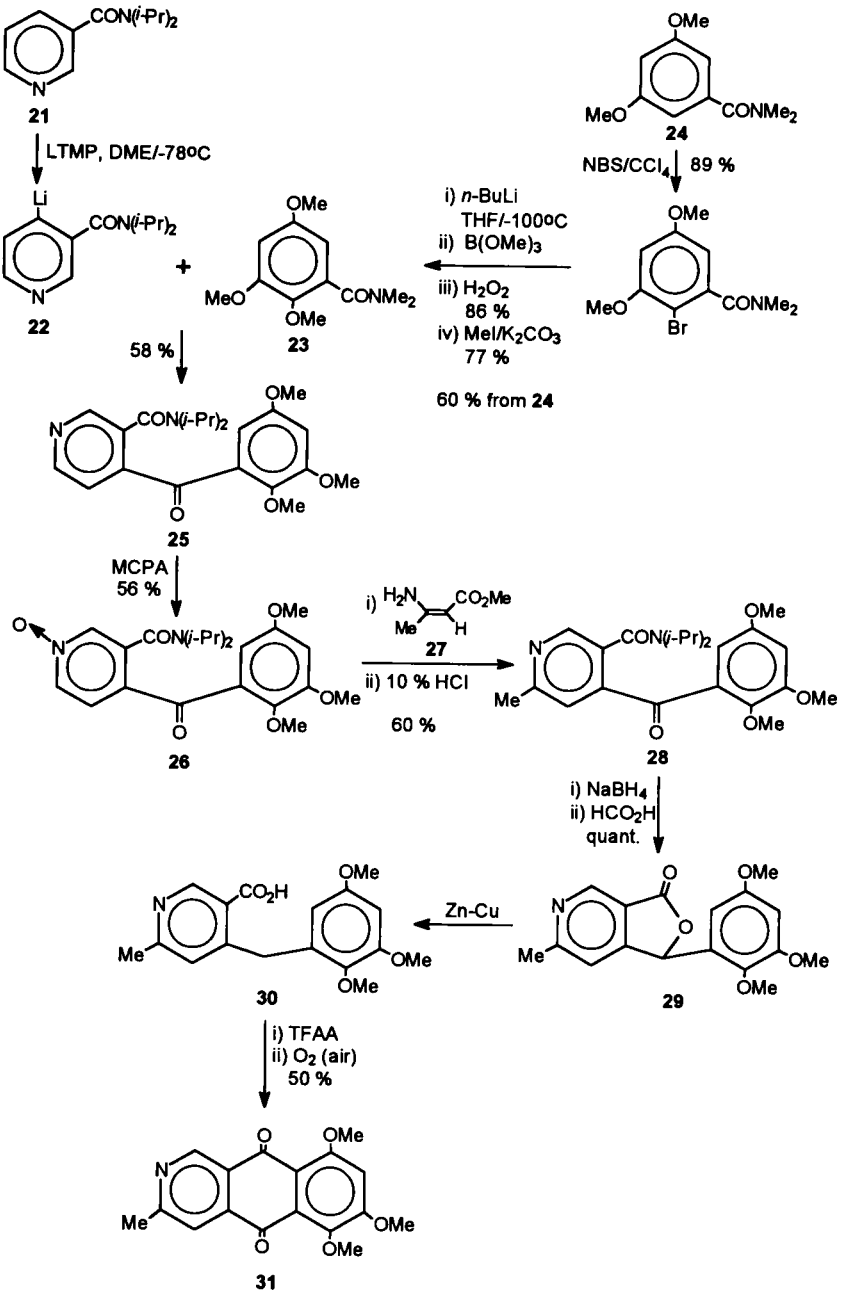
dimethoxyethane which gave a mixture of two regioisomers **15** and **16** in a 7:1 ratio, respectively. Conversion of the azaanthraquinone **16** to 8-*O*-methylbostrycoidin (**3**) was achieved by irradiation with a 400 W medium pressure mercury lamp and selective demethylation of **3** with boron trichloride provided bostrycoidin (**2**) in excellent yield.

Cameron *et al.* also reported a different approach to the synthesis of bostycoidin (**2**) and 8-*O*-methylbostrycoidin (**3**) (Scheme 2) *(18, 19)*, through acylation of the pyridine **17** with 3,5-dimethoxybenzaldehyde **18** using iron(II) sulfate and *t*-butyl hydroperoxide. The aldehyde radical generated by the action of iron(II) sulfate and *t*-butyl hydroperoxide attacked the pyridine **17** at the 2- and 4-positions to yield **19** and **20**, respectively. Treatment of **20** dissolved in 1,2-dichloroethane with hydrogen chloride gas, and then with boiling aqueous ammonia, led to the formation of **16**, which is an intermediate in their previous synthesis (Scheme 1) *(17)*.

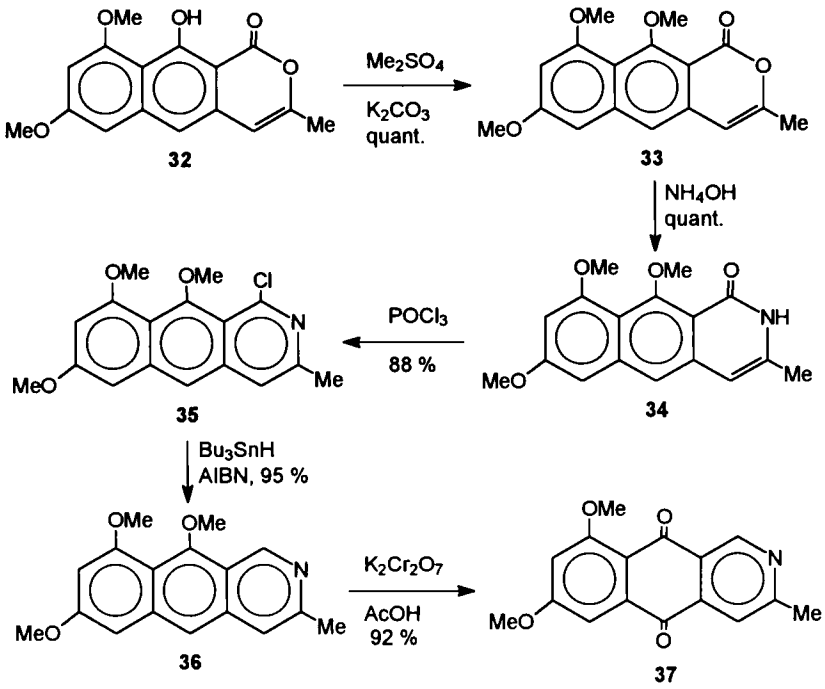


SCHEME 2. Synthesis of 8-*O*-methylbostrycoidin (**3**) and bostrycoidin (**2**) *(18, 19)*.

In 1987, Watanabe *et al.* reported their rather long synthesis of bostrycoidin involving directed lithiation of a tertiary nicotinamide (Scheme 3) *(20)*. Selective lithiation of *N,N*-diisopropylnicotinamide (**21**) at the 4- position was achieved using LTMP, prepared from *n*-BuLi and 2,2,6,6-tetramethylpiperidine at -78°C. The lithiated product was then treated with *N,N*-dimethyl-2,3,5-trimethoxybenzamide (**23**), which was prepared from **24** in five steps (Scheme 3), to give *N,N*-diisopropyl-4-(2,3,5-trimethoxybenzoyl) nicotinamide (**25**). Treatment of the keto-amide **25** with *m*-chloroperbenzoic acid (MCPBA) yielded the *N*-oxide **26**. Reaction of **26** with methyl 3-aminocrotonate (**27**) in the presence of benzenesulfonyl chloride was followed by hydrolysis of the crude adduct with 10 % HCl to give the 6-methylated compound **28**. The ket one group of **28** was reduced to an alcohol with $NaBH_4$, and then treatment with formic acid yielded **29**, the lactone of which was



SCHEME 3. Formal total synthesis of bostrycoidin (2) *(20)*.



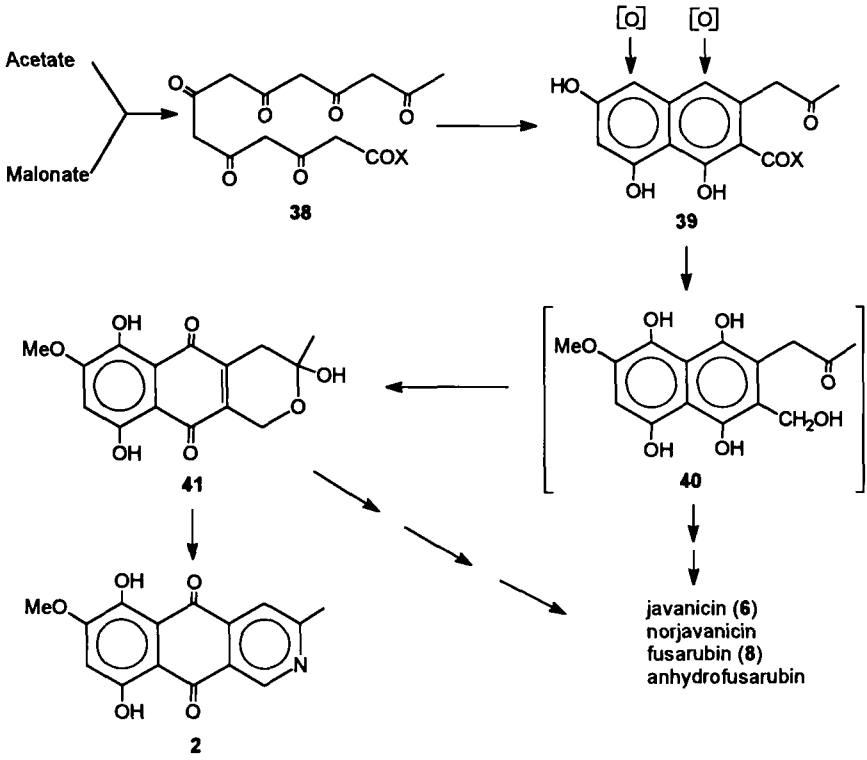
SCHEME 4. Formal total synthesis of bostrycoidin (**2**) *(21)*.

reductively cleaved by zinc-copper couple to afford the acid **30**. Cyclization of **30** was carried out in trifluoroacetic anhydride (TFAA), and then air oxidation of the product furnished bostrycoidin dimethylether (**31**). As bostrycoidin dimethylether had already been converted to bostrycoidin *(17-19)*, a formal total synthesis was completed.

The latest formal total synthesis of bostrycoidin (**2**) was reported by Deshpande *et al.* in 1996 (Scheme 4) *(21)*. Their synthesis was initiated by methylation of hydroxynaphthopyrone **32** with $MeSO_4$ which yielded **33**. Treatment of **33** with aqueous ammonia afforded the isoquinolone **34**, the lactam ring of which was aromatized by treatment with $POCl_3$ to obtain the isoquinoline **35**. Dehalogenation of **35** with tributyltin hydride and AIBN gave **36** which was oxidized with potassium dichromate in acetic acid to afford the aza-anthraquinone **37**. As the photochemical hydroxylation and subsequent demethylation of **37** had been reported, this constituted a formal total synthesis of bostrycoidin.

3. Biosynthesis

Based on a series of studies *(22-24)*, Kurobane *et al.* proposed a biosynthetic pathway for bostycoidin (**1**) which had the ketide **38** as a starting point (Scheme 5).



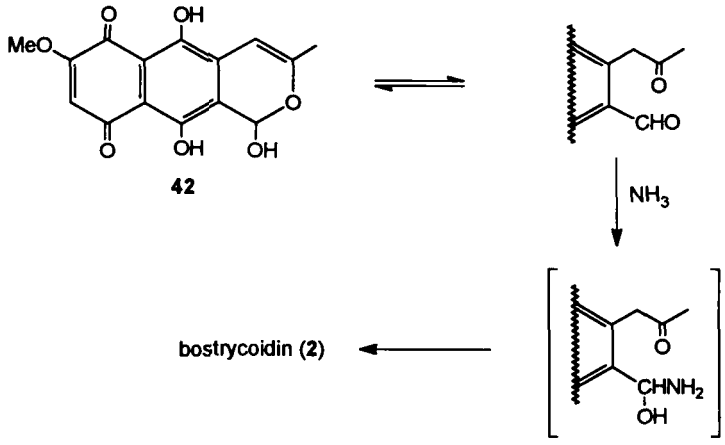
SCHEME 5. Biogenesis of bostrycoidin (2) *(22)*.

The ketide undergoes a sequence of modifications including hydroxylation of **39** to form the intermediate **40**, and ring closure on **40** leads to the formation of the lactone **41**. Amination of **41** would result in the biosynthesis of bostrycoidin (**2**). Some natural products such as javanicin (**6**), norjavanicin, fusarubin (**8**) and anhydrofusarubin could also originate from the intermediates **40** and **41**.

The hypothesis on the biosynthesis of bostrycoidin from dihydrofusarubin (**41**) in the presence of ammonia was investigated by Parisot *et al. (25)* with anhydrofusarubin lactol (**42**), the hemiacetal of fusarubin aldehyde identified from *Fusarium solani (26)* (Scheme 6). When the lactol **42** was treated with ammonia (conc.) in refluxing benzene, 100% conversion into bostrycoidin was observed. Furthermore, slow and complete conversions at room temperature and at 60°C, respectively, were also performed.

4. Biological activity

Bostrycoidin (**2**) was reported to exhibit antibiotic properties and be active against the tubercle bacillus, strain H-37, *in vitro (8)*. In bacterial cells, it stimulated



SCHEME 6. Formation of bostrycoidin (2) from anhydrofusarubin lactol 42 *(25)*.

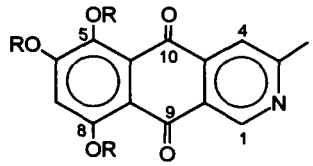
the oxygen consumption and induced cyanide-insensitive oxygen consumption *(27)*. Bostrycoidin acted as the electron acceptor for bacterial diaphorase and also stimulated the generation of superoxide anion and hydrogen peroxide.

B. TOLYPOCLADIN

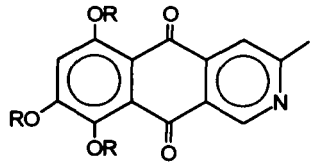
1. Isolation and structure elucidation

In 1990, Gräfe *et al.* reported the isolation of a new aza-anthraquinone type alkaloid called tolypocladin (**43**) from the mycelium of *Tolypocladium inflatum* DSM 915 *(28)*. It differs from bostrycoidin (**2**) *(8)* only with the hydroxyl group at C-6 in place of the methoxy group in bostrycoidin. Its production was greatly effected by the presence of 4-8 mg $ZnSO_4.7H_2O$/l in the medium, and water-soluble fluorescent aluminium complexes.

Tolypocladin (**43**): reddish-brown amorphous powder; soluble in DMF, DMSO and water pH > 9, insoluble in *n*-hexane and water pH< 7, moderately soluble in methanol acetone and chloroform; mp >300°C (dec); sublimes *in vacuo* (230-260°C); MS *m/z* 271.047 M^+; IR λ 3600-2905 (br), 1623, 1585, 1456, 1412, 1309, 1118, 928, 763 cm^{-1}; UV (acid form in MeOH) ν 505, 545, 460, 320, <250 nm; (trisodium salt in methanol) 560, 525, 420, 290 nm; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.23 (1H, s, H-1), 7.88 (1H, s, H-4), 6.69 (1H, s, H-7), 2.70 (3H, s, CH_3), 11.55 (1H, s, OH), 12.77 (1H, s, OH), 13.32 (1H, s, OH); ^{13}C-NMR (100 MHz, MSO-d_6) δ 25.05, 105.38, 110.69, 113.29, 118.12, 124.61, 138.71, 148.37, 150.36, 157.76, 161.04, 165.33, 183.22, 186.27.



tolypocladin **43** R=H
triacetyl-tolypocladin **44** R=Ac

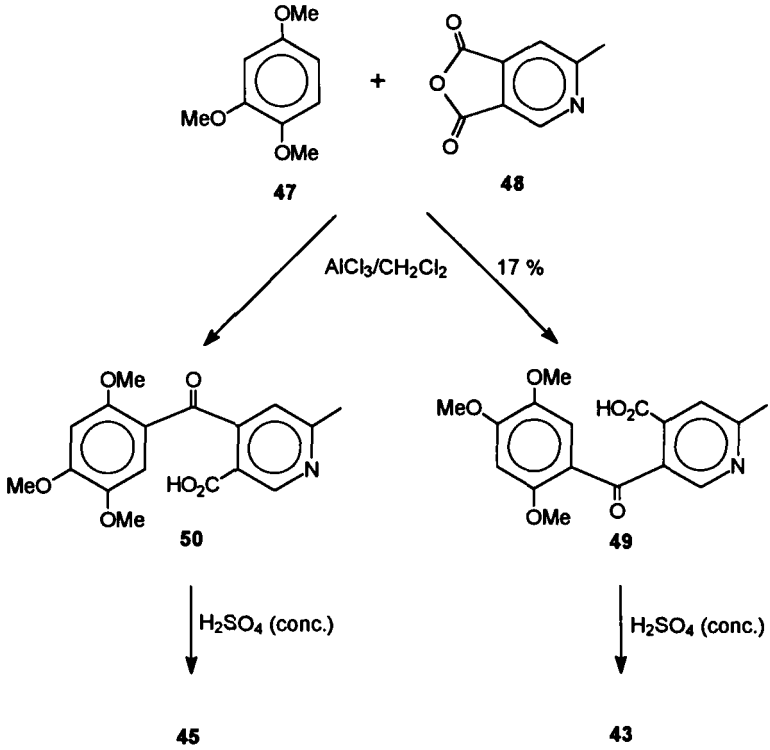


isotolypocladin **45** R=H
triacetyl-isotolypocladin **46** R=Ac

2. Synthesis

In 1997, a simple synthesis of tolypocladin (**43**) (and its isomer isotolypocladin (**45**)) was reported by Werner *et al.* (Scheme 7) *(29)*.

Condensation of trimethoxybenzene **47** with 2-methylpyridine-4,5-dicarboxylic acid anhydride (**48**) in the presence of aluminum chloride yielded the products **49** and **50** which were separated by recrystallization. Cyclization and



SCHEME 7. Total synthesis of tolypocladin (**43**) and its isomer isotolypocladin (**45**) *(29)*.

demethylation of **49** and **50** in sulfuric acid gave the desired products tolypocladin (**43**) and its isomer isotolypocladin (**45**), respectively. Because of their low solubility, purification of the products **43** and **45** could not be achieved. On the other hand, their tri-*O*-acetyl derivatives **44** and **46** were easily purified, by recrystallization or chromatography.

3. Biological activity

Tolypocladin (**43**) was reported to be inactive against Gram-positive bacteria (*Bacillus subtilis* ATCC 6633) and the fungi (*Phoma spp., Penicillium spp.*) *(28)*. On the other hand, because of its metal-chelating properties, tolypocladin was thought to act as a scavenger of trace elements or as a detoxifying ligand for high concentrations of heavy metals *(28, 29)*.

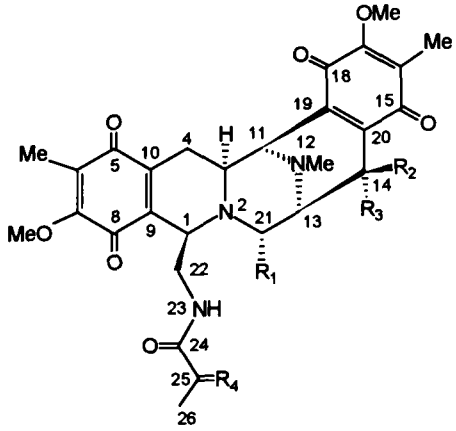
III. Saframycin Type Alkaloids

A. SAFRAMYCINS

1. Isolation and structure elucidation

In 1977, Arai *et al.* reported the isolation of a five-component antibiotic complex from the culture broth of *Streptomyces lavendulae* No. 314 *(30)*, which is the source of the satellite antibiotics chlorocarcins A, B, and C *(31)* and mimosamycin (**208**) *(106)*. Isolation of the complex was achieved following the same procedure as applied to chlorocarcins, and the five components were characterized as saframycins A (**51**), B (**52**), C (**53**), D (**63**) and E (**65**). Among them, due to the instability of the molecule, the structure of saframycin E was suggested in 1995 by Kubo *et al. (32)*. During their synthetic studies they synthesized the triacetate form of **65** which was proved to be identical with the triacetyl derivative of natural saframycin E (Scheme 14). The complete structure and stereochemistry of saframycin C (**53**) was established by a single crystal X-ray diffraction analysis of its hydrobromide, and the structure of the closely related saframycin B (**52**) was then assigned on the basis of experimental evidence *(33)*.

The structure of saframycin A *(51)*, which possesses a nitrile group at C-21, was deduced by intense spectroscopic studies, including high field NMR analysis on the conformations of saframycins A and C *(34-36)*. In order to increase the production of this highly active antibiotic, sodium cyanide was added to the broth, which resulted in remarkable increase in the amount of saframycin A *(37, 38)*. The incorporation of cyanide into the nitrile moiety of saframycin A was proved by addition of $Na^{14}CN$ to the broth. This gave saframycin A labeled with ^{14}C. It was understood that incorporation of cyanide into saframycin A is a stoichiometric reaction, that is addition of 1 mole of ^{14}C into the broth corresponded to an increment of 1 mol of saframycin A. Treatment of saframycin A (**51**) with acid caused hydrolysis of the nitrile functionality and produced saframycin S (**56**), decyanosaframycin A, which is expected to exist in two forms, the iminium salt and the α-carbinolamine. Furthermore, treatment of saframycin S with NaCN in neutral or acidic solution formed saframycin A (Scheme 8) *(37-39)*.

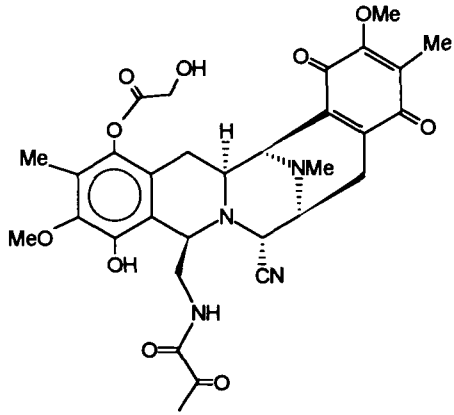


Saframycin A **51** R_1=CN, R_2=R_3=H, R_4=O
(AR_2) B **52** R_1=R_2=R_3=H, R_4=O
C **53** R_1=R_2=H, R_3=OMe, R_4=O
G **54** R_1=CN, R_3=OH, R_2=H, R_4=O
H **55** R_1=CN, R_2=R_3=H, R_4=OH, CH_2COCH_3
S **56** R_1=OH, R_2=R_3=H, R_4=O

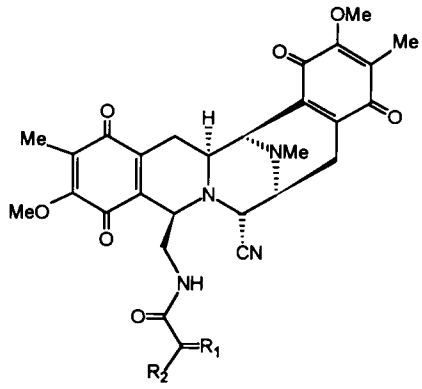
Saframycin AH_1 **57**
AH_2 (=AR_1) **58**
} R_1=CN, R_2=R_3=H, R_4=OH,H

AH_1Ac **59**
AH_2Ac **60**
} R_1=CN, R_2=R_3=H, R_4=H, $OCOCH_3$

AR3 (=BH_1) **61** R_1=R_2=R_3=H, R_4=H, OH

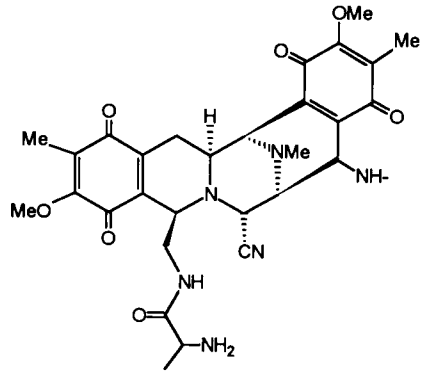


Saframycin R **62**



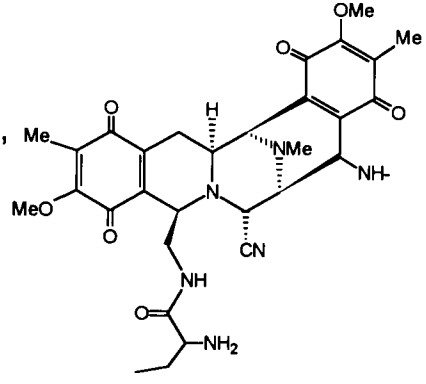
Saframycin Y3 **66** R_1=NH_2, H, R_2=Me
Yd-1 **67** R_1=NH_2, H, R_2=CH_2CH_3
Yd-2 **68** R_1=NH_2, H, R_2=H
Ad-1 **69** R_1=O, R_2=CH_2CH_3

Saframycin Y2b **70** R_1= H,



R_2=Me

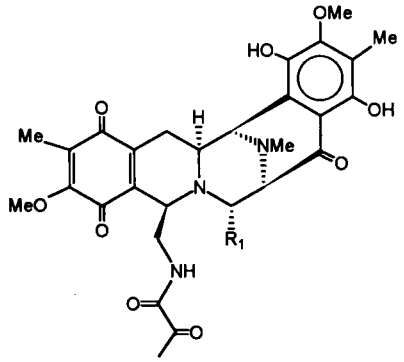
Y2b-d **71** R_1= H,



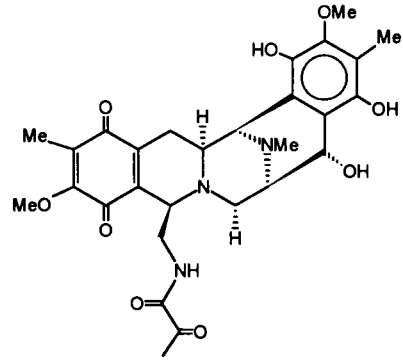
R_2=CH_2CH_3

In 1982, Arai *et al.* reported that saframycin A (**51**) was converted to 25-dihydrosaframycin A (**58**) (AH_2 or AR_1), saframycin B (**52**) (AR_2) and 21-decyano-25-dihydrosaframycin A (**61**) (AR_3 or BH_1) by *R. amidophilus* IFM 144 *(40)*. When the conversion was examined in various types of *Actinomycetes*, such as *Mycobacterium*, *Nocardia* and *Streptomyces*, only saframycin $AR_1$was converted by the *Mycobacterium* type, AR_1, AR_2 and AR_3 by *Nocardia* type and no conversion by the *Streptomyces* type were observed *(41)*.

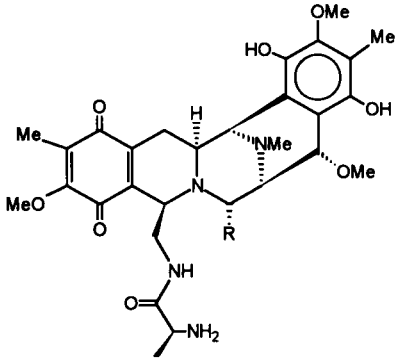
Also in 1982, a new saframycin, saframycin R (**62**) was isolated by Arai *et al.* during the course of a study on the minor components in the culture of *Streptomyces lavendulae* No. 314. It differs from other saframycins in having reduced carbonyl groups in one of the quinone rings, i.e. it is a hydroquinone derivative *(42)*. Its full structure and confirmation by high field NMR (^1H and ^{13}C) was then disclosed in 1983 *(43)*.



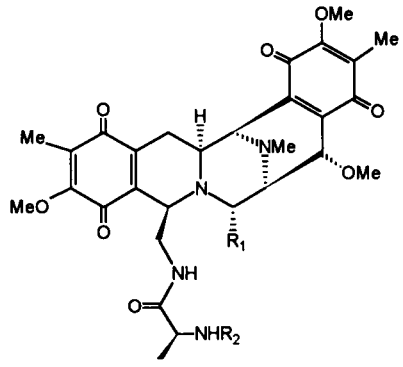
Saframycin D **63** R_1=H
F **64** R_1=CN



Saframycin E **65**

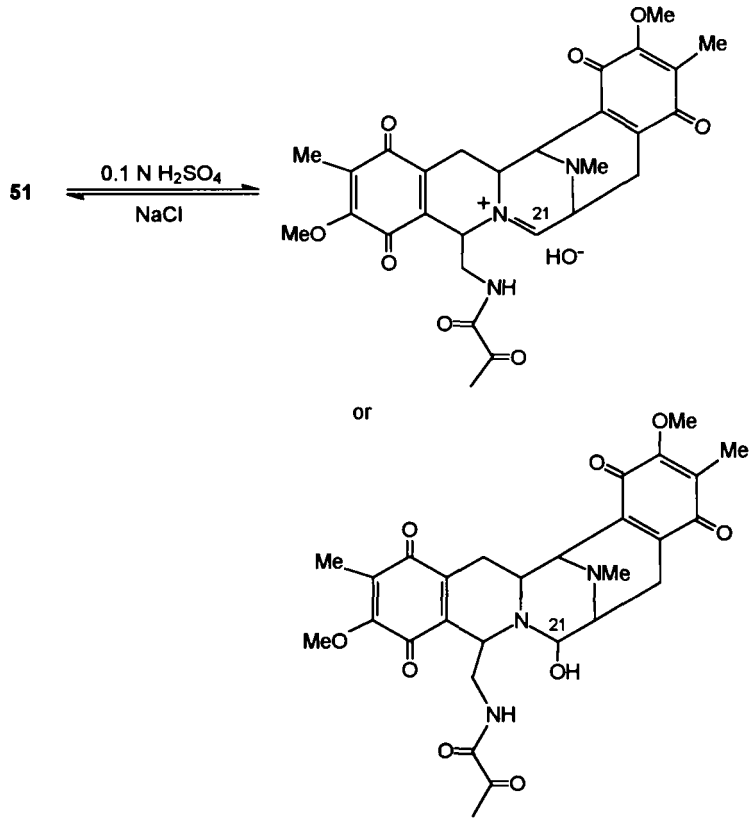


Saframycin Mx1 **72** R=OH
Mx2 **73** R=H



Saframycin Mx3 **74** R_1=OH, R_2=Ac
75 R_1=CN, R_2=H

Although the isolation and spectroscopic data of saframycin D (**63**) were reported in 1977 when the streptomycins were first isolated, its structure was disclosed later in 1984, along with other saframycins F (**64**), G (**54**), H (**55**), AH_1 (**57**), AH_1Ac (**59**) and AH_2Ac (**60**) *(44)*. The detailed spectroscopic studies, particularly on the explanation of structures of saframycins D *(45)*, F, G and H *(46)*, then appeared in the literature. Saframycin D (**63**) and F (**64**) differ from the rest of the saframycins, having one of the benzoquinones reduced to hydroquinone as for saframycin R (**62**) and both have a carbonyl group at C-14. The difference between saframycin D and F is the presence of cyano group attached to C-21 in saframycin F; that is saframycin F could be called 21-cyanosaframycin D. Saframycin G (**54**) and H (**55**) are basically similar to saframycin A, and only differ having an α-equatorial hydroxy functionality attached to C-14 and hydroxy and β-ketopropyl groups at C-25, respectively. Saframycins AH_1Ac (**59**) and AH_2Ac (**60**) were obtained by acetylation of saframycin AH_1 (**57**) and AH_2 *(44)*.



Saframycin S **56**

SCHEME 8. Production of saframycin S (**56**) *(35-37)*.

In 1985, Arai *et al.* reported that, considering the biosynthesis of saframycin, they attempted to prepare some derivatives altered in the side chains by precursor directed-biosynthesis *(47)*. In this way, initially the synthesis of saframycins Y3 (**66**), Yd-1 (**67**) and Yd-2 (**68**) were achieved by supplementing the *S. lavendulae* resting cells with either alanine and glycine or alanylglycine for the production of Y3, 2-amino-*n*-butyric acid and glycine or 2-amino-*n*-butyrylglycine for Yd-1 and with glycylglycine for Yd-2. In 1986, during the course of a similar study, the same group isolated three more saframycin derivatives *(48)*. When they supplemented the resting cells with tyrosine, methionine, 2-amino-*n*-butyric acid and glycine or 2-amino-*n*-butyrylglycine, Yd-1 (**67**), Ad-1 (**69**) and Y2b-d (**71**) were produced, and when the supplementation was carried out with alanine and glycine, or alanylglycine in addition to methionine and tyrosine, saframycin Y3 (**66**) and Y2b (**70**) were isolated. Y2b was characterized as a dimer of Y3, which is a dehydrogenation product of two molecules of Y3, and saframycinY2b-d is a dimerof saframycinYd-1.

In 1988, Kienast *et al.* reported the isolation and characterization of two new antibiotics from the culture broth of the myxobacterium, *Myxococcus xanthus* strain Mxx48 *(49)*. These structurally similar compounds to the saframycins were called saframycin Mx1 (**72**) and Mx2 (**73**), which mainly differ from the *Streptomyces* products in their side chain by having alanine rather than pyruvic acid *(49, 50)*. Compounds with an alanine side chain could also be obtained from *Streptomyces*, but only by mutasynthesis *(47)*. Like saframycins D (**63**) and F (**64**), one of the benzoquinone rings of saframycins Mx1 (**72**) and Mx2 (**73**) is reduced to a hydroquinone, which was oxidized back to benzoquinone during the conversion of Mx1 to its corresponding acetyl Mx3 (**74**) and cyano (**75**) derivatives *(49)*.

A reversed-phase HPLC procedure was later developed for a good separation of mixtures of saframycins *(51, 52)*. Although a thin-layer chromatographic method was available, this method did not suffice for identification and precise quantification. A technique was also developed for polarographic and voltammetric determination of the individual saframycins *(52)*. This technique allows classification of the different saframycins into four groups in terms of their structures.

Saframycin A (**51**): $C_{29}H_{30}N_4O_8$; Yellow powder; mp 122-126°C; Mass *m/z* 562 M^+; $[\alpha]^{20}_D$ +18.2° (*c* 0.9, MeOH); IR ($CHCl_3$) ν 3400, 1716, 1685, 1660, 1615 cm^{-1}; UV (MeOH, log ε) λ_{max} 267 (4.34), λ_{min} 230 (3.88) nm; ^{1}H-NMR ($CDCl_3$) δ 3.89 (1H, ddd, *J*=4.5, 2.9, 1.5 Hz, H-1), 3.14 (1H, ddd, *J*=11.7, 2.6, 2.6 Hz, H-3), 2.28 (1H, dd, *J*=17.6, 2.6 Hz, Ha-4), 1.29 (1H, ddd, *J*=17.6, 11.7, 2.9 Hz, Hb-4), 4.01 (1H, d, *J*=2.6 Hz, H-11), 3.43 (1H, ddd, *J*=8.2, 2.9, 1.9 Hz, H-13), 2.83 (1H, dd, *J*=20.9, 8.2 Hz, Ha-14), 2.24 (1H, dd, *J*=20.9, 2.9 Hz, Hb-14), 4.07 (1H, d, *J*=1.9 Hz, H-21), 3.84 (1H, ddd, *J*=14.3, 8.8, 1.5 Hz, Ha-22), 3.27 (1H, ddd, *J*=14.3, 4.5, 4.5 Hz, Hb-22), 6.86 (1H, dd, *J*=8.8, 4.5 Hz, NH), 4.03 and 4.02 (2x3H, s, 2xOCH_3), 2.32 (3H, s, NCH_3), 1.99 and 1.93 (2x3H, s, 2xCH_3), 2.25 (3H, s, $COCH_3$); ^{13}C-NMR ($CDCl_3$) δ 54.0 or 54.3 (C-1 or 11), 54.6 or 56.3 (C-3 or 13), 25.1 (C-4, t), 21.6 (C-14, t), 58.3 (C-21, d), 40.7 (C-22, t), 185.2 or 186. 5 (C-5 or C-15), 128.3 or 129.2 (C-6 or C-16), 155.6 or 155.9 (C-7 or C-17); 180.8 or 183.4 (C-8 or C-18), 135.6 (C-9 and C-19), 141.2 or 141.6 (C-10 or C-20), 8.7 (-CH_3 6 and 16), 61.0 or 61.1 (-OCH_3 7 or 17), 42.6 (N-CH_3), 160.2 (NHCO), 196.7 and 24.3 ($COCH_3$), 116.7 (CN).

Saframycin B (**52**): $C_{28}H_{31}N_3O_8$; orange yellow prisms; mp 108-109°C; Mass *m/z* 537 M^+; $[\alpha]^{20}_D$ -54.4° (*c* 1.0, MeOH); IR ($CHCl_3$) ν 3430, 1720, 1690, 1660, 1620 cm^{-1}; CD (MeOH): 275 nm (Δε -14.8); UV (MeOH, log ε) λ_{max} 269 (4.35), 368 (3.13), λ_{min} 232 (3.86), 330 (3.10); ^{1}H-NMR ($CDCl_3$) δ 3.66 (1H, ddd, *J*=4.2, 2.9, 1.3 Hz, H-1), 2.74 (1H, ddd, *J*=10.7, 3.5, 2.4 Hz, H-3), 2.76 (1H, dd, *J*= 16.7, 3.5 Hz, Ha-4), 1.28 (1H, ddd, *J*=16.7, 10.7, 2.9 Hz, Hb-4), 4.03 (1H, dd, *J*=2.4, 0.5 Hz, H-11), 3.17 (1H, dddd, *J*=7.3, 2.4, 2.0, 0.5 Hz, H-13), 2.82 (1H, dd, *J*=10.7, 2.0 Hz, Ha-21), 2.98 (1H, dd, *J*=10.7, 2.4 Hz, Hb-21), 3.70 (1H, ddd, *J*=14.1, 9.8, 1.3 Hz, Ha-22), 3.20 (1H, ddd, *J*=14.1, 4.2, 3.5 Hz, Hb-22), 6.90 (1H, dd, *J*=9.8, 3.5 Hz, NH), 4.01 and 4.00 (2x3H, s, OCH_3), 2.00 and 1.90 (2x3H, s, CH_3), 2.28 (3H, s, NCH_3), 2.24 (3H, s, $COCH_3$); ^{13}C-NMR ($CDCl_3$) δ 185.7 or 187.0 (C-5 or C-15), 127.7 or 129.2 (C-6 or C-16), 155.5 or 156.1 (C-7 or C-17), 181.3 or 182.8 (C-8 or C-18), 136.3 or 136.6 (C-9 or C-19), 141.6 or 142.8 (C-10 or C-20), 8.6 (-CH_3 6 and 16), 60.9 (-OCH_3 7 and 17), 41.2 (N-CH_3), 52.2 or 54.8 (C-1 or C-11), 56.9 or 57.4 (C-3 or C-13), 25.6 (C-4, t), 22.7 (C-14, t), 58.7 (C-21, t), 40.4 (C-22, t), 160.1 (NHCO), 196.5 and 24.2 (*COCH*$_3$).

Saframycin C (**53**): $C_{29}H_{33}N_3O_9$; orange needles; mp 143-146°C; Mass *m/z* 567 M^+; $[\alpha]^{20}_D$ -20.8° (*c* 1.0, MeOH); CD (MeOH): 273 nm (Δε -28.3); IR ($CHCl_3$) ν 3400, 1720, 1685, 1655, 1615 cm^{-1}; UV (MeOH, log ε) λ_{max} 266.5 (4.32), 368 (3.19), λ_{min} 230 (3.86), 330 (3.16) nm; ^{1}H-NMR (100 MHz, $CDCl_3$) δ 1.86 (3H, s), 2.00 (3H, s), 2.38 (3H, s), 2.44 (3H, s), 3.46 (3H, s), 3.96 (6H, s), 6.60 (1H, br) (for detailed ^{1}H-NMR studies see ref. *35*); ^{13}C-NMR ($CDCl_3$) δ 185.5 or 186.6 (C-5 or C-15), 127.9 or 130.7 (C-6 or C-16), 155.4 or 156.1 (C-7 or C-17), 181.3 or 183.2 (C-8 or C-18), 136.6 (C-9 and C-19), 141.5 or 141.6 (C-10 or C-20), 8.7 or 9.0 (-CH_3 6 or 16), 60.9 or 61.0 (-OCH_3 7 or 17), 42.3 (N-CH_3), 55.2 or 55.7 (C-1 or C-11), 57.6 or 58.0 (C-3 or C-13), 25.5 (C-4, t), 71.9 (C-14, d), 59.3 (-OCH_3 14), 55.7 (C-21, t), 40.7 (C-22, t), 160.2 (NHCO), 196.5 and 24.3 (*COCH*$_3$).

Saframycin D (**63**): $C_{28}H_{31}N_3O_9$; yellow needles; mp 150-154°C; Mass *m/z* 553 M^+; $[\alpha]^{20}_D$ + 141.0° (*c* 1.0, MeOH); IR ($CHCl_3$) ν 3560, 3400, 1720, 1685, 1660, 1630 cm^{-1}; UV (MeOH, log ε) λ_{max} 243 (4.14), 274 (4.24), 369 (3.75), λ_{min} 231 (4.08), 253 (4.06), 319 (3.30) nm; ^{1}H-NMR ($CDCl_3$) δ 3.68 (1H, ddd, *J*=4.0, 3.0, 1.5 Hz, H-1), 2.93 (1H, ddd, *J*=11.0, 3.0, 3.0 Hz, H-3), 2.96 (1H, dd, *J*=18.0, 3.0 Hz, Ha-4), 1.58 (1H, ddd, *J*=18.0, 11.0, 3.0 Hz, Hb-4), 4.31 (1H, dd, *J*=3.0, 0.5 Hz, H-11), 3.28 (1H, ddd, *J*=3.0, 2.0, 0.5 Hz, H-13), 3.28 (1H, dd, *J*=10.5, 3.0 Hz, Ha-21), 2.93 (1H, dd, J=10.5, 2.0 Hz, Hb-21), 3.71 (1H, ddd, *J*=14.0, 9.0, 1.5 Hz, Ha-22), 3.06 (1H, ddd, *J*=14.0, 4.0, 3.0 Hz, Hb-22), 6.28 (1H, dd, *J*=9.0, 4.0 Hz, NH), 4.02 and 3.93 (2x3H, s, 2xOCH_3), 2.43 (3H, s, NCH_3), 2.15 and 1.89 (2x3H, s, 2xCH_3), 2.26 (3H, s, $COCH_3$); ^{13}C-NMR ($CDCl_3$) δ 57.6 (C-1, d), 57.0 (C-3, d), 24.5 (C-4, t), 186.1 (C-5), 153.3 (C-15), 127.5 (C-6), 118.7 (C-16), 156.3 (C-7), 154.9 (C-17), 181.2 (C-8), 139.4 (C-18), 136.6 (C-9), 112.2 (C-19), 141.8 (C-10), 118.2 (C-20), 57.4 (C-11, d), 65.5 (C-13, d), 203.7 (C-14), 54.8 (C-21, t), 40.8 (C-22, t), 160.3 (C-23), 195.8 (C-24), 24.3 (C-25, q), 8.9 and 8.6 (CH_3), 61.2 and 61.0 (OCH_3), 42.4 (NCH_3).

Saframycin E (**65**): $C_{28}H_{33}N_3O_9$; yellow powder; mp 146-148°C; $[\alpha]^{20}_D$ -37.3° (*c* 0.53, MeOH); IR (KBr) ν 3380, 1720, 1685, 1655, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 272 (4.10), 368 (2.98), λ_{min} 241 (3.84), 340 (2.96) nm; ^{1}H-

NMR (100 MHz, pyridine-d_5) δ 1.83 (3H, s), 2.17 (3H, s), 2.30 (3H, s), 2.48 (3H, s), 3.82 (3H, s), 3.95 (3H, s), 5.22 (1H, s).

Saframycin F (**64**): $C_{29}H_{30}N_4O_9$; light yellow powder; mp 134-136°C (dec); EIMS *m/z* 578 M^+; $[\alpha]^{22}_D$ +28.4° (*c* 0.1, MeOH); IR ($CHCl_3$) ν 3550, 3400, 1720, 1690, 1660, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 231 (3.98), 277 (4.11), 375 (3.65) nm; ^{1}H-NMR ($CDCl_3$) δ 3.98 (1H, bs, H-1), 3.33 (1H, ddd, *J*=11.3, 3.2, 3.2 Hz, H-3), 3.07 (1H, dd, *J*=17.8, 3.3 Hz, Ha-4), 1.53 (1H, ddd, *J*=17.8, 11.3, 2.6 Hz, Hb-4), 4.37 (1H, dd, *J*=3.2, 1.5 Hz, H-11), 3.47 (1H, dd, *J*=2.9, 1.5 Hz, H-13), 4.26 (1H, d, *J*=2.9 Hz, Ha-21), 3.71 (1H, ddd, *J*=14.6, 9.5, 1.5 Hz, Ha-22), 3.05 (1H, ddd, *J*=14.6, 3.8, 3.8 Hz, Hb-22), 6.12 (1H, dd, *J*=9.5, 3.8 Hz, NH), 4.04 and 3.94 (2x3H, s, 2xOCH_3), 2.47 (3H, s, NCH_3), 2.17 and 1.83 (2x3H, s, 2xCH_3), 2.25 (3H, s, $COCH_3$); ^{13}C-NMR ($CDCl_3$) δ 56.2 (d, C-1), 53.4 (d, C-3), 24.3 (t, C-4), 185.7 (s, C-5), 127.9 (s, C-6), 156.2 (s, C-7), 180.6 (s, C-8), 139.8 (s, C-9), 141.1 (s, C-10), 54.4 (d, C-11), 59.0 (d, C-13), 198.9 (s, C-14), 153.4 (s, C-15), 119.3 (s, C-16), 155.3 (s, C-17), 141.1 (s, C-18), 111.8 (s, C-19), 56.8 (d, C-21), 40.8 (t, C-22), 195.8 (s, *C*OCH_3), 160.3 (s, NHCO), 117.1 (s, CN), 61.1 and 60.8 (2xq, OCH_3-7 and 17), 42.6 (q, NCH_3), 23.9 (q, CO*C*H_3), 9.3 and 8.7 (2xq, CH_3-6 and 16).

Saframycin G (**54**): $C_{29}H_{30}N_4O_9$; yellow powder; mp 134-136°C (dec); EIMS *m/z* 578 M^+; $[\alpha]^{22}_D$ -28.0° (*c* 0.1, MeOH); IR ($CHCl_3$) ν 3560, 3400, 1685, 1655, 1610 cm^{-1}; UV (MeOH, log ε) λ_{max} 266 (4.19) nm; ^{1}H NMR ($CDCl_3$) δ 3.96 (1H, bs, H-1), 3.07 (1H, ddd, *J*=11.5, 2.9, 2.9 Hz, H-3), 2.86 (1H, dd, *J*=17.3, 2.9 Hz, Ha-4), 1.21 (1H, ddd, *J*=17.3, 11.5, 3.2 Hz, Hb-4), 4.13 (1H, dd, *J*=2.9, 1.6 Hz, H-11), 3.46 (1H, bs, H-13), 4.14 (1H, d, *J*=3.1 Hz, Ha-21), 3.69 (1H, ddd, *J*=14.3, 9.5, 1.6 Hz, Ha-22), 3.33 (1H, ddd, *J*=14.3, 4.4, 4.4 Hz, Hb-22), 6.63 (1H, dd, *J*=9.5, 4.4 Hz, NH), 4.04 and 4.03 (2x3H, s, 2xOCH_3), 2.48 (3H, s, NCH_3), 2.00 and 1.92 (2x3H, s, 2xCH_3), 2.26 (3H, s, $COCH_3$); ^{13}C-NMR ($CDCl_3$) δ 56.1 (d, C-1), 53.3 (d, C-3), 25.0 (t, C-4), 185.2 (s, C-5), 128.3 (s, C-6), 155.9 (s, C-7), 180.7 (s, C-8), 140.9 (s, C-10), 55.0 (d, C-11), 56.1 (d, C-13), 62.1 (d, C-14), 188.3 (s, C-15), 129.1 (s, C-16), 182.7 (s, C-18), 135.7 (s, C-19), 141.1 (s, C-20) 56.4 (d, C-21), 40.7 (t, C-22), 196.8 (s, *C*OCH_3), 160.2 (s, NHCO), 116.3 (s, CN), 61.0 (q, OCH_3-7 and 17), 42.5 (q, NCH_3), 8.7 (q, CH_3-6 and 16).

Saframycin H (**55**): $C_{32}H_{36}N_4O_9$; light yellow needles; mp 184-186°C (dec); EIMS *m/z* 620 M^+; $[\alpha]^{22}_D$ -7.0° (*c* 0.1, MeOH); IR ($CHCl_3$) ν 3420, 1675, 1655, 1610 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.19) nm; NMR ($CDCl_3$) δ 4.02 (1H, bs, H-1), 3.15 (1H, ddd, *J*=11.5, 3.6, 3.6 Hz, H-3), 2.87 (1H, dd, *J*=17.3, 3.6 Hz, Ha-4), 1.43 (1H, ddd, *J*=17.3, 11.0, 3.2 Hz, Hb-4), 3.92 (1H, dd, bs, H-11), 3.45 (1H, d, *J*=8.3 Hz, H-13), 2.84 (1H, dd, *J*=20.8, 8.3 Hz, Ha-14), 2.24 (1H, d, *J*=20.8 Hz, Hb-14), 4.09 (1H, d, *J*=2.4 Hz, Ha-21), 3.84 (1H, ddd, *J*=13.9, 10.3, 1.6 Hz, Ha-22), 2.95 (1H, ddd, *J*=13.9, 3.4, 3.4 Hz, Hb-22), 6.75 (1H, dd, *J*=10.3, 3.4 Hz, NH), 4.05 and 4.00 (2x3H, s, 2xOCH_3), 2.33 (3H, s, NCH_3), 1.95, 1.88 and 0.91 (3x3H, s, 3xCH_3), 2.12 (3H, s, $COCH_3$), 2.93 and 2.41 (2x2H, d and d, *J*=17.8 and 17.8 Hz, 2xmethylene); ^{13}C-NMR ($CDCl_3$) δ 56.5 (d, C-1), 53.6 (d, C-3), 25.2 (t, C-4), 185.5 (s, C-5), 127.2 (s, C-6), 156.4 (s, C-7), 180.7 (s, C-8), 140.0 (s, C-10), 54.2 (d, C-11), 54.5 (d, C-13), 21.6 (t, C-14), 186.5 (s, C-15), 129.0 (s, C-16), 155.7 (s, C-17), 183.7 (s, C-18), 136.1 (s, C-19), 141.4 (s, C-20) 58.1 (d, C-21),

40.2 (t, C-22), 211.6 (s, $COCH_3$), 175.1 (s, NHCO), 116.7 (s, CN), 61.0 (q, OCH_3-7 and 17), 41.6 (q, NCH_3), 25.8 (q, $COCH_3$), 8.5 (q, CH_3-6 and 16), 74.4 (s, $C(OH)CH_2$), 49.0 (t), 31.3 (q, CH_3).

Saframycin R (**62**): $C_{31}H_{34}N_4O_{10}$; dark brown powder; mp 184-186°C (dec); IR ($CHCl_3$) ν 3400, 1770, 1685, 1660, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 270 (3.95) nm; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 6.30 (1H, t, -NH), 4.55 (2H, s, $COCH_2OH$), 4.05 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 2.28 (3H, s, NCH_3), 2.20 (6H, s, $-CH_3$ and $COCH_3$), 1.92 (3H, s, $-CH_3$); (for detailed ^{1}H-NMR studies see ref. *43*); ^{13}C-NMR (acetone-d_6) δ 60.8 or 60.9 (C-1 or 11), 57.3 or 57.5 (C-3 or 13), 25.1 (C-4), 149.1 or 150.4 (C-5 or 7), 116.8 (C-6), 136.7 or 137.1 (C-8 or 19), 123.2 or 124.9 (C-9 or 10), 21.3 (C-14), 182.1 (C-15), 128.3 (C-16), 156.8 (C-17), 186.5 (C-18), 142.4 (C-20), 55.4 (C-21), 40.8 (C-22), 118.4 (CN), 196.8 ($NCOCOCH_3$), 161.4 ($NCOCOCH_3$), 171.7 ($OCOCH_2OH$), 8.5 (CH_3), 9.5 (CH_3), 24.2 ($COCOCH_3$), 41.4 (NCH_3), 55.4 (OCH_3), 59.5 (OCH_3), 61.0 ($OCOCH_2OH$).

Saframycin S (**56**): $C_{28}H_{31}N_3O_9$; dark yellow powder; mp 107-115°C (dec); IR ($CHCl_3$) ν 3400, 1720; 1680;1650 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.21), λ_{min} 234 (3.92) nm; for ^{1}H-NMR see ref *38*; ^{13}C-NMR ($CDCl_3$) δ 55.0 (d), 54.1 (d), 52.9 (d) and 49.7 (d) (C-1, C-11, C-3 and C-13), 25.2 (t, C-4), 188.6 (s) and 185.6 (s) (C-5 and C-15), 129.5 (s) and 127.8 (s) (C-6 and C-16), 156.0 (s) (C-7 and 17), 182.9 (s) and 180.9 (s) (C-8 and C-18), 137.3 (s) and 135.1 (s) (C-9 and C19), 141.1 (s) and 133.9 (s) (C-10 and C-20), 21.0 (t, C-14), 81.6 (d, C-21), 41.1 (t, C-22), 196.7 (s, $COCH_3$), 160.0 (s, NHCO), 61.1 (q, OCH_3-7 and 17), 42.5 (q, NCH_3), 24.3 (q, $COCH_3$), 8.8 (q, CH_3-6 and 16).

Saframycin AH_1 (**57**): $C_{29}H_{32}N_4O_5$; yellow powder; mp 164-167°C; Mass *m/z* 564 M^+; $[\alpha]^{25}_D$ -1.8° (MeOH); IR ($CHCl_3$) ν 3440, 1660, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.11) nm; ^{1}H-NMR (100 MHz, $CDCl_3$) δ 1.18 (3H, d, *J*=6 Hz), 1.86 (3H, s), 1.90 (3H, s), 2.30 (3H, s), 3.94 (3H, s), 4.00 (3H, s); ^{13}C-NMR δ 186.6 or 185.7 (C-5 or 15), 182.5 or 180.9 (C-8 or 18), 174.4 (NHCO), 156.2 or 155.3 (C-7 or 17), 141.8 or 140.7 (C-10 or 20), 136.3 or 135.7 (C-9 or 19), 129.0 or 127.7 (C-6 or 16), 116.9 (CN), 68.4 ($CH(OH)CH_3$, d), 61.2 (OCH_3 7 and 17, q), 58.5 (C-21, d), 56.9, 54.6, 54.5 or 54.3 (C-1, 3, 11 or 13, d), 41.7 (NCH_3, q), 40.1 (C-22, t), 25.3 (C-4, t), 21.7 ($CH(OH)CH_3$, q), 21.3 (C-14, t), 8.9 (CH_3 6 and 16, q).

Saframycin AR_1 (AH_2) (**58**): $C_{29}H_{32}N_4O_5$; yellow powder; mp 142-145°C; Mass *m/z* 564 M^+; $[\alpha]^{25}_D$ +21.4° (MeOH); IR ($CHCl_3$) ν 3430, 1660, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.01) nm; ^{1}H-NMR (100 MHz, $CDCl_3$) δ 0.92 (3H, d, *J*=6 Hz, $COCHCH_3$), 1.86 (3H, s, $-CH_3$), 1.90 (3H, s, $-CH_3$), 2.28 (3H, s, NCH_3), 3.94 (3H, s, OCH_3), 4.00 (3H, s, $-OCH_3$); ^{13}C-NMR δ 186.6 or 185.6 (C-5 or 15), 182.5 or 180.9 (C-8 or 18), 174.5 (NHCO), 156.4 or 155.6 (C-7 or 17), 141.7 or 140.3 (C-10 or 20), 136.4 or 135.9 (C-9 or 19), 128.8 or 127.6 (C-6 or 16), 117.0 (CN), 68.1 ($CHOHCH_3$, d), 21.6 ($CH(OH)CH_3$, q), 61.1 (OCH_3-7 and 17, q), 58.2 (C-21, d), 56.4, 54.6, 54.3 or 53.8 (C-1, 3, 11 or 13, d), 41.7 (NCH_3, q), 40.1 (C-22, t), 25.5 (C-4, t), 21.0 (C-14, t), 8.8 (CH_3-6 and 16, q).

Saframycin AR_3 (BH_1) (**61**): $C_{28}H_{33}N_3O_8$; yellow powder; mp 123-126°C; Mass *m/z* 539 M^+; $[\alpha]^{25}_D$ -76.7° (MeOH); IR ($CHCl_3$) ν 3400, 1660, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 269 (4.12) nm; ^{1}H-NMR (100 MHz, $CDCl_3$) δ 0.95 (3H, d, *J*=6

Hz, $COCHCH_3$), 1.88 (3H, s, $-CH_3$), 1.92 (3H, s, $-CH_3$), 2.29 (3H, s, NCH_3), 4.00 (3H, s, $-OCH_3$), 4.02 (3H, s, $-OCH_3$); ^{13}C-NMR δ 187.1 or 186.0 (C-5 or 15), 182.7 or 181.4 (C-8 or 18), 174.6 (NHCO), 156.8 or 155.7 (C-7 or 17), 142.8 or 140.6 (C-10 or 20), 137.4 or 136.8 (C-9 or 19), 128.6 or 126.9 (C-6 or 16), 68.2 ($CH(OH)CH_3$, d), 22.8 ($CH(OH)CH_3$, q), 60.9 (OMe-7 and 17, q), 58.6 (C-21, t), 58.2, 56.8, 55.0 or 52.3 (C-1,3,11 or 13, d), 41.3 (NCH_3, q), 40.7 (C-22, t), 26.1 (C-4, t), 21.0 (C-14, t), 8.5 (CH_3-6 and 16).

Saframycin Y3 (**66**): $C_{29}H_{33}N_5O_7$; yellow powder; mp 143-146°C; Mass (field desorption) *m/z* 563 M^+; $[\alpha]^{20}_D$ -46.1° (*c* 0.07, MeOH); IR ($CHCl_3$) ν 3380,1655, 1615, 1515 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.27), 340 (sh); λ_{min} 232 (3.77) nm; ^{1}H-NMR ($CDCl_3$) δ 3.94 (1H, bs, C-1), 3.15 (1H, ddd, C-3), 2.88 (1H, dd, C-4), 1.37 (1H, ddd, C-4), 4.07 (1H, bs, C-11), 3.45 (1H, dd, C-13), 2.80 (1H, dd, C-14), 2.27 (1H, d, C-14), 4.00 (1H, d, C-21), 1.92 (3H, s, $-CH_3$), 1.88 (3H, s, $-CH_3$), 4.03 (3H, s, $-OCH_3$), 4.06 (3H, s, $-OCH_3$), 2.33 (3H, s, NCH_3), 3.83 (2H, ddd, CH_2) 3.00 (2H, ddd, CH_2), 7.23 (1H, dd, NH), 3.27 (1H, q, $CH(NH_2)$), 0.92 (3H, d, CH_3); ^{13}C-NMR ($CDCl_3$) δ 56.8 (C-1, d), 52.6 (C-3, d), 25.4 (C-4, t), 185.7 or 185.4 (C-5 or 15), 127.0 or 128.4 (C-6 or 16), 155.5 or 155.1 (C-7 or 17), 180.4 or 182.0 (C-8 or 18), 136.4 or 135.9 (C-9 or 19), 139.7 or 141.7 (C-10 or 20), 54.3 (C-11, d), 54.5 (C-13, d), 21.6 (C-14, t), 58.2 (C-21, d), 116.8 (CN), 8.5 (CH_3, q), 8.6 (CH_3, q), 61.0 (OCH_3, q), 61.1 (OCH_3, q), 41.7 (NCH_3, q), 39.9 (C-22, t), 174.5 (CO), 50.1 (C-25, d), 21.3 (CH_3, q).

Saframycin Yd-1 (**67**): yellow powder; mp 124-127°C; Mass (field desorption) *m/z* 577 M^+; $[\alpha]^{20}_D$ -43.5° (*c* 1.0, MeOH); IR ($CHCl_3$) ν 3380, 1660, 1615, 1515 cm^{-1}; UV (MeOH, log ε) λ_{max} 269 (4.26); λ_{min} 233 (3.77) nm; ^{1}H-NMR ($CDCl_3$) δ 3.91 (1H, bs, C-1), 3.14 (1H, ddd, C-3), 2.86 (1H, dd, C-4), 1.35 (1H, ddd, C-4), 4.03-4.05 (1H, C-11), 3.45 (1H, dd, C-13), 2.81 (1H, dd, C-14), 2.32 (1H, d, C-14), 4.01 (1H, d, C-21), 1.89 (3H, s, $-CH_3$), 1.93 (3H, s, $-CH_3$), 4.03 (3H, s, $-OCH_3$), 4.05 (3H, s, $-OCH_3$), 2.32 (3H, s, NCH_3), 3.83 (2H, ddd, CH_2) 3.06 (2H, ddd, CH_2), 7.23 (1H, dd, NH), 3.05 (1H, t, $CH(NH_2)$), 1.06 (m), 1.48 (m), 0.47 (t) (CH_2CH_3); ^{13}C-NMR ($CDCl_3$) δ 56.8 (C-1, d), 53.6 (C-3, d), 25.4 (C-4, t), 185.5 or 185.4 (C-5 or 15), 127.2 or 128.3 (C-6 or 16), 156.6 or 155.3 (C-7 or 17), 180.8 or 182.5 (C-8 or 18), 136.5 or 135.9 (C-9 or 19), 139.7 or 141.7 (C-10 or 20), 54.2 (C-11, d), 54.5 (C-13, d), 21.5 (C-14, t), 58.1 (C-21, d), 116.8 (CN), 8.5 (CH_3, q), 8.7 (CH_3, q), 61.0 (2xOCH_3, q), 41.6 (NCH_3, q), 39.9 (C-22, t), 174.7 (CO), 56.0 (C-25, d), 27.9 and 10.3 (CH_2CH_3).

Saframycin Yd-2 (**68**): $C_{28}H_{31}N_5O_7$; yellow powder; mp 144-148°C; Mass (field desorption) *m/z* 549 M^+; IR ($CHCl_3$) ν 3380, 1660, 1615, 1515 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.23); λ_{min} 233 (3.77) nm; ^{1}H-NMR ($CDCl_3$) δ 3.39 (1H, bs, C-1), 3.14 (1H, ddd, C-3), 2.88 (1H, dd, C-4), 1.38 (1H, ddd, C-4), 4.05 (1H, bs, C-11), 3.44 (1H, dd, C-13), 2.82 (1H, dd, C-14), 2.31 (1H, d, C-14), 4.00 (1H, d, C-21), 1.90 (3H, s, $-CH_3$), 1.93 (3H, s, $-CH_3$), 4.03 (6H, s, 2x-OCH_3), 2.32 (3H, s, NCH_3), 3.82 (2H, ddd, CH_2) 3.10 (2H, ddd, CH_2), 7.23 (1H, dd, NH), 2.97 (d), 3.11 (d).

Saframycin Y2b (**70**): $C_{58}H_{64}N_{10}O_{14}$; orange yellow powder; mp >200°C (dec); FAB-MS *m/z* 1,133 (MH^++8); SIMS *m/z* 1,127 (MH^++2); $[\alpha]^{22}_D$ -42.2° (*c*

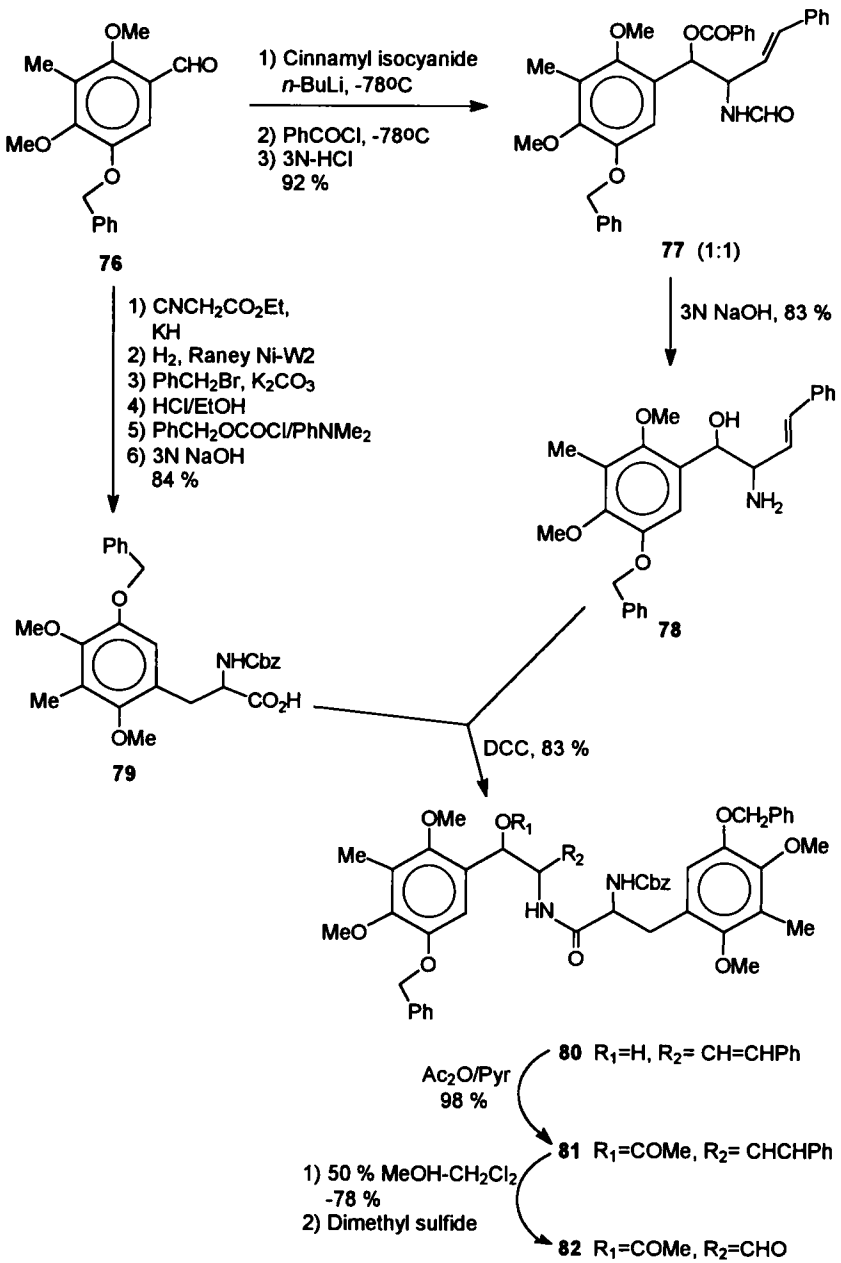
1.0, MeOH); IR ($CHCl_3$) ν 3360, 1655, 1615, 1450 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.66) nm; for ^{1}H-1 and ^{13}C-NMR, see ref. *48*.

Saframycin Y2b-d (**71**): $C_{60}H_{68}N_{10}O_{14}$; orange yellow powder; FABMS *m/z* 1,161 (MH^++8); SI-MS *m/z* 1,155 (MH^++2); $[\alpha]^{22}_D$ -41.9° (*c* 1.0, MeOH); IR ($CHCl_3$) ν 3360, 1655, 1615, 1450 cm^{-1}; UV (MeOH, log ε) λ_{max} 269 (4.64) nm; for ^{1}H-1 and ^{13}C-NMR, see ref. *48*.

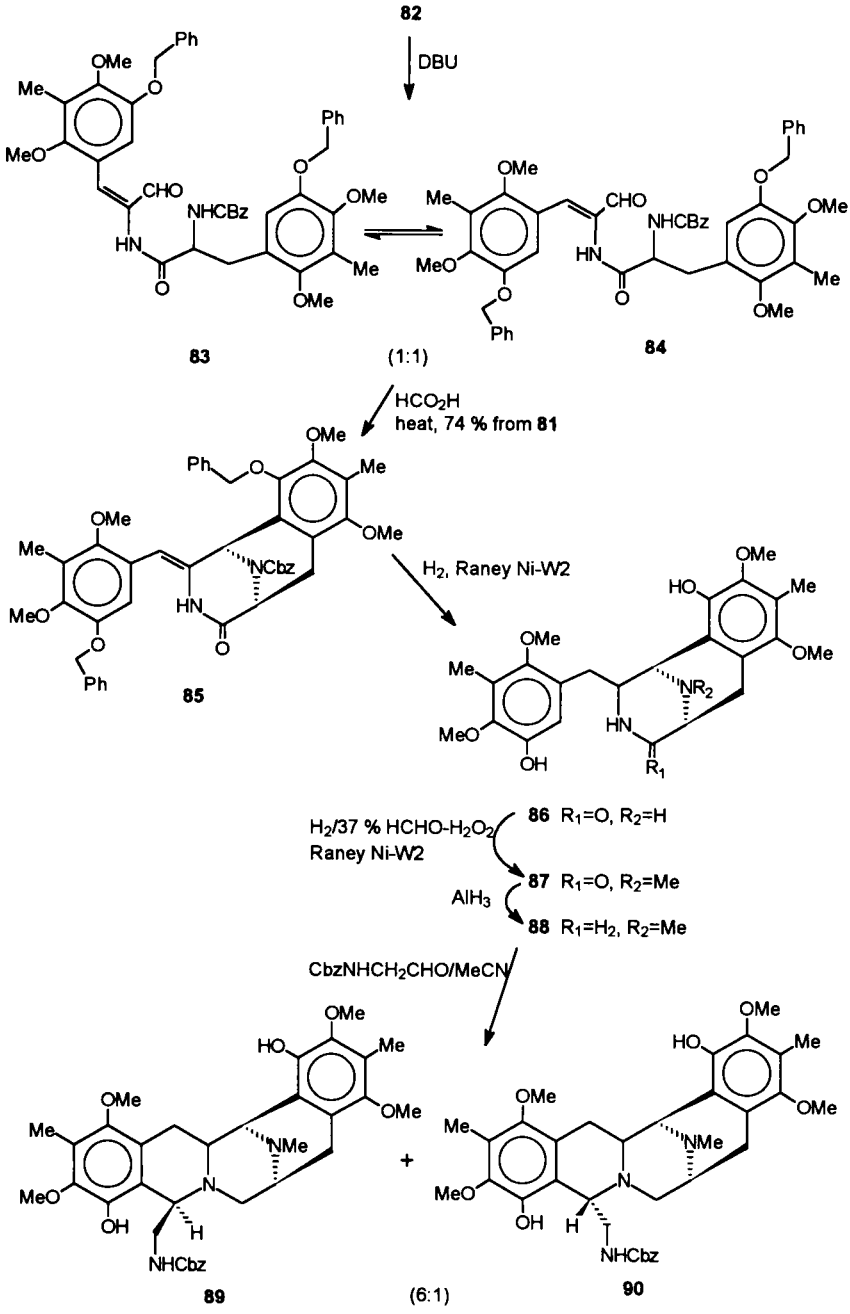
Saframycin Ad-1 (**69**): $C_{30}H_{32}N_4O_8$; amorphous yellow powder; mp 124-128°C; MI-MS *m/z* 578 (M^++2), 576 (M^+); IR ($CHCl_3$) ν 3400, 1710, 1685, 1660, 1615 cm^{-1}; UV (MeOH, log ε) λ_{max} 268 (4.30); for ^{1}H-1 and ^{13}C-NMR, see ref. *48*.

Saframycin Mx1 (**72**):$C_{29}H_{38}N_4O_9$; IR (film) ν 3200, 2900, 1657, 1619, 1459 cm^{-1}; UV (MeOH, log ε) λ_{max} 273 (3.95); $[\alpha]^{20}_D$ -70.7° (*c* 0.5, MeOH); ^{1}H-NMR (methanol-d_4) δ 4.33 (1H, m, H-1), 3.50 (1H, ddd, H-3), 3.12 (1H, dd, Ha-4), 1.51 (1H, ddd, Hb-4), 4.75 (1H, dd, H-11), 4.20 (1H, d, H-13), 4.82 (1H, d, H-14), 4.78 (1H, s, H-21), 3.77 (1H, dd, Ha-22), 3.25 (1H, dd, Hb-22),3.69 (1H, q, H-25), 0.93 (3H, d, CH_3-26), 4.02 (3H, s, OCH_3-7) 3.75 (3H, s, OCH_3-17), 3.72 (3H, s, OCH_3-14), 2.84 (3H, s, NCH_3), 2.25 (3H, s, CH_3-16), 1.93 (3H, s, CH_3-6); ^{13}C-NMR (methanol-d_4) δ 54.63 (d, C-1), 51.80 (d, C-3), 25.60 (t, C-4), 186.95 (s, C-5), 129.33 (s, C-6), 157.23 (s, C-7), 182.77 (s, C-8), 143.1 (s, C-9), 138.43 (s, C-10), 58.19 (d, C-11), 59.98 (d, C-13), 72.98 (d, C-14), 147.16 (s, C-15), 123.44 (s, C-16), 148.07 (s, C-17), 142.74 (s, C-18), 112.20 (s, C-19), 119.36 (s, C-20), 89.23 (d, C-21), 41.39 (t, C-22), 170.85 (s, C-24), 50.02 (d, C-25), 17.16 (q, C-26), 8.76 (q, CH_3-6), 9.78 (q, CH_3-16), 61.45 (q, OCH_3-7), 61.02 (q, OCH_3-17), 58.19 (q, OCH_3-14), 42.15 (q, NCH_3).

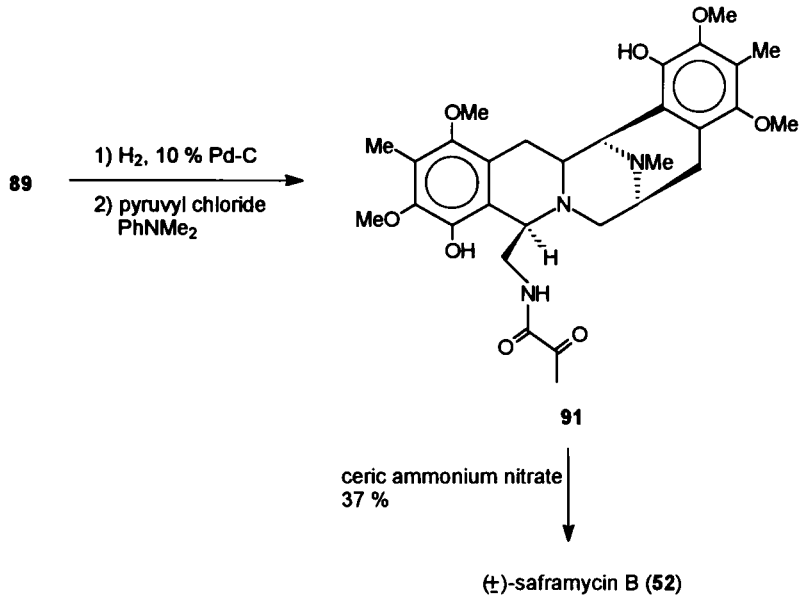
Saframycin Mx2 (**73**):$C_{29}H_{38}N_4O_8$; IR (film) ν 3200, 1680, 1656, 1461 cm^{-1}; UV (MeOH, log ε) λ_{max} 273 (3.95); $[\alpha]^{20}_D$ -119.8° (*c* 0.5, MeOH); ^{1}H-NMR (methanol-d_4) δ 3.59 (1H, m, H-1), 3.12 (1H, ddd, H-3), 3.04 (1H, ddd, Ha-4), 1.59 (1H, ddd, Hb-4), 4.90 (1H, dd, H-11), 4.13 (1H, d, H-13), 4.95 (1H, d, H-14), 3.11 (1H, dd, Ha-21), 3.71 (d, Hb-21), 4.09 (1H, dd, Ha-22), 3.24 (1H, dd, Hb-22),3.68 (1H, q, H-25), 0.82 (3H, d, CH_3-26), 4.02 (3H, s, OCH_3-7) 3.75 (3H, s, OCH_3-17), 3.69 (3H, s, OCH_3-14), 2.88 (3H, s, NCH_3), 2.26 (3H, s, CH_3-16), 1.93 (3H, s, CH_3-6); ^{13}C-NMR (methanol-d_4) δ 61.11 (d, C-1), 57.07 (d, C-3), 26.19 (t, C-4), 187.17 (s, C-5), 128.87 (s, C-6), 157.37 (s, C-7), 182.91 (s, C-8), 143.01 (s, C-9), 137.63 (s, C-10), 58.15 (d, C-11), 58.38 (d, C-13), 75.39 (d, C-14), 146.58 (s, C-15), 123.36 (s, C-16), 147.75 (s, C-17), 143.04 (s, C-18), 113.04 (s, C-19), 120.75 (s, C-20), 54.44 (t, C-21), 39.36 (t, C-22), 170.65 (s, C-24), 49.85 (d, C-25), 17.08 (q, C-26), 8.81 (q, CH_3-6), 9.98 (q, CH_3-16), 61.04 (q, OCH_3-7), 61.44 (q, OCH_3-17), 58.07 (q, OCH_3-14), 41.39 (q, NCH_3).



SCHEME 9. Total synthesis of (±)-saframycin B (**52**) *(53)*.



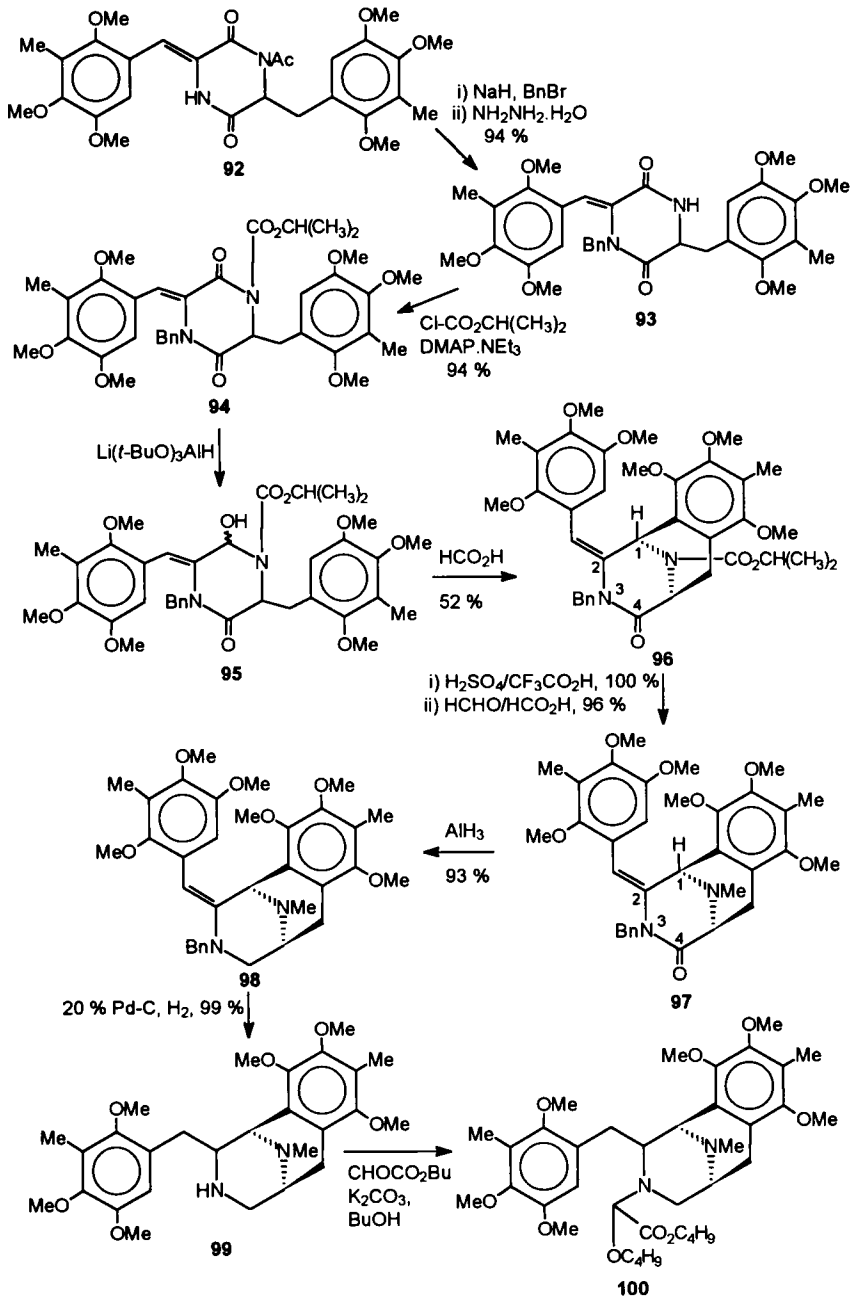
SCHEME 9. *Continued*



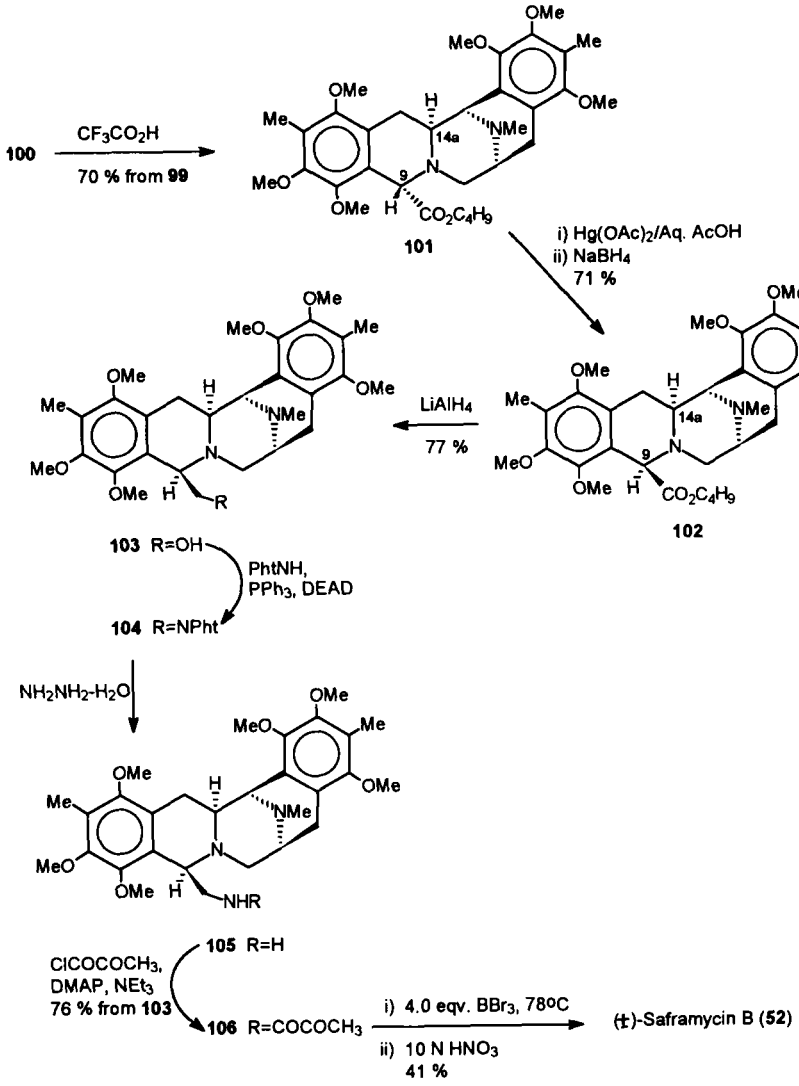
SCHEME 9. *Continued*

2. *Synthesis*

The first total synthesis of saframycins appeared as a total synthesis of (±)-saframycin B (**52**) in 1982 (Scheme 9) *(53)*. This elegant synthesis was reported by Fukuyama *et al.*, and initially involved the preparation of the highly substituted benzaldehyde **76** in seven steps. Compound **76** was then used as a starting material for the syntheses of **78** and **79** in four and six steps, respectively. Treatment of the aldehyde **76** with cinnamyl isocyanide in the presence of *n*-butyllithium at -78°C was followed by the addition of benzoyl chloride at -78°C. Hydrolysis of the product with 3N HCl afforded **77** as a 1:1 diastereomeric mixture of formamides. Compound **78** was obtained after basic treatment of the mixture **77** with 3N sodium hydroxide. The second precursor **79** was synthesized in six steps (Scheme 9). Condensation of the acid **79** and the amine **78** in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) gave the amide **80**, the hydroxyl group of which was then acetylated with acetic anhydride to obtain **81**. The olefin unit of **81** was converted to an aldehyde on careful ozonolysis and then treatment with dimethyl sulfide, which yielded an unstable diastereomeric mixture **82**. Treatment of **82** with DBU produced a mixture of *cis*- and *trans*-α,β-unsaturated aldehydes **83** and **84** in a 1:1 ratio. Closure of the first ring was achieved by heating the mixture in formic acid to give the desired bicyclic compound **85**. Hydrogenation of the olefin group of **85** over Raney Ni took place from the less hindered side which gave **86** as the sole

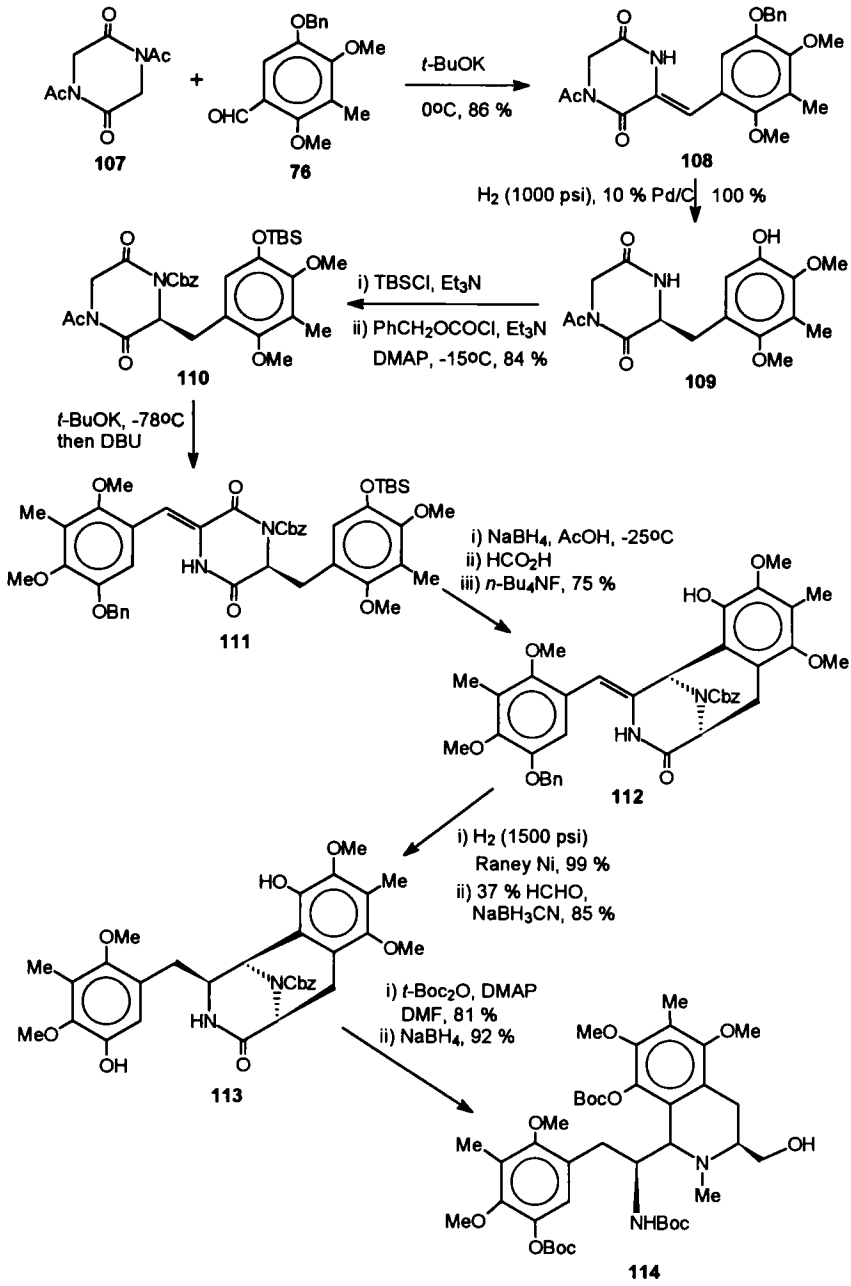


SCHEME 10. Total synthesis of (±)-saframycin B (**52**) *(57)*.

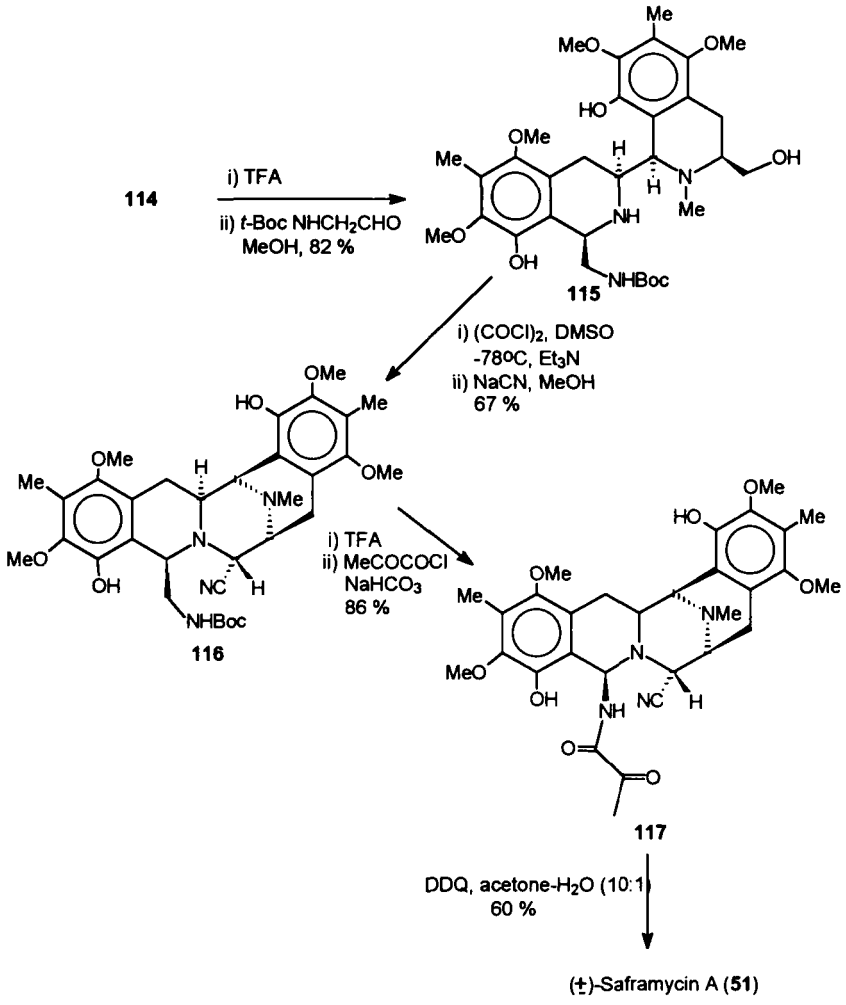


SCHEME 10. *Continued*

product. Formation of the second ring was achieved in three steps. Compound **86** was reductively alkylated over Raney Ni using formaldehyde-H_2O as the alkylating agent to give **87**, the lactam group of which was then reduced to the amine with



SCHEME 11. Total synthesis of (±)-saframycin A (**51**) *(59)*.

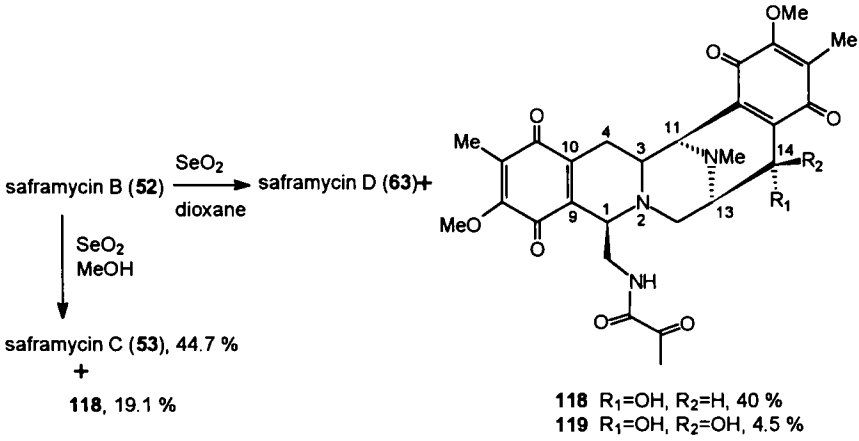


SCHEME 11. *Continued*

aluminum hydride to yield **88**. Reaction of **88** with the aldehyde, $CbzNHCH_2CHO$, afforded the desired cyclized compound **89** and its epimer **90** in a 6:1 ratio. Hydrogenation of **89** over Pd-C (10 %) to remove the amine protecting group, was followed by addition of pyruvyl chloride to give **91**, which was converted to (±)-saframycin B (**52**) on treatment with ceric ammonium nitrate.

The second stereoselective total synthesis of (±)-saframycin B (**52**), which was based upon extensive synthetic studies *(54-56)*, was reported by Kubo *et al.* in 1988 (Scheme 10) *(57)*. Their initial step involved the utilization of the 2,5-piperazinedione **92** as a starting material *(58)*. The lactam nitrogen of **92** was benzylated with benzyl bromide and then treatment of the product with hydrazine

hydrate removed the acetyl protecting group to give **93**. Protection of the lactam nitrogen of **93** with isopropyl chloroformate in the presence of DMAP and triethylamine yielded **94**, the activated amide carbonyl group of which was regioselectively reduced using lithium tri-*tert*-butoxyaluminum hydride to afford the alcohol **95**. Cyclization of **95** was carried out in formic acid to give **96**. Its *E*-stereochemical assignment was based on ^{1}H NMR studies which indicated that the proton at the C-1 was positioned in the deshileding zone (6.77 ppm) of the aromatic ring and the carbonyl group. Removal of the amine protecting group was performed using trifluoroacetic acid and H_2SO_4. Methylation of the corresponding product with formaldehyde and formic acid gave the tricyclic lactam **97**, the stereochemical structure of which was confirmed by a single crystal X-ray diffraction analysis *(57)*. The lactam carbonyl functionality of **97** was reduced with aluminum hydride which yielded the unstable enamine **98**. Reduction of **98** over palladium-carbon cleanly gave hydrogenation from the α-face, along with the removal of the benzyl protecting group. The secondary amine group of the product **99** was then reacted with a large excess of butyl glyoxalate to obtain the *O,N*-acetal **100**. Treatment of **100** with trifluoroacetic acid led to the formation of the desired pentacyclic molecule **101**. X-Ray crystallographic studies of its ethyl ester derivative revealed that it was an epimeric mixture at C-9. Then, epimerization was performed using mercury acetate in aqueous acetic acid which was followed by reduction with sodium borohydride. Hydride attack from the less hindered α-face yielded the desired ester **102** which was converted to the alcohol **103** with lithium aluminum hydride. Treatment of **103** with diethyl azodicarboxylate (DEAD), triphenylphosphine and phthalimide furnished the imide **104**. Removal of the phthaloyl group was carried out with hydrazine hydride to give the primary amine **105**, which was subsequently reacted with pyruvoyl chloride to obtain the pyruvamide **106**. It was then converted to (±)-saframycin B by demethylation with 4.0 equiv. of boron tribromide followed by oxidation with 10N HNO_3.



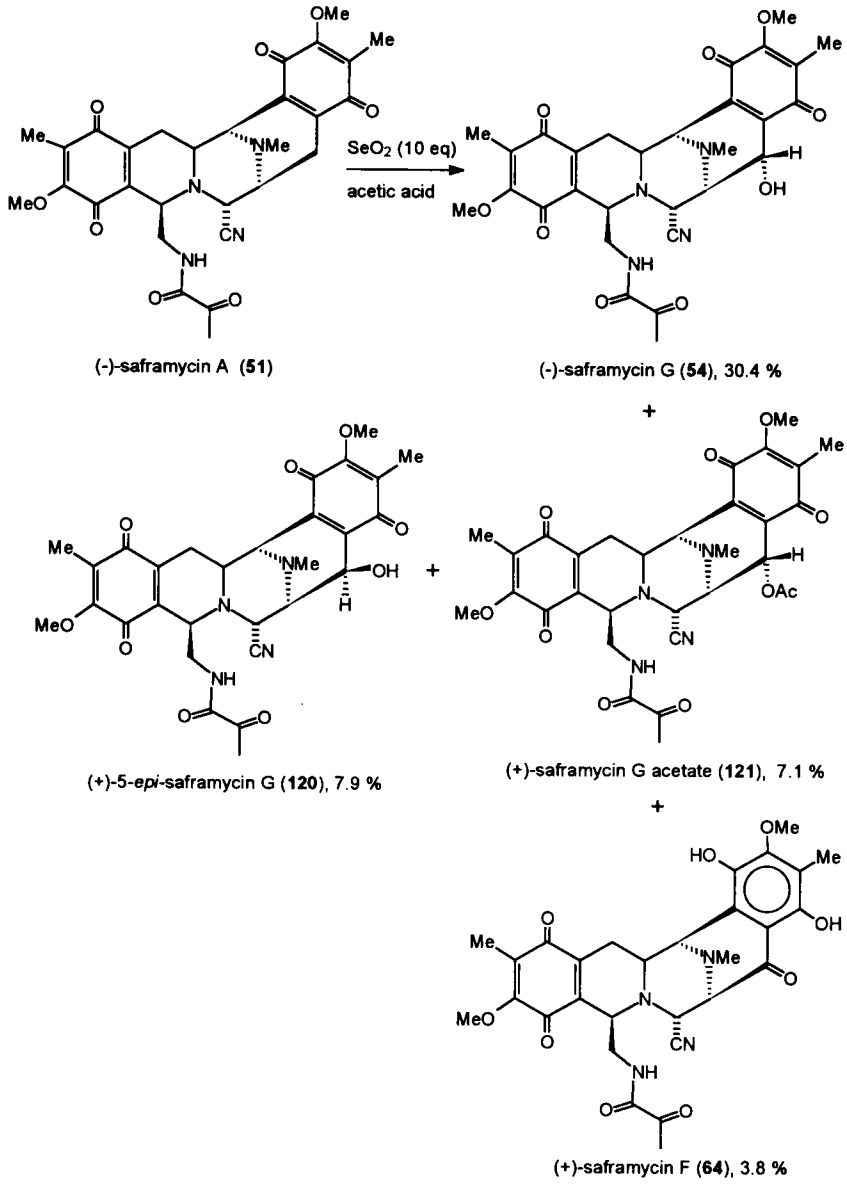
SCHEME 12. Synthesis of saframycin C (**53**) and D (**63**) from saframycin B (**52**) *(60, 61)*.

In 1990, the first total synthesis of (±)-saframycin A (**51**) was reported by Fukuyama *et al.* (Scheme 11) *(59)*. Their elegant synthesis started with the

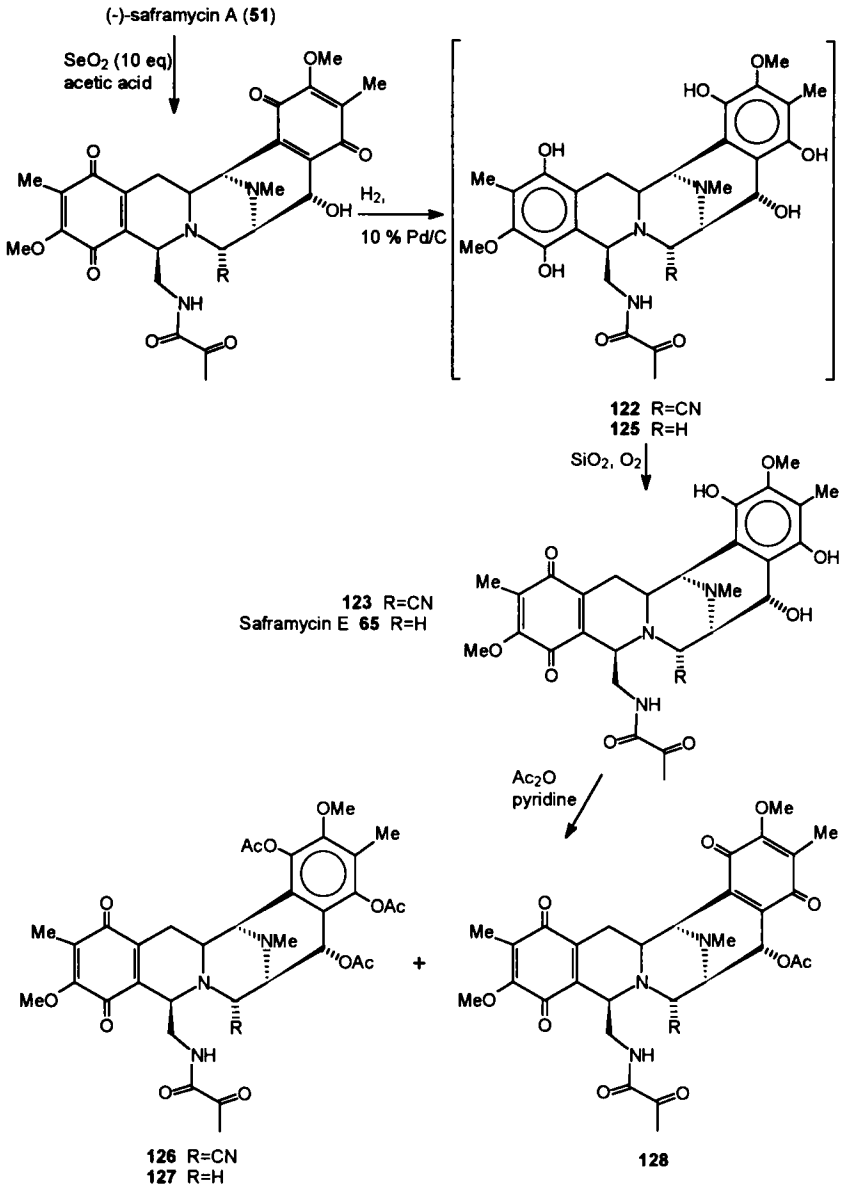
condensation of the aldehyde **76** *(53)* with the readily available *N,N'*-diacetylpiperazinedione (**107**) to give **108**, the olefin in which was hydrogenated on palladium-carbon to yield the phenol **109**. Protection of the phenolic hydroxyl and the amide nitrogen was carried out with *t*-butyldimethylsilyl chloride and carbobenzyloxy chloride, respectively, to afford **110**. A second condensation of the piperazinedione **110** with the aldehyde **76** furnished arylidenepiperazinedione **111**. Selective reduction of the activated ring carbonyl functionality with sodium borohydride was followed by ring formation in formic acid. Deprotection of the phenolic silyl group of the corresponding product with tetrabutylammonium fluoride furnished the desired bicyclic compound **112**. Hydrogenation of **112** over Raney Ni reduced the olefin functionality from the less hindered *exo* side and removed the amine protecting group Cbz. Subsequent reductive methylation of the product using formaldehyde and sodium cyanoborohydride yielded **113**. Treatment of **113** with di-*tert*-butyldicarbonate to protect the phenolic hydroxyl groups and activate the lactam ring was followed by reductive cleavage of the amide bond with sodium borohydride to obtain the alcohol **114**. Deprotection of Boc groups of **114** with trifluoroacetic acid and reaction of the corresponding amine with the *N*-(*tert*-butoxycarbonyl) protected aldehyde, *t*-BocNHCH$_2$CHO, yielded the desired tetrahydroquinoline **115**. The alcohol **115** was carefully oxidized and treatment of the resultant unstable aminal with sodium cyanide furnished closure of the last remaining ring, **116**. The protecting Boc group was removed with trifluoroacetic acid, and the free amine was reacted with pyruvylchloride to obtain the pyruvamide **117** which was oxidatively demethylated with DDQ to give (±)-saframycin A (**51**).

In 1990, Kubo *et al.* reported that they successfully transformed (±)-saframycin B (**52**) to (±)-saframycin C (**53**) and saframycin D (**63**) by utilizing selenium oxide oxidation in the synthesis (Scheme 12) *(60, 61)*. The presence of methoxy and carbonyl functionalities at C-14 of saframycin C and saframycin D, respectively, was provided by oxidation of saframycin B. Thus, treatment of saframycin B with SeO_2 in dioxane furnished a carbonyl group at C-14 to give saframycin D (**63**) along with the alcohols **118** and **119**. When saframycin B was treated with SeO_2 in methanol a methoxy functionality at C-14 was obtained, that is, saframycin B was transformed to C, along with the same side product **118** obtained in the transformation of saframycin B (**52**) to saframycin D (**63**).

Using the same technique of selenium oxide oxidation, Kubo *et al.* transformed (-)-saframycin G (**54**) to (+)-5-*epi*-saframycin G (**120**), (-)-saframycin G acetate (**121**) and (+)-saframycin F (**64**) (Scheme 13) *(32, 62)*. This was achieved by treating saframycin A with 10 equivalents of selenium oxide in acetic acid at room temperature. (-)-Saframycin G (**54**) was then converted into saframycin Mx derivative **123** (Scheme 14) *(32, 62)*. Hydrogenation of (-)-saframycin G (**54**) on palladium-carbon produced the unisolatable intermediate **122** which, after removal of the solvent *in vacuo*, was treated with SiO_2 in the presence of oxygen to obtain the saframycin Mx derivative **123**. Because this compound was easily oxidized by light and oxygen, and above pH 7 to the *bis*-quinone saframycin G (**54**), **123** was acetylated with acetic anhydride to give the stable triacetate **126**.



SCHEME 13. Transformation of (-)-saframycin A to (-)-saframycin G (**54**), (+)-*epi*-saframycin G (**120**), (-)-saframycin G acetate (**121**) and (+)-saframycin F (**64**) *(62)*.



SCHEME 14. Transformation of (-)-saframycin A (**51**) and (±)-saframycin B (**52**) to Mx type saframycin **123** and saframycin E (**65**), respectively *(32, 62)*.

Kubo *et al.* successfully applied this strategy to the transformation of (±)-saframycin B (**52**) to (±)-saframycin E (**65**) (Scheme 14) (*32, 62*). Treatment of (±)-

saframycin B with SeO_2 in dioxane produced the alcohol **124**. Hydrogenation of **124** on palladium-carbon yielded again the intermediate **125** which was not isolated. After removal of the solvent, subsequent oxidation of **125** with oxygen in the presence of SiO_2 gave (±)-saframycin E (**65**). Acylation of **65** with acetic anhydride furnished the triacetate derivative of **65** and the bisquinone **128**. It was determined that the triacetate **127** was identical with the triacyl derivative of natural saframycin E (**65**) *(30)*.

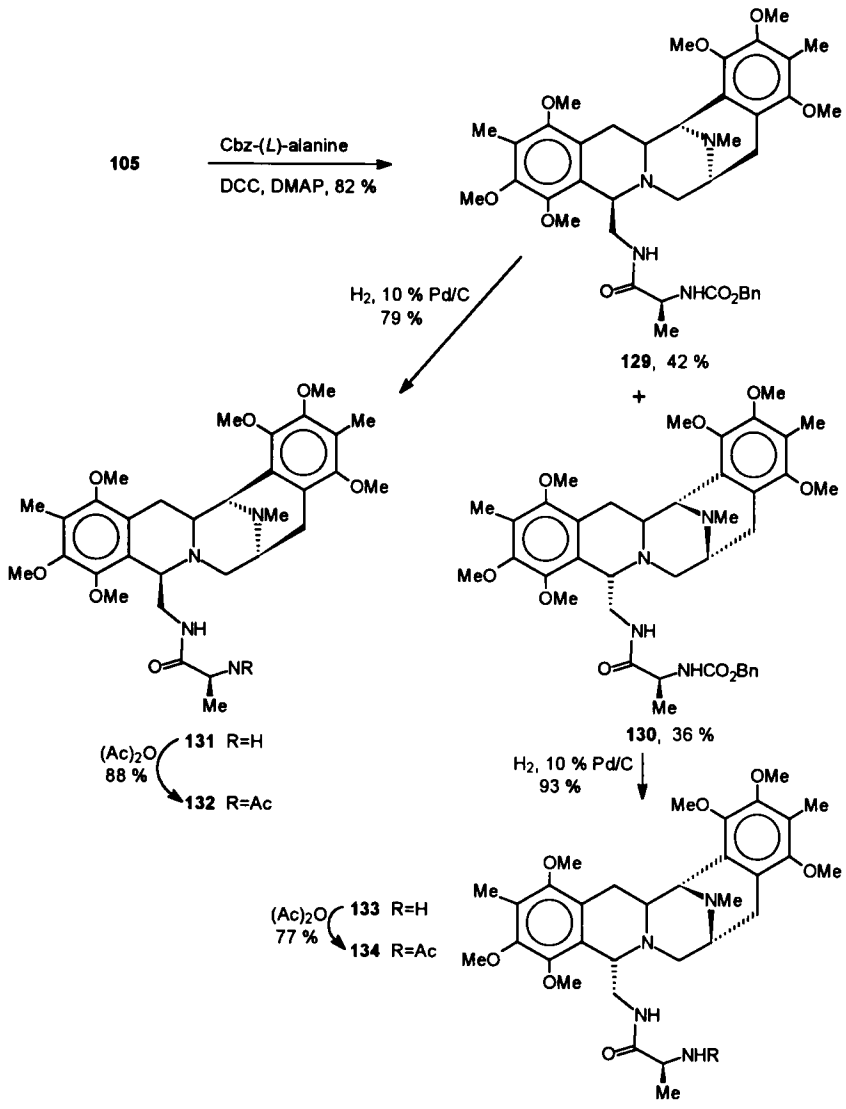
In 1995, Kubo *et al.* reported the synthesis of (-)-*N*-acetylsaframycin Mx2 (**139**) and its *epi*-(+)-enantiomer (**141**) using their key intermediate, **105**, in saframycin B (**52**) synthesis *(57)* as starting material (Scheme 15) *(63)*. Condensation of the amine **105** with Cbz-(*L*)-alanine in the presence of DCC gave the amides **129** and **130**. Hydrogenation of **129** and its *epi*-isomer **130** on palladium-carbon produced the amines **131** and **133**, which were acetylated with acetic anhydride to furnish the amides **132** and **134**, respectively. Partial demethylation of **132** with boron tribromide, and then oxidative demethylation, gave the *bis*-quinone **135**. Introduction of a methoxy group at C-14 was accomplished by reacting **135** with SeO_2 in methyl alcohol which yielded **136**, along with the 14-hydroxy compound **137**. Hydrogenation of **136** on palladium-carbon produced the *bis*-hydroquinone **138** which, after removal of the solvent *in vacuo*, was partially oxidized with oxygen in the presence of SiO_2 to obtain (-)-*N*-acetylsaframycin Mx2 (**139**). This sensitive compound to light and oxygen was converted to its stable form **140** by acetylation with acetic anhydride. Following the same strategy, Kubo *et al.* transformed **134** to the *epi*-(+)-enantiomer **141** of (-)-*N*-acetylsaframycin Mx2 (**139**). Like **139**, light and oxygen sensitive **141** was acetylated with acetic anhydride to obtain its stable form **142**.

3. Biosynthesis

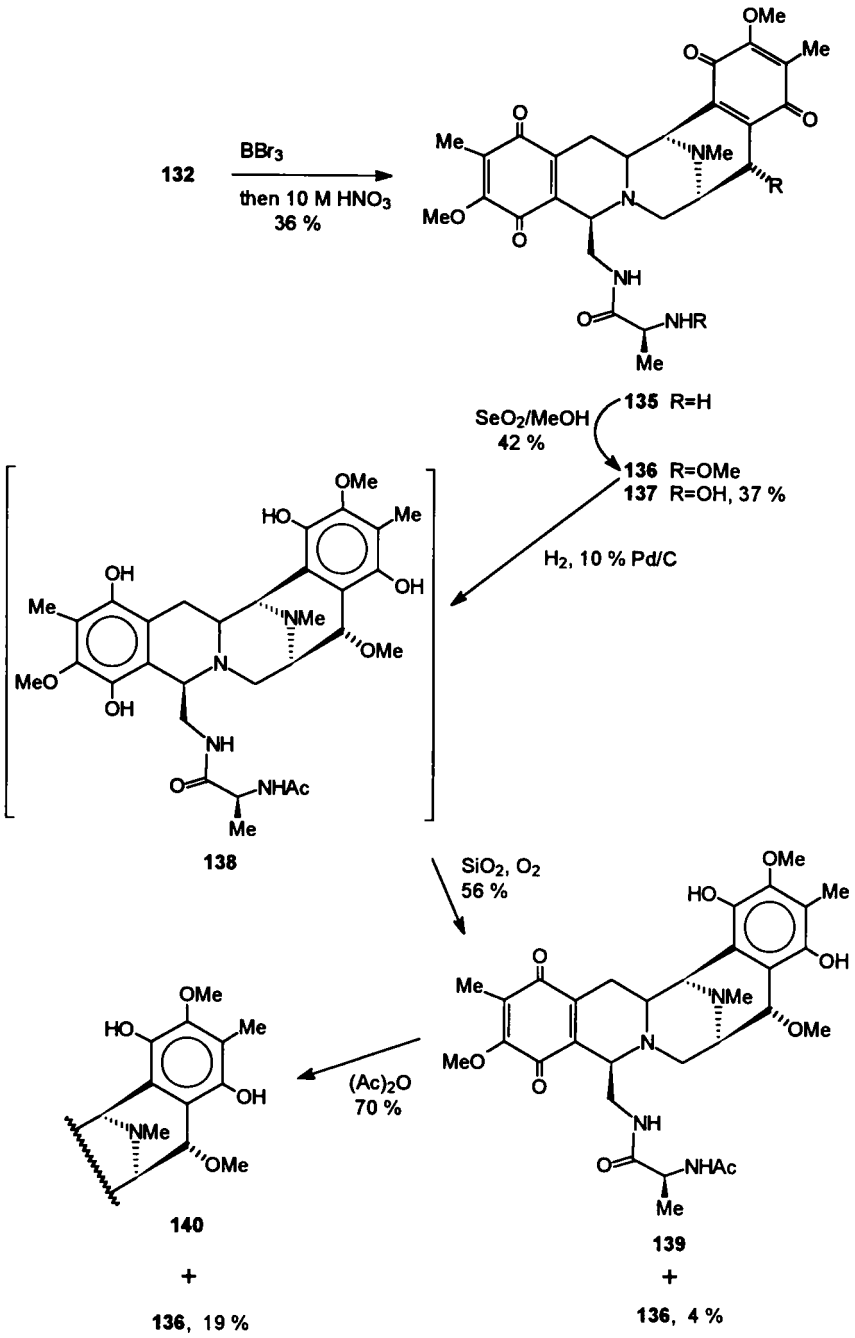
In 1995, Mikami *et al.* reported their ^{14}C-labeled experiments indicating that the biosynthesis of saframycin A (**51**) involves the amino acids tyrosine, glycine, alanine and methionine (Scheme 16) *(73, 74)*. Their experiments suggested that cyclization of two tyrosine molecules led to the formation of the dimeric skeleton of saframycin A. They also indicated that the incorporation of two O-, two C- and one N-methyl carbons is from methionine units, and that glycine and alanine are the precursors for the pyruvoylamine side chain attached to C-1.

Considering the pathway, their attempt to biosynthesize new saframycin derivatives using various tyrosine analogues failed *(73)*. However, they succeeded in altering the side chain to prepare new saframycin derivatives (See section "*1. Isolation and structure elucidation*").

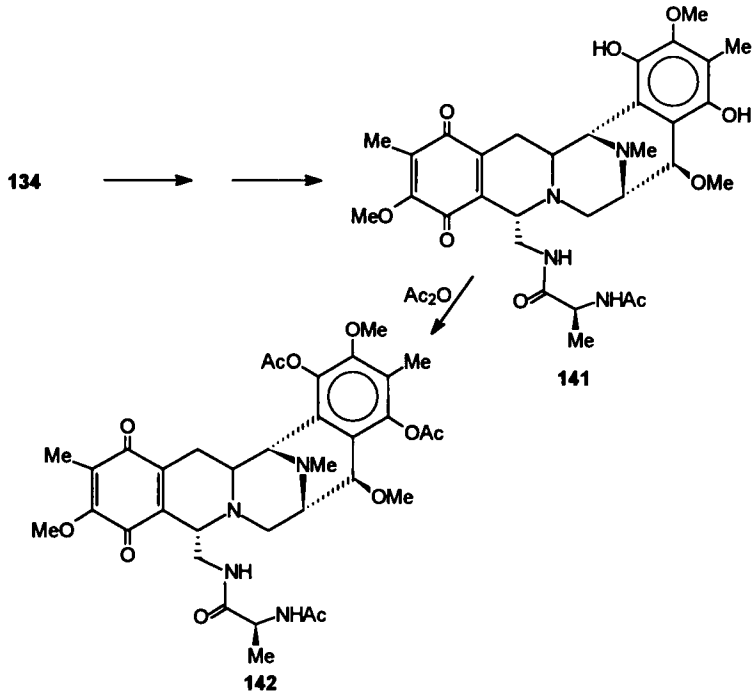
Further studies on the biosynthesis of the saframycins were reported by Pospiech *et al.* in 1995 *(75)* and 1996 *(76)* with respect to the involvement of the peptide synthetases and an *O*-methyltransferase.



SCHEME 15. Total synthesis of (-)-*N*-acetylsaframycin Mx2 (**119**) and its *epi*-(+)-enantiomer (**121**) *(34)*.



SCHEME 15. *Continued*



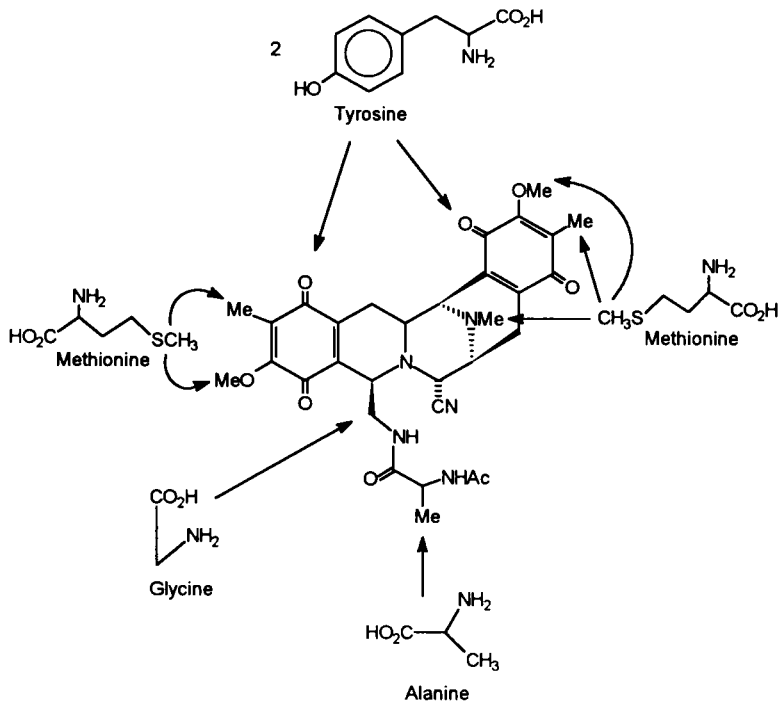
SCHEME 15. *Continued*

4. Biological activity

Of the saframycins, saframycin A (**51**) was reported to possess the highest antitumor and antibacterial activity. Arai *et al.* indicated that saframycin S (**56**) has also quite comparable antimicrobial and antitumor activities to those of saframycin A *(37, 39)*.

Saframycins were found to be active only on Gram-positive bacteria and while saframycin A is the most active one, D and E showed the lowest activity *(30)*.

Cytotoxic activity studies carried out by Kishi *et al.* revealed that saframycins such as A (**51**), S (**56**), AH_1 (**57**) and AH_2 (**58**), which possess the α-cyanoamine or the α-carbinolamine functionalities at C-21, exhibited potent cytotoxicity with ID values of 0.0056, 0.0053, 0.0080 and 0.0061 μM, respectively *(44)*. On the other hand, saframycins such as G (**54**), H (**55**), F (**64**), AH_1Ac (**59**) and AH_2Ac (**60**) with an α-cyanoamine group at C-21 and bulky substituents either at C-14 or at C-25 showed lower cytotoxic activities than saframycin A, with ID values of 0.030, 0.033, 0.59, 0.025 and 0.027 μM, respectively. The saframycins such as B (**52**), C (**53**), D (**63**) and AR_3 (**11**) without the α-cyanoamine and α-carbinolamine groups at C-21 showed much lower cytotoxic activities. Their ID values were 0.80, 3.9, 4.8 and 0.65 μM, respectively.



SCHEME 16. Biosynthesis of saframycin A (**51**) *(73, 74)*.

Saframycin A (**51**) and C (**53**) were examined against different tumor systems in mice *(77)*. Although both inhibited the growth of L1210 cells completely in suspension culture, saframycin A showed good cytotoxicity against cultured L1210 leukemia cells at the concentration of 0.02 μg/ml, while complete inhibition of the growth was achieved at a 1.0 μg/ml of saframycin C. Saframycin A was found to be highly active against Ehrlich ascites carcinoma and P388 leukemia and moderately active against L1210 leukemia and B16 melanoma. On the other hand, saframycin C was found to have far less biological activity *in vitro* and *in vivo*. When a 1 mg/kg/day dose of saframycin A was used from days 1 to 4, or from days 1 to 6 to inhibit Ehrlich ascites carcinoma in ddY mice the percentage of 60-day survivors were 60% and 70%, respectively, all of the control mice died within 21 days when 1.0×10^6 cells were implanted. However, 60-day survivors were only 20% at the dose of 15 mg/kg/day of saframycin C from days 1 to 20.

Tumor rejection experiments revealed that the mice cured by saframycin A develop a clear resistance against the same tumor *(77)*.

Saframycin A showed a direct cytotoxicity against cultured HeLa S_3 cells which were inhibited at a concentration of 0.02 μg/ml *(78)*. Investigation of the effects of saframycin A on solid tumors in mice bearing sarcoma 180 showed a

marked inhibition of tumor growth (T/C, treated over control, 54-11%). However, its effects were less on body weight (T/C, 94-43%).

Saframycin S (**56**) which has comparable antimicrobial and antitumor activities to those of saframycin A (**51**) showed a marked activity against Ehrlich ascites tumors with the doses of 0.5 to 0.75 mg/kg/day for 10 days, and the rate of survivors was 80 to 90% *(37, 39)*. However, this antibiotic was found to be less effective against P388 leukemia compared to saframycin A. The LD_{50} of saframycin S was determined to be 3.2 mg/kg (*i.p.*) for ddY mice.

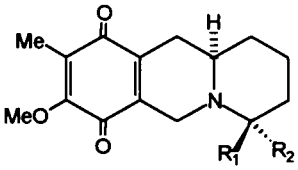
Of the microbial conversion products, saframycin AR_1 (**58**) and AR_3 (**61**), AR_1 was found to be more active against Gram-positive bacteria and did not show any activity against fungi *(40)*. Among the tested microorganisims, *Corynebacterium diphtheriae* is the most sensitive one to AR_1, and it was completely inhibited by 0.01 μg/ml. Saframycin AR_1 is 5 to 10 times less active against microorganisims than saframycin A, and saframycin AR_3 was determined to be 10 to 50 times less active than saframycin AR_1.

Against the L1210 mouse leukemia cell line, the ED_{50} values of saframycin A, AR_1 and AR_3 were found to be 0.003 μg/ml, 0.004 μg/ml and 0.35 μg/ml, respectively. Preliminary *in vivo* study of AR_1 and AR_3 against L1210 mouse leukemia showed ILS (increase in life span) of 156.8% and 143.2% at a daily doses of 1.5 mg/kg and 1.0 mg/kg of AR_1. However, AR_3 did not show any antitumor activity for a daily dose of 5.0 mg/kg *(40)*.

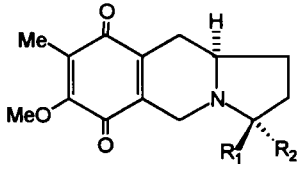
Saframycin R (**62**) was reported to be as effective as saframycin A against L1210 cultured cells, Ehrlich ascites tumor and P388 leukemia *(42)*. It showed antimicrobial activity and its acute toxicity to mice was less than ten times that of saframycin A.

Arai *et al.* suggested that the side-chain of saframycins plays an important role in their antitumor activities *(47)*. They prepared 15 acyl, 9 alkyl and 3 carbamoyl derivatives of saframycin Y3 and water-soluble saframycin Yd-1 hydrochloride *(79)*. Their studies indicated that the presence of a bulky group in the side-chain drastically decreases the activity. However, when a bulky group is introduced into the side-chain in *N*-alkyl derivatives, no decrease in the activity was observed. This could be interpreted as that *N*-alkyl derivatives sustain the basic character of the side chain, whereas it disappears in *N*-acyl and *N*-carbamoyl derivatives. The basic character may therefore play an important role in the release of the cyano group and then formation of the α-carbinolamine functionality which is essential for the binding to DNA.

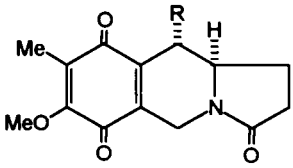
Of these side-chain modified derivatives of saframycin Y3, pivaloyl- and *n*-caproylsaframycin Y3, and water soluble saframycin Yd-1.HCl showed marked antitumor activity against L1210 mouse leukemia cells *(79)*. Further studies showed that these three derivatives are also active against B16-F10 melanoma, and saframycin Yd-1.HCl established the greatest prolongation of survival time. When mice were treated with these new saframycins, a marked inhibition of spontaneous metastasis of Lewis lung carcinoma was observed.



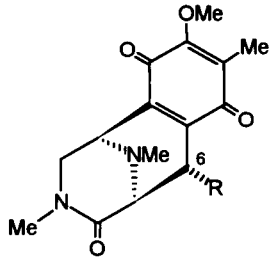
143 R_1=H, R_2=CN
144 R_1=CN, R_2=H



145 R_1=H, R_2=CN
146 R_1=CN, R_2=H



147 R=H
148 R=OH
149 R=OMe

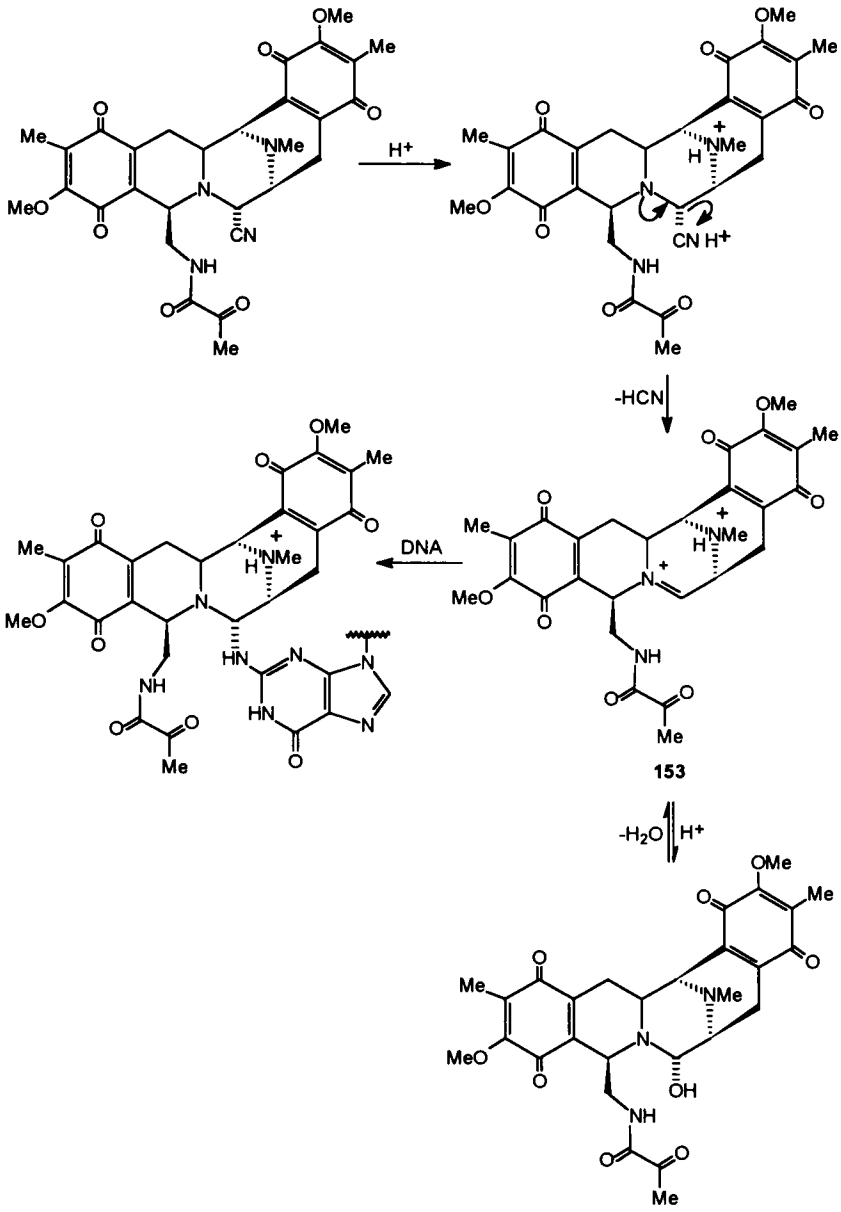


150 R=H
151 R=OH
152 R=OMe

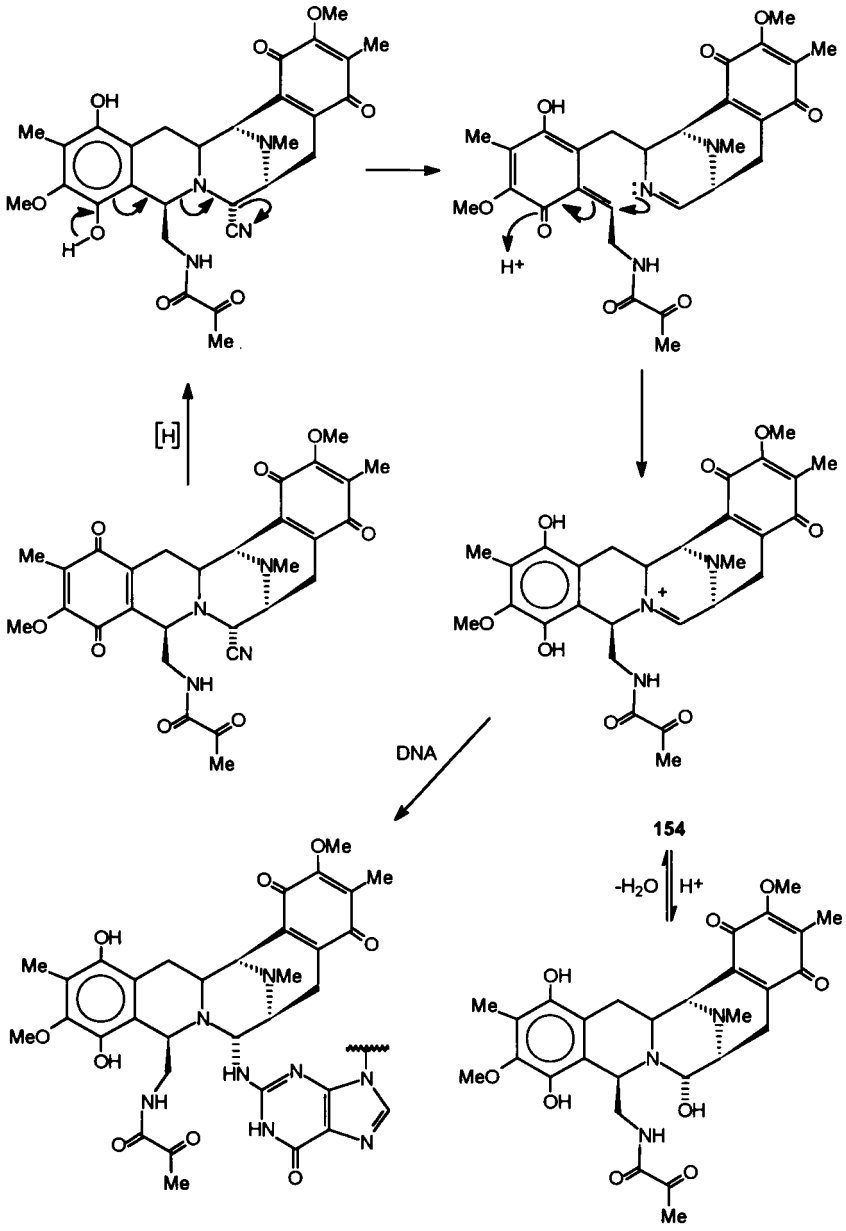
Antimicrobial spectra of saframycin Y3 (**66**), Yd-1 (**67**), Yd-2 (**68**), Ad-1 (**69**), Y2b (**70**) and Y2b-d (**71**) were found to be very similar to that of saframycin A, even though they had minor differences in their activities against some microorganisims *(80)*. However, cytotoxic activities were different, with that of Yd-2 drastically reduced.

Saframycin Mx1 (**51**) was reported to be a rather efficient inhibitor of several Gram-positive organisims and of halobacteria *(80)*. Tests of saframycin Mx1 with *S. aureus* showed that at a concentration 0.5 μg/ml DNA, RNA and protein synthesis were inhibited immediately. However, in a cell-free system from *Escherichia coli* no inhibition of protein synthesis was observed up to 50 μg/ml, while the activity of RNA-polymerase of *E. coli* was inhibited *in vitro*.

Some efforts have also been devoted to the preparation of simple structures which might mimic the biological activity of saframycins. Kubo *et al.* reported that the compounds **143-149** did not show any significant cytotoxic activity (**143**: ED_{50}= 3.0 μg/kg, **144**: ED_{50}= 4.0 μg/kg, **145**: ED_{50}= 3.0 μg/kg, **146**: ED_{50}: 3.0 μg/kg, **147**: ED_{50}= 2.0 μg/kg, **148**: ED_{50}= 3.5 μg/kg, **149**: ED_{50}= 3.0 μg/kg) *(81, 82)*. On the other hand **150** and **151** exhibited low cytotoxic potency (**150**: ID_{50} = 0.22 μg/ml, **151**: ID_{50}= 8.8 μg/ml), while introduction of a methoxy group at C-6 increased the efficiency (**152**: ID_{50}= 0.158 μg/ml) *(83)*. Antimicrobial activity studies demonstrated that **143** is active against *Bacillus subtilis* (MIC= 16.0 μg/ml) *(81)*. Compounds **145** and **146** were found to be active against the fungus *Trichophyton mentagrophytes* with relatively low MIC values and interestingly, the compound **145** with an α-cyano group showed four times higher antifungal activity against dermatophytes compared to that with a β-cyano **146** (**145**: MIC= 6.25 μg/ml, **146**: MIC= 25 μg/ml) *(82)*.



SCHEME 17. Acid promoted covalent binding of streptomycin A to guanine in the minor groove of DNA *(84-90)*.



SCHEME 18. Reducing cofactors promoted covalent binding of streptomycin A to guanine in the minor groove of DNA *(84-90)*.

The mechanism of action of the saframycins has been investigated in depth by various groups *(84-90)*. Saframycin A (**51**) was found to be a potent inhibitor of nucleic-acid synthesis of both eukaryotes and prokaryotes *(84)*. The synthesis of RNA is more sensitive to saframycin A in L1210 cells, while it is more effective against the synthesis of DNA in *B. subtilis*.

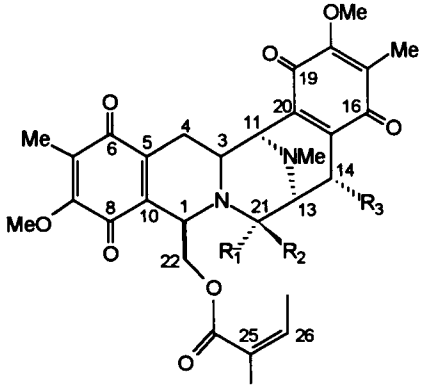
Studies indicated that saframycins present three types of mode of binding to the nucleotides: i) a reversible noncovalent binding, ii) acid-promoted covalent binding to the 2-amino group of guanine in the minor groove of DNA (Scheme 17), and iii) reducing cofactors, such as dithiothreitol, promoted covalent binding to the 2-amino group of guanine in the minor groove of DNA (Scheme 18). In the latter two bindings, the presence of a leaving group such as CN or OH is vital. As is shown in Schemes 10 and 11, release of the leaving group leads to the formation of cyclic immonium ions **153** and **154**, respectively, which act as an electrophile toward the nucleophilic center in DNA, the 2-amino group of guanine.

In addition to these processes, single strand scission of DNA mediated by reactive oxygen species was also reported *(87)*. These reactive oxygen species are produced by the redox cycling of the quinone moieties of the saframycins.

B. RENIERAMYCINS

1. Isolation and Structure Elucidation

In 1982, Faulkner *et al.* reported the isolation of renieramycins A-D (**155-158**) as minor metabolites, along with other metabolites such as renierone (**232**),



renieramycin A **155** R_1=R_2=H, R_3=OH
B **156** R_1=R_2=H, R_3=OEt
C **157** R_1, R_2=O, R_3=OH
D **158** R_1, R_2=O, R_3=OEt
E **159** R_1=OH, R_2=R_3=H
F **160** R_1=OH, R_2=H, R_3=OMe
G **161** R_1, R_2=O, R_3=H

mimosamycin (**208**), *N*-formyl-1,2-dihydrorenierone (**235**), *O*-demethylrenierone (**233**), 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**), 2,5-

dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**237**) from a bright-blue sponge, *Reniera* sp. collected near Isla Grande, Mexico *(91)*. Having an isoquinolinequinone unit is the common structural similarity among these metabolites, except the latter one (**237**) which has an isoindolequinone structure and is reported to be the first naturally-occurring isoindole.

Although initially the geometry of the renieramycins was defined on the basis of a series of nuclear Overhauser enhancement difference spectra (NOEDS) studies, single crystal X-ray diffraction analysis of saframycin C revealed a different picture at C-1, the point of attachment of the side chain, and the structures of renieramycins were reassigned in 1989 by the same group *(92)*. In the same report where reassignments were made, the isolation and the structural elucidation of two new metabolites, renieramycins E (**159**) and F (**160**), from the same sponge were also disclosed. The renieramycins were understood to have identical stereochemical and similar dimeric structures to those of the saframycins (**51-53**) *(30)*.

In 1992, Davidson reported that the isolation of renieramycin G (**161**) from the hard blue Fijian sponge *Xestospongia caycedoi*, along with three other known metabolites mimosamycin (**208**), renierol (**250**) and *N*-formyl-1,2-dihydrorenierone (**235**) *(93)*.

Renieramycin A (**155**): $C_{30}H_{34}N_2O_9$; Mass *m/z* 566 M^+; IR ($CHCl_3$) ν 3160, 1720, 1660, 1650, 1645, 1625 cm^{-1}; UV (MeOH, ε) λ 268 (15800), 365 (1370) nm; $[\alpha]^{20}_D$ -36.3° (*c* 0.16, MeOH); ^{1}H-NMR ($CDCl_3$) δ 3.60 (1H, m, H-1), 2.64 (1H, ddd, *J*=11, 2.5 and 2.5 Hz, H-3), 1.26 (1H, ddd, *J*=17, 11 and 3 Hz, Ha-4), 2.75 (1H, ddd, *J*=17, 2.5 and 1 Hz, Hb-4), 1.91 and 1.92 (2x3H, s, CH_3-7 and 17), 4.00 and 4.01 (2x3H, s, OCH_3-8 and 18), 4.04 (1H, dd, *J*=2.5 and 1 Hz, H-11), 3.18 (1H, bs, H-13), 4.44 (1H, d, *J*=2 Hz, H-14), 3.18 (1H, bd, *J*=11 Hz, Ha-21), 2.71 (1H, dd, *J*=11 and 3.5 Hz, Hb-21), 4.49 (1H, dd, *J*=11.5 and 3 Hz, Hb-22), 4.19 (1H, dd, *J*=11.5 and 2 Hz, Hb-22), 1.55 (3H, brs, CH_3), 1.78 (3H, d, *J*=7 Hz, CH_3), 5.92 (1H, brq, *J*=7 Hz, H-26), 2.43 (3H, s, NCH_3); ^{13}C-NMR (C_6D_6) δ 166.0, 156.8, 156.4, 143.1, 141.9, 139.8, 137.7, 137.1, 130.8, 130.0, 126.3, 64.2, 62.8, 61.5, 61.0, 59.5, 57.1, 56.7, 42.8, 26.9, 20.9, 16.2, 9.0, 8.8.

Renieramycin B (**156**): $C_{32}H_{38}N_2O_9$; HRMS *m/z* 594 M^+; IR ($CHCl_3$) ν 1715, 1660, 1645, 1620 cm^{-1}; UV (MeOH, ε) λ 268 (17400), 365 (1460) nm; $[\alpha]^{20}_D$ -32.3° (*c* 0.15, MeOH); ^{1}H-NMR ($CDCl_3$) δ 3.61 (1H, brs, H-1), 2.62 (1H, ddd, *J*=11, 3 and 2.5 Hz, H-3), 1.23 (1H, ddd, *J*=18, 11 and 3 Hz, Ha-4), 2.74 (1H, dd, *J*=18 and 3 Hz, Hb-4), 1.90 and 1.93 (2x3H, s, CH_3-7 and 17), 4.00 and 3.95 (2x3H, s, OCH_3-8 and 18), 4.04 (1H, dd, *J*=3 and 1 Hz, H-11), 3.19 (1H, bs, H-13), 4.05 (1H, s, H-14), 3.10 (1H, dd, *J*=11 and 2 Hz, Ha-21), 2.76 (1H, dd, *J*=11 and 3.5 Hz, Hb-21), 4.32 (1H, dd, *J*=11.5 and 3 Hz, Ha-22), 4.27 (1H, dd, *J*=11.5 and 2.5 Hz, Hb-22), 1.56 (3H, brs, CH_3), 1.79 (3H, d, *J*=7 Hz, CH_3), 5.91 (1H, brq, *J*=7 Hz, H-26), 2.48 (3H, s, NCH_3), 1.19 (3H, t, *J*=7 Hz, OCH_2CH_3), 3.76 (2H, q, *J*=7 Hz, OCH_2CH_3).

Renieramycin C (**157**): $C_{30}H_{32}N_2O_{10}$; HRMS *m/z* 580 M^+; IR ($CHCl_3$) ν 3100, 1720, 1680, 1660, 1650, 1620 cm^{-1}; UV (MeOH, ε) λ 266 (14900), 360 (21600) nm; $[\alpha]^{20}_D$ -89.2° (*c* 0.065, MeOH); ^{1}H-NMR ($CDCl_3$) δ 5.48 (1H, brs, H-1), 3.86 (1H, ddd, *J*=12, 3.5 and 2.5 Hz, H-3), 1.41 (1H, ddd, *J*=17, 12 and 2 Hz, Ha-4), 3.02 (1H, dd, *J*=17 and 2.5 Hz, Hb-4), 1.93 and 1.95 (2x3H, s, CH_3-7 and

17), 4.01 and 4.05 (2x3H, s, OCH_3-8 and 18), 4.19 (1H, dd, *J*=3.5 and 1 Hz, H-11), 3.73 (1H, s, H-13), 4.78 (1H, brs, H-14), 4.36 (1H, dd, *J*=11.5 and 2.5 Hz, Ha-22), 4.72 (1H, dd, *J*=11.5 and 2.5 Hz, Hb-22), 1.50 (3H, brs, CH_3), 1.69 (3H, d, *J*=7 Hz, CH_3), 5.90 (1H, brq, *J*=7 Hz, H-26), 2.61 (3H, s, NCH_3).

Renieramycin D (**158**): $C_{32}H_{36}N_2O_{10}$; HRMS *m/z* 608 M^+; IR ($CHCl_3$) ν 1720, 1680, 1665, 1645, 1620 cm^{-1}; UV (MeOH, ε) λ 264 (16100), 370 (1450) nm; $[\alpha]^{20}_D$ -100.7° (*c* 0.092, MeOH); ^{1}H-NMR ($CDCl_3$) δ 5.49 (1H, brs, H-1), 3.87 (1H, H-3), 1.41 (1H, ddd, *J*=17, 12 and 1 Hz, Ha-4), 3.02 (1H, dd, *J*=17 and 2.5 Hz, Hb-4), 1.92 and 1.95 (2x3H, s, CH_3-7 and 17), 3.97 and 4.04 (2x3H, s, OCH_3-8 and 18), 4.22 (1H, d, *J*=2 Hz, H-11), 3.67 (1H, brs, H-13), 4.36 (1H, brs, H-14), 4.42 (1H, dd, *J*=12 and 2.5 Hz, Ha-22), 4.63 (1H, dd, *J*=12 and 2.5 Hz, Hb-22), 1.48 (3H, brs, CH_3), 1.68 (3H, d, *J*=7 Hz, CH_3), 5.89 (1H, brq, *J*=7 Hz, H-26), 2.62 (3H, s, NCH_3), 1.23 (3H, t, *J*=7 Hz, OCH_2CH_3), 3.87 (2H, q, *J*=7 Hz, OCH_2CH_3).

Renieramycin E (**159**): $C_{30}H_{33}N_2O_8$; amorphous yellow powder (unstable); HRMS *m/z* 549 M^+; IR ($CHCl_3$) ν 3390, 1700, 1655, 1615 cm^{-1}; UV (MeOH, ε) λ 266 (17000) nm; ^{1}H-NMR ($CDCl_3$) δ 4.44 (1H, m, H-1), 3.16 (1H, dt, *J*=11.5 and 2.5 Hz, H-3), 2.75 (1H, dd, *J*=16.9 and 2.5 Hz, Hα-4), 1.31 (1H, ddd, *J*=16.9, 11.5 and 2.9 Hz, Hβ-4), 1.91 (2x3H, s, CH_3-7 and 17), 3.98 and 4.00 (2X3H, s, OCH_3-8 and 18), 3.92 (1H, brd, *J*=2.5 Hz, H-11), 3.21 (1H, brd, *J*=7.6 Hz, H-13), 2.66 (1H, dd, *J*=20.9 and 7.6 Hz, Hα-14), 2.21 (1H, d, *J*=20.9 Hz, Hα-14), 4.44 (1H, m, Hβ-21), 4.45 (1H, dd, *J*=11.2 and 3.2 Hz, H-22), 4.15 (1H, dd, *J*=11.2 and 1.8 Hz, H-22), 1.57 (3H, m, CH_3-25), 1.79 (3H, dq, *J*=7.2 and 1.4 Hz, CH_3-26), 5.92 (1H, qq, *J*=7.2 and 1.4 Hz, H-26), 2.25 (3H, s, NCH_3).

Renieramycin F (**160**): $C_{31}H_{35}N_2O_9$; amorphous yellow powder (unstable); HRMS *m/z* 579 M^+; IR ($CHCl_3$) ν 3300, 1710, 1660, 1615 cm^{-1}; UV (MeOH, ε) λ 265 (12000) nm; ^{1}H-NMR ($CDCl_3$) δ 4.33 (1H, m, H-1), 3.11 (1H, brd, *J*=11 Hz, H-3), 2.75 (1H, dd, *J*=16.8 and 2.5 Hz, Hα-4), 1.20 (1H, dd, *J*=16.8, 11.5 and 2.9 Hz, Hβ-4), 1.90 and 1.94 (2x3H, s, CH_3-7 and 17), 3.97 and 4.00 (2x3H, s, OCH_3-8 and 18), 4.00 (1H, m, H-11), 3.29 (1H, brd, *J*=2.2 Hz, H-13), 3.76 (1H, s, Hα-14), 4.57 (1H, brd, *J*=10.1 Hz, Hβ-21), 4.28 (1H, dd, *J*=11.2 and 2.5 Hz, H-22), 4.20 (1H, dd, *J*=11.2 and 3 Hz, H-22), 1.57 (3H, m, CH_3-25), 1.80 (3H, dq, *J*=7.2 and 1.4 Hz, CH_3-26), 5.94 (1H, qq, *J*=7.2 and 1.4 Hz, H-26), 3.53 (3H, s, OCH_3), 2.46 (3H, s, NCH_3).

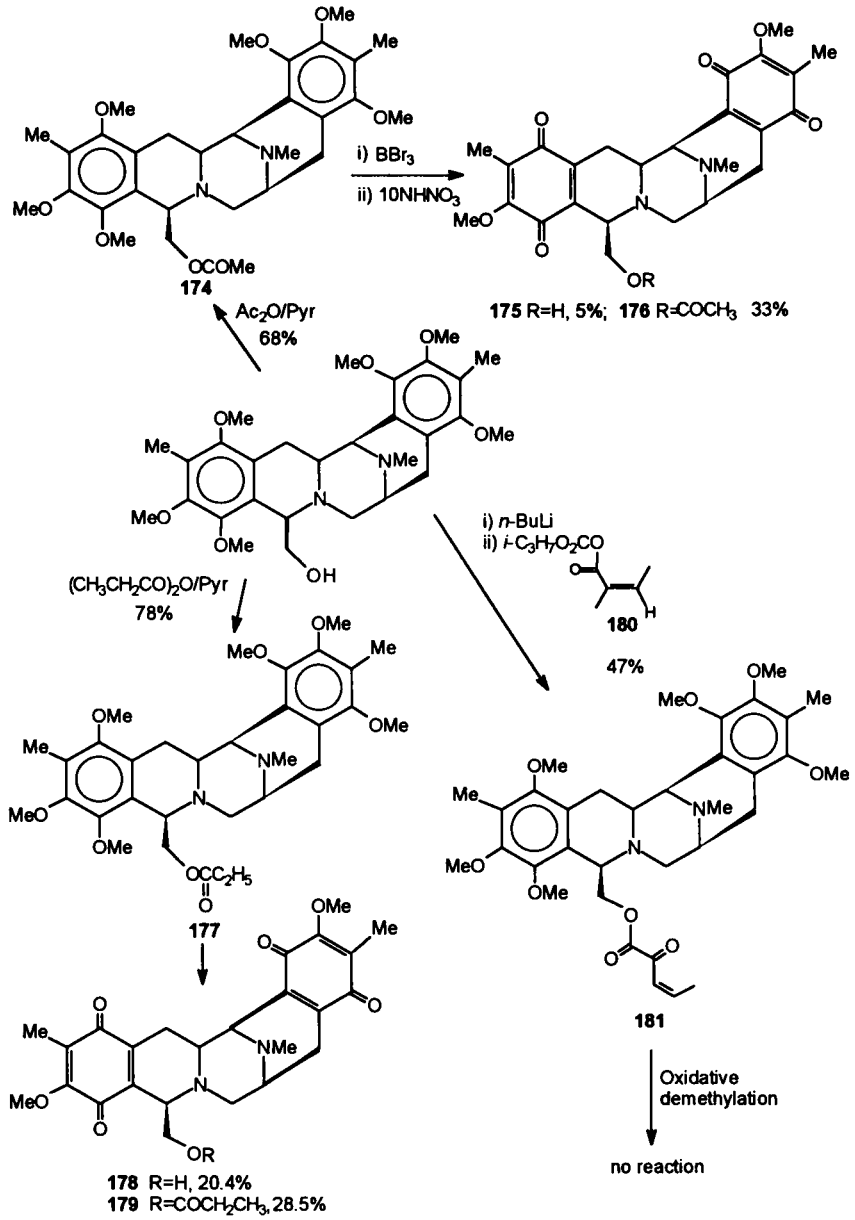
Renieramycin G (**161**): FABMS *m/z* 565 M^++H; IR (film) ν 2923, 1713, 1658, 1652, 1308, 1230, 1150 cm^{-1}; UV (MeOH, ε) λ 269 (20000) nm; ^{1}H-NMR (CD_2Cl_2) δ 5.40 (1H, brs, H-1), 3.85 (1H, dt, *J*=12.2 and *J*=3.0 Hz, H-3), 3.01 (1H, dd, *J*=16.5 and 3.0 Hz, Ha-4), 1.49 (1H, ddd, *J*=16.5, 12.2 and 1.6 Hz, Hb-4), 1.93 (2x3H, s, 2xArMe), 3.98 and 4.01 (2x3H, s, OCH_3), 4.12 (1H, brd, *J*=4.0 Hz, H-11), 3.67 (1H, d, *J*=7.1 Hz, H-13), 2.87 (1H, dd, *J*=20.6 and 6.1 Hz, Ha-14), 2.64 (1H, d, *J*=20.6 Hz, Hb-14), 4.67 (1H, dd, *J*=11.7 and 2.8 Hz, Ha-22), 4.32 (1H, dd, *J*=11.7 and 2.6 Hz, H-22), 5.90 (1H, m, H-26), 1.52 (3H, t, *J*=1.6 Hz, CH_3-25),1.68 (3H, dq, *J*=7.3 and 1.6 Hz, CH_3-26), 2.36 (3H, s, NCH_3); ^{13}C-NMR (CD_2Cl_2) δ 50.52 (C-1), 56.63 (C-3), 26.09 (C-4), 142.15 (C-5), 185.58 (C-6), 129.63 and 128.79 (C-7 and 17), 155.99 (C-8), 180.84 (C-9), 136.59 (C-10), 53.2 (C-11), 59.60 (C-13), 24.00 (C-14), 142.56 (C-15), 186.64 (C-16), 156.56 (C-18), 182.90 (C-19), 135.45 (C-20), 170.71 (C-21), 63.34 (C-22), 167.30 (C-24), 127.26

(C-25), 139.50 (C-26), 15.60 (CH_3-25), 20.51 (CH_3-26), 8.76 (ArMe), 61.28 and 61.24 (2xOCH_3),40.06 (NCH_3).

2. Synthesis

Of the renieramycins A-G (**155-161**), the total synthesis of (±)-renieramycin A (**155**) was reported by Fukuyama *et al.* in 1990 (Scheme 19) *(94)*. Their elegant synthesis initially involved the preparation of the benzaldehyde **163** in ten steps and overall 55% yield. Condensation of the aldehyde **163** with *N,N'*-diacetylpiperazinedione (**162**) in the presence of *t*-BuOK exclusively gave the *Z*-isomer **164**. Hydrogenation of the olefin **164** over palladium-carbon was followed by introduction of a carbobenzoxy group in order to activate the piperazinedione ring. Compound **165** was then condensed with 5-benzyloxy-2,4-dimethoxy-3-methylbenzaldehyde (**166**) in the presence of *t*-BuOK and then DBU to furnish **167** which was converted to the tricyclic compound **168** in three steps. Initially, the ring carbonyl functionality of **168** was selectively reduced with $NaBH_4$, and then cyclization in formic acid via acyliminium-ion formation was followed by hydrolysis of the resulting formate with sodium hydroxide to obtain the desired product **168**. Reduction of the olefin **168** using Raney nickel, which took place from the less hindered *exo* side, gave the *endo*-compound, the bridgehead amine of which was then protected as a base-sensitive urethane ($ClCO_2(CH_2)_2CO_2Me$). Subsequent benzylation of phenolic groups of the resultant product yielded **169**. The primary alcohol functionality of **169** was removed by performing Swern oxidation to obtain the desired phenol **170**. Conversion of **170** to **171** was achieved in four steps. Treatment of **170** with DDQ in aqueous THF gave the desired alcohol, the phenol group of which was methylated with methyl iodide and the urethane protecting group was removed using DBU through retro-Michael reaction. The resultant amine was then reductively methylated to afford **171**. Treatment of **171** initially with AlH_3 to reduce the lactam, and then hydrogenolysis of the product over palladium-carbon to remove the benzyl protecting groups, furnished the phenol **172**. Conversion of the tricyclic compound **172** to a pentacyclic molecule was achieved by reacting **172** with glycolaldehyde angelate which gave a 5:1 mixture of **173** and its minor α-epimer. Oxidation of **173** with DDQ gave (±)-renieramycin A (**155**).

In 1991, Kubo *et al.* reported the synthesis of renieramycin congeners (Scheme 20) *(95)*. Starting from the key intermediate **103** in saframycin B (**52**) synthesis (Scheme 10) *(57)*. Acetylation of the alcohol **103** with acetic anhydride in pyridine gave **174**. Treatment of **174** with 4 equiv. of boron tribromide was then followed by oxidation with 10 N HNO_3 to give the quinones **175** and **176** in 5% and 33% yields, respectively. In the other sequence, the alcohol **103** was reacted with propionic anhydride in pyridine to obtain **177**. Similarly, partial demethylation and oxidative demethylation of **177** furnished the quinones **178** and **179** in 20.4% and 28.5% yields, respectively. Lastly, treatment of the alcohol **103** with *n*-BuLi and addition of the anhydride **180** provided the desired compound **181**. Unfortunately, oxidative demethylation of **181** failed to give any product, and only starting material was recovered. Partial demethylation of **181** with boron tribromide gave large amounts of degradation products.



174

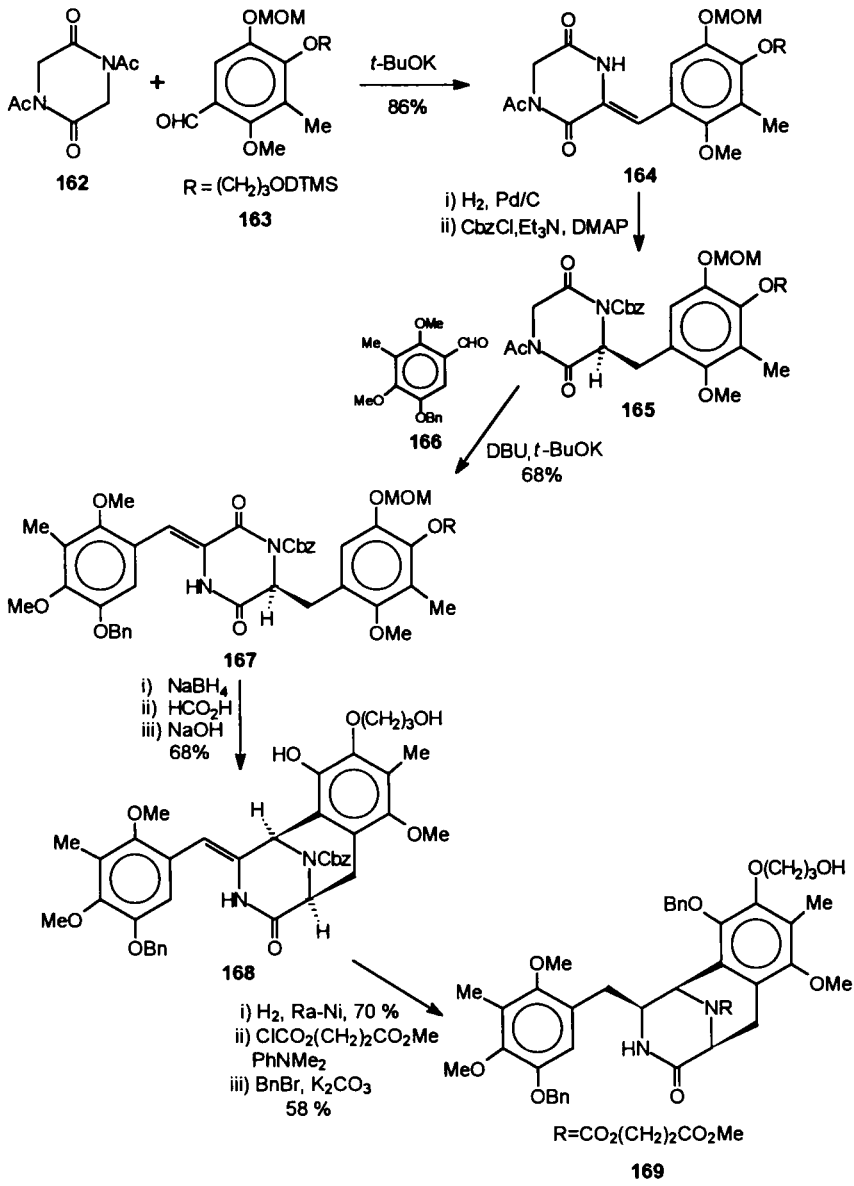
175 R=H, 5%; **176** R=$COCH_3$ 33%

177

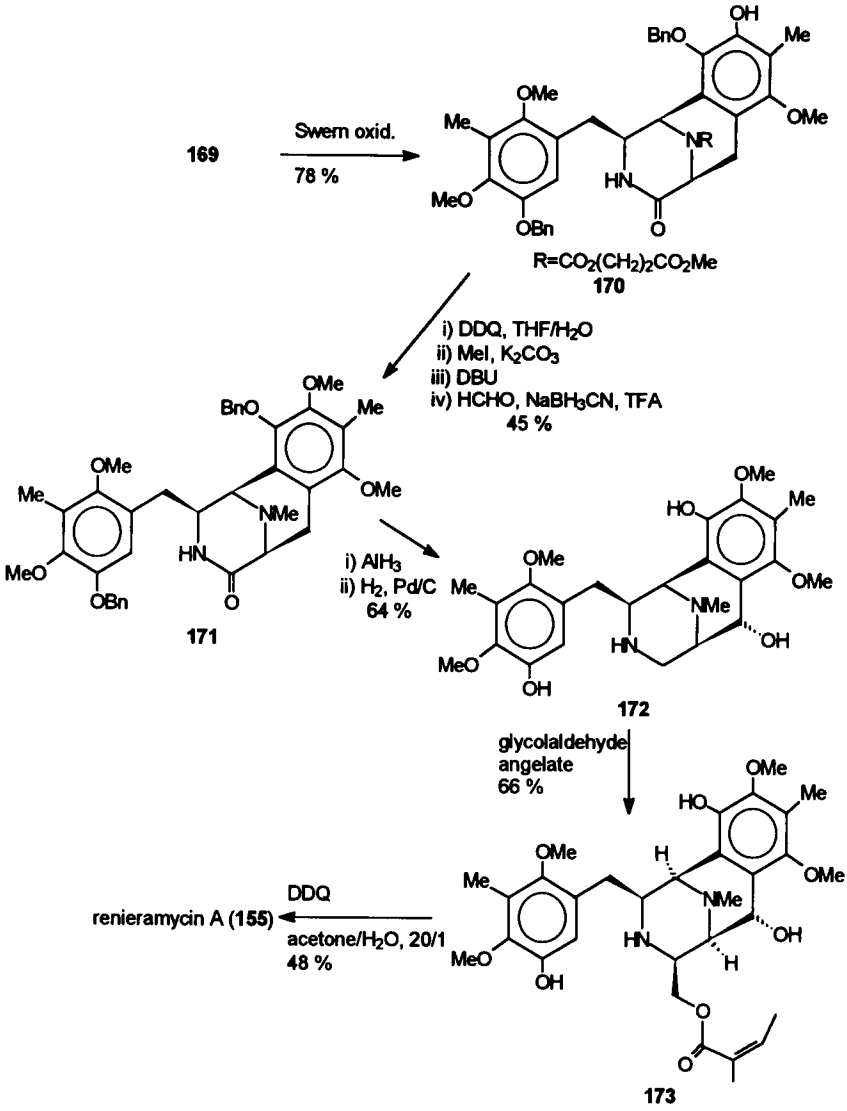
178 R=H, 20.4%
179 R=$COCH_2CH_3$, 28.5%

180

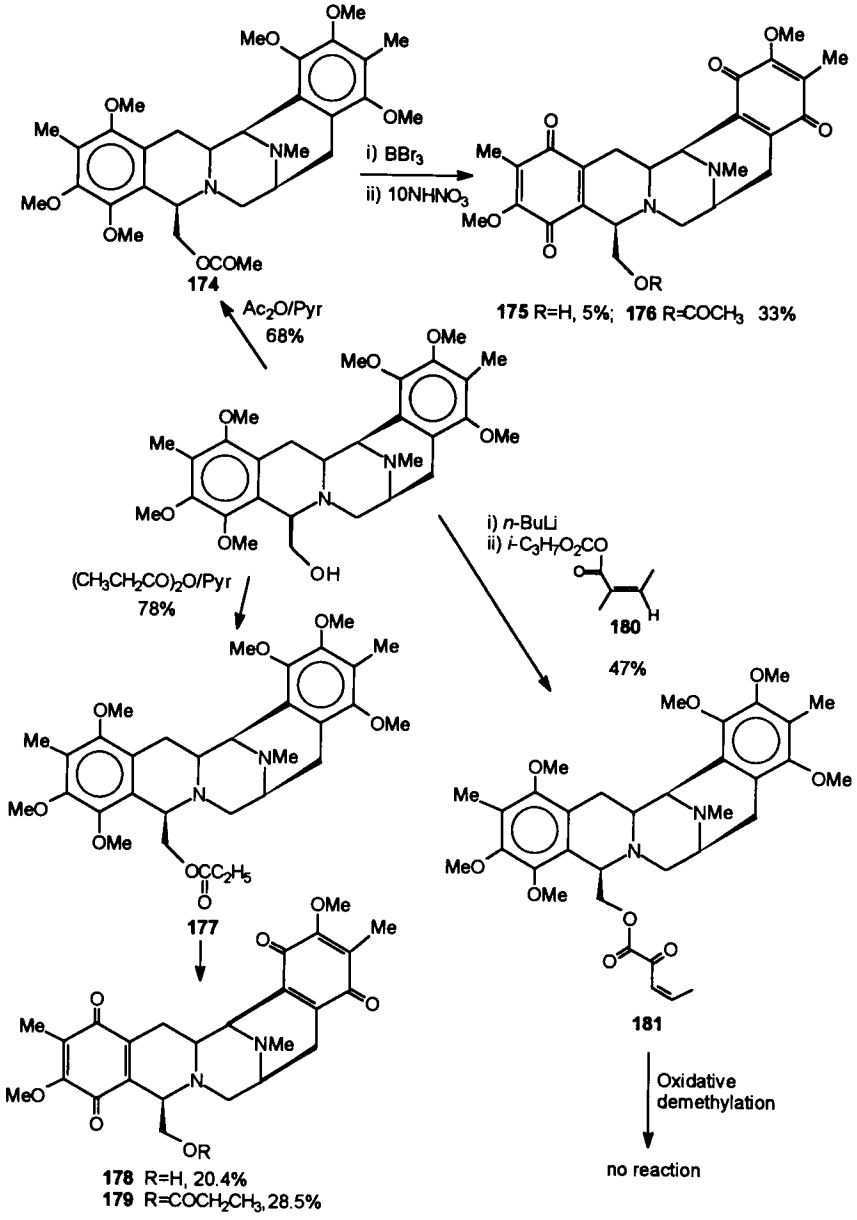
181



SCHEME 19. The total synthesis of (±)-renieramycin (155) by Fukuyama *et al. (94)*.



SCHEME 19. *Continued*



SCHEME 20. Synthesis of renieramycin congeners *(95)*.

3. Biological activity

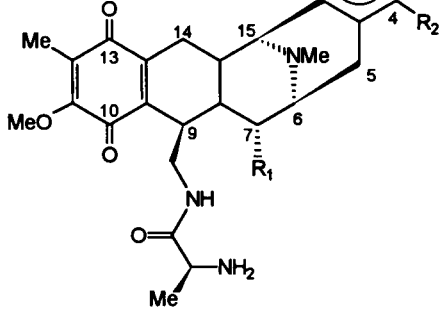
Renieramycins A-D (**155-158**) were reported to show antimicrobial activity against a variety of terrestrial and marine microorganisms *(91)* while renieramycin G (**161**) displayed moderate activity with the MIC values of 0.5 and 1.0 μg/ml against the KB and LoVo cell lines, respectively *(93)*.

C. SAFRACINS

1. Isolation and Structure Elucidation

In 1983, Ikeda *et al.* reported the isolation of safracin A (**182**) and safracin B (**183**) from a culture broth of *Pseudomonas fluorescens* A2-2 *(96)*, which were initially named as Y-16482β and Y-16482α, respectively *(97)*. Their conventional spectroscopic measurements suggested very similar structures to the saframycins **51-53** *(30)*. However, the pyruvamide side chain and one of the *p*-quinone rings of the saframycins are substituted by an alanyl amide side chain and a monophenol ring in the safracins, respectively. The absolute configuration of the safracins was explained on the basis of single crystal X-ray diffraction measurements of brominated safracin A (**184**) *(98)*. In the same year, Cooper *et al.* isolated safracin B from a culture broth of *Pseudomonas fluorecens* SC 12695 and called it EM5519 *(99)*. It was later understood that EM5519 is identical to safracin B (**183**) *(99, 100)*.

Safracin A (**182**): $C_{28}H_{36}N_4O_6$; pale yellow needles; as dihydrochloride monohydrate, mp>300°C (dec.); MS *m/z* 524 M^+; $[\alpha]^{20}_D$ -144° (*c* 0.5, MeOH); IR (KBr) ν 1690, 1680, 1660, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 271 (3.93)nm.

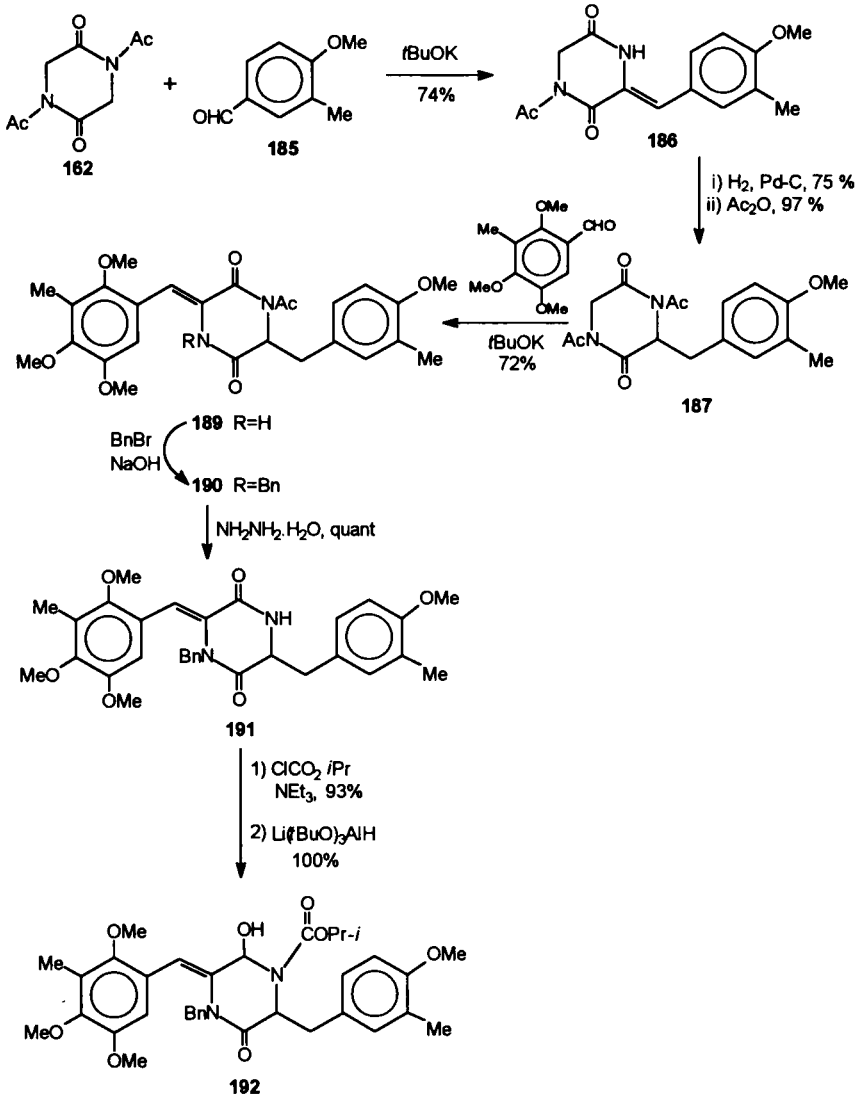


safracin A **182** R_1=R_2=H
safracin B **183** R_1=OH, R_2=H
C **184** R_1=H, R_2=Br

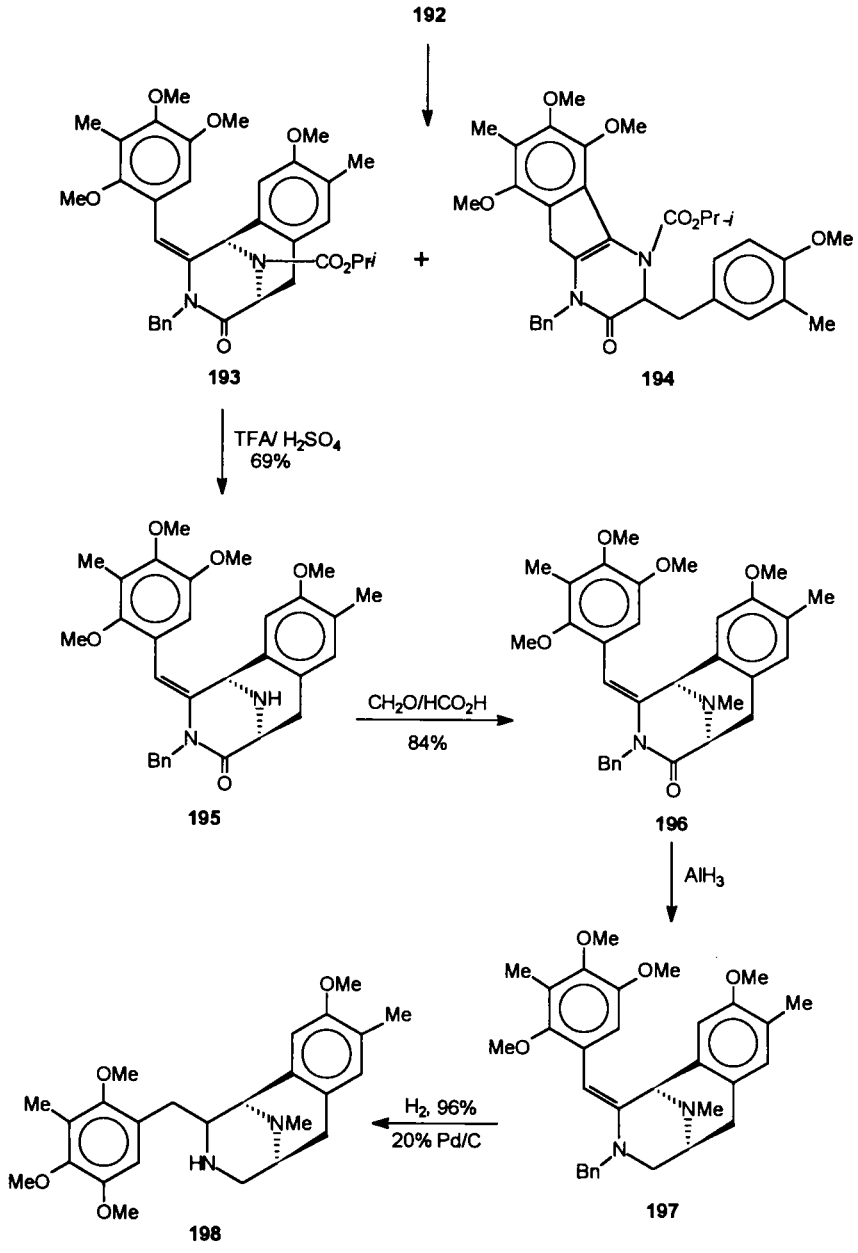
Safracin B (**183**): $C_{28}H_{36}N_4O_7$; pale yellow needles; as dihydrochloride monohydrate, mp>260°C (dec.); MS *m/z* 540 M^+; $[\alpha]^{20}_D$ -106° (*c* 0.5, MeOH); IR (KBr) ν 1690, 1680, 1660, 1620 cm^{-1}; UV (MeOH, log ε) λ_{max} 270 (3.89).

2. Synthetic Studies

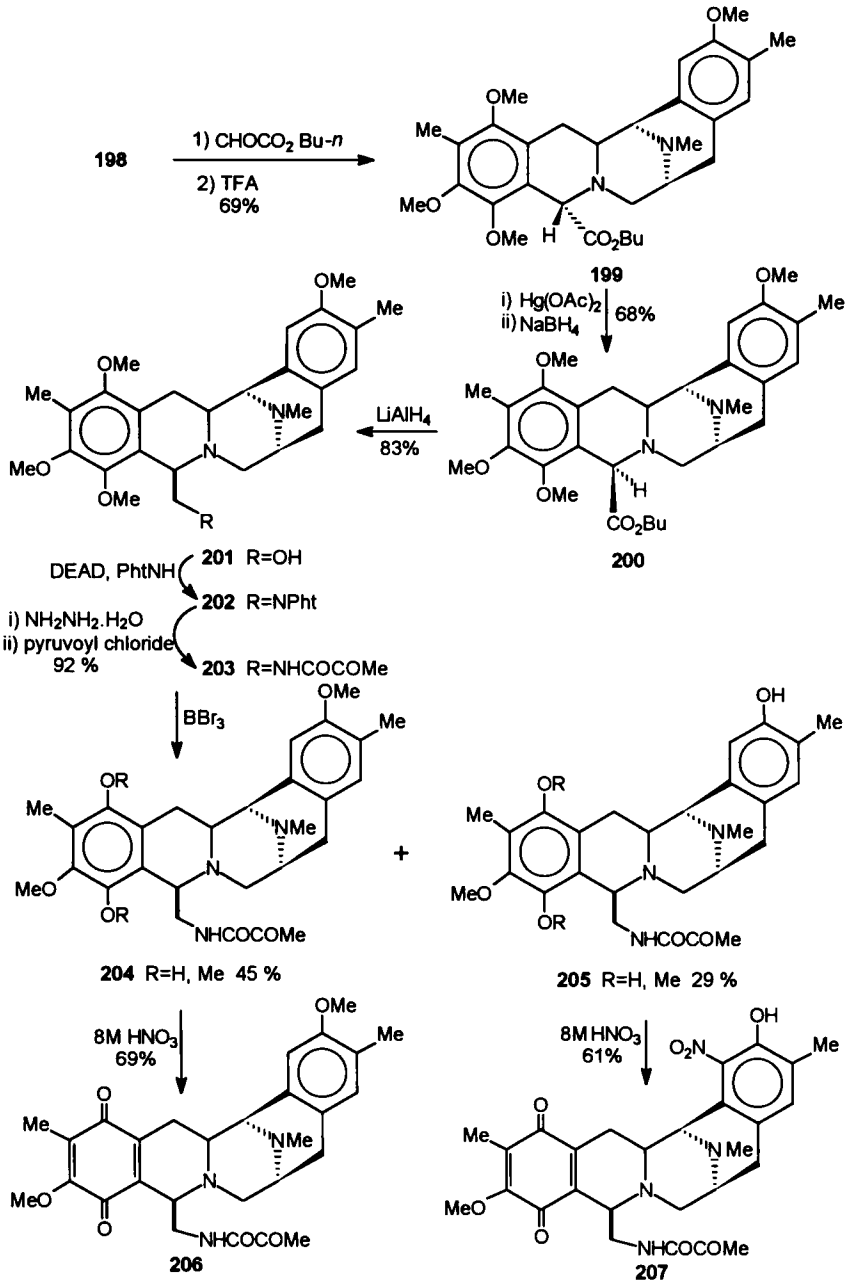
Although total syntheses of the safracins have not been achieved yet, some reports on partial syntheses and studies toward the total synthesis of safracin A have appeared in the literature. The efforts devoted by Kubo *et al.* succeeded so far in a partial synthesis (Scheme 21) *(102, 103)*. Their synthesis started with the condensation of commercially available 1,4-diacetyl-2,5-piperazinedione (**162**) with 4-methoxy-3-methylbenzaldehyde (**185**) in the presence of *tert*-butoxide to give the arylidenepiperazinedione **186**. Hydrogenation of **186** over palladium-carbon was followed by acetylation using acetic anhydride to furnish **187**. Condensation of **187** and 2,4,5-trimethoxy-3-methylbenzaldehyde (**188**) in the presence of potassium *tert*-butoxide gave **189**. Benzylation of **189** with benzyl bromide to obtain **190** was followed by treatment with hydrazine hydrate to yield **191**. Activation of the piperazine ring was achieved by introduction of a 2-propyloxycarbonyl group, and the selective reduction of the carbonyl group with lithium tri-*tert*-butoxyaluminum hydride furnished a diastereomeric mixture of the alcohol **192**. Treatment of **192** with formic acid gave the desired molecule **193** in 86% yield, and the undesired one in 20% yield, along with the precursor **191** in 8% yield. When this reaction was performed using $TiCl_4$, **193** was obtained in 86% and **194** in 4% yield, and when the reagent was changed to $BF_3.OEt_2$, **193** was obtained in 32% and **194** in 61% yield. Deprotection of the desired molecule **193** with trifluoroacetic acid and H_2SO_4 gave **195** which was methylated with formaldehyde and formic acid to yield the lactam **196**. Conversion of **196** to the amine **197** was achieved by reduction of the carbonyl group of the lactam with aluminum hydride. Hydrogenation of **197** over 20% palladium-carbon cleanly reduced the double bond from the α-face, and the tricyclic product **198** was converted to the pentacyclic compound **199** in two steps, i) reaction with butyl glyoxylate in the presence of K_2CO_3, and then ii) treatment of the product with trifluoroacetic acid for the ring closure. Epimerization of the butyl carboxylate group of **199** was achieved using mercury acetate in 5% aqueous AcOH to give **200** which was subsequently reduced with lithium aluminum hydride to obtain the alcohol **201**. Treatment of **201** with diethyl azodicarboxylate, triphenylphosphine and phthalimide performed the replacement of the oxygen of the hydroxyl functionalty of **201** with nitrogen. The reaction of **202** with hydrazine hydrate to convert the imide to an amine was followed by acetylation with pyruvoyl chloride to give the pyruvamide **203**. Employment of a partial demethylation and oxidative demethylation sequence developed by Kubo *et al.* resulted in the formation of the mono-*p*-quinone system *(57)*. Treatment of **203** with boron tribromide yielded the phenols **204** and **205** in 45% and 29%, respectively. Oxidative demethylation was performed using 8 M HNO_3 for both **204** and **205**, which gave the *p*-quinones **206** and **207**, respectively. All attempts to introduce hydroxyl groups at C-1 to continue the total synthesis failed.



SCHEME 21. Synthetic studies on safracin A (**182**) *(102, 103)*.



SCHEME 21. *Continued*



SCHEME 21. *Continued*

In 1985, Ikeda reported his findings on the factors affecting the production of safracins *(101)*. The presence of $FeSO_4$ and $FeCl_3$ at a concentration of 0.01%,

under highly aerobic condition, increased safracin B production, while at the 0.1% concentration, production of safracin A increased. Because the production of safracin B always preceded A, it was suggested that safracin A was derived from safracin B. An increase in the production of safracin A was observed without any decrease in safracin B production at higher concentrations of Fe ions during the fermentation. The experiments indicated that amino acids such as lysine with 0.1% of $FeSO_4.7H_2O$ increased the production of safracins A and B, while 0.5% of alanine had the opposite effect. 0.1% of L-tyrosine, L-alanine, L-tryptophan and L-phenylalanine with 0.01% of $FeCl_3.6H_2O$ increased safracin B production.

3. Biological Activity

Safracins A and B showed *in vitro* activity against Gram-positive and Gram-negative bacteria, and the superiority of safracin B to safracin A was indicated. However, no therapeutic activity in mice infected with *Staphylococcus aureus* was observed *(104)*.

The morphological changes of *E. coli* were reported at safracin A and B concentrations varying from 25 to 100 μg/ml and from 0.20 to 3.12 μg/ml, respectively, various abnormal forms of *E. coli* such as tadpole or starfish appeared. Cell growth was completely inhibited at concentrations of safracin A over 100 μg/ml and by safracin B at over 6.25 μg/ml, and abnormal forms were not observed. On the other hand, *E. coli* cells changed to filamentous forms with safracins A and B in HIB without supplementation of sucrose and $MgSO_4.7H_2O$.

Both safracin A and B showed antitumor activity against L1210 and P388 leukemias and B16 melanoma, although the toxic and effective doses of safracin B were found to be much lower than those of safracin A *(104,105)*. Greater effectiveness for safracin B over safracin A, in terms of antitumor activity and of prolonging the life span of tumor-bearing mice, was taken to mean that the α-carbinolamine structure of safracin B plays an important role, as indicated in other antitumor antibiotics with similar functionality, such as the saframycins and renieramycins.

IV. Isoquinolinequinone Type Alkaloids

A. MIMOSAMYCIN

1. Isolation and Structure Elucidation

In 1976, Arai *et al.* reported the isolation of a new antibiotic, mimosamycin (**208**), from the culture filtrate of the microorganism *Streptomyces lavendulae* No.314 *(106)*. The structure was disclosed in 1977 on the basis of single crystal X-ray analysis and total synthesis *(107)*. The full report of the single crystal X-ray analysis was released in 1978 *(108)*. The large scale production of mimosamycin from the same source was also described by the same group *(109)*.

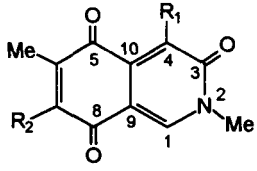
Later, isolation of mimosamycin from various sources, such as sponge identified as a *Reniera* sp. in 1982 *(91)*, blue sponges of the genus *Xestospongia* in 1987 *(110)*, the blue marine sponge *Cribrochalina* sp. in 1992 *(111)* and the bright-blue sponge *Petrosia* sp. in 1993 *(112)* were reported by Faulkner *et al.*, Ireland *et al.*, Pettit *et al.* and Venkateswarlu *et al.*, respectively.

In 1994, Kobayashi *et al.* identified the two isomers 4-aminomimosamycin (**209**) and 7-amino-7-demethoxymimosamycin (**210**) from a blue-colored sponge, *Petrosia* sp., collected off the east coast of India *(113)*.

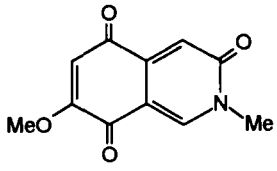
Mimosamycin (**208**): $C_{12}H_{11}NO_4$; mimosa-yellow prisms; gives positive reaction with Ehrlich's reagent (orange) and positive reaction with ninhydrin and Dragendorff's reagents; mp 227-231°C; LRMS *m/z* 233 M^+; $[\alpha]^{24}_D$ -1.8° (*c* 1.0, CH_2Cl_2); IR (KBr) ν 1685, 1655, 1635, 1585 cm^{-1}; UV (MeOH, log ε) λ_{max} 230 sh (4.16), 317 (4.14), 396 (3.56), λ_{min} 277 (3.78), 370 (3.53); ^{1}H-NMR (60 MHz, $CDCl_3$) δ 2.10 (3H, s), 3.69 (3H, s), 4.20 (3H, s), 7.12 (1H, s), 8.28 (1H, s); ^{13}C-NMR ($CDCl_3$) δ 142.1 (C-1), 162.8 (C-3), 116.7 (C-4), 183.5 (C-5), 133.6 (C-6), 159.5 (C-7), 177.3 (C-8), 111.3 (C-9), 138.9 (C-10), 9.6 (CH_3), 61.3 (OCH_3).

4-Aminomimosamycin (**209**): $C_{12}H_{12}N_2O_4$; deep-red needles; mp 250-252°C (most of the crystals sublime below the melting point); EIMS *m/z* 248 M^+; UV (MeOH, log ε) λ_{max} 483 (3.58), 350 (3.34), 282 sh (3.58); ^{1}H-NMR ($CDCl_3$) δ 7.60 (1H, s, H-1), 6.25 and 8.87 (2x1H, br, NH_2), 2.07 (3H, s, CH_3-6), 3.64 (3H, s, NCH_3), 4.06 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$) δ 128.1 (C-1), 159.2 (C-3), 140.0 (C-4), 186.4 (C-5), 135.1 (C-6), 158.4 (C-7), 178.4 (C-8), 106.6 (C-9), 112.6 (C-10), 38.1 (NCH_3), 9.5 (CH_3), 60.8 (OCH_3).

7-Amino-2,6-dimethylisoquinoline-3,5,8-trione (**210**): $C_{11}H_{10}N_2O_3$; light brown solid; mp>300°C;); EIMS *m/z* 218 M^+; UV (MeOH, log ε) λ_{max} 434 (2.91), 362 sh (3.49), 327 (3.73), 250 sh (3.67); ^{1}H-NMR ($CDCl_3$) δ 8.26 (1H, s, H-1), 5.20 and 7.30 (2x1H, br, NH_2), 2.00 (3H, s, CH_3-6), 3.65 (3H, s, NCH_3), 7.15 (1H, s, H-4); ^{13}C-NMR (C_5D_5N) δ 143.0 (C-1), 163.2 (C-3), 115.6 (C-4), 179.5 (C-5), 113.9 (C-6), enveloped by solvent signal (C-7), 177.6 (C-8), 111.1 (C-9), 141.3 (C-10), 37.5 (NCH_3), 10.3 (CH_3).



mimosamycin



211

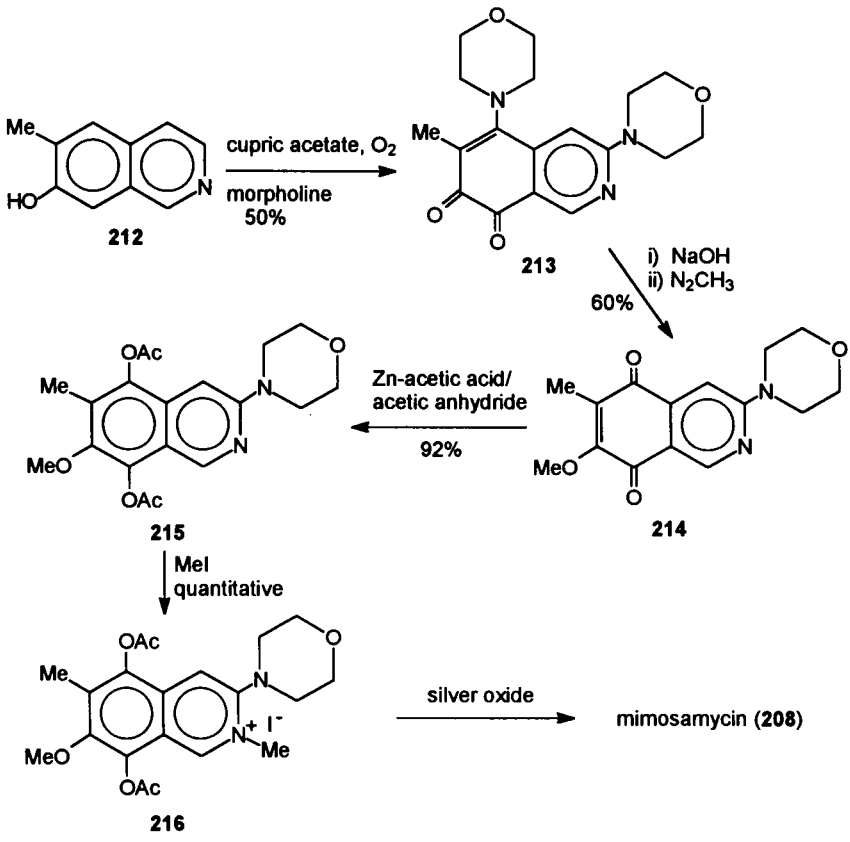
4-aminomimosamycin

7-amino-7-demethoxymimosamycin

208 R_1=H, R_2=OMe

209 R_1=NH_2, R_2=OMe

210 R_1=H, R_2=NH_2

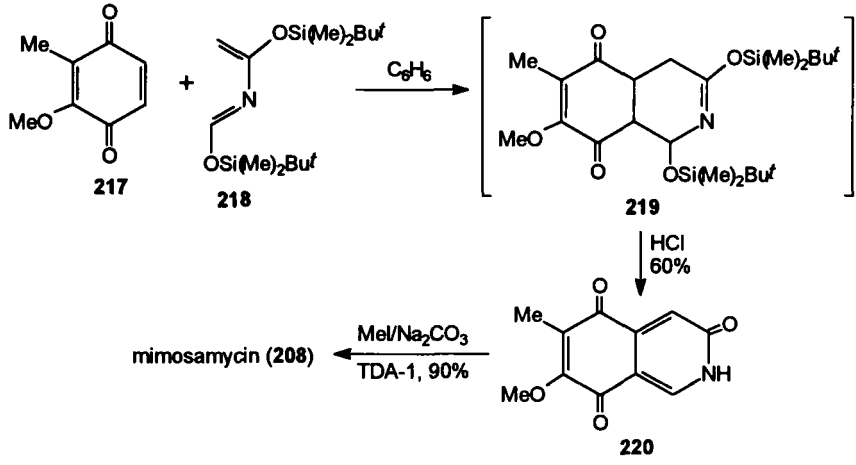


SCHEME 22. The total synthesis of mimosamycin (**208**) by Fukumi *et al. (107)*.

2. Synthesis

The synthesis of mimosamycin (**208**) was first disclosed by Fukumi *et al.* in 1977 *(107)*. Using the same methodology, they later reported various syntheses of mimosamycin *(114, 115)*, including its analogue 7-methoxy-2-methyl-3,5,8-isoquinolinetrione (**211**) *(116)*.

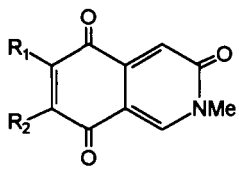
Their synthesis started with the readily accessible 6-methyl-7-isoquinolinol (**212**) *(117-119)*. Oxidation of **212** with oxygen in the presence of cupric acetate and morpholine afforded the quinone **213**. Methoxy-*p*-quinone **214** was obtained by treating **213** first with sodium hydroxide and then with diazomethane. All attempts to methylate **214** directly failed. This difficulty was circumvented by reductive acetylation using zinc-acetic acid/acetic anhydride, which gave the hydroquinone diacetate **215**. Mimosamycin was obtained on methylation of **215** with methyl iodide to yield **216**, followed by treatment with silver oxide to afford **208**.



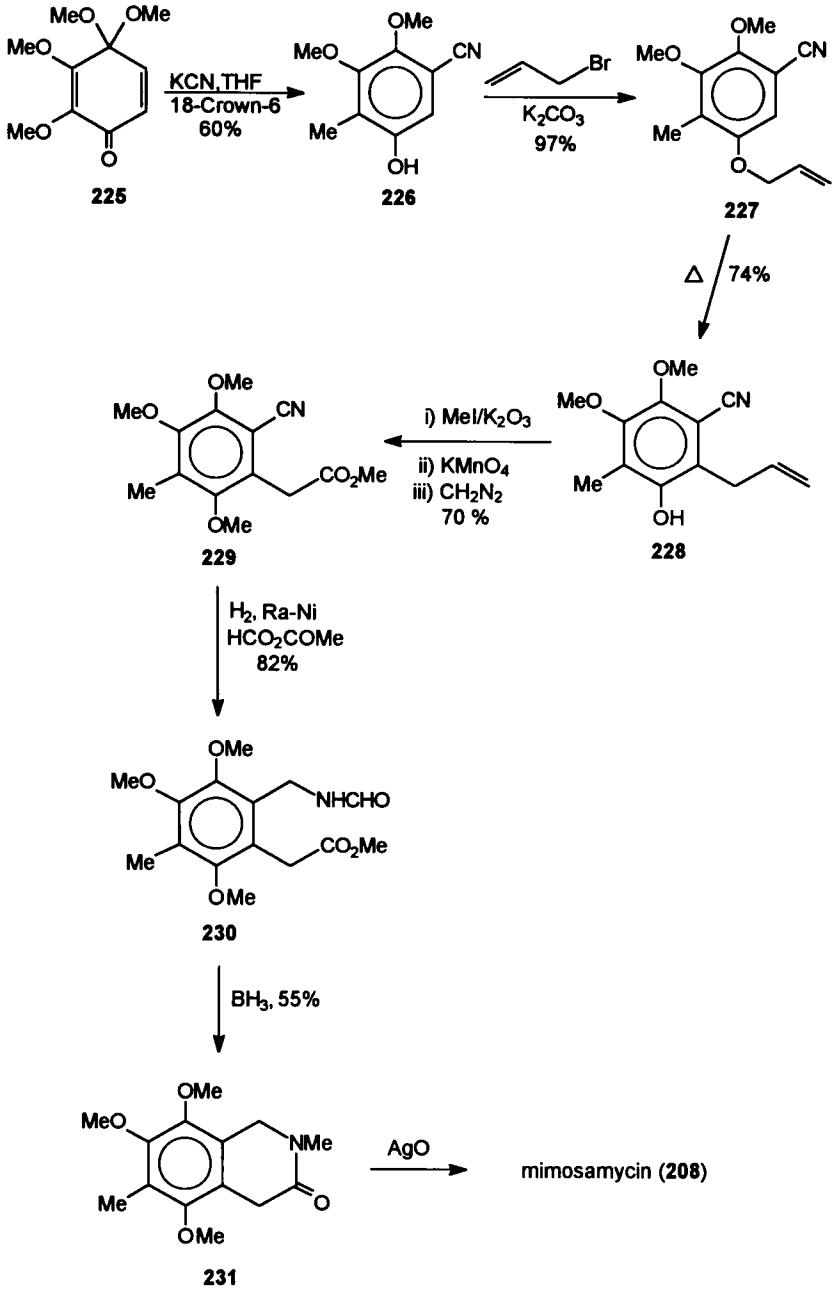
SCHEME 23. The total synthesis of mimosamycin (**208**) by McKillop *et al. (120).*

In 1987, McKillop *et al.* reported their two-step synthesis of mimosamycin (**208**) (Scheme 23) *(120)*. Their first step involved Ghosez's heterodiene cycloaddition approach *(121)*. Condensation of the quinone **217** with the diene **218** in a minimum amount of benzene gave the cycloadduct **219** which was treated directly with aqueous hydrochloric acid to afford the trione **220**. Methylation of **220** with methyl iodide using TDA-1 gave mimosamycin (**208**) in 90% yield. Using the same methodology McKillop *et al.* synthesized a number of analogues of mimosamycin (**221-224**) *(120)*.

In 1988, a rather long synthesis of mimosamycin was released by Parker *et al.* (Scheme 24) *(122)*. Their synthesis was initiated by conversion of the quinone ketal **225** to benzonitrile **226**, using potassium cyanide in the presence of crown-6. Addition of allyl bromide to **226** produced the ether **227** which was then subjected to Claisen rearrangement to give the phenol **228**. Methylation of the phenol with methyl iodide followed by oxidation of the olefin yielded an acid which was converted to its methyl ester on treatment with diazomethane to give **229**. Hydrogenation of **229** over Raney nickel in acetic-formic anhydride yielded the *N*-formyl ester **230**. The ring closure on **230** was achieved by diborane reduction to obtain the isoquinolinone **231**, which was then converted to mimosamycin on oxidative demethylation using AgO in dioxane/nitric acid.



221 $R_1=R_2=Me$
222 $R_1=R_2=CH=CH-CH=C$
223 $R_1=(CH_3)_3C$, $R_2=H$
224 $R_1=H$, $R_2=(CH_3)_3C$
} 1:



SCHEME 24. The total synthesis of mimosamycin (**208**) by Parker *et al. (122).*

3. Biological Activity

The neutral antibiotic mimosamycin (**208**) was found to be mainly active against *Mycobacterium tuberculosis* and inactive on the murine tumors *(123)*. Kobayashi *et al.*, reported that mimosamycin showed aldose reductose inhibitory effect (34.6 %) at 10 $\mu M/dm^3$, and 7-amino-7-demethoxymimosamycin (**210**) showed a cAMP phosphodiesterase inhibitory effect (26.3 %) at 100$\mu M/dm^3$ *(113)*. Mimosamycin was found to have significant activity against the P388 leukemia cell line (ED_{50} 0.73 μg/ml) *(111)*.

B. RENIERONE

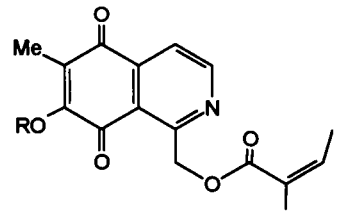
1. Isolation and Structure Elucidation

In 1979, Faulkner *et al.* reported the isolation of a metabolite from a bright blue sponge, *Reniera* sp., collected near Isla Grande, Mexico *(124)*. On the basis of spectroscopic and single crystal X-ray diffraction measurements, its structure was explained to be an isoquinolinequinone and named renierone (**232**). Further studies on *Reniera* sp. by the same group resulted in the isolation of three more renierone-related metabolities, *O*-demethylrenierone (**233**), and *N*-formyl-1,2-dihydrorenierones (**235a**) and (**235b**), along with mimosamycin (**208**), 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**), 2,5-dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**237**), and four renieramycins A-D (**155-158**) *(91)*.

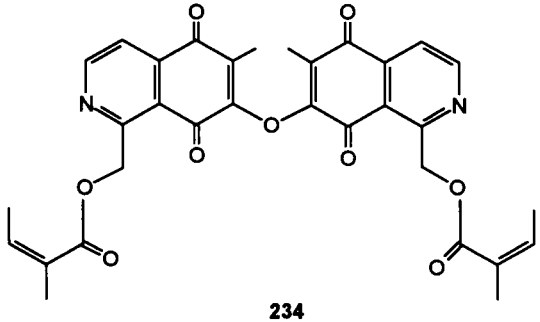
In 1992, isolation of *O*-demethylrenierone (**233**) and its dimer (**234**) from the sponge, *Haliclona* sp., collected from Gujarat coast, India was reported by Parameswaran *et al. (125)*, and in 1996, Proksch *et al.* reported an *N*-ethylene methyl ketone derivative of renierone isolated from a blue sponge, *Xestospongia* sp., collected in the Philippines, along with mimosamycin (**208**), renierone (**232**) and 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**) *(126)*.

Renierone (**232**) and *O*-demethylrenierone (**233**) were also isolated from the blue marine sponge, *Cribrochalina* sp., collected in the channel between Guradu and Madu Islands of South Male Atoll, Republic of the Maldives *(111)*.

Renierone (**232**): $C_{17}H_{17}NO_5$; mp 91.5-92.5°C; HRMS *m/z* 315; ^{1}H-NMR ($CDCl_3$) δ 8.93 (1H, d, *J*=4.8 Hz; H-3), 7.89 (1H, d, *J*=4.8 Hz, H-4), 2.12 (3H, s, H-11), 4.15 (3H, s, H-12), 5.80 (2H, s, H-13), 6.15 (1H, qq, *J*=7.2 and 1.4 Hz, H-16), 1.97 (3H, dq, *J*=1.5, 1.5 Hz, CH_3-17), 2.01 (dq, *J*=7.3, 1.5 Hz, CH_3-18); ^{13}C-NMR ($CDCl_3$) δ 159.0 (s, C-1), 154.3 (d, C-3), 118.7 (d, C-4), 184.9 (s, C-5), 123.1 (s, C-6), 157.2 (s, C-7), 182.2 (s, C-8), 128.3 (s, C-9), 139.4 (s, C-10), 8.0 (q, C-11), 60.9 (q, C-12), 65.7 (t, C-13), 168.0 (s, C-14), 131.1 (s, C-15), 138.0 (d, C-16), 20.7 (q, C-17), 15.8 (q, C-18).

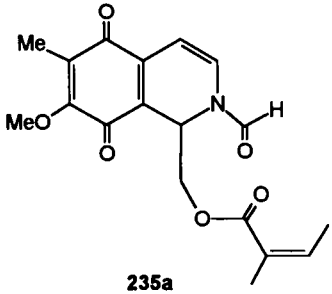


renierone **232** R=Me
O-demethylrenierone **233** R=H

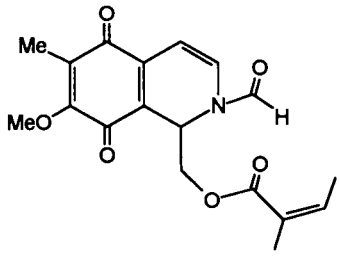


234

dimer of *O*-demethylrenierone

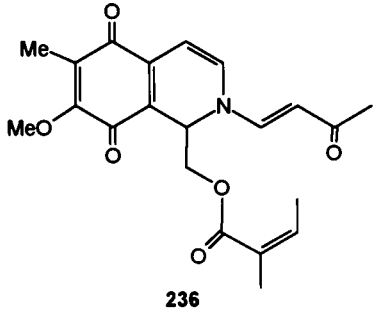


235a

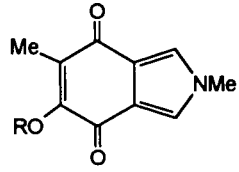


235b

N-formyl-1,2-dihydrorenierone



236



237

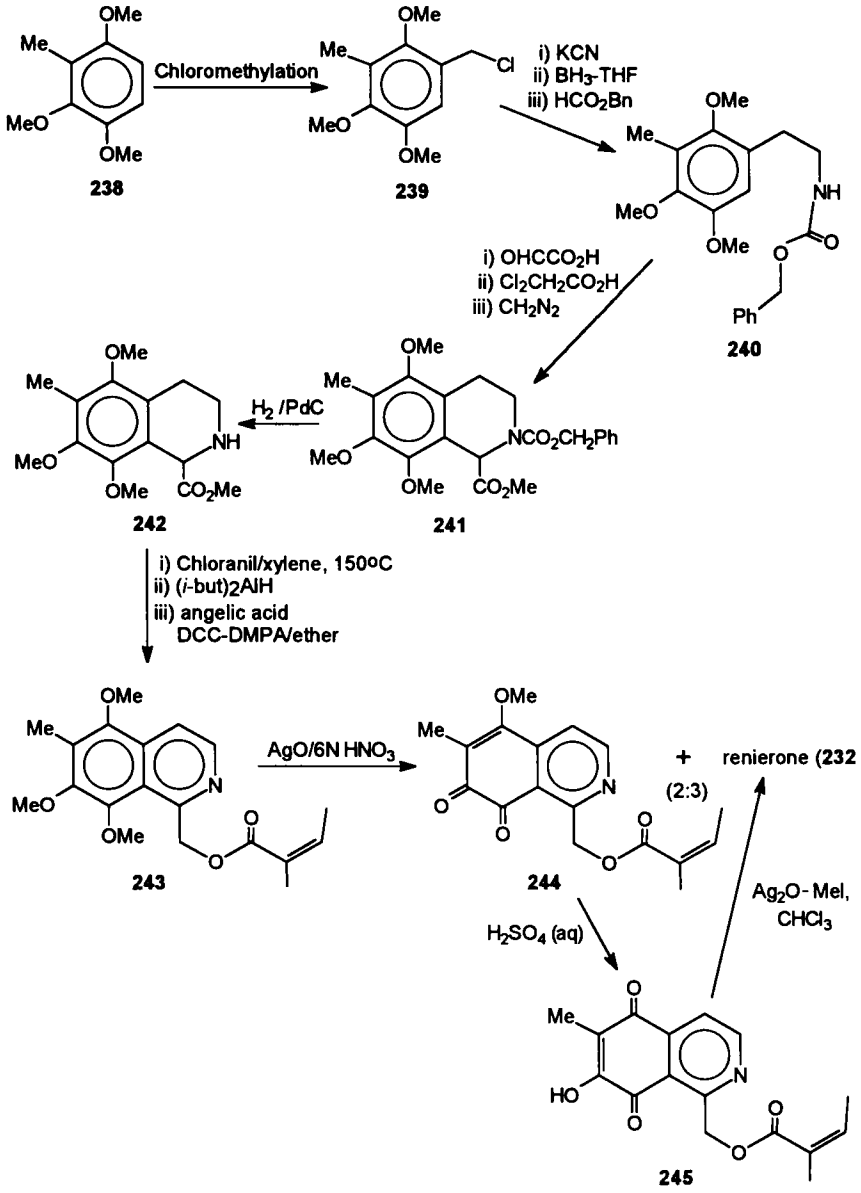
N-ethylenemethyl ketone derivative of renierone

O-Demethyl renierone (**233**): $C_{16}H_{15}NO_5$; yellow solid, mp 135-136°C; EIMS *m/z* 301 M^+; IR (KBr) ν 3324, 2922, 1713, 1648, 1576, 1388, 1328, 1238, 9, 1166, 1103, 1063, 747 cm^{-1}; UV (MeOH, ε) λ 215 (29543), 252 (11715), 297 (6065), 317 (5320), 376 (2830); ^{1}H-NMR ($CDCl_3$) δ 8.98 (1H, d, *J*=4.8 Hz), 7.95 (1H, d, *J*=4.8 Hz), 6.13 (1H, brq, *J*=6.8 Hz), 5.80 (2H, s), 2.1 (3H, s), 2.01 (3H, d, *J*=7.1 Hz), 1.97 (3H, brs); ^{13}C-NMR ($CDCl_3$) δ 183.6, 181.3, 167.9, 157.1, 155.3, 153.8, 140.03, 138.2, 127.8, 120.7, 119.1, 65.2, 20.6, 15.7, 8.5.

Dimer of *O*-demethylrenierone (**234**): red crystals; mp 174°C; IR (KBr); ν 3412, 3314, 1710, 1673, 1606, 1570, 1410, 1354, 1274, 1233, 1158, 1058, 746 cm^{-1}; UV (MeOH, ε) λ 231 (31850), 256 (29972), 304 (6247), 324.5 (8000), 379.5 (5141), 485.5 (2170) nm; ^{1}H-NMR ($CDCl_3$) δ 8.90 (1H, d, *J*=4.9 Hz), 7.93 (1H, d, *J*=4.94 Hz), 6.11 (1H, qq, *J*=7.23 and 1.28 Hz), 5.77 (2H, s), 2.01 (3H, s), 2.01 (3H, dq, *J*=7.14 and 1.5 Hz), 1.98 (3H, brq, *J*=1.5 Hz).

N-Formyl-1,2-dihydrorenierone (**235**): $C_{18}H_{19}NO_6$; non-crystalline red solid; HRMS *m/z* 345; IR (CH_2Cl_2) ν 1715, 1650 cm^{-1}; UV (MeOH, ε) λ 216 (31000), 265 (16500), 340 (6000), 515 (3500) nm; ^{1}H-NMR (**235a**, $CDCl_3$) δ 5.99 (1H, dd, *J*=4 and 3 Hz), 6.92 (1H, d, *J*=8 Hz), 6.03 (1H, d, *J*=8 Hz), 4.37 (1H, dd, *J*=12 and 4 Hz), 4.21 (1H, dd, *J*=12 and 3 Hz), 8.43 (1H, s), 1.77 (3H, brs), 6.06 (1H, q, *J*=7), 1.91 (3H, d, *J*=7 Hz), 1.95 (3H, s, Me), 4.05 (3H, s, OMe); ^{13}C-NMR (**235a**, $CDCl_3$) δ 184.6, 180.1, 167.2, 161.9 (d), 156.1, 153.6, 140.4 (d), 133.1 (d), 126.9, 126.5, 123.8, 102.7 (d), 62.9 (t), 60.9 (q), 47.3 (d), 20.3 (q), 15.5 (q), 8.4 (q); ^{1}H-NMR (**235b**, $CDCl_3$) δ 5.37 (1H, dd, *J*=9 and 4), 7.45 (1H, d, *J*=8 Hz), 6.25 (1H, d, *J*=8 Hz), 4.24 (1H, dd, *J*=12 and 9 Hz), 3.91 (1H, dd, *J*=12 and 4 Hz), 8.22 (1H, s), 1.87 (3H, brs), 6.15 (1H, q, *J*=7), 2.00 (3H, d, *J*=7 Hz), 1.98 (3H, s, Me), 4.07 (3H, s, OMe); ^{13}C-NMR (**235b**, $CDCl_3$) δ 184.0, 180.0, 166.5, 161.1 (d), 155.8, 154.0, 139.5 (d), 129.2 (d), 126.8, 126.4, 123.0, 100.7 (d), 62.9 (t), 60.9 (q), 49.6 (d), 20.3 (q), 15.7 (q), 8.5 (q)

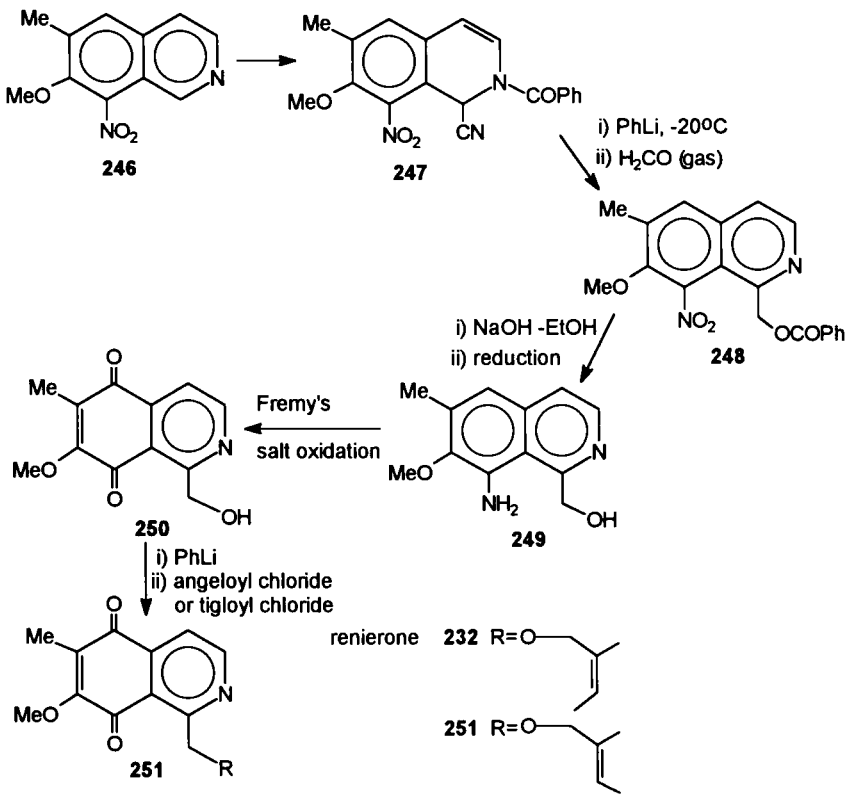
N-Ethylene methyl ketone derivative of renierone (**236**): $C_{21}H_{23}NO_6$; CIMS *m/z* 385 M^+, 386 M^++1; EIMS *m/z* 385; IR (KBr) ν 3432 (br), 2927, 1717, 1648, 1527, 1452, 1376, 1263, 1230, 1189, 1135, 949, 744 cm^{-1}; UV (MeOH, ε) λ 223 (27000), 312 (23000), 330 (21000) nm; CD (EtOH) Δε -17.5 (225 nm), Δε -13.0 (268 nm), Δε +12.5 (342 nm); ^{1}H-NMR ($CDCl_3$) δ 5.53 (1H, br dd, $\Sigma J \approx 10.0$ Hz, H-1), 6.85 (1H, d, *J*=7.9 Hz), 5.98 (1H, d, *J*=7.8 Hz, H-4), 1.96 (3H, s, H-11), 4.04 (3H, s, H-12), 4.30 (1H, dd, *J*=12.0 and 6.2 Hz, H-13a), 4.12 (1H, dd, *J*=12.0 and 4.5 Hz, H-13b), 6.11 (1H, qq, *J*=7.2 and 1.5 Hz, H-16), 1.82 (3H, dq, *J*=1.5 and 1.5 Hz, CH_3-17), 1.96 (3H, dq, *J*=7.2 and 1.5 Hz, CH_3-18), 7.42 (1H, d, *J*=13.8 Hz, H-19), 5.93 (1H, d, *J*=13.8 Hz, H-20), 2.21 (3H, s, CH_3-22); ^{13}C-NMR ($CDCl_3$) δ 51.8 (d, C-1), 138.3 (d, C-3), 99.7 (d, C-4), 185.6 (s, C-5), 127.8 (s, C-6), 156.8 (s, C-7), 180.6 (s, C-8), 120.4 (s, C-9), 136.3 (s, C-10), 8.7 (q, C-11), 61.4 (q, C-12), 61.2 (t, C-13), 167.5 (s, C-14), 127.4 (s, C-15), 140.2 (d, C-16), 20.7 (q, C-17), 15.9 (q, C-18), 145.9 (d, C-19), 105.7 (d, C-20), 195.9 (s, C-21), 29.1 (q, C-22).



SCHEME 25. Total synthesis of renierone (232) *(127)*.

2. *Synthesis*

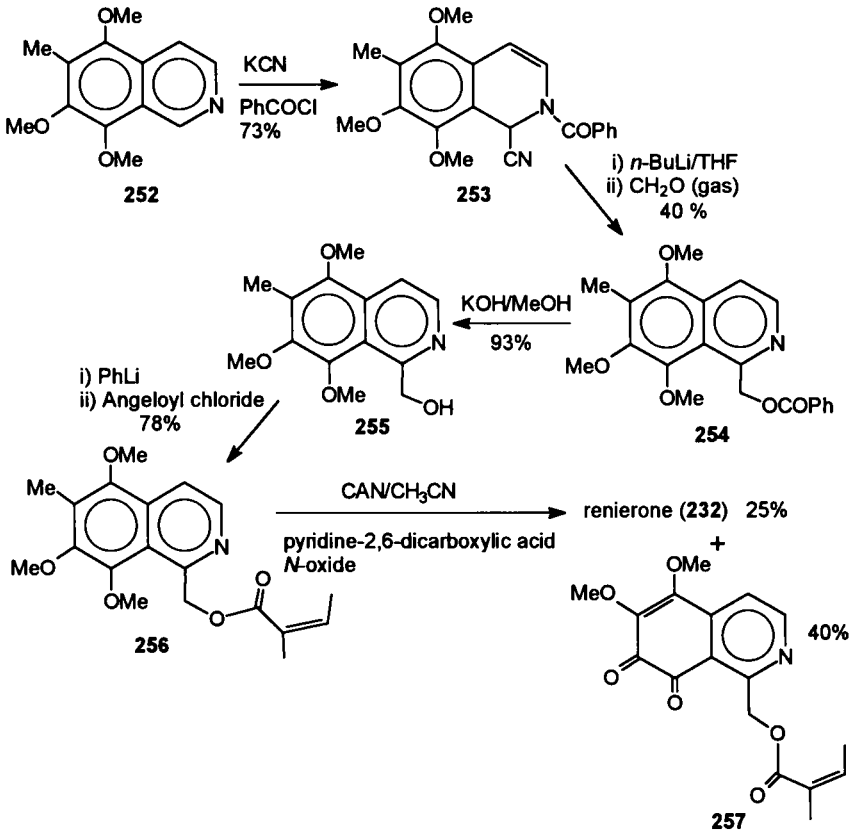
The first total synthesis of renierone (**232**) was reported by Danishefsky *et al.* in 1980 (Scheme 25) *(127)*. Their synthesis started with chloromethylation of **238** under standard conditions to obtain **239** which was converted to **240** in three steps; i) replacement of chlorine with cyanide, ii) reduction of cyanide using borane-THF, and iii) the reaction of the corresponding amine with benzyl chloroformate. Treatment of **240** with glyoxylic acid, and then subsequently with dichloroacetic acid, afforded the ring closure, and the acid group of the product was methylated with diazomethane to obtain the isoquinoline **241**. The *N*-protecting group of **241** was removed by hydrogenolysis with H_2/PdC in methanol to give **242**. The quinoline **242** was converted to **243** on, i) treatment with chloranil/xylene to aromatize the system, ii) reduction of the ester group with di-isobutylaluminum hydride, and then iii) reaction of the corresponding alcohol with angelic acid according to the conditions described by Ziegler *(128)*. With the compound **243** in hand. the next step, oxidation with AgO/6N HNO_3, gave the desired product



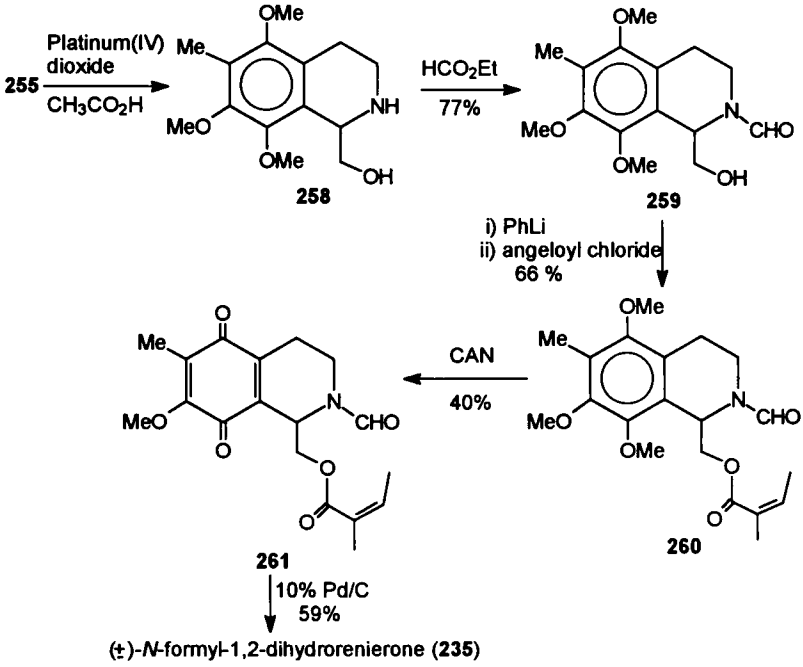
SCHEME 26. Total synthesis of renierone (**232**) and its isomer (**251**) *(129)*.

renierone along with the undesired dione **244** in 3:2 ratio, respectively. In order to recycle **244** into renierone, **244** was treated with dilute sulfuric acid in dioxane-acetone to yield the isoquinolinequinone **245** which was reacted with methyl iodide to afford renierone **232**.

In 1981, the second total synthesis of renierone was reported by Kubo *et al.* (Scheme 26) *(129)*. Their starting material, the 8-nitroisoquinoline **246**, was converted to 1-cyano-8-nitroisoquinoline **247** using Uff's procedure *(130)*. The lithium salt of **247**, prepared with phenyllithium in dioxane-ether, was reacted with formaldehyde (gas) to yield **248**, the benzoyl group of which was hydrolyzed with sodium hydroxide, and then its nitro group was catalytically reduced to the amine **249**. Treatment of 8-aminoisoquinoline **249** with Frémy's salt afforded the isoquinoline-5,8-quinone **250** which was converted to renierone (**232**) and its isomer **251** by lithiation with phenyllithium, and then reacting with angeloyl chloride and tigloyl chloride, respectively. Following this methodology, Kubo *et al.* synthesized various derivatives of renierol (around 16 derivatives) with different R groups *(131)*.



SCHEME 27. Total synthesis of renierone (**232**) *(131, 132)*.



SCHEME 28. Total synthesis of (±)-*N*-formyl-1,2-dihydrorenierone (**235**) *(131, 132)*.

Employing a different methodology, another synthesis of renierone (**232**) (Scheme 27) and a synthesis of (±)-*N*-formyl-1,2-dihydrorenierone (**235**) (Scheme 28) were also reported by Kubo *et al. (131, 132)*. Treatment of the isoquinoline **252**, which was synthesized in five steps, with potassium cyanide and benzoyl chloride afforded **253**, the lithium salt of which was reacted with formaldehyde (gas) to yield the methyl benzoate **254**. Hydrolysis of the benzoyl group with potassium hydroxide gave the alcohol **255** which was converted to **256** after lithiation with phenyllithium followed by reaction with angeloyl chloride. Oxidative methylation of **256** with ceric ammonium nitrate (CAN) in the presence of pyridine-2,6-dicarboxylic acid *N*-oxide gave renierone (**232**) along with the isomer **257**, which is reported to be recyclable to renierone.

For the synthesis of (±)-*N*-formyl-1,2-dihydrorenierone (**235**) (Scheme 28) *(131, 132)*. Kubo *et al.* catalytically hydrogenated the intermediate **255** from the renierone synthesis (Scheme 27) with platinum(IV) dioxide in acetic acid to yield the tetrahydroisoquinoline **258**. *N*-Formylation of **258** using ethyl formate gave **259**, the lithium salt of which was reacted with angeloyl chloride to afford **260**. Oxidative demethylation of **260** with CAN gave the *p*-quinone **261** without formation of any isomer. Treatment of **261** with 10% palladium furnished (±)-*N*-formyl-1,2-dihydrorenierone (**235**).

During the course of their synthetic studies on isoquinolinequinone type alkaloids, Kubo *et al.* reported a methodology for the synthesis of renierone (**232**) and mimocin (**262**), which involved the synthesis of the tetrahydroisoquinoline nucleus through cyclization of an *O, N*-acetal (Scheme 32) *(133)*.

3. Biological Activity

Renierone (**232**) was reported to show strong antimicrobial activity against *S. aureus, B. subtilis,* and *C. albicans (124)*, while it showed only weak insecticidal activity toward the larvae of *S. littoralis*, and no inhibition for *E. coli (126)*. Renierone also did not exhibit fungicidal activity against the fungus *Cladosporium cucumerimum (126)*. Renierone and *N*-formyl-1,2-dihydrorenierone (**235**) were reported to inhibit cell division in the fertilized sea urchin egg assay *(91)*.

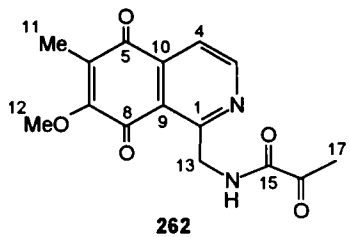
The *N*-ethylenemethyl ketone derivative of renierone (**236**) was found to be active against the Gram-positive bacteria *Bacillus subtilis* and *Staplyloccoccus aureus,* while weakly active toward the larvae of *S. littoralis*. It did not show any fungicidal activity against the fungus *Cladosporium cucumerinum*, or any activity against *E. coli (126)*.

O-Demethylrenireone (**233**) was reported to be active against the bacteria *B. polymyxa* and *Flavobacterium* sp. On the other hand, its dimer **234** showed slightly stronger activity against these organisms, and also inhibited *B. subtilis, K. pneumoniae* and *V. parahaemolyticus*. Both *O*-demethylrenierone and its dimer did not inhibit the growth of the yeast cultures, such as *Candida tropicalis* MTCC-230, *Rhodotorula rubra* MTCC-248 and *Saccharomyces cerevisiae* MTCC-249 *(125)*.

C. MIMOCIN

1. Isolation and Structure Elucidation

In 1980, Kubo *et al.* reported the isolation of a new isoquinolinequinone antibiotic, mimocin (**262**), from the fermentation broth of *Streptomyces lavendulae* No.314 *(134)*, from which mimosamycin (*208*) had been isolated by the same group in 1974 *(106)*. Further studies on its minor metabolites resulted in the isolation of **262**. Its structure was explained on the basis of spectroscopic studies and confirmed by its synthesis*(134, 135)*.

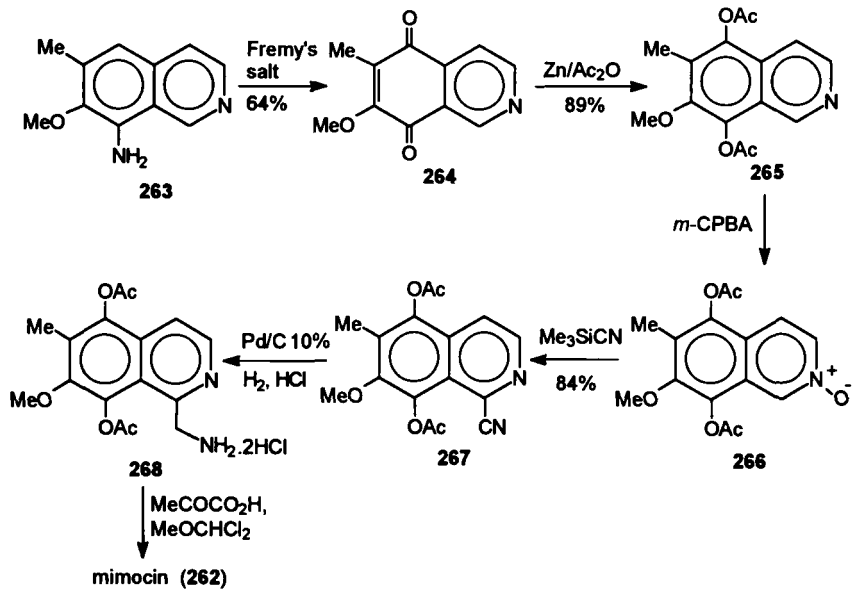


262

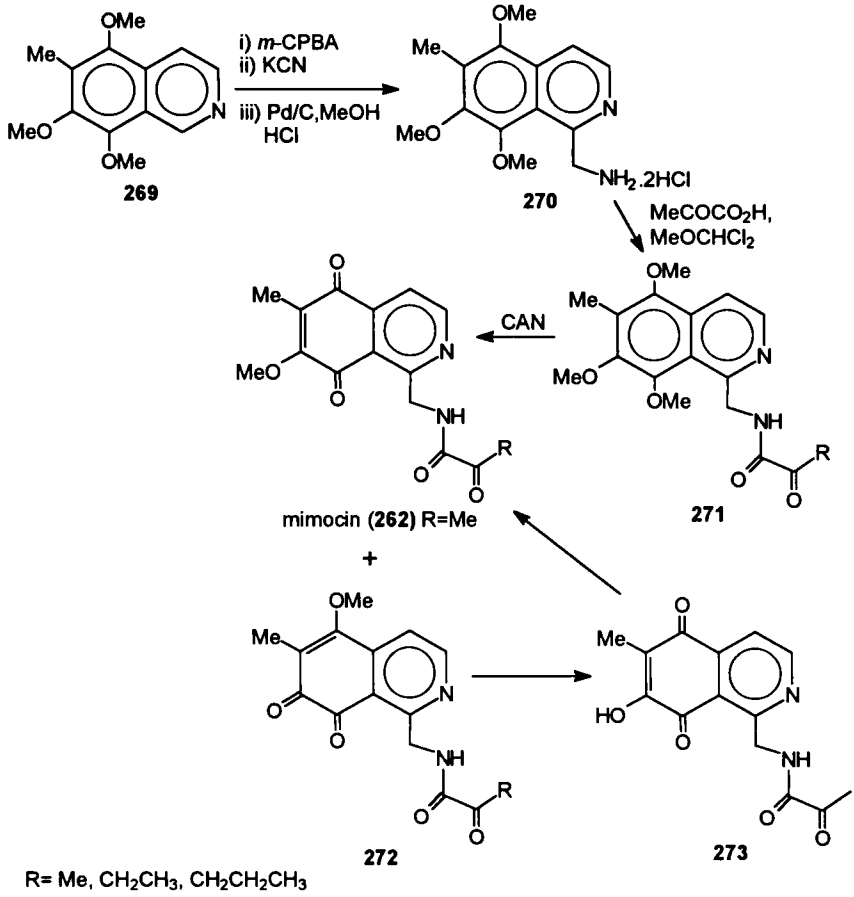
Mimocin (**262**): $C_{15}H_{14}N_2O_5$; yellow prisms; mp 189-191°C (dec., ether); MS *m/z* 302 M^+; IR ($CHCl_3$) ν 3380, 1720, 1670 cm^{-1}; UV (MeOH) λ 243, 322 nm; ^{1}H-NMR ($CDCl_3$) δ 7.92 (1H, d, *J*=5 Hz), 8.94 (1H, d, *J*=5 Hz), 8.57 (1H, brs, NH), 5.10 (2H, d, *J*=5 Hz), 2.09 (3H, s, CH_3), 2.52 (3H, s, $COCH_3$), 4.17 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$) δ 155.8 (C-1), 153.4 (C-3), 118.3 (C-4), 184.0 (C-5), 130.4 (C-6), 158.2 (C-7), 181.4 (C-8), 122.3 (C-9), 139.1 (C-10), 9.1 (C-11), 61.3 (C-12), 44.1 (C-13), 160.1 (C-15), 196.5 (C-16), 24.6 (C-17).

2. *Synthesis*

The first total synthesis of mimocin (**262**) was achieved by the same group who isolated the antibiotic to confirm its structure (Scheme 29) *(133-134, 136-137)*. Their synthesis started with 7-methoxy-6-methyl-8-nitroisoquinoline *(131)* which was converted to the 8-aminoquinoline **263** by a catalytic reduction. Frémy's salt oxidation of **263** gave the 5,8-isoquinolinequinone **264** which was acetylated by the reductive acetylation using zinc in acetic anhydride to afford **265**. Its *N*-oxide **266** was obtained by treating **265** with *m*-chloroperoxybenzoic acid. Reaction of **266** with trimethylsilyl cyanide in *N*-methyl-2-pyrrolidone gave cleanly 1-cyanoisoquinoline **267**, the cyano group of which was catalytically hydrogenated with 10% palladium/charcoal in methanol containing hydrochloric acid to afford the sensitive 1-aminomethylisoquinoline dihydrochloride **268**. Compound **268** was then treated with pyruvic acid to furnish mimocin **262**.



SCHEME 29. Total synthesis of mimocin (**262**) (*136*).

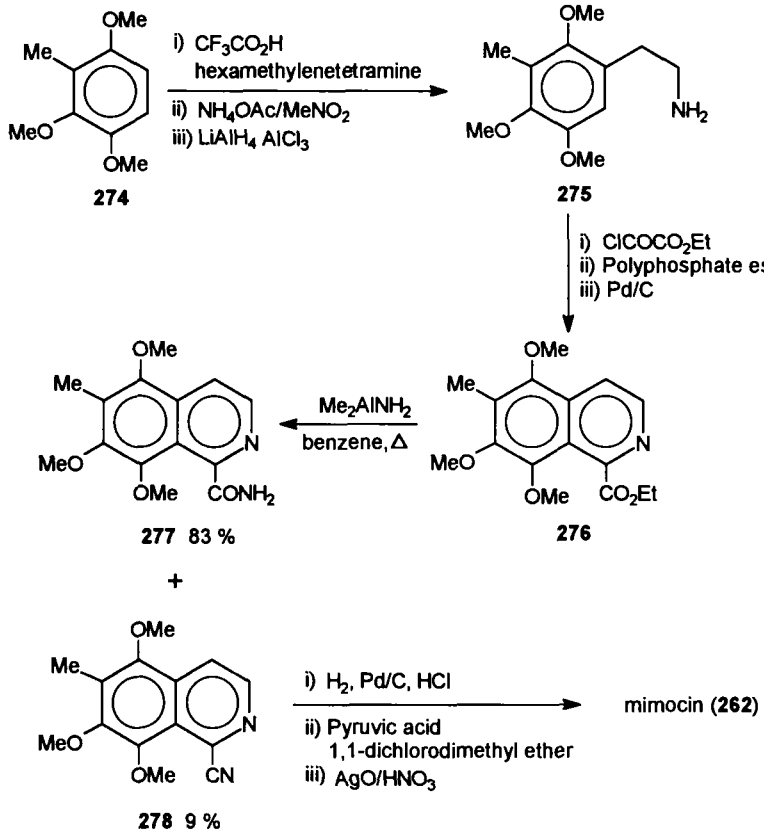


SCHEME 30. Synthesis of mimocin (**262**) and its analogues *(133, 136, 137)*.

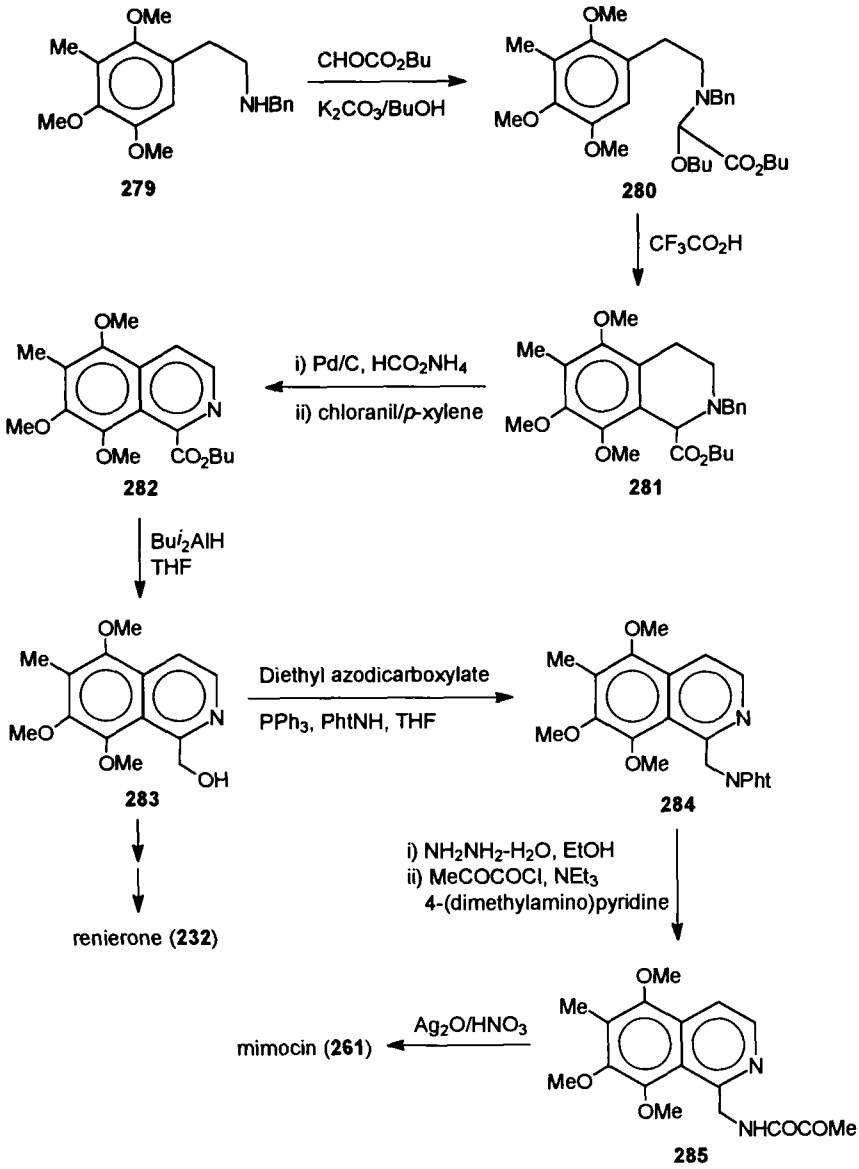
The same group also achieved the syntheses of mimocin analogues, and mimocin itself, following almost the same route, but slightly modifying it using 5,7,8-trimethoxy-6-methylisoquinoline (**269**) as the starting material (Scheme 30) *(133, 136-137)*. Compound **269** was converted to **270** in three steps: i) treatment with *m*-CPBA, ii) reacting the product with potassium cyanide, and iii) reduction with 10% Pd/C in methanol containing hydrochloric acid. In a similar fashion, the free base **270** was reacted with pyruvic acid to obtain 1-pyruvoylaminomethylisoquinoline **271**. The oxidative demethylation of **271** using ceric ammonium nitrate (CAN) gave mimocin **262**. The corresponding *o*-quinone isomer **272** was converted to **262** by treatment with sulfuric acid to give **273** and then subsequent methylation with methyl iodide in the presence of Ag_2O.

The second total synthesis of mimocin (**262**) was reported by Matsuo *et al.* (Scheme 31) *(138, 139)*. Their rather long synthesis involved the construction of the

corresponding isoquinoline **276** in six steps. Reaction of their starting material, 2,3,6-trimethoxytoluene (**274**), with i) hexamethylenetetramine and trifluoroacetic acid, ii) nitromethane in the presence of ammonium acetate, and iii) lithium aluminum hydride-aluminum chloride gave the amine **275**. It was then converted to the isoquinoline **276** in additional three steps, i) addition of ethyl oxalyl chloride, ii) ring closure in polyphosphate ester, and iii) aromatization with 5% Pd/C in decalin at 160-170°C. The ester group of **276** was transformed to the nitrile **278** along with the minor product amide **277**, using dimethylaluminum amide in refluxing benzene. Hydrogenation of the nitrile over 5% Pd/C in methanol containing hydrochloric acid was subsequently followed by treatment with pyruvic acid. The product was then subjected to oxidative-demethylation with silver oxide-nitric acid to furnish mimocim (**262**).



SCHEME 31. Synthesis of mimocin (**262**) *(138, 139)*.



SCHEME 32. Synthesis of mimocin (**262**) and renierone (**232**) *(133)*.

In 1989, Kubo *et al.* reported a new methodology for the synthesis of isoquinolines which could lead to the synthesis of various isoquinolinequinone alkaloids (Scheme 32) *(133)*. This method included the synthesis of a 1,2,3,4-

tetrahydroisoquinoline such as **281**. Their starting material, *N*-benzylamine **279** *(140)*, was reacted with butyl glyoxylate in butanol in the presence of anhydrous potassium carbonate to give the unstable *O,N*-acetal **280** which was stirred with trifluoroacetic acid without purification to yield the tetrahydroisoquinoline **281**. The isoquinoline **282** was obtained on treatment of **281** with anhydrous ammonium formate in the presence of Pd/C (10%) to remove the benzyl protecting group, and then with chloranil in *p*-xylene to aromatize the system. The ester group of **282** was reduced to an alcohol with diisobutylaluminum hydride in THF to obtain **283**, the alcohol functionality of which was transformed to an imide by reacting with phthalimide in the presence of diethyl azodicarboxylate and triphenylphosphine (Mitsunobu conditions) to afford **284**. The isoquinolinequinone **284** with imide functionality was converted to **285** in two steps, i) cleavage of the phthaloyl group with hydrazine and ii) acylation of the corresponding amine with pyruvoyl chloride. Compound **285** was then reacted with Ag_2O to obtain mimocin (**262**).

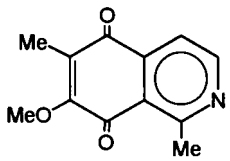
3. Biological Activity

Mimocin (**262**) was reported to be strongly active against *B. subtilis* and *C. albicans (134)*.

D. 1,6-DIMETHYL-7-METHOXY-5,8-DIHYDROISOQUINOLINE-5,8-DIONE

1. Isolation and Structure Elucidation

Further studies, by Faulkner *et al.* in 1982, on the sponge *Reniera* sp. collected near Isla Grande, Mexico, and from which renierone (**232**) was also isolated *(124)*, led to the isolation of a new alkaloid 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**), along with mimosamycin (**208**), *N*-formyl-1,2-dihydrorenierone (**235**), *O*-demethylrenierone (**233**), 2,5-dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**237**) and renieramycins A-D (**155-158**) *(91)*. In 1996, Proksch *et al.* also reported the isolation of **286** from a different source, sponges of the genus *Xestospongia* collected off the shores of Mindoro Island, Philippines *(126)*.



286

1,6-Dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**): $C_{12}H_{11}NO_3$; mp 188-190°C (dec); HRMS *m/z* 217; IR ν (CH_2Cl_2) 1675 cm^{-1}; UV (MeOH) λ 223, 276, 283, 292 nm; ^{1}H-NMR ($CDCl_3$) δ 2.04 (3H, s), 2.94 (3H, s),

4.15 (3H, s), 7.94 (1H, d, J=5 Hz), 8.76 (1H, d, J=5 Hz); ^{13}C-NMR ($CDCl_3$) δ 183.0, 180.0, 158.0 (2xC), 153.7, 139.0, 130.2, 122.0, 117.4, 61.3, 29.7, 9.1.

2. Synthesis

Synthesis of 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**) was carried out by Kubo *et al.* following various reaction schemes (Scheme 33) *(131, 132)*. In one of their methods, the trimethoxyisoquinolylmethanol **287** was tosylated by lithiation with phenyllithium followed by addition of *p*-toluenesulfonyl chloride to obtain **288** which was reduced with lithium triethylborohydride to yield dimethylisoquinoline **289**. Oxidative demethylation of **289** using ceric ammonium nitrate afforded **286** together with a side product **290**.

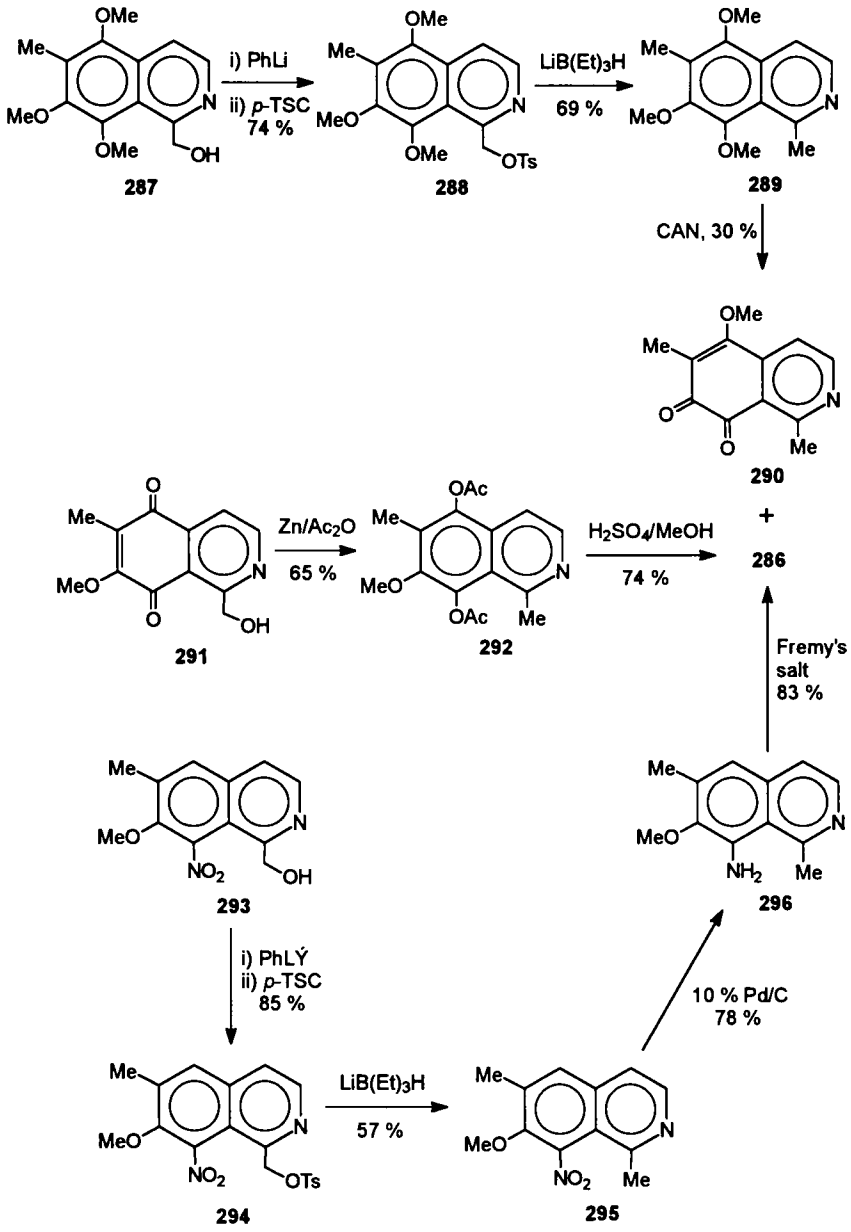
Synthesis of **286** was also achieved without formation of any side product. In this synthesis Kubo *et al.* treated the *p*-quinone **291** with zinc in acetic anhydride to give the di-acetoxyisoquinoline **292** which was converted to **286** on reaction with sulfuric acid.

In their other method the nitroalcohol **293** was tosylated in the same way as described to synthesize **288**, and the product **294** was reduced with lithium triethylborohydride to obtain 7-methoxy-1,6-dimethyl-8-nitroisoquinoline (**295**). Reduction of the nitro group of **295** with 10% palladium-carbon to the give amine **296** was followed by Frémy's salt oxidation to furnish **286** *(131, 132)*.

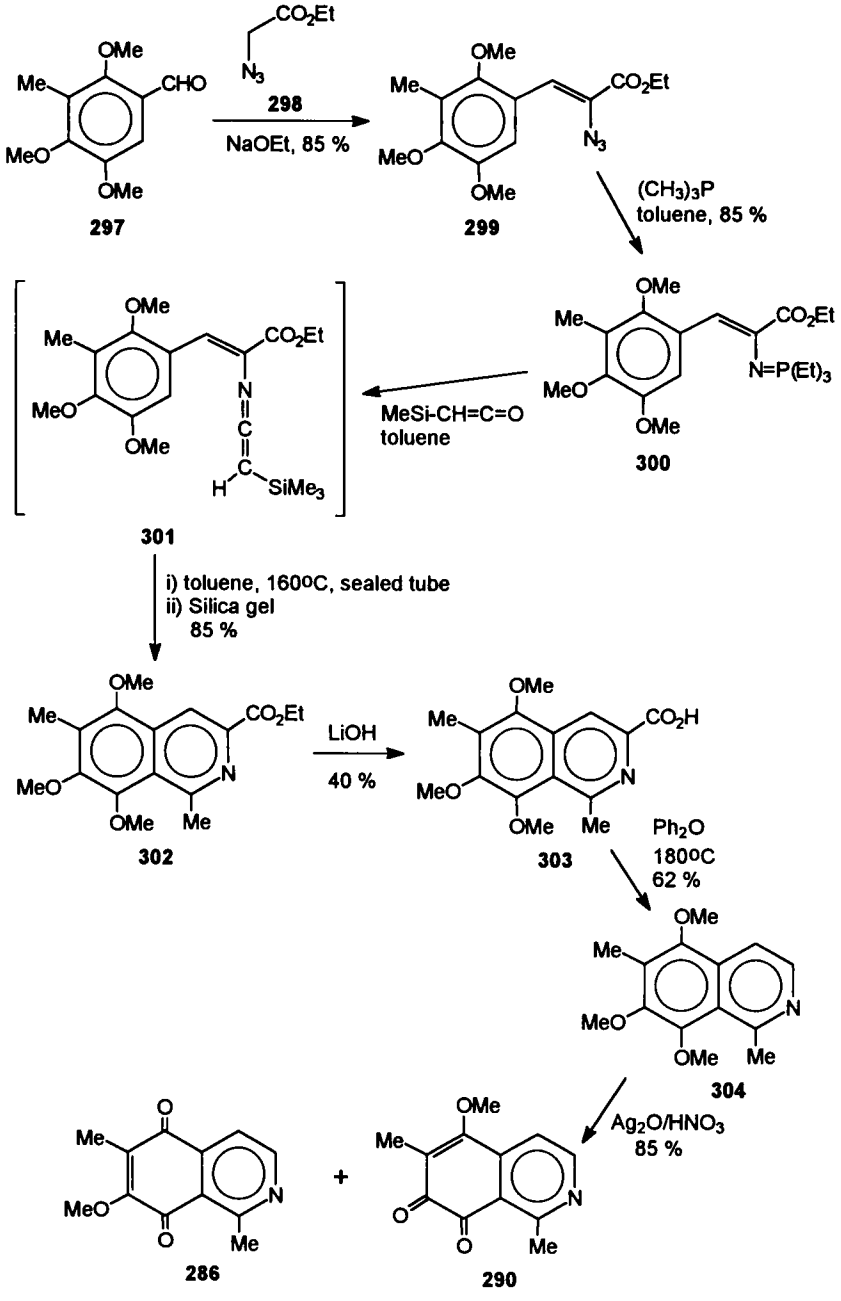
In 1997, Molina *et al.* reported the synthesis of **286** using the chemistry of electrocyclization of a β-arylvinyl ketenimine (Scheme 34) *(141)*. Their synthesis started with the condensation of the benzaldehyde **297** with ethyl azidoacetate **298** to give α-azidocinnamate **299**, which was converted to **302** in a one-flask process by sequential treatment with trimethylphosphine for the synthesis of **300** and then with (trimethylsilyl) ethenone for the formation of the intermediate ketenimine **301**. This compound underwent electrocyclic ring-closure on heating at 160^{0}C. Isolation of **302** was achieved by silica gel column chromatography. Lithium hydroxide treatment of **302** gave the isoquinoline **303** with the carboxylic acid functionality at C-3. Decarboxylation of **303** was carried out in diphenyl ether at 180^{0}C to afford the isoquinoline **304** which was then converted to **286** by oxidation with Ag_2O, which also resulted in the formation of a side product *o*-quinone **290**.

3. Biological Activity

Alkaloid **286** was reported to show insecticidal activity. It inhibited the growth of the larvae of *S. littoralis* by 93% and was found to have EC_{50} for growth inhibition and LC_{50} as 35 ppm [± 0.43 (SE)] and 521 ppm [± 0.65 (SE)],



SCHEME 33. Total synthesis of **286** *(131, 132)*.



SCHEME 34. Total synthesis of **286** *(141)*.

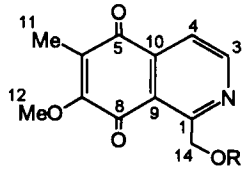
respectively *(126)*. Alkaloid **286** was found to be active against the Gram-positive bacteria, however, it did not show any activity against *E. coli*, and no fungicidal activity against the fungus *Cladosporium cucumerinum* was observed.

It was reported that **286** inhibits human immunodeficiency virus (HIV) reverse transcriptase and avian myeloblastosis virus (AMV) reverse transcriptase, while it was not found to inhibit the activity of DNA polymerases α and β *(142-144)*.

E. RENIEROL

1. Isolation and Structure Elucidation

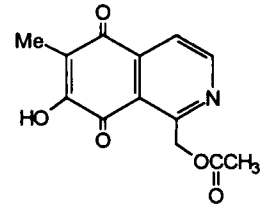
In 1987, Ireland *et al.* reported the isolation of a new metabolite, renierol (**250**), from a hard blue sponge *Xestospongia caycedoi* collected at Sand Island, Suva Harbor, Fiji at a depth of 2m *(110)*. Structure elucidation revealed its obvious resemblance to renierone (**232**) and 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline (**286**). Later, isolation of the derivatives of **250**, renierol acetate (**305**), renierol propionate (**306**), *N*-formyl-1,2-dihydrorenierol acetates (**307a**) and (**307b**) and *N*-formyl-1,2-dihydrorenierol propionates (**308a**) and (**308b**) also appeared *(145)*.



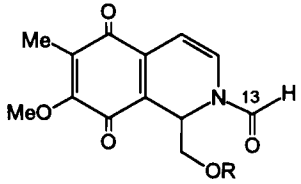
renierol **250** R=H

renierol acetate **305** R= $-C(=O)-CH_3$

renierol propionate **306** R= $-C(=O)-CH_2CH_3$

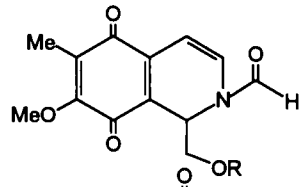


O-demethylrenierol acetate **309**



N-formyl-1,2-dihydrorenierol acetate **307a** R= $-C(=O)-CH_3$

N-formyl-1,2-dihydrorenierol propionate **308a** R= $-C(=O)-CH_2CH_3$



307b R= $-C(=O)-CH_3$

308b R= $-C(=O)-CH_2CH_3$

In 1993, a new renierol derivative *O*-demethylrenierol acetate (**309**) was isolated by Venkateswarlu from a bright-blue sponge identified as *Petrosia* sp. collected on the Mandapam coast, India *(112)*.

Renierol (**250**): $C_{12}H_{11}NO_4$; reddish brown powder; HRMS *m/z* 233 M^+; EIMS *m/z* 233 M^+ IR ν 3600-2850, 1665 cm^{-1}; UV (MeOH, ε) λ 314.8 (4100), 241.4 (13000), 105.6 (15000) nm; ^{1}H-NMR ($CDCl_3$) δ 8.93 (1H, d, *J*=4.61 Hz), 7.93 (1H, d, *J*=4.61 Hz), 4.85 (1H, s, OH), 5.19 (2H, s, OCH_2), 4.15 (3H, s, OCH_3), 2.10 (3H, s, CH_3); ^{13}C-NMR ($CDCl_3$) δ 159.87, 184.18, 184.05, 157.74, 152.32 (d), 138.62, 130.27, 121.28, 117.77 (d), 63.64 (t), 60.88 (q), 8.64 (q).

Renierol acetate (**305**): $C_{14}H_{13}NO_5$; mp 118-119°C; HRMS *m/z* 275; IR (KBr) ν 1750, 1670, 1650 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.87 (1H, d, *J*=5 Hz), 8.89 (1H, d, *J*=5 Hz), 5.77 (2H, s), 2.13 (3H, s), 2.27 (3H, s), 4.23 (3H, s).

Renierol propionate (**306**): ^{13}C-NMR ($CDCl_3$) δ 156.77 (C-1), 153.95 (C-3), 118.42 (C-4), 184.46 (C-5), 130.54 (C-6), 158.47 (C-7), 181.71 (C-8), 122.70 (C-9), 138.93 (C-10), 9.13 or 9.04 (C-11 or 17), 61.23 (C-12), 65.37 (C-14), 174.33 (C-15), 27.43 (C-16).

O-Demethylrenierol acetate (**309**): $C_{13}H_{11}NO_5$; oil; MS *m/z* 261 M^+; IR ν 3500, 1735 and 1680 cm^{-1}; UV (ε) λ 300 (4094), 226 (19400) nm; ^{1}H-NMR δ 7.97 (1H, d, *J*=5 Hz), 9.02 (1H, d, *J*=5 Hz), 2.15 (3H, s, CH_3), 2.25 (3H, s, $COCH_3$), 5.75 (2H, s, OCH_2).

N-Formyl-1,2-dihydrorenierol acetate (**307**): $C_{15}H_{15}NO_6$; HRMS *m/z* 305 M^+; IR (KBr) ν 2957, 1744, 1702, 1648, 1615, 1552, 1440, 1390, 1324, 1286, 1264, 1224, 1186, 1150, 1047, 947, 747, 718 cm^{-1} ; UV (MeOH, log ε) λ 269 (3.99), 340 (3.54), 500 (3.19) nm; (**307a**) ^{1}H-NMR ($CDCl_3$) δ 5.94 (1H, dd, *J*=4.9 and 3.7 Hz, H-1), 6.92 (1H, d, *J*=7.6 Hz, H-3), 6.06 (1H, d, *J*=7.6 Hz, H-4), 1.96 (3H, s, H-11), 4.07 (3H, s, H-12), 8.42 (1H, s, H-13), 4.18 (1H, dd, *J*=11.9 and 3.7 Hz, H-14), 4.24 (1H, dd, *J*=11.9 and 4.9 Hz, H-14), 1.97 (3H, s, H-16); ^{13}C-NMR ($CDCl_3$) δ 47.28 (C-1), 133.35 (C-3), 100.96 (C-4), 184.91 (C-5), 127.31 (C-6), 156.28 (C-7), 180.24 (C-8), 123.81 (C-9), 135.58 (C-10), 8.66 (C-11), 61.16 (C-12), 162.17 (C-13), 63.09 (C-14), 170.79 (C-15), 20.73 (C-16); (**307b**) ^{1}H-NMR ($CDCl_3$) δ 5.31 (1H, dd, *J*=9.8 and 4.0 Hz, H-1), 7.43 (1H, d, *J*=7.6 Hz, H-3), 6.23 (1H, dd, *J*=7.6 and 1.2 Hz, H-4), 1.98 (3H, s, H-11), 4.05 (3H, s, H-12), 8.23 (1H, brs, H-13), 3.81 (1H, dd, *J*=11.3 and 4.0 Hz, H-14), 4.16 (1H, dd, *J*=11.3 and 9.8 Hz, H-14), 2.08 (3H, s, H-16); ^{13}C-NMR ($CDCl_3$) δ 49.61 (C-1), 129.36 (C-3), 102.88 (C-4), 184.70 (C-5), 128.12 (C-6), 156.02 (C-7), 180.27 (C-8), 123.13 (C-9), 136.28 (C-10), 8.76 (C-11), 61.16 (C-12), 161.28 (C-13), 61.27 (C-14), 170.14 (C-15), 20.64 (C-16).

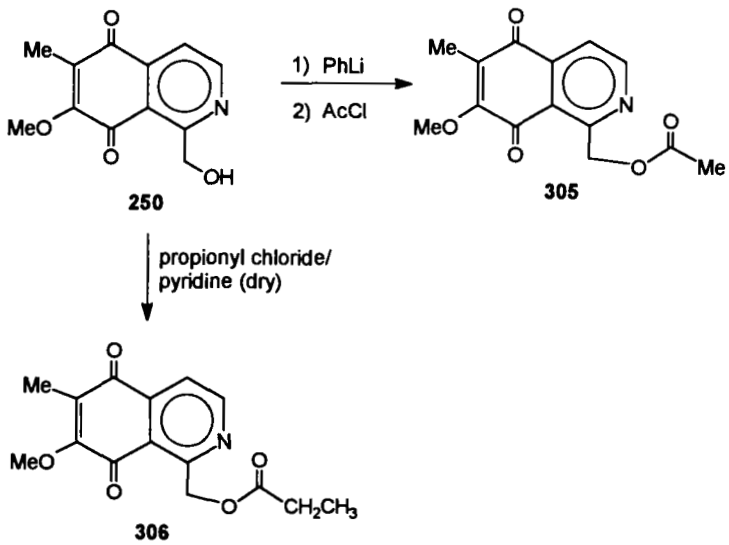
N-Formyl-1,2-dihydrorenierol propionate (**308**): $C_{16}H_{17}NO_6$; HRMS *m/z* 319 M^+; IR (KBr) ν 2947, 1742, 1702, 1648, 1617, 1554, 1440, 1385, 1324, 1286, 1266, 1204, 1188, 1146, 1090, 947, 747, 720 cm^{-1} ; UV (MeOH, log ε) λ 268 (4.00), 340 (3.57), 500 (3.24) nm; (**308a**) ^{1}H-NMR ($CDCl_3$) δ 5.95 (1H, dd, *J*=4.9 and 3.4 Hz, H-1), 6.92 (1H, d, *J*=7.6 Hz, H-3), 6.05 (1H, d, *J*=7.6 Hz, H-4), 1.96 (3H, s, H-11), 4.07 (3H, s, H-12), 8.42 (1H, s, H-13), 4.18 (1H, dd, *J*=11.9 and 3.4 Hz, H-14), 4.27 (1H, dd, *J*=11.9 and 4.9 Hz, H-14), 2.24 (2H, q, *J*=7.6 Hz, H-16), 1.06 (3H, t, *J*=7.6 Hz, H-17); ^{13}C-NMR ($CDCl_3$) δ 47.33 (C-1), 133.36 (C-3), 100.91 (C-4), 184.86 (C-5), 127.18 (C-6), 156.24 (C-7), 180.19 (C-8), 123.81 (C-

9), 135.50 (C-10), 8.59 or 8.91 (C-11 or 17), 61.13 (C-12), 162.09 (C-13), 63.07 (C-14), 174.15 (C-15), 20.39 (C-16); (**308b**) ^{1}H-NMR ($CDCl_3$) δ 5.32 (1H, dd, *J*=9.5 and 3.7 Hz, H-1), 7.43 (1H, d, *J*=7.6 Hz, H-3), 6.22 (1H, dd, *J*=7.6 and 1.2 Hz, H-4), 1.98 (3H, s, H-11), 4.05 (3H, s, H-12), 8.22 (1H, brs, H-13), 3.82 (1H, dd, *J*=11.3 and 3.7 Hz, H-14), 4.21 (1H, dd, *J*=11.3 and 9.5 Hz, H-14), 2.35 (2H, q, *J*=7.6 Hz, H-16), 1.14 (3H, t, *J*=7.6 Hz, H-17); ^{13}C-NMR ($CDCl_3$) δ 49.64 (C-1), 129.32 (C-3), 102.82 (C-4), 184.66 (C-5), 128.05 (C-6), 155.97 (C-7), 180.23 (C-8), 123.11 (C-9), 136.23 (C-10), 8.70 or 8.86 (C-11 or 17), 61.13 (C-12), 161.24 (C-13), 61.11 (C-14), 173.55 (C-15), 27.30 (C-16).

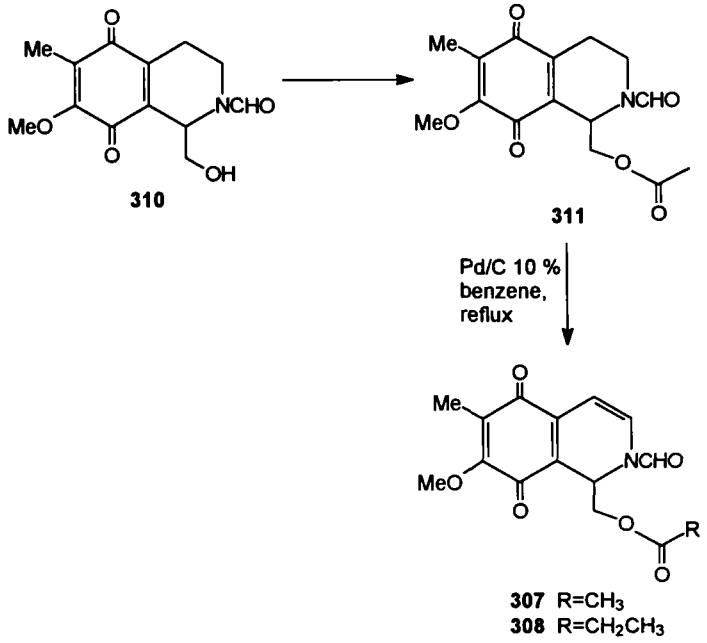
2. *Synthesis*

Synthesis of renierol (**250**) had already been achieved by Kubo *et al.* as an intermediate during the synthesis of renierone (**232**) (Scheme 26) *(129, 131, 135)* before the natural renierol was isolated *(110)*. Treatment of renierol (**250**) first with phenyllithium and then with acetyl chloride furnished renierol acetate (**305**), while reacting renierol with propionyl chloride gave renierol propionate (**306**) (Scheme 35) *(145)*.

For the synthesis of *N*-formyldihydro renierols **307** and **308**, Kubo *et al.* acetylated or propionated the intermediate **310** obtained during the synthesis of renierone derivatives *(131)* to yield **311** which was converted to the corresponding (±)-*N*-formyl-1,2-dihydrorenierol acetate (**307**) or (±)-*N*-formyl-1,2-dihydrorenierol propionate (**308**), respectively, on dehydrogenation with 10% palladium on carbon (Scheme 36) *(145)*.



SCHEME 35. Synthesis of renierol acetate (**305**) and renierol propionate (**306**) *(145)*.

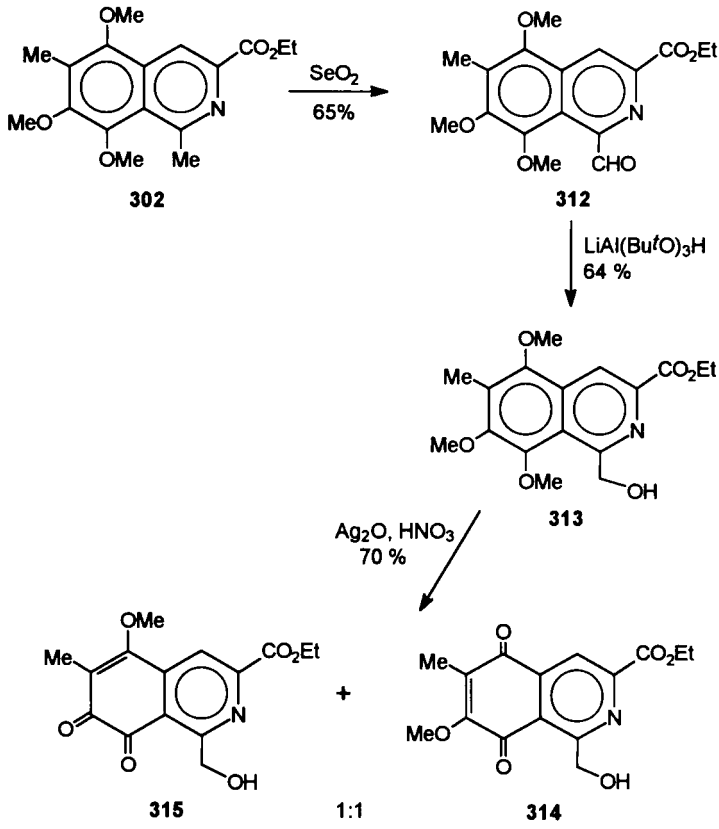


SCHEME 36. Synthesis of (±)-*N*-formyl-1,2-dihydrorenierol acetate (**307**) and (±)-*N*-formyl-1,2-dihydrorenierol propionate (**308**) *(145)*.

Synthesis of the 3-ethoxycarbonylrenierol (**314**) derivative of renierol was reported by Molina *et al.* in 1997 (Scheme 37) *(141)*. Intermediate **302** from the synthesis of 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**) (Scheme 34) was oxidized with SeO_2 to obtain the 1-formylisoquinoline **312**, the formyl group of which was reduced to the alcohol with lithium tri-*tert*-butoxyaluminum hydride to give **313**. Oxidative demethylation of **313** with Ag_2O/HNO_3/dioxane furnished 3-ethoxycarbonyl-renierol (**314**), along with its *o*-quinone isomer **315**, in 1:1 ratio.

3. Biological Activity

Renierol (**250**), renierol acetate (**305**), renierol propionate (**306**), *N*-formyl-1,2-dihydrorenierol acetate (**307**) and *N*-formyl-1,2-dihydrorenierol propionate (**308**) were reported to be active against *Staphylococcus aureus (110, 145)*. The last four compounds **305**, **306**, **307** and **308** were also found to be active against *Bacillus subtilis (145)*, and renierol showed mild cytotoxicity against the L1210 cell line with IC_{50} 3.0 μg/ml *(110)*.

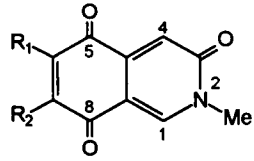


SCHEME 37. Synthesis of 3-ethoxycarbonylrenierol (**314**) *(141)*.

F. PERFRAGILIN

1. Isolation and Structure Elucidation

In 1990, Schmitz *el al.* reported the isolation of perfragilin from the methanol and chloroform-methanol extracts of a bryozoan, *Membranipora perfragilis* collected at Stenhouse Bay in South Australia (*146*). Single crystal X-ray diffraction analyses of perfragilins A (**316**) and B (**317**) were later disclosed by Rizvi *et al. (147)*. Although isolation of such an alkaloid was reported in 1990, its full spectral and X-ray diffraction data were made available in the literature only in 1993 (*147, 148*).



Perfragilin A **316** R_1=MeS, R_2=NH_2
Perfragilin B **317** R_1=R_2=SMe
Perfragilin C **318** R_1=SMe, R_2=H

In the same year, isolation of another derivative of perfragilin, 2-methyl-6-methylthioisoquinoline-3,5,8(2*H*)-trione (**318**), along with perfragilin B (**317**), from the bryozoan *Biflustra perfragilis* collected in the Bass Strait, was reported *(159)*.

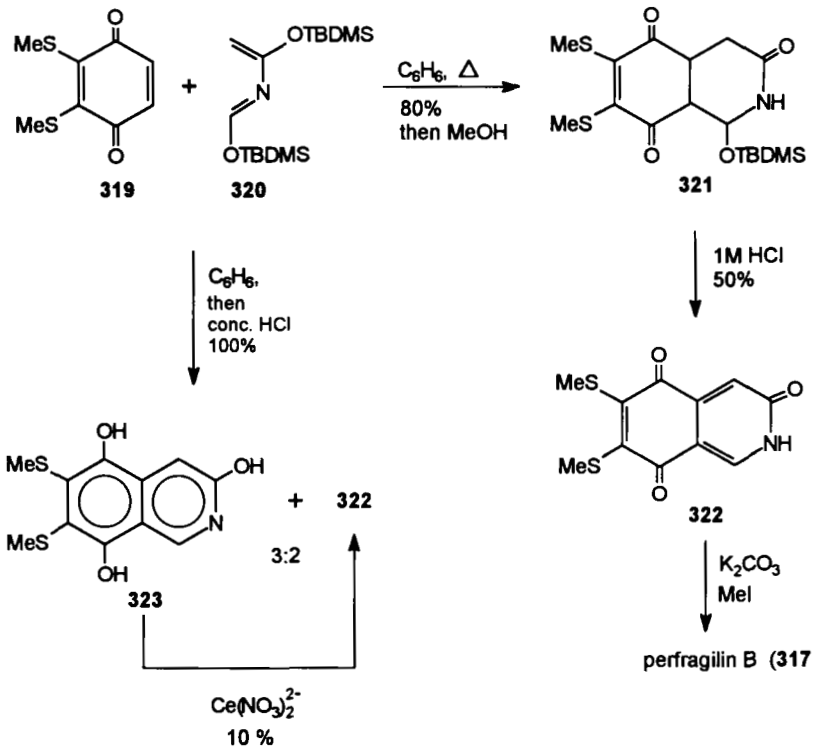
Perfragilin A (**316**): Red needles; mp 219-220°C; FABMS *m/z* 251 M^++1; IR (thin film) ν 3423, 3322, 1675, 1645, 1572 cm^{-1}; UV (95 % EtOH, ε) λ 222 (12616), 248 (7000), 330 (15140), 362 (8785), 440 (2224); ^{1}H-NMR (CD_3OD) δ 8.56 (1H, s, H-1), 6.98 (1H, s, H-4), 2.26 (3H, s, SMe), 3.64 (3H, s, NMe); ^{13}C-NMR ($CD_3OD/CDCl_3$) δ 144.89 (C-1), 165.37 (C-3), 116.64 (C-4), 178.84 (C-5), 112.61 (C-6 and 9), 155.54 (C-7), 176.83 (C-8), 142.76 (C-10), 16.86 (SMe), 38.52 (NMe).

Perfragilin B (**317**): Red needles; mp 163°C; EIMS *m/z* 281 M^+; IR (thin film) ν 1670, 1623 cm^{-1}; UV (EtOH, ε) λ 214 (14941), 235 (10504), 332 (8227), 382 (5946), 465 (1848); ^{1}H-NMR ($CDCl_3$) δ 8.23 (1H, s, H-1), 7.07 (1H, s, H-4), 2.67 and 2.73 (2x3H, s, SMe), 3.64 (3H, s, NMe); ^{13}C-NMR ($CDCl_3$) δ 142.44 (C-1), 162.49 (C-3), 117.43 (C-4), 176.66 (C-5), 147.36 (C-6 or 7), 150.67 (C-7 or 6), 175.66 (C-8), 111.92 (C-9), 139.68 (C-10), 18.14 (SMe), 18.68 (SMe), 38.42 (NMe).

Perfragilin C (**318**): $C_{11}H_9NO_3S$; yellow powder; HRMS *m/z* 235 M^+; IR (KBr) ν 3400, 3000, 2950, 1689, 1653, 1602, 1554, 1539, 1481, 1458, 1417, 1384, 1332, 1311, 1286, 1138, 1105, 1062, 1026, 811, 611 cm^{-1}; UV (MeOH, ε) λ 367 (10594), 217 (16675); ^{1}H-NMR ($CDCl_3$) δ 8.36 (1H, s, H-1), 7.12 (1H, s, H-4), 6.6 (1H, s, H-7), 3.66 (NMe), 2.38 (3H, s, SMe); ^{13}C-NMR ($CDCl_3$) δ 143.9 (C-1), 160 (C-3), 118 (C-4), 179.6 or 178.8 (C-5 or 8), 131.6 (C-6), 128.3 (C-7), 111.8 (C-9), 139.8 (C-10), 14.7 (SMe), 39.3 (NMe).

2. Synthesis

Total synthesis of perfragilin B (**317**) was reported by Schmitz *et al.* in 1993 (Scheme 38) *(150)*, who had also isolated the original material *(146)*. Their synthesis involved a similar strategy to that used for mimosamycin (**208**) *(120)*. Cycloaddition of the quinone **319** *(151)* with the diene **320** *(121, 152)* in refluxing benzene gave the isoquinolinequinone **321** after the addition of methanol. Treatment of **321** with 1M HCl vielded **322**, which on methylation with methyl iodide produced perfraglin



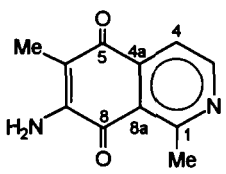
SCHEME 38. Schmitz's total synthesis of perfragilin B (**317**) *(150)*.

B (**317**). When the cycloaddition reaction was followed by the addition of conc. HCl instead of the addition of methanol, a mixture of **323** and **322**, in a 3:2 ratio, was obtained. The hydroquinone **323** was converted to **322** by oxidation with ceric nitrate.

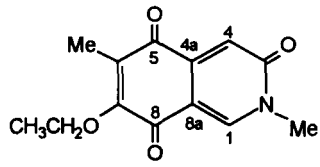
3. Biological Activity

Perfragilins A (**316**) and B (**317**) were reported to be cytotoxic against P-388 murine leukemia cells, with **317** being significantly more potent (ED_{50} 0.8, 0.07 μg/ml, respectively) *(148)*.

Perfragilin B (**317**) was reported to cause significant mortality in the brine shrimp bioassay at a level of $3\text{-}5\text{x}10^{-4}$ mmol/l, and it also inhibited the growth of cultured marine bacteria *(149)*. However, it did not show *in vitro* antibacterial activity against human bacterial pathogens.



cribrostatin 1 **324**



cribrostatin 2 **325**

G. CRIBROSTATINS

1. Isolation and Structure Elucidation

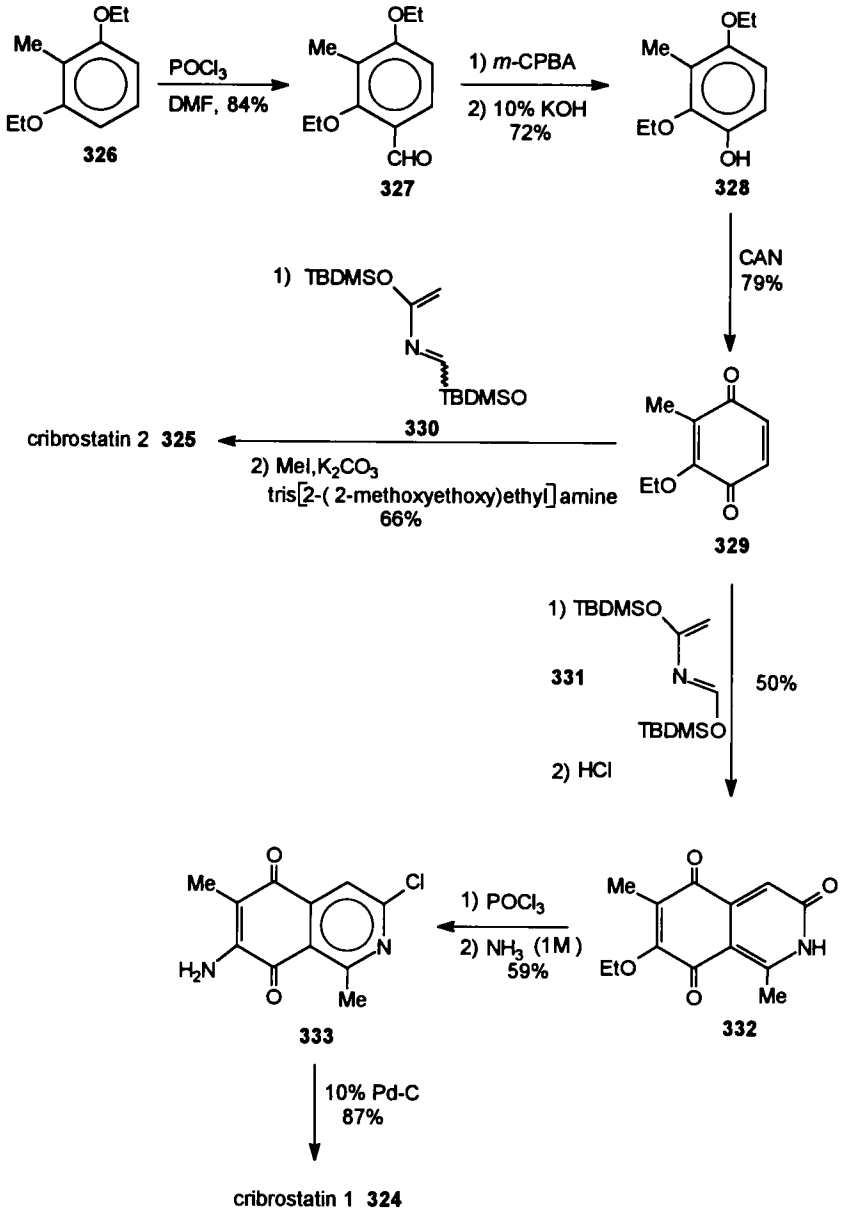
In 1992, Pettit *et al.* isolated two new compounds, cribrostatin 1 (**324**) and cribrostatin 2 (**325**), from the blue marine sponge, *Cribrochalina* sp., collected in the channel between the Guradu and Madu Islands of South Malé Atoll, Republic of the Maldives *(111)*. On the basis of spectroscopic and X-ray single crystal measurements, its structure was determined to be closely related to 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**286**) *(91, 126)* and mimosamycin (**208**) *(106)*. Cribrostatin 1 (**324**) only differs from **286** by having an amino group attached to C-7 in place of a methoxy group, while cribrostatin 2 (**325**) has an ethoxy group at C-7 in place of the methoxy group of mimosamycin (**208**).

Cribrostatin 1 (**324**): $C_{11}H_{10}N_2O_2$; red-orange crystals; mp 220-235°C (dec); HREIMS *m/z* 202 M^+; IR (NaCl) ν 3405, 3300, 1681, 1635, 1602, 1558 cm^{-1}; UV (MeOH, ε) λ 207 (8730), 232 (4700), 265 (4170), 272 (4160), 324 (537) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.83 (1H, d, *J*=4.9 Hz, H-3), 7.86 (1H, d, *J*=4.9 Hz, H-4), 2.98 (3H, s, CH_3-1), 2.01 (3H, s, CH_3-6), 5.20 (2H, brs, NH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 159.58 (C-1), 154.14 (C-3), 117.70 (C-4), 140.77 (C-4a), 180.90 (C-5), 111.80 (C-6), 146.94 (C-7), 181.85 (C-8), 122.38 (C-8a), 25.69 (CH_3-1), 9.23 (CH_3-6).

Cribrostatin 2 (**325**): golden-yellow solid; mp 194-195°C (dec); EIMS *m/z* 247 M^+; IR (KBr) ν 2953, 2854, 1682, 1643, 1609, 1548 cm^{-1}; UV (MeOH, ε) λ 209 (5829), 328 (4126) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.25 (1H, s, H-1), 7.10 (1H, H-4), 3.66 (3H, s, NCH_3), 2.07 (3H, s, CH_3-6), 1.39 (3H, t, *J*=7.0 Hz, OCH_2CH_3), 4.48 (2H, q, *J*=7.0 Hz, OCH_2CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 142.02 (C-1), 162.79 (C-3), 116.6 (C-4), 138.95 (C-4a), 183.52 (C-5), 133.71 (C-6), 159.14 (C-7), 177.37 (C-8), 111.29 (C-8a), 28.37 (CH_3-2), 9.66 (CH_3-6), 16.07 and 69.78 (OCH_2CH_3).

2. Synthesis

Total synthesis of the cribrostatins was reported by Kubo *et al.* in 1995 (Scheme 39) *(153)*. 1,3-Diethoxytoluene (**326**), which is the ethylated form of commercially available methylresorcinol, was formylated with phosphorous oxychloride in *N,N*-dimethylformamide to yield the benzaldehyde **327**. Treatment of



SCHEME 39. Synthesis of cribrostatins 1 (**324**) and 2 (**325**) *(153)*.

327, first with *m*-chloroperoxybenzoic acid and then with 10% KOH, gave the phenol **328**. Oxidative demethylation of **328** with ceric(IV)ammonium nitrate afforded the 1,4-benzoquinone **329** which was subjected to the hetero Diels-Alder reaction with both 1,3-bis(*tert*-buthyldimethylsilyloxy)-2-azabuta-1,3-diene (**330**) and 2,4-bis(*tert*-buthyldimethylsilyloxy)-3-azapenta-1,3-diene (**331**). The [4+2] cycloadduct from the reaction between **329** and **330** was methylated with methyl iodide in the presence of potassium carbonate and tris[2-(2-methoxyethoxy)ethyl]amine to give cribrostatin 2 (**325**), and treatment of the [4+2] cycloadduct from the reaction between **329** and **331** with concentrated hydrochloric acid furnished the 3-quinolonequinone **332**. Aromatization of **332** with phosphorous oxychloride was followed by replacement of the ethoxy group at C-7 by an amine using 1M ammonia to give the aminoquinolinequinone **333**. Removal of the 3-chloro group of **333** with 10% palladium/carbon afforded cribrostatin 1 (**324**).

3. Biological Activity

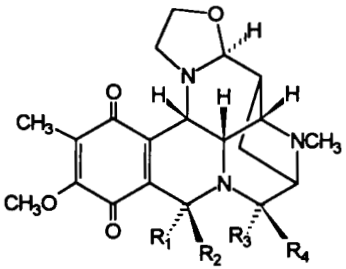
Cribrostatins 1 (**324**) and 2 (**325**) were reported to be active against the P388 lymphocytic leukemia cell line (ED_{50} 1.58 and 2.73 μg/ml, respectively) *(153)*.

V. Naphthyridinomycin Type Alkaloids

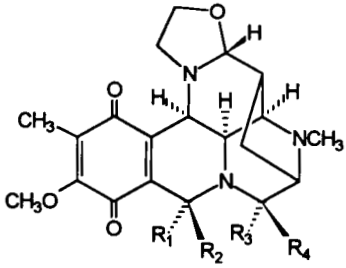
A. NAPHTHYRIDINOMYCIN, CYANONAPHTHYRIDINOMYCIN (CYANOCYCLINE A), SF-1739 HP AND NAPHTHOCYANIDINE (CYANOCYCLINE F)

1. Isolation and Structure Elucidation

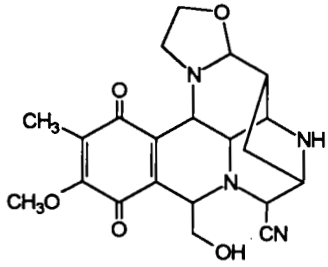
In 1974 Kluepfel *et al.* reported the isolation of a ruby red crystalline antibiotic, naphthyridinomycin (**334**) from the culture filtrate of *Streptomyces lusitanus* AY B-1026 *(154, 155)*. Single-crystal X-ray diffraction analysis of this highly congested molecule was disclosed by Brisse and Hanessian *(156)*. During the course of biosynthetic studies in 1982, due to its lack of stability, naphthyridinomycin was converted to cyanonaphthyridinomycin by treatment with sodium cyanide *(157)*. Almost at the same time, Hayashi *et al.* independently isolated the same compound from a culture broth of *Streptomyces flavogriseus* No:49, and named it cyanocycline A (**335**) *(158)*. Its single-crystal X-ray diffraction analysis was later reported by the same group on the basis of the corresponding dihydrobromide salt *(159)*. Although X-ray single crystal structures of naphthyridinomycin and cyanocycline were disclosed, depending on the biosynthetic studies by Gould *et al. (160, 161)*, and the total synthesis of (+)-cyanocycline A, Fukuyama *et al.* claimed that naphthyridinomycin and cyanocycline A have the structures **334**' and **335**', respectively *(6)*.



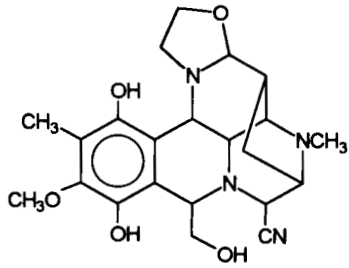
334 R_1=CH_2OH, R_2=R_3=H, R_4=OH
335 R_1=CH_2OH, R_2=R_3=H, R_4=CN



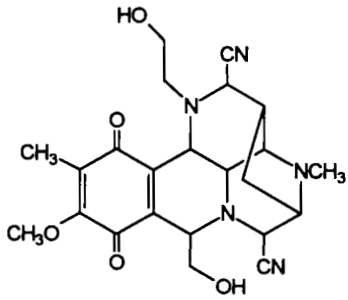
334' R_1=R_4=H, R_2=CH_2OH, R_3=OH
335' R_1=R_4=H, R_2=CH_2OH, R_3=CN



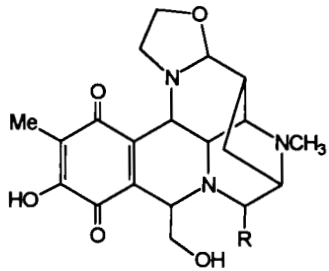
cyanocycline B **336**



cyanocycline C **337**



cyanocycline D **338**



SF-1739 HP
naphthocyanidine (cyanocycline F)

339 R=OH
340 R=CN

It was reported that during the isolation of naphthyridinomycin, a complex incorporating naphthyridinomycin A as a major product and naphthyridinomycin B as a minor product, was obtained *(162)*. The chemical structure of the minor product has not yet been disclosed in the literature.

In 1993, Gould *et al.* reported the isolation of new derivatives of naphthyridinomycin and cyanonaphthyridinomycin, cyanocycline B (**336**), C (**337**) and D (**338**), from a cyanide-treated broth of *Streptomyces lusitanus (163)*. They were characterized using modern spectroscopic techniques, such as COSY and long-range HETCOSY.

In 1976, Watanabe *et al.* isolated a new antibiotic, SF-1739, from the culture filtrate of *Streptomyces* sp. strain SF-1739, which is an isolate from a soil sample collected at Boroda, India *(164)*. Its structure was determined in 1982 after converting into SF-1739 HP and naphthocyanidine by treating SF-1739 first with mineral acid and then with potassium cyanide, respectively *(165)*. The spectroscopic studies and single crystal X-ray diffraction analyses of these semisynthetic compounds revealed the structures **339** and **340** for SF-1739 HP and naphthocyanodine (cyanocycline F), respectively *(165)*. Their close resemblance to naphthyridinomycin and cyanonaphthyridinomycin suggested that **339** and **340** are demethylnaphthyridinomycin and demethylcyanonaphthyridinomycin, respectively, and the original unstable compound SF-1739 is actually naphthyridinomycin.

Naphthyridinomycin (**334**): $C_{21}H_{27}N_3O_6$; ruby red crystalline; mp 108-110°C (dec); soluble in water, methanol, acetone, chloroform, dichloromethane, ethyl acetate and ether; insoluble in hexane; $[\alpha]^{25}_D$ +69.4° (*c* 1, $CHCl_3$); IR (CH_2Cl_2) ν 3000, 2940, 2880, 2845, 1715, 1690, 1650, 1604, 1495 cm^{-1}; UV (MeOH) λ 270, 248.5 nm; for ^1H and ^{13}C-NMRs, see refs. *154* and *157*.

Cyanonaphthyridinomycin (Cyanocycline A) (**335**): $C_{22}H_{26}N_4O_5$; orange-red needles; mp 168-170°C (dec); soluble in chloroform, ethyl acetate and alcohol, and barely soluble in water, acetone and petroleum ether; MS *m/z* 426 M^+; $[\alpha]^{25}_D$ +82° (*c* 1, $CHCl_3$); IR (KBr) ν 3440, 3380, 3240, 2220, 1670, 1655, 1636, 1619, 1228, 1130, 910 cm^{-1}; UV (MeOH, ε) λ 268 (10990) nm; (0.1 N HCl-MeOH, ε) λ 263 (9457) nm; (0.1 N NaOH-MeOH, ε) λ 283 (10437) nm; for ^1H and ^{13}C-NMRs, see refs. *157* and *191*.

Cyanocycline B (**336**): $C_{21}H_{24}N_4O_5$; HRFABMS *m/z* 412 M^+; IR (KBr) ν 3303, 2944, 2360, 2247, 1654, 1450, 1310, 912, 732 cm^{-1}; UV (85 % MeCN/H_2O) λ 210, 266, 375 nm; ^1H-NMR (400 MHz, $CDCl_3$) δ 4.66 (1H, brs), 4.17 (1H, brs), 4.03 (3H, s), 4.03 (1H, m), 3.91 (1H, m), 3.88 (1H, dd, *J*=11.4, 2.6 Hz), 3.81 (1H, brd, *J*=2.9 Hz), 3.80 (1H, brs), 3.73 (1H, m), 3.63 (1H, m), 3.48 (1H, dd, *J*=6.0, 3.1 Hz), 3.03 (1H, m), 3.01 (1H, m), 2.79 (1H, brd, *J*=3.0 Hz), 2.66 (1H, ddd, *J*=12.9, 6.9, 6.0 Hz), 2.26 (1H, ddd, *J*=12.9, 12.9, 6.9 Hz), 1.98 (1H, s), 1.72 (1H, dd, *J*=12.9, 6.9 Hz); ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 186.39, 181.16, 156.69, 142.94, 140.71, 128.29, 117.13, 93.12, 61.57, 61.15, 57.27, 56.40, 55.65, 53.28, 52.80, 52.28, 50.06, 47.55, 38.89, 31.61, 8.86.

Cyanocycline C (**337**): Unstable, for dimethyl derivative, see ref. *163*.

Cyanocycline D (**338**): $C_{23}H_{27}N_5O_5$; mp 112-114°C (recryst. from EtOAc/hexane); HRFABMS *m/z* 412 M^++1; IR (KBr) ν 3356, 2846, 2306, 2227, 1656, 1450, 1238, 1151, 905, 739 cm^{-1}; UV (85 % MeCN/H_2O) λ 204, 268, 370

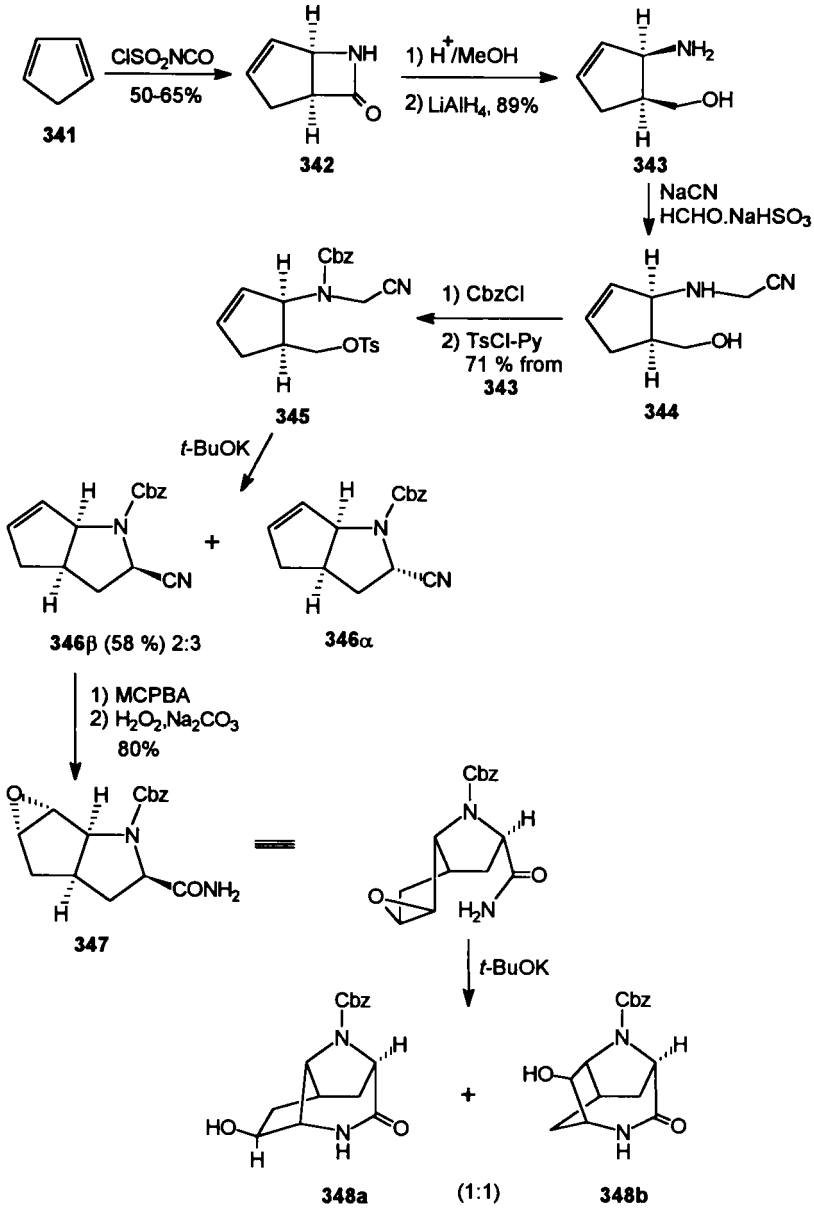
nm; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.67 (1H, brs), 4.56 (1H, brd, *J*=9.1 Hz, exchangeable with D_2O), 4.21 (1H, d, *J*=2.6 Hz), 4.05 (3H, s), 3.96 (d, *J*=3.4 Hz), 3.88 (1H, dd, *J*=11.4, 2.6 Hz), 3.87 (3H, s), 3.72 (1H, m), 3.66 (1H, m), 3.62 (1H, brd, *J*=11.4 Hz), 3.45 (1H, m), 3.27 (1H, dd, *J*=4.5, 4.4 Hz), 2.91 (1H, dd, *J*=11.0, 5.6 Hz), 2.90 (1H, s), 2.69 (1H, m), 2.60 (1H, m), 2.46 (3H, s), 2.38 (1H, dd, *J*=13.3, 7.1 Hz), 2.27 (1H, m, exchangeable with D_2O), 1.99 (3H, s), 1.79 (1H, dd, *J*=13.0, 6.1 Hz); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 184.90, 180.80, 155.70, 140.70, 128.60, 116.70, 116.20, 62.80, 61.10, 60.90, 59.11, 59.09, 56.55, 53.63, 53.41, 53.02, 51.79, 51.73, 41.15, 36.81, 28.50, 8.87.

SF-1739 HP (**339**): $C_{20}H_{25}N_3O_6$; purple powder; soluble in H_2O and methanol; insoluble in CH_2Cl_2, ethyl acetate and ethyl ether; gives positive Lemieux, and iodine and negative ninhydrin, Molisch and biuret reactions; $[\alpha]^{25}_D$ +64° (*c* 0.5 MeOH); IR (KBr) ν 3400, 2950, 2900, 1650, 1530, 1390, 1240, 1180, 1000 cm^{-1}; UV (MeOH) λ 275 (165), 330 (sh); ^{13}C-NMR (25 MHz, D_2O) δ 186.8, 185.3, 169.2, 146.6, 137.9, 114.1, 93.8, 79.3, 66.5, 62.4, 60.6, 60.5, 53.4, 51.3, 50.0, 48.0, 40.5, 34.2, 28.7, 8.5; for ^{1}H-NMR, see ref. *165*.

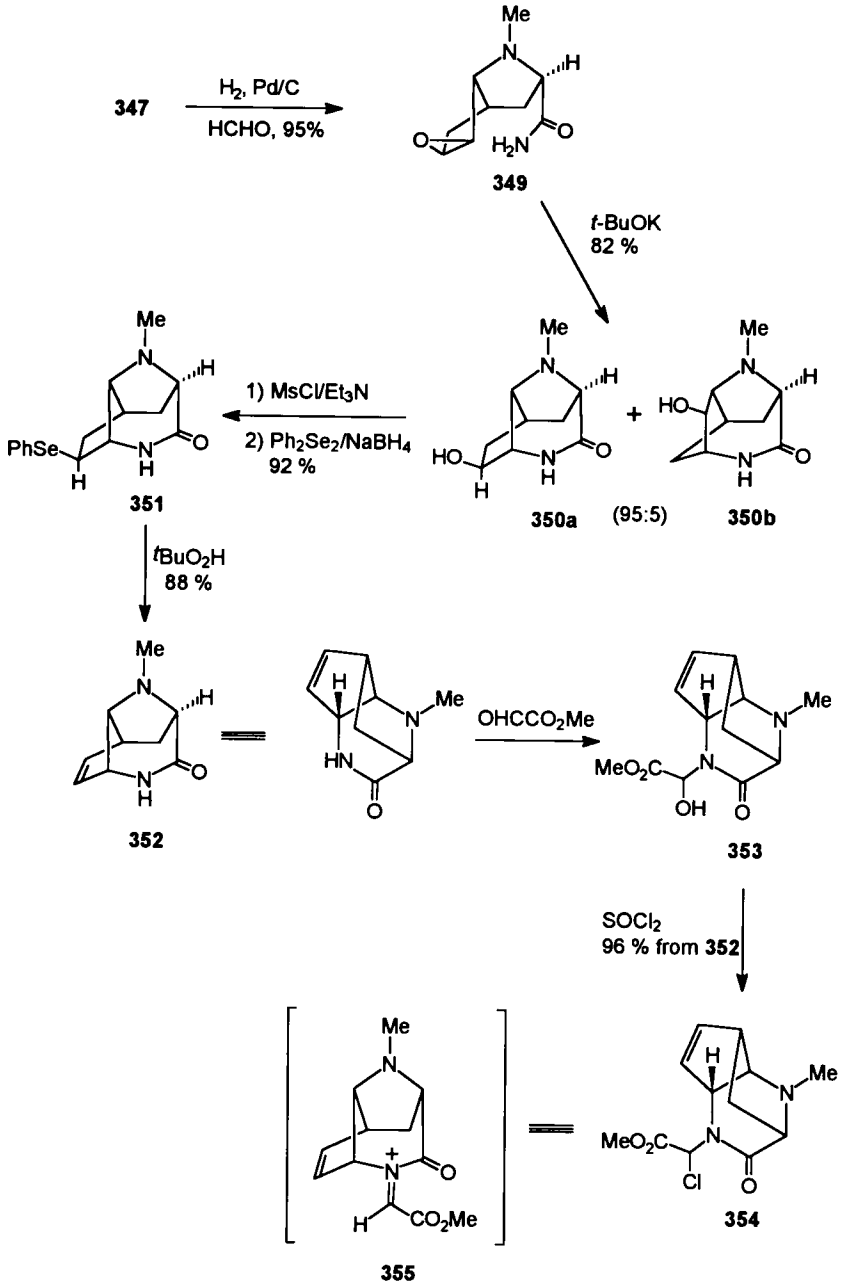
Naphthocyanidine (cyanocycline F) (**340**): $C_{21}H_{24}N_4O_5$; reddish yellow crystal; soluble in H_2O and methanol; insoluble in CH_2Cl_2, ethyl acetate and ethyl ether; gives positive Lemieux, and iodine and negative ninhydrin, Molisch and biuret reactions; $[\alpha]^{25}_D$ +76° (*c* 0.5 MeOH); IR (KBr) ν 3400, 2970, 2900, 2240, 1670, 1550, 1400, 1240, 1180, 1050 cm^{-1}; UV (MeOH) λ 273 (300), 395 (32); ^{13}C-NMR (25 MHz, $CDCl_3$) δ 186.4, 183.9, 164.3, 146.0, 136.6, 117.6, 113.8, 93.4, 62.7, 61.8, 60.6, 59.9, 56.6, 54.4, 53.5, 50.1, 48.1, 41.2, 35.3, 29.2, 8.1; for ^{1}H-NMR, see ref. *165*.

2. Synthesis

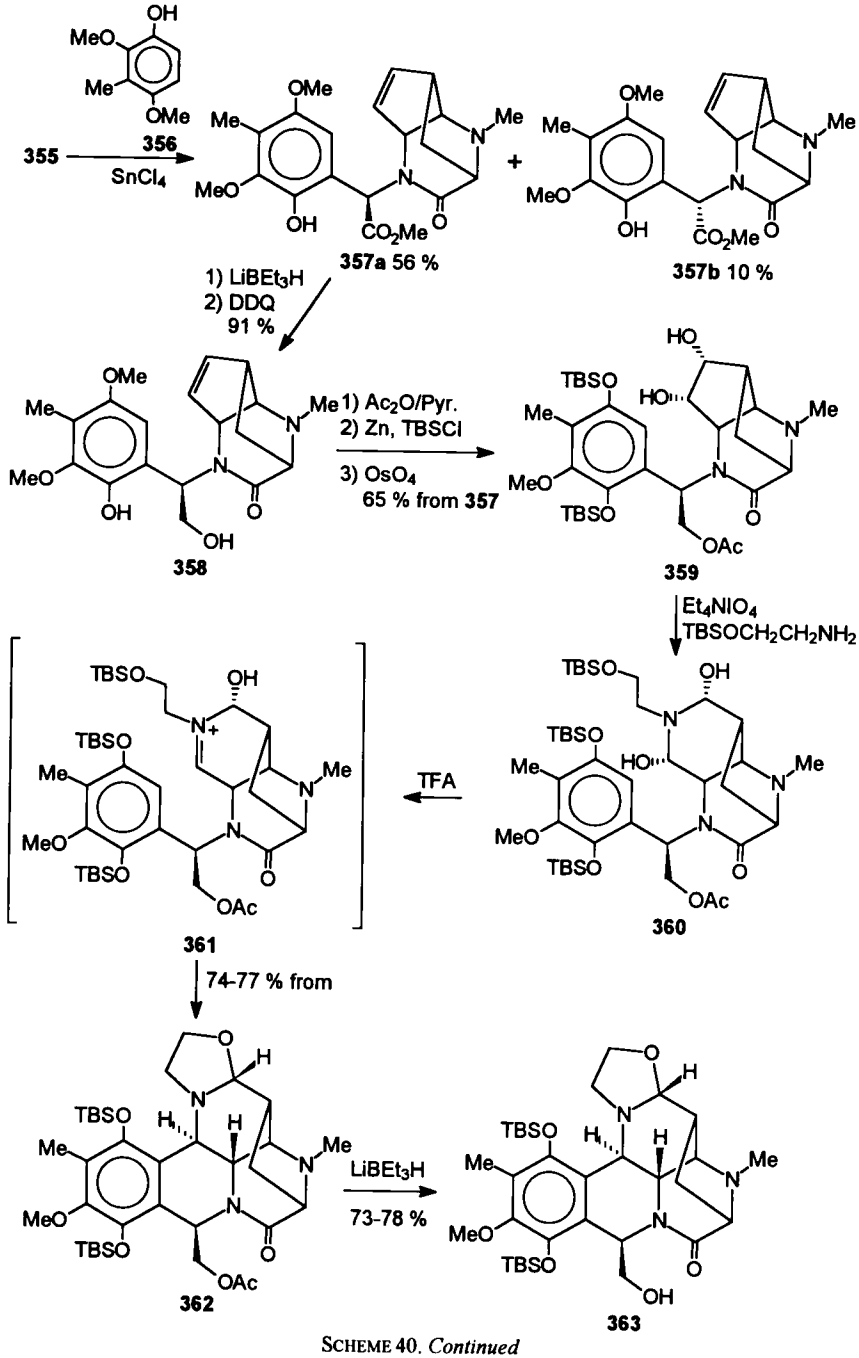
The first total synthesis of the challenging alkaloid cyanocycline A (**335**) was reported by Evans *et al.* in 1986 *(166)*, more then ten years after its isolation (Scheme 40) *(155)*. Their elegant synthesis can be divided into two parts. The first part comprises the synthesis of the core molecule, the tricyclic lactam **350**, which was achieved in fifteen steps *(167)*. Their initial reaction, cycloaddition of chlorosulfonyl isocyanate to cyclopentadiene (**341**), gave the β-lactam **342** with desired stereochemistry. The amide bond of **342** was acid cleaved and reduction with lithium aluminum hydride furnished the amino alcohol **343**. The amine part of **343** was converted to an amino nitrile using sodium cyanide and formaldehyde. The selective protection of the amine functionality of **344** with a carbobenzoxy group and of the hydroxyl functionality with a tosyl group yielded **345**. Treatment of **345** with *t*-BuOK yielded an epimeric mixture of **346α** and **346β** (2:3). The undesired isomer **346β** was recycled by subjecting to potassium *t*-butoxide catalyzed equilibration, which gave an overall 58% yield. Epoxidation of the olefin **346α** with *m*-chloroperbenzoic acid and treatment with hydrogen peroxide furnished the compound **347** with β-epoxide and amide functionalities. Attack from the amide nitrogen to the epoxide in the presence of *t*-butoxide proceeded without regioselectivity, which resulted in the formation of **348a** and **348b** in a 1:1 ratio. This difficulty was overcome by hydrogenating **347** in the presence of formaldehyde



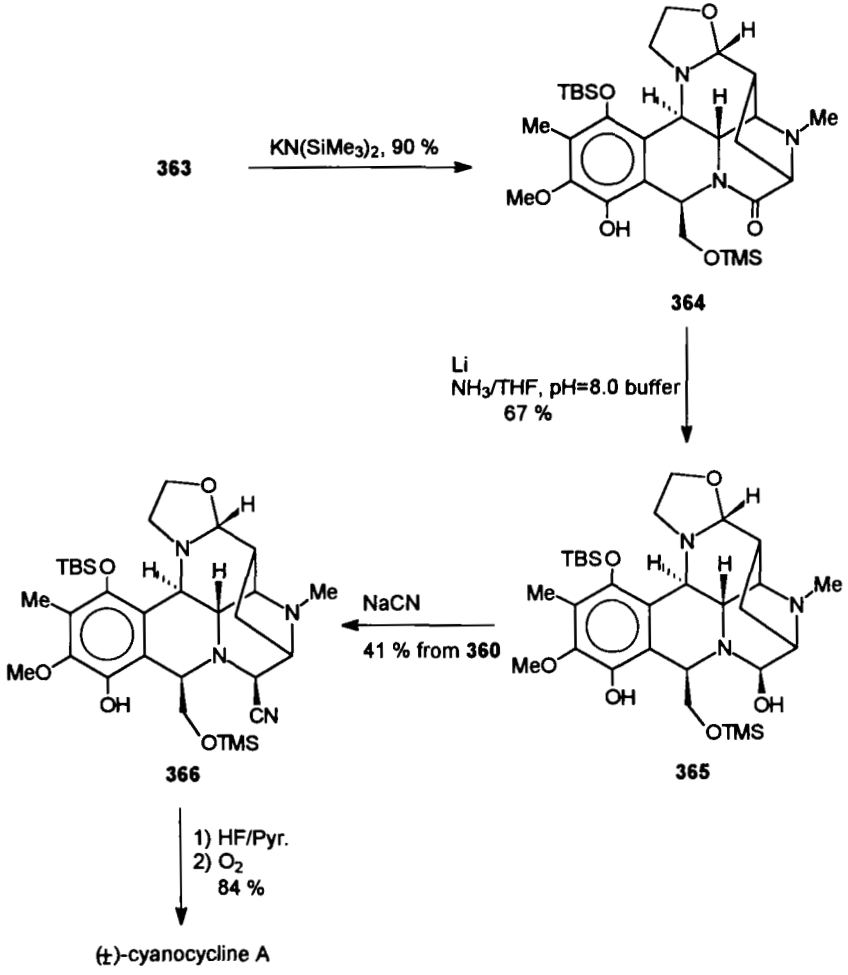
SCHEME 40. Evans *et al.* total synthesis of (±)-cyanocycline A *(166-168).*



SCHEME 40. *Continued*



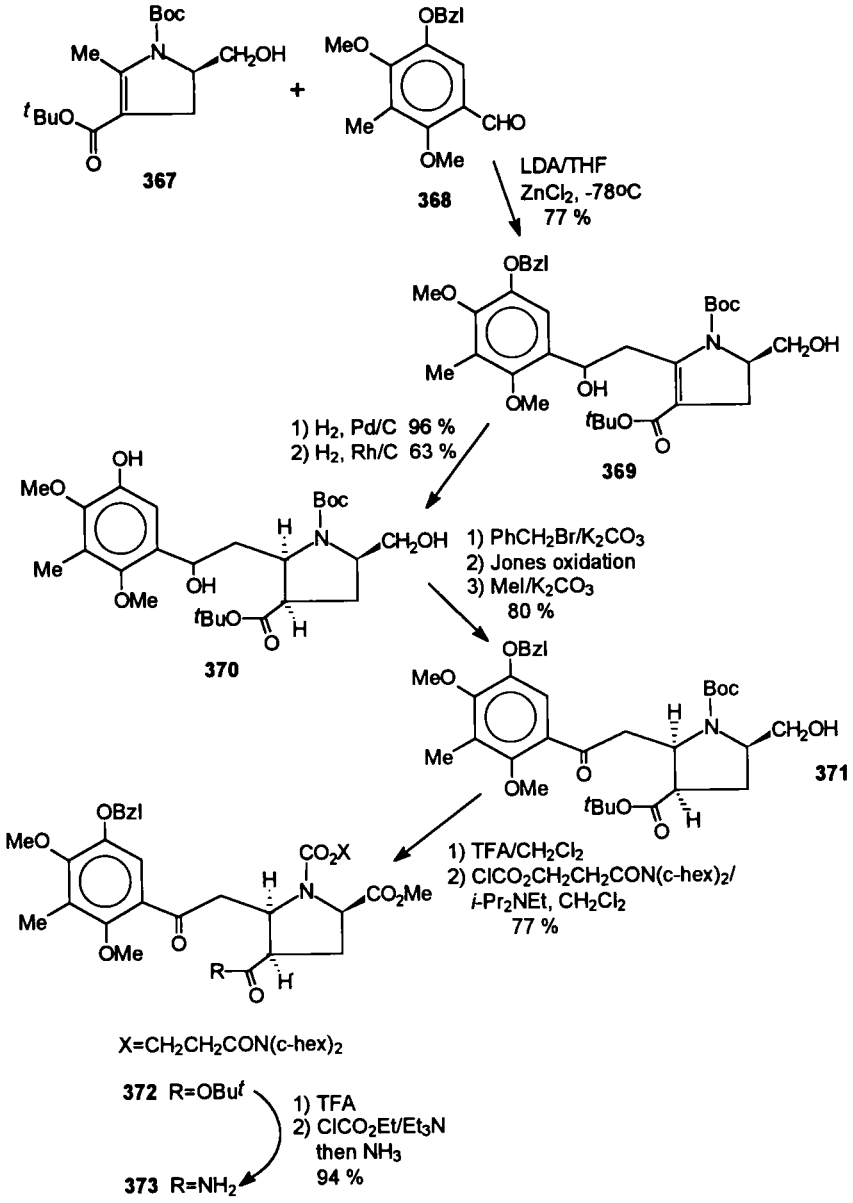
SCHEME 40. *Continued*



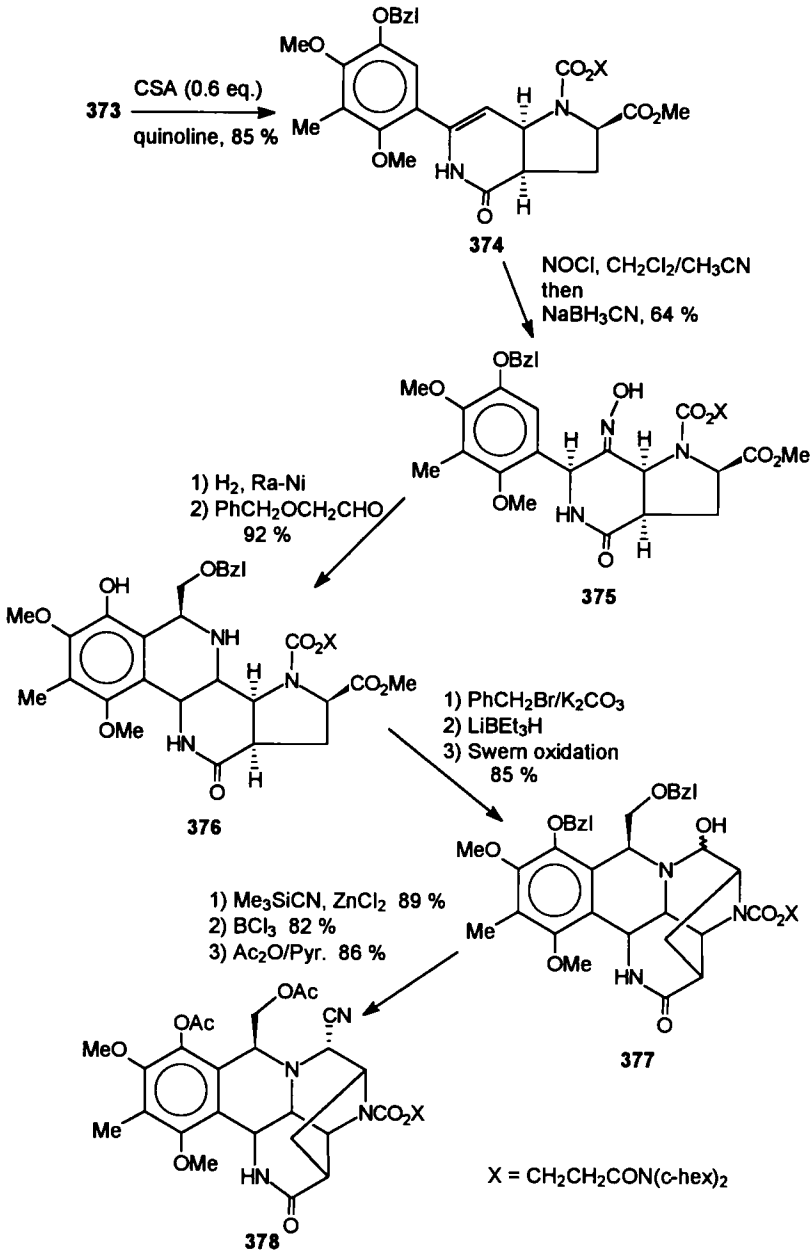
SCHEME 40. *Continued*

(95%), that is, the Cbz group was replaced by a methyl group. The *N*-methylamine **349** then underwent the ring closure with the desired regioselectivity, 95 ≥ 5 **350a** and **350b**, respectively. Mesylation of the alcohol **350a** was followed by reaction with sodium phenylselenide which furnished **351**. The selenide of the trifluoroacetate salt of **351** was oxidatively eliminated with *t*-butyl hydroperoxide to obtain the desired tricyclic olefin **352**.

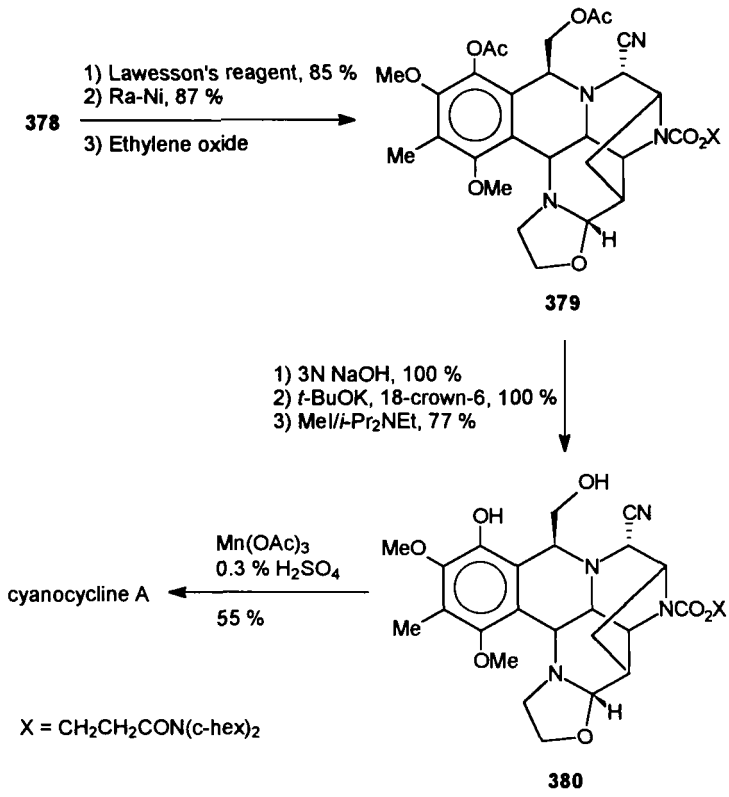
After successfully achieving the first part of the synthesis, the second part, which comprises the coupling of the tricyclic compound **352** with the quinone



SCHEME 41. Fukuyama's total synthesis of (±)-cyanocycline A *(169, 170)*.

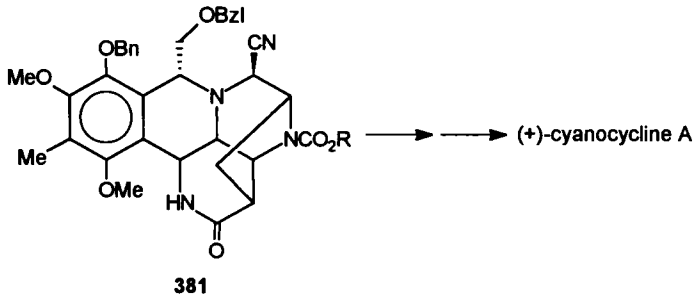


SCHEME 41. *Continued*



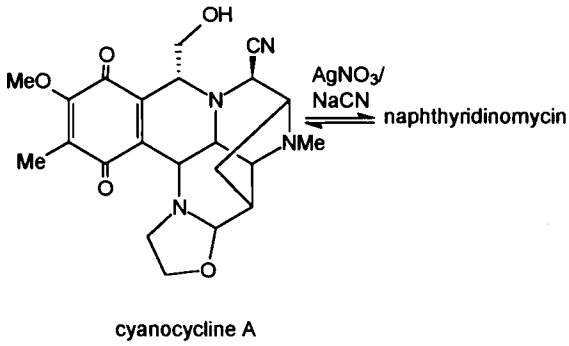
SCHEME 41. *Continued*

portion, was performed *(166, 168)*. In the initial step, the tricyclic lactam **352** was condensed with methyl glyoxylate to give **353**, the hydroxyl functionality of which was exchanged for chlorine using thionyl chloride. Aromatic substitution of 2,4-dimethoxy-3-methylphenol with **354** was activated by tin tetrachloride. The isolation of 56% of the desired isomer **357a** suggested that preferential attack of the phenol **357** took place from the α-face of the acyliminium ion **355**. Only 10% of the isomer **357b** was isolated. The selective reduction of the ester portion of **357a** using lithium triethylborohydride was followed by oxidation of the *p*-methoxyphenol with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) which furnished benzoquinone **358**. The hydroxyl portion of **358** was acylated with acetic anhydride, and then treatment with activated zinc in the presence of *t*-butyldimethylsilyl chloride (TBS) gave the reductively silylated product, the olefin portion of which was catalytically osmylated to yield the diol **359**. Oxidation of the diol **359** with tetraethylammonium periodate in the presence of *t*-butyldimethylsilyl protected ethanolamine and strictly excluding moisture gave **360**. The elegant double cyclization was performed by treatment of



SCHEME 42. Synthesis of (+)-cyanocycline A *(6)*.

360 with trifluoroacetic acid (TFA) which gave the desired hexacyclic compound **362** via iminium **361**. Reduction of the lactam group proved to be the most challenging step of the synthesis. It was found that the best solution was to use dissolved metal reduction conditions to avoid the reduction of the oxazolidine ring at the same time. The acetate of **362** was first converted to the alcohol **363** with $LiBEt_3H$. Using potassium hexamethyldisilylamide, the phenol **364** was obtained to avoid the Birch reduction of the aromatic ring in the next step. The amide reduction on **364** was then performed with a large excess of lithium (100 equiv.) in NH_3/THF which yielded **365** successfully. The crude **365** was directly converted to the more stable nitrile **366** with sodium cyanide in a buffer solution (pH=8.0 Tris buffer). In the last step, treatment of **366** with HF to remove TBS was followed by air oxidation of the resultant hydroquinone to give the racemic cyanocycline A.



SCHEME 43. Synthesis of naphthyridinomycin *(6)*.

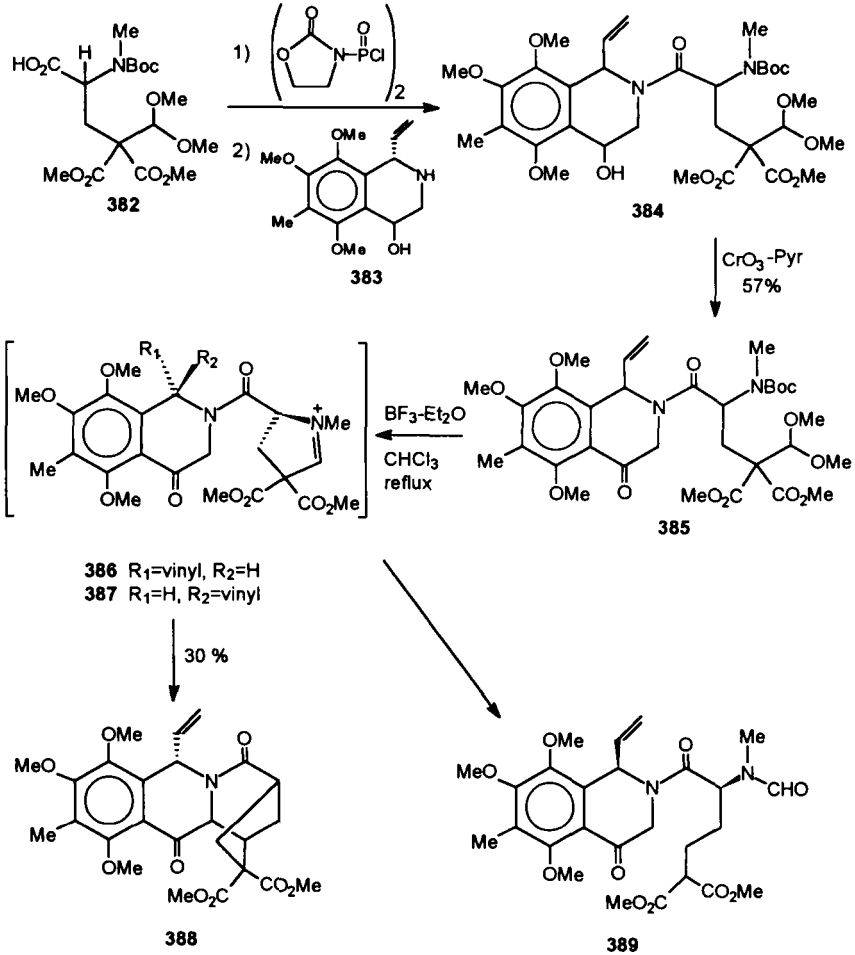
In 1987, a second total synthesis of cyanocycline A was reported by Fukuyama *et al.* (Scheme 41) *(169, 170)*. Their initial key reaction, addition of dihydropyrrole **367** to 5-(benzyloxy)-2,4-dimethoxy-3-methylbenzaldehyde **368**, was successfully achieved by first treating the pyrrole **367** with LDA and then with zinc chloride to generate a dienolate which was reacted with **368** to furnish the desired compound **369**. Hydrogenolysis of **369** to remove the benzyl group using Pd/C as catalyst and then hydrogenation of the olefin functionality using Rh/C, resulted in the formation of pyrrolidine **370**. Controlling the stereochemistry of the pyrrolidine ring, synthesis of the keto ester **371** was achieved in three steps, i) addition of benzyl bromide to protect the phenolic hydroxyl group, ii) Jones oxidation of the hydroxymethylene to an acid, and finally iii) methylation of the acid functionality to the methyl ester. Selective deprotection of *N*-Boc group with 2% TFA-CH_2Cl_2 was followed by treatment with *N,N*-dicyclohexyl-3-chlorocarboxypropanamide to give the urethane **372**. The *tert*-butyl ester group of **372** was converted into the amide in two steps, i) hydrolysis of *t*-Bu using TFA, and ii) treatment with $ClCO_2Et$ and then addition of ammonium, which gave the amide **373**. Cyclization of **373** was carried out using camphorsulphonic acid (CSA) to give the ene lactam **374**. The vital stereoselective conversion of **374** to the oxime **375** was achieved by treatment with nitrosyl chloride, the α-chloro oxime group of which was in turn reduced with $NaBH_3CN$ to give the oxime **375**. Catalytic hydrogenation of the oxime group of **375** was followed by treatment of the resultant aminophenol with glycolaldehyde benzyl ether to yield tetrahydroisoquinoline **376**. The epimeric mixture of aminal **377** was obtained in three steps, i) protection of phenol with benzyl bromide, ii) reduction of the methyl ester functionality to an alcohol with $LiBEt_3H$, and iii) Swern oxidation of the resultant alcohol. The single isomer of the aminonitrile was obtained on treatment of **377** with trimethylsilyl cyanide. The corresponding compound was reacted with BCl_3 and then with acetic anhydride to obtain **378**. The lactam **378** was converted to a thiolactam on treatment with Lawesson's reagent, which was then carefully desulfurized with Ra-Ni to give the stable imine. Conversion of the resultant imine to the oxazolidine **379** was accomplished using ethylene oxide. Hydrolysis of the acetyl groups with NaOH, removal of the amino protection group using *t*-BuOK, and then methylation of the amine portion with MeI furnished **380**. Finally, oxidation of the phenolic group with manganese triacetate afforded (±)-cyanocycline A (**335**).

Synthesis of (+)-cyanocycline A was also achieved by Fukuyama *et al.* using the same reaction conditions developed during the synthesis of racemic cyanocycline A. The optically pure intermediate **381** was converted to (+)-cyanocycline A (Scheme 42) *(6)*.

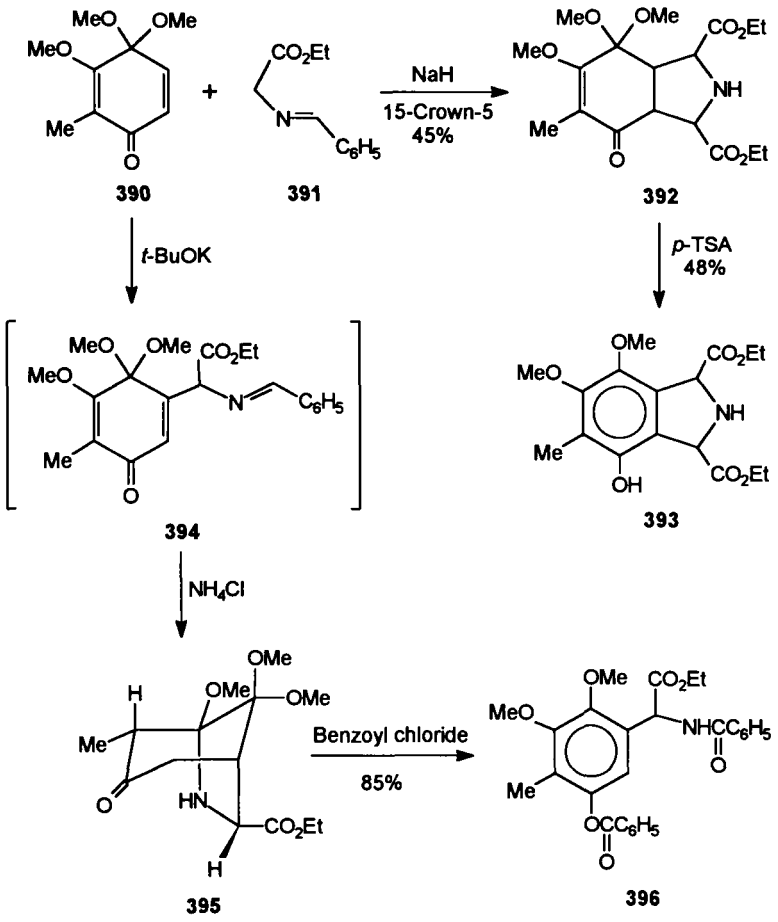
Fukuyama *et al.* also disclosed that treatment of cyanocycline A with ten equivalents of silver nitrate gave an unstable compound. The ^{1}H-NMR spectrum, mass spectral data and conversion of the new compound back to cyanocycline A on treatment with sodium cyanide in a buffered solution strongly suggested the synthesis of naphthyridinomycin (Scheme 43) *(6)*.

An approach to the synthesis of naphthyridinomycin was reported by Danishefsky *et al.* in 1984 (Scheme 44) *(171, 172)*. As in the total syntheses, Danishefsky's approach alsocomprises the synthesis of two important parts and their

coupling. These two portions, the acid **382** and the racemic tetrahydroisoquinolinol **383** were connected by the method of Palomo-Coll to give the diastereomeric mixture **384** *(173)*. Oxidation of **384** using Collins reagent afforded the keto amide **385**. Treatment of **385** with boron trifluoride etherate in refluxing chloroform gave two products, the tetracyclic compound **388** and the formyl transferred compound **389**. It is believed that the intermediate comprised a mixture of **386** and **387**, and that the failed cyclization could be because of the repulsion between the vinyl group and the iminium ring of **387**.



SCHEME 44. Danishefsky's approach to naphthyridinomycin *(171, 172)*.

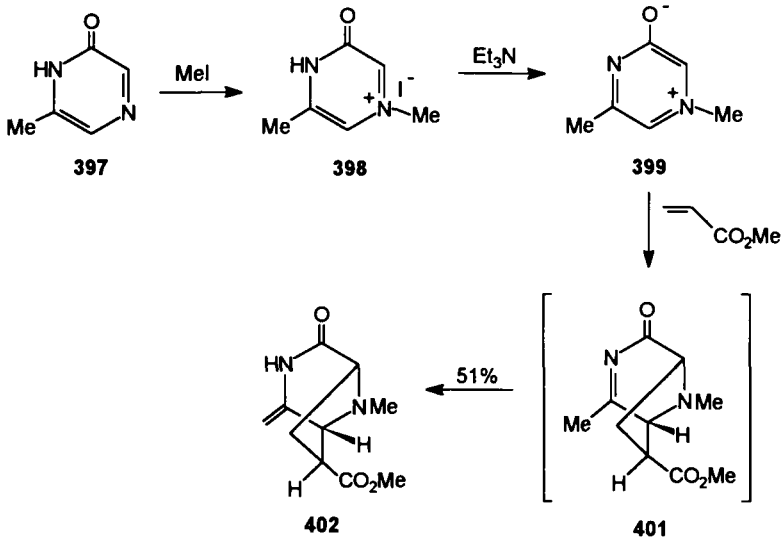


SCHEME 45. Parker's partial synthesis of naphthyridinomycin *(174)*.

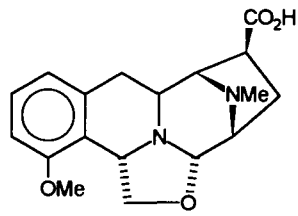
A partial synthesis of naphthyridinomycin was described by Parker *et al.* in 1984 (Scheme 45) *(174)*. When the readily available quinone monoacetal **390** *(175, 176)* was treated with **391** in the presence of sodium hydride and 15-crown-5, an isoindole **392** was obtained as a mixture of diastereoisomers. The mixture was aromatized with *p*-toluenesulphonic acid to afford a single diastereomer **393**. When the reaction between **390** and **391** was promoted using *t*-butoxide, the adduct **394** was isolated, along with the starting materials. Treatment of crude **394** with ammonium chloride resulted in the isolation of the bicyclic compound **395**. Aromatized and derivatized **396** was obtained when **395** was heated with excess benzoyl chloride. Although this approach has not yet been elaborated further in the

synthesis of naphthyridinomycin, it was later used in the total synthesis of mimosamycin (**208**) *(122)*.

Two attractive and closely related approaches to the construction of the 3,8-diazobicyclo[3.2.1]octane moiety of naphthyridinomycin were reported by Joule *et al.* and Garner *et al.* in 1987 (Scheme 46) *(177)* and 1988 (Scheme 47) *(179, 180)*, respectively. In their partial synthesis, Joule *et al.* quaternized the readily available 6-methylpyrazine-2-one (**397**) using methyl iodide to obtain **398** (Scheme 46). Treatment of **398** with triethylamine gave the zwitterion 3-oxidopyrazinium **399**. 1,3-Dipolar cycloaddition to **399** with methyl acrylate **400** gave exclusively the *exo*-compound **402** via the possible intermediate **401**. Later, this strategy was further elaborated in the synthesis of quinocarcin (**403**) *(178)*.

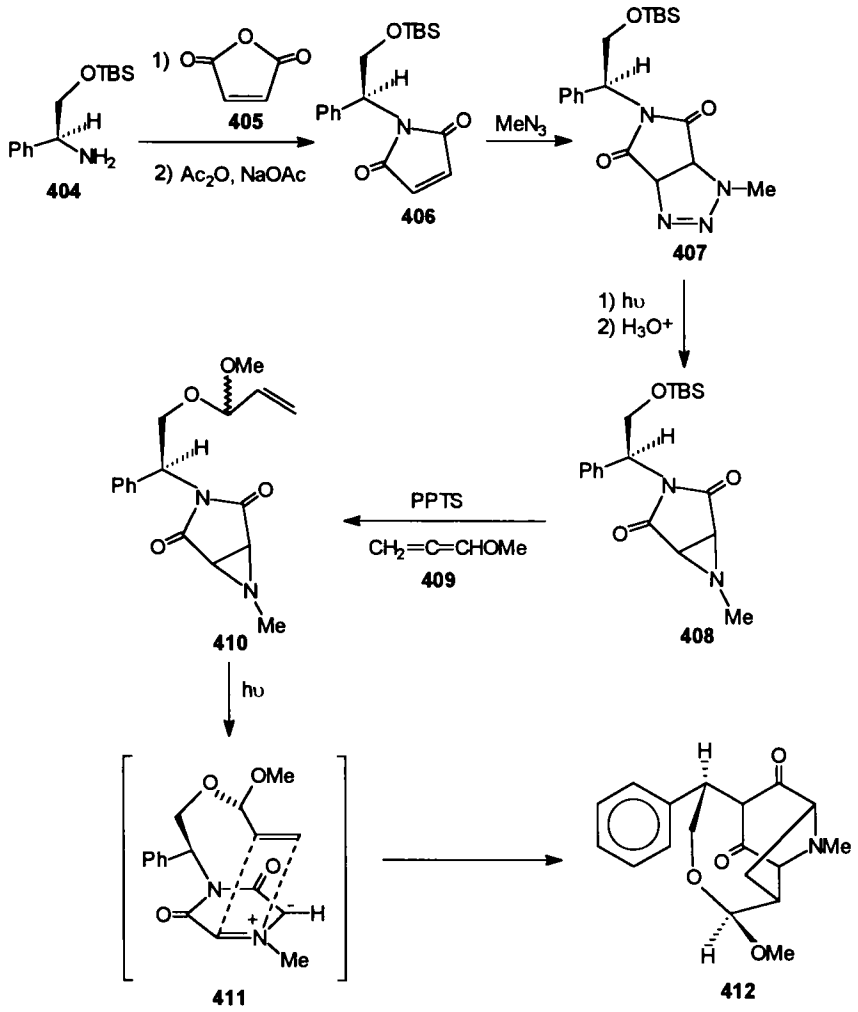


SCHEME 46. Joule's synthetic studies on naphthyridinomycin *(177)*.

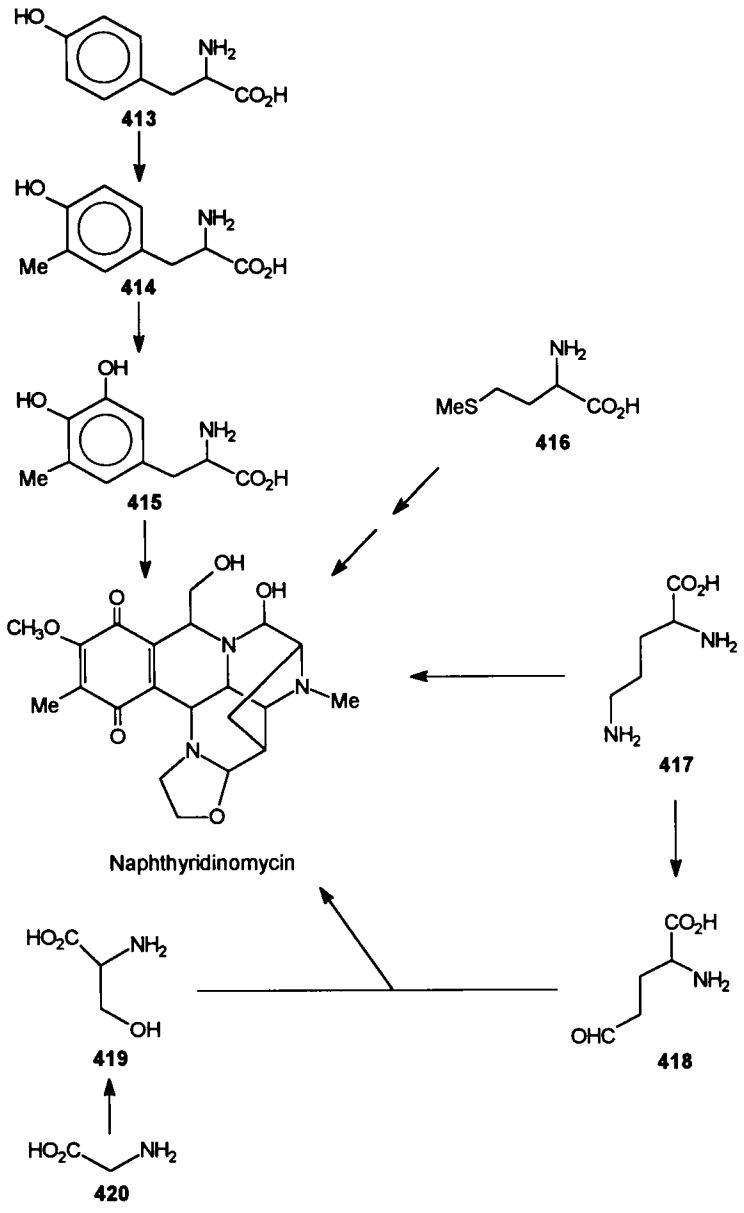


403

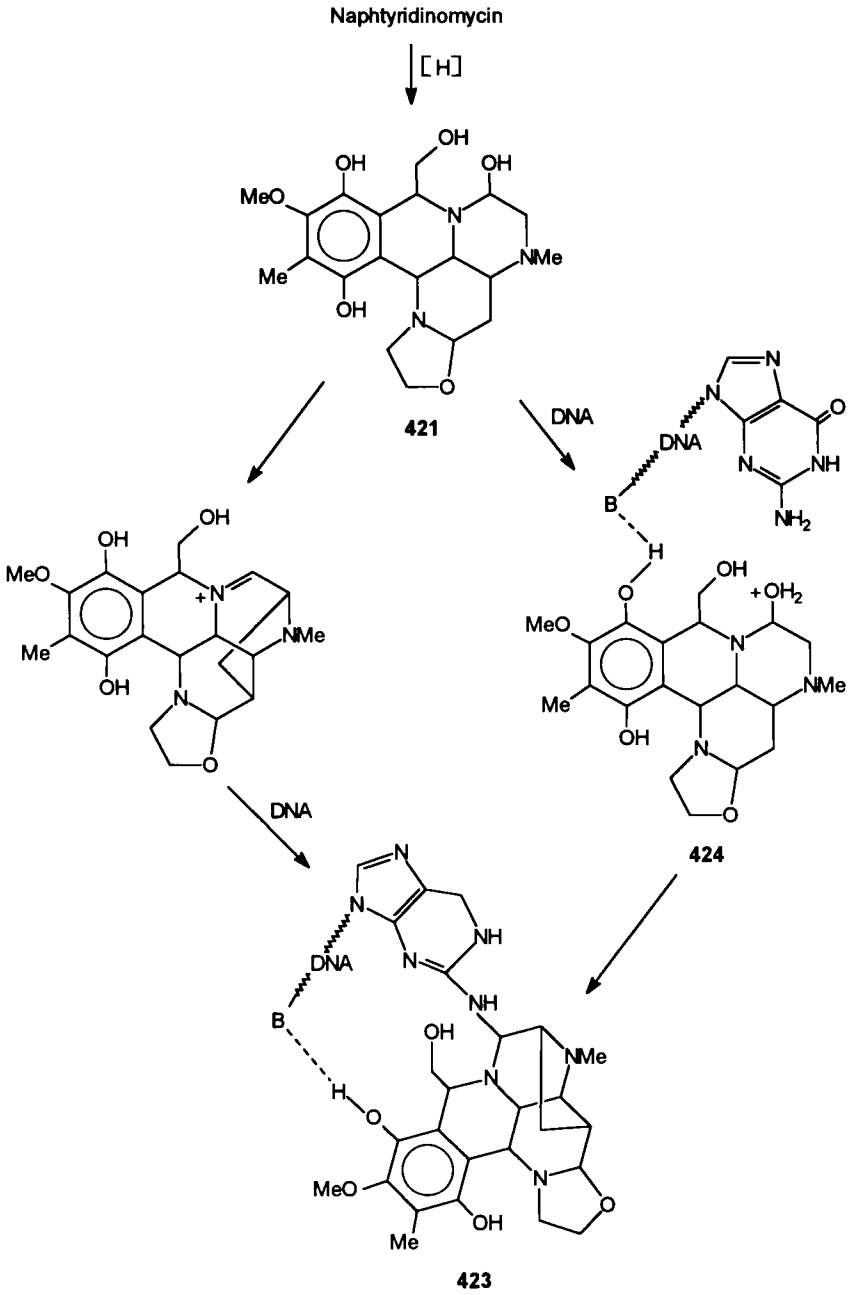
Garner *et al.* reported their improved diastereoselective synthesis through an intramolecular 1,3-dipolar cycloaddition (Scheme 47) *(179, 180)*. Reaction of the readily available 5-phenylglycinol derivative **404** with maleic anhydride **405** was



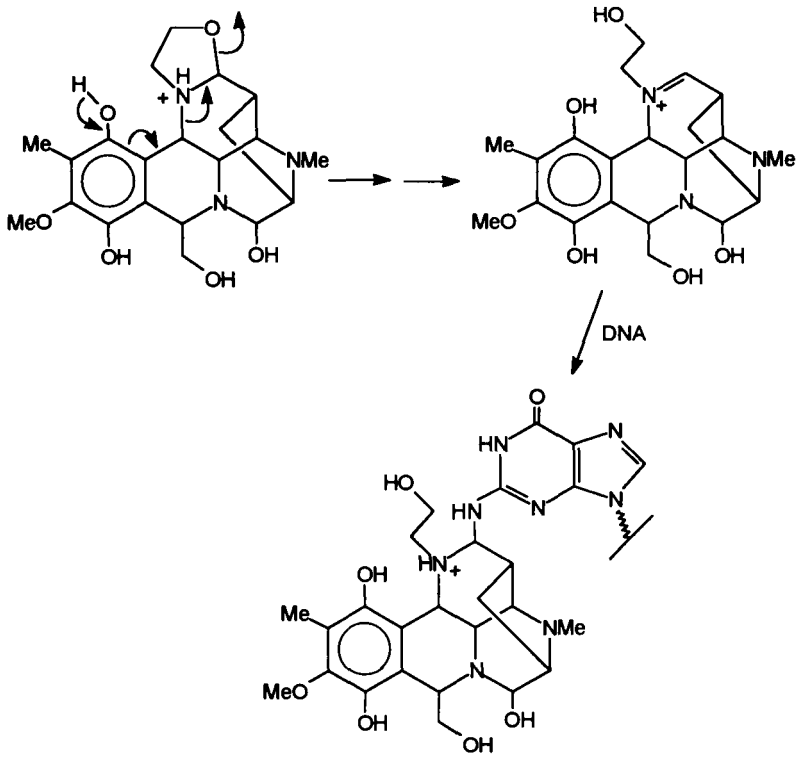
SCHEME 47. Garner's synthetic studies on naphthyridinomycin *(179, 180)*.



SCHEME 48. Biosynthetic pathway of naphthyridinomycin *(160, 182-184)*.



SCHEME 49. Proposed mechanisim by Zmijewski *et al.* for the binding of naphthyridinomycin with DNA *(187)*.



SCHEME 50. Alternative mechanisim by Remers *et al.* for binding of naphthyridinomycin with DNA *(188)*.

followed by ring closure using acetic anhydride/sodium acetate to afford the maleimide **406**. Treatment of **406** with methyl azide resulted in the formation of the triazoline **407**, which was radiated and then treated with acid to give the aziridine **408**. On reacting **408** with methoxy-allene **409**, a 1:1 diastereomeric mixture of acetal **410** was obtained. Photolysis of **410** gave intramoleculer cycloaddition reaction only through the *S*-configuration **411**, which resulted in the formation of a tricyclic product **412**. Further elaboration of this strategy was later carried out during the synthetic studies of quinocarcin (**403**) *(181)*.

3. Biosynthesis

Because of the instability of naphthyridinomycin (**334**), biosynthetic studies were generally carried out on cyanonaphthyridinomycin. In 1982, Zmijewski *et al.* reported that the amino acids tyrosine, glycine, ornithine and the *S*-methyl group of methionine were efficiently incorporated into cyanonaphthyridinomycin (Scheme 48) *(182)*.

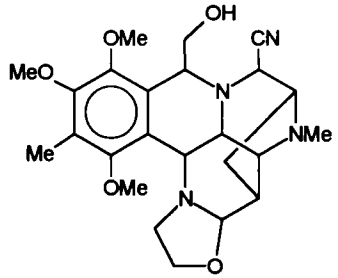
On the basis of their findings, Zmijewski and Gould proposed that a serine (**419**) metabolite derived from glycine (**420**) *(183)* condenses with glutamate semi-aldehydride intermediate **418**, an ornithine (**417**) metabolite, on the way to the biosynthesis of naphthyridinomycin (Scheme 48) *(184)*. Methyl group attached to the sulfur of methionine (**416**) provides the three methyl groups *(160, 182-184)*. It was determined that the entire carbon skeleton of tyrosine (**413**) is incorporated in naphthyridinomycin through the metabolites **414** and **415** *(160)*.

4. Biological activity

Naphthyridinomycin was found to be lethal to proliferating cultures of *Escherichia coli*. It induced the elongation in *Escherichia coli* cells at low concentrations, such as 0.1 μg/ml, and inhibited the incorporation of ^{14}C-thymidine into DNA and ^{3}H-leucine into proteins at concentrations equal to or lower than the MIC *(185)*. However, degradation of cellular DNA was not observed. Its primary effect was reported to be the inhibition of DNA synthesis. This irreversible action of naphthyridinomycin was explained by its strong binding ability to the DNA template of the cell *(185-187)*. Its effects on RNA and protein syntheses were observed at higher concentrations. It was found that binding of naphthyridinomycin to DNA was greatly stimulated by the addition of dithiothreitol (DTT) into the incubation mixture *(186)*, but it is slightly inhibitory at concentrations above 10 mM. Zmijewski *et al.* postulated two mechanisms, according to which naphthyridinomycin (**334**) binds to dG-dC base pairs in the minor groove of double stranded DNA (Scheme 49) *(187)*. Reduced naphthyridinomycin **421** can easily form the iminium salt **422**, which reacts with the 2-amino group of guanine in the minor groove of double stranded DNA to form **423**. The same product **423** could be obtained in the second mechanism via the intermediate **424**, that is, protonation of the carbonyl and elimination of water leads to the formation of **423**.

Later, Remers *et al.* suggested an alternative mechanism for the binding of naphthyridinomycin and cyanocycline A to DNA, based on the opening of the oxazolidine ring and alkylation by the amino group of a guanine residue in the DNA strand (Scheme 50) *(188)*.

Potent inhibition of nucleic acid synthesis by cyanocycline A in both eukaryotic and prokaryotic cells was also reported. Experiments in Meth A cells showed that cyanocycline A is more effective on RNA synthesis than that of DNA, and vice versa in *E. coli*. On binding studies of cyanocycline A to DNA, it was observed that its antimicrobial activity, against *M. luteus* was diminished by DNA (herring sperm) *(189)*. Considering this and the other similar results, it was concluded that cyanocycline A may not function as an intercalating agent. Reducing cyanocycline A to hydroxycyanocycline A with 2 mM dithiothreitol gave almost the same antimicrobial activity. The medium with herring sperm DNA and 2 mM dithiothreitol did not exert any effect on the antimicrobial activity and binding to DNA of cyanocycline A. These results indicated that its binding mechanism to DNA is different from that of naphthyridinomycin *(189)*. In their studies on the interaction of cyanocycline A with DNA, Zmijewski *et al.*, contrary to the previous study



425

suggested that cyanocycline is extremely dependent on the presence of dithiothreitol. The binding of cyanocycline A to DNA occurs with the loss of cyanide. They suggested that this is not promoted by the acidic pH, but by the presence of dithiothreitol *(190)*. Their results showed that the antibiotic does not bind well to DNA until the cyanide is released.

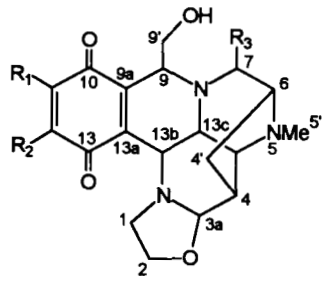
Semisynthetic derivatives of naphthyridinomycin SF-1739 HP (**339**) and naphthocyanidine (**340**) were reported to have more biological activity than naphthyridinomycin (**334**). They showed marked activity against leukemia P388 with the maximum ILS of > 368.6% (SF-1739 HP) and 183% (naphthocyanidine) *(165)*. This enhancement of biological activities by the replacement of methoxy with either hydroxyl or amino groups was also confirmed by Zmijewski's studies on the derivatives of cyanonaphthyridinomycin *(191)*. These findings were also supported by molecular modeling structure-activity studies, which showed that the models with OH and NH_2 in place of a methoxy group have stronger drug-DNA complexes through extra hydrogen bonding *(192, 193)*.

Cyanocyclines B (**336**), C (**337**) and D (**338**) were found to be active against a variety of bacteria *(163)*. On the other hand, dimethylcyanocycline C (**425**) showed a lack of biological activity. This was interpreted that the benzoquinone moiety of naphthyridinomycin type antibiotics plays an important role in the mechanism of their binding to DNA.

B. DNACINS

1. Isolation and Structure Elucidation

In1980, the isolation of the new antibiotics, dnacin A_1 (**426**) and dnacin B_1 (**427**), from the culture broth of *Nocarda* sp.No.C-14482 was reported *(194)*. Although its partial structure could be explained by the same group *(195)*, the full picture was disclosed byHida *et al.*in 1994,fourteen years after its first isolation *(196)*.



dnacin A_1 **426** R_1=NH_2, R_2=H, R_3=CN
dnacin B_1 **427** R_1=NH_2, R_2=H, R_3=OH

With its close structural resemblence to naphthyridinomycin, dnacin A_1 and B_1 differ only in their R_1 and R_2 groups at the 11- and 12-positions in the benzoquionone part. Both dnacin A_1 and B_1 have an amino functionality at the 11 and a hydrogen at the 12 positions, while A_1 has a cyano and B_1 has a hydroxyl at position 7, the same as cyanocycline A and naphthyridinomycin, respectively.

Dnacin A_1 (**426**): $C_{20}H_{23}N_5O_4$; dark red needles; mp>300°C (dec); FDMS *m/z* 379 M^+; $[\alpha]^{20}_D$ +125° (*c* 0.06 $CHCl_3$); IR (KBr) ν 3430, 1680, 1650, 1625, 1600 cm^{-1}; UV (MeOH, ε) λ 213 (22300), 281 (9000), 496 (2100) nm; ^{1}H-NMR ($CDCl_3$) δ 2.95 (1H, m, H-1), 3.12 (1H, ddd, *J*=12.4, 7.2, 2.5 Hz, H-1), 3.71 (1H, ddd, *J*=8.8, 7.2, 2.5, H-2), 4.01 (1H, dt, *J*=9.6, 7.2 Hz, H-2), 4.73 (1H, s, H-3a), 2.95 (1H, m, H-4), 1.71 (1H, dd, *J*=13.3, 6.8, H-4'), 2.40 (1H, dt, *J*=13.3, 6.8 Hz, H-4'), 3.18 (1H, m, H-4a), 2.42 (3H, s, H-5'), 3.39 (1H, m, H-6), 3.94 (1H, d, *J*=3.4 Hz, H-7), 4.18 (1H, brs, H-9), 3.65 (1H, dd, *J*=11.4, 1.0 Hz, H-9'), 3.89 (1H, dd, *J*=11.4, 2.5 Hz, H-9'), 5.77 (1H, s, H-12), 3.81 (1H, s, H-13b), 2.85 (1H, d, *J*=2.9 Hz, H-13c), 5.25 (2H, brs, NH_2), 5.98 (1H, br, OH); ^{13}C-NMR ($CDCl_3$) δ 50.1 (C-1), 61.1 (C-2), 93.1 (C-3a), 35.1 (C-4), 29.0 (C-4'), 60.2 (C-4a), 41.3 (C-5'), 62.4 (C-6), 54.2 (C-7), 56.7 (C-9), 59.8 (C-9'), 138.5 (C-9a), 182.0 (C-10), 146.9 (C-11), 101.4 (C-12), 183.8 (C-13), 145.1 (C-13a), 47.6 (C-13b), 53.1 (C-13c), 117.4 (CN).

Dnacin B_1 (**427**): $C_{19}H_{24}N_4O_5$; dark red needles; mp>300°C (dec); FDMS *m/z* 370 M^+-H_2O; $[\alpha]^{20}_D$ +50° (*c* 0.06 $CHCl_3$); IR (KBr) ν 3580, 3420, 3175, 1685, 1650, 1610 cm^{-1}; UV (MeOH, ε) λ 213 (24300), 283 (9300), 496 (2100) nm; ^{1}H-NMR (CD_3OD) δ 2.92 (1H, dt, *J*=12.6, 9.5 Hz, H-1), 3.10 (1H, ddd, *J*=12.6, 7.2, 2.5 Hz, H-1), 3.70 (1H, ddd, *J*=9.5, 7.2, 2.5, H-2), 3.96 (1H, dt, *J*=9.5, 7.2 Hz, H-2), 4.71 (1H, s, H-3a), 2.87 (1H, dt, *J*=12.5, 6.4 Hz, H-4), 1.54 (1H, dd, *J*=12.5, 6.4, H-4'), 2.36 (1H, dt, *J*=12.5, 7.1 Hz, H-4'), 3.13 (1H, m, H-4a), 2.37 (3H, s, H-5'), 3.44 (1H, m, H-6), 4.17 (1H, d, *J*=3.6 Hz, H-7), 4.44 (1H, brs, H-9), 3.49 (1H, dd, *J*=10.9, 1.4 Hz, H-9'), 4.03 (1H, dd, *J*=10.9, 2.7 Hz, H-9'), 5.69 (1H, s, H-12), 3.74 (1H, s, H-13b), 2.98 (1H, d, *J*=3.1 Hz, H-13c); ^{13}C-NMR (CD_3OD) δ 50.9 (C-1), 62.8 (C-2), 94.8 (C-3a), 35.2 (C-4), 29.6 (C-4'), 61.5 (C-4a), 41.0 (C-5'), 61.8 (C-6), 89.3 (C-7), 54.4 (C-9), 60.7 (C-9'), 140.3 (C-9a), 183.8 (C-10), 150.8 (C-11), 100.1 (C-12), 185.3 (C-13), 146.9 (C-13a), 49.1 (C-13b), 53.3 (C-13c).

2. Biological Activity

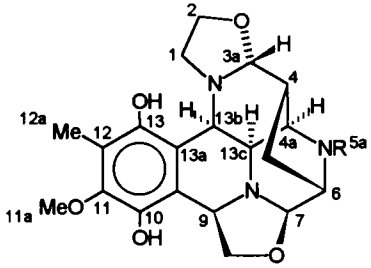
Dnacins were reported to show strong activity against Gram-negative, Gram-positive and acid-fast bacteria, but weak activity against fungi. Dnacin B_1 (0.1 μg/ml) suppressed the growth of *E. coli* K-12 in aerobic conditions, and hardly effected the growth at levels up to 0.5 μg/ml under anaerobic conditions. Dnacin A_1 gave similar results as dnacin B_1 *(194)*. Both dnacins A_1 and B_1 showed preferential activity against the Hfr strain at high temperature *(197)*. They suppressed the growth of TE120 at 0.1 μg/ml (dnacin A_1) and 0.05 μg/ml (dnacin B_1) concentrations at 42°C, while the growth of TE144 was not affected even by double the level of each compound.

In 1982, Tanida *et al.* published their results on the mechanism of action of dnacin B_1 in the *E. coli* system *(198)*. Their findings showed that the antibiotic was preferentially able to inhibit DNA synthesis and to interfere with cell division at a sublethal concentration. Inhibition of [^{3}H]thymidine incorporation at a dnacin B_1 concentration of 0.2 μg/ml, and to some extent inhibition of [^{14}C]uracil incorporation were observed, while no effects on the incorporation of [^{14}C]leucine and *N*-acetyl[^{14}C]glucosamine were detected. When *E. coli* NA-15 cells were exposed to dnacin B_1 at a 0.04 μg/ml level, which is a sublethal concentration, the cells became extremely elongated. It did not cause any filamentous growth at a lethal concentration of 0.4 μg/ml *(198)*.

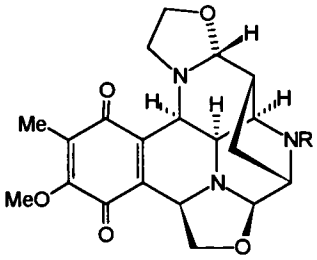
Considering that thermal denaturation of DNA is stabilized by the bound antitumor agents, Tanida *et al.* performed thermal denaturation studies. They observed increases in the melting temperatures and the hyperchromicities of bacterial DNA in the presence of dnacin B_1. Their results also suggested that the binding of dnacin B_1 on this DNA strain could be due to the guanine and cytosine residues. Their agarose gel electrophoresis experiments confirmed the ability to cleave DNA strands. Use of reducing agents such as dithiothreitol (DTT), 2-mercaptoethanol and NADPH facilitated the single-strand scission of PM2 DNA. Even at a dnacin B_1 concentration of 0.5 μg/ml, the DNA-cleaving activity was observed. It was determined that the activity is dependent on the concentration of the antibiotic.

Scavengers such as D-mannitol, dimethyl sulfoxide and 2-mercaptoethylamine for hydroxyl radicals inhibited the DNA-cleaving activity. Then, it was claimed that the hydroxyl radical, which was formed by the reaction of superoxide with H_2O_2, could be involved in the DNA cleavage process caused by dnacin B_1, as the reduced dnacin B_1 catalyzes the generation of superoxide. It was reported that dnacin B_1 prolonged the life-span of mice with leukemia P388, which could be due to the generation of oxygen free radicals in tumor cells.

In a separate report in 1994, Tanida *et.al.* stated that dnacins A_1 and B_1 inhibit the activity of cdc25B phosphatase *(199)*.



bioxalomycin α1 **428** R=H
bioxalomycin α2 **429** R=Me



bioxalomycin β1 **430** R=H
bioxalomycin β2 **431** R=Me

C. BIOXALOMYCINS

1. Isolation and Structure Elucidation

In 1994, Bernan *et al.* reported the isolation of four new antibiotics, bioxalomycins α_1, α_2, β_1, and β_2, from fermantations of *Streptomyces viridodiastaticus* subsp. *"litoralis"* LL-31F508 *(200, 201)*. The difference between the α and β forms is the presence of hydroquinone and benzoquinone rings, respectively, and the β forms are distinguished from the antibiotic naphthyridinomycin (**334**) by the presence of a second oxazolidine ring. Although the attempts to isolate naphthyridinomycin from the culture mixture failed, considering the findings and comparing the ^1H and ^{13}C NMR spectra with those of synthetic naphthyridinomycin, it was claimed that the true naturally occurring form of naphthyridinomycin is bioxalomycin β_2 (**431**) *(202)*.

Bioxalomycin α_1 (**428**): $C_{20}H_{25}N_3O_5$; HRFABMS *m/z* 387.

Bioxalomycin α_2 (**429**): $C_{21}H_{27}N_3O_5$; HRFABMS *m/z* 401; $[\alpha]^{25}_D$ +31° (Me); UV (H_2O) λ_{max} 294 nm; UV (MeOH, ε) λ_{max} 290 (2729) nm.

Bioxalomycin β_1 (**430**): $C_{20}H_{23}N_3O_5$; HRFABMS *m/z* 385.

Bioxalomycin β_2 (**431**): $C_{21}H_{25}N_3O_5$; HRFABMS *m/z* 399; UV (acidic aqueous soln.) λ_{max} 270 nm; UV (MeOH) λ_{max} 370 nm.

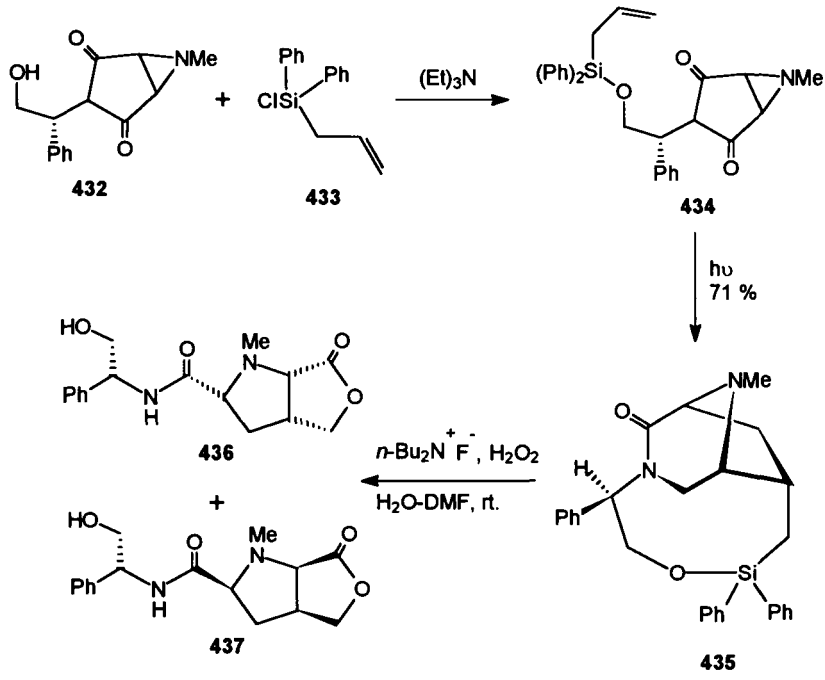
For ^1H- and ^{13}C-NMR studies, see ref. *201*.

2. Synthesis

Synthesis of an intermediate during the synthetic studies toward the bioxalomycins was reported by Garner *et al.* (Scheme 51) *(203, 204)*. Their synthesis of the asymmetric intermediate started with the treatment of the aziridine alcohol **432** with silyl chloride **433** to obtain the allyl silyl ether **434**. Photolysis of **434** gave the desired *endo-re* diastereomers **435** (ds=16/1). When the diastereomeric mixture **435** was treated with tetrabutylammonium fluoride and hydrogen peroxide, cleavage of the silyl ether and lactonization took place to yield **436** (minor) and **437** (major).

3.Biological Activity

Bioxalomycins were reported to display potent antimicrobial activity against Gram-negative and Gram-positive bacteria, and they were also found to be active against tumor cell lines *(200, 201)*. An extensive study on the biological activity of bioxalomycin α_2 was carried out by Singh *et al.* in 1994 *(204)*. It was found to have excellent activity against Gram-positive organisims with the MIC range from ≤ 0.002 to 0.25 μg/ml, while it was less active against Gram-negative organisims (MIC 0.5-4 μg/ml). Among DNA, RNA and protein syntheses, bioxalomycin α_2 was determined to be more effective on the inhibition of DNA synthesýs, which was reduced by 56% in 10 min after the introduction of bioxalomycin; RNA and protein syntheses were inhibited by 2 and 11%, respectively. Their studies suggested that DNA is the primary target of these alkaloids.



SCHEME 51. Synthesis of an intermediate towards the synthesis of the bioxalomycins (**428-431**) *(203, 204)*.

Bioxalomycin α_2 was found to be able to protect mice from a lethal challenge with *S. aureus*, but it was not effective to protect mice against an *E. coli* infection. It was found to be more effective when administered by the parenteral route than the oral route *(204)*.

VI. Summary

Alkaloids containing the isoquinolinequinone unit isolated to date have been reviewed in depth, in terms of their isolation and structure elucidation, synthesis, biosynthesis and biological activities.

Because of their complex and challenging structures, as well as interesting biological activities, widespread effort has been devoted to an understanding of their properties. These efforts include development of highly created synthetic pathways to these compounds and their analogues, an in depth understanding of their biological activities and of their modes of action and biosynthesis. These studies have been examined and presented in this review.

Acknowledgments

I thank Dr J. A. Joule (University of Manchester) for his useful comments on the draft of this manuscript.

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CHAPTER 4

NITROGEN-CONTAINING METABOLITES FROM MARINE BACTERIA

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I. Introduction

This chapter is meant to comprehensively review, as of August 1998, the nitrogen-containing secondary metabolites of marine Eubacteria. Excluded from this chapter, but planned for a future companion chapter, are the nitrogen-containing metabolites of marine cyanobacteria. While in some senses it could seem desirable to

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0099-9598/00 $30.00

have a single chapter reviewing both of these groups, in fact they are quite different topics in terms of the metabolic themes present within their natural products. Marine bacterial metabolites have much in common with their terrestrially-derived counterparts, particularly those that contain nitrogen. In many cases, the compounds reported from these "marine species", perhaps better articulated as "marine isolates", have strong precedents in the terrestrial world, and much insight into the biosynthetic pathways responsible for their occurrence is available from experimentally-derived information with the terrestrial analog. However, the chemistry of marine cyanobacteria is quite diverse, and for the most part, unlike that of any other class of organism, including other marine bacteria. For this reason, we have felt it most appropriate to conceive of a separate chapter to review this latter topic.

The overall organization of this chapter on the nitrogen-containing metabolites of Eubacteria is along taxonomic lines. Subdivisions within this chapter are at the genus level. Coverage of each natural product story has attempted to indicate something of where the bacterium was isolated, both in terms of habitat and geographical location, as well as any other particular features of note such as chemical ecology or human intoxications. As alluded to above, a major issue in the chemistry of marine bacteria is whether or not they are truly marine, or do they represent terrestrial species that have been carried into the sea and are able to survive there for a time, but do not thrive and reproduce in a sustained fashion? In other words, their continued presence in the sea requires continuous "reinoculation" from terrestrial sources. For this reason, it has been of particular interest to note the conditions (salt content, enrichment) of culture for these "marine-isolated" bacteria. This is followed by a brief outline of how the compounds of interest were detected and then isolated and elucidated as to structure. Biological activities of the isolated compounds are summarized, and an indication of the biosynthetic pathway or biosynthetic subunits is given. In those cases where specific biosynthetic experimentation has been undertaken, the relevant biosynthetic processes are discussed at greater length. A comprehensive coverage of chemical syntheses of the covered metabolites has not been attempted.

In general, the structural and biosynthetic diversity represented by the marine bacterial nitrogen-containing metabolites covered in this review is striking. They include such diverse substances as novel purines, macrolides containing lactam rings, diketopiperazine dipeptides, macrocyclic peptides, sphingolipids, fatty acid amides, amino sugars, indole and anthranilic acid derivatives, phenazines, simple amides, and complex polycyclic alkaloids deriving from various amino acid subunits. Of the 72 unique alkaloidal substances reported in this review of marine bacteria, 11 (15%) possess a recognizable indole nucleus, 15 (21%) are of a cyclic peptide constitution, and 3 (4%) are linear peptides. A total of 8 (44%) of these 18 peptides contain polyketide, fatty acid, or ketide extended amino acid residues as components of their structures.

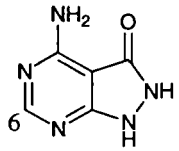
There are a number of excellent reviews on the chemistry and biological activity of metabolites from marine microorganisms. Given the exceptional contributions their laboratory has given to this field, it is very appropriate that Fenical

and Jensen of Scripps have written several reviews with the major focus on the richness of these life forms for the discovery of structurally-diverse and pharmaceutically-valuable natural products (*1-3*). Additional perspectives on the more general topic of natural products from cultured marine microorganisms, including microalgae, have been provided by Davidson (*4*), and on recent achievements and prospects with secondary metabolites from diverse marine microorganisms by Pietra (*5*).

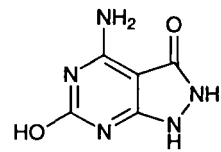
II. Alkaloidal Chemistry of the Eubacteria

A. *AGROBACTERIUM*

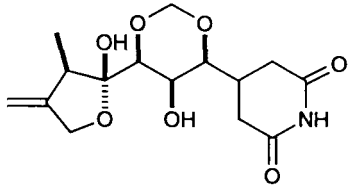
Members of the genus *Agrobacterium* have been a source of two types of nitrogen-containing natural products. A collection program of seawater-derived marine bacteria from several tropical islands in Okinawa prefecture, Japan, yielded a new species of *Agrobacterium*, *A. aurantiacum* (*6*). Diluted and nutrient enriched seawater cultures of this new bacterium yielded a broth which was highly inhibitory to xanthine oxidase, a key enzyme in purine catabolism and the site of action of the anti-gout drug allopurinol. Bioassay-guided isolation of this water soluble inhibitor, akalone (**1**) utilized ion exchange chromatography and HPLC, and its structure was determined by a standard interplay of spectroscopic data, including ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC. From the structure of akalone (**1**), a pyrazole ring analog of a normal purine, it likely acts similarly to allopurinol; following metabolic activation at C-6 it forms a non-productive complex with the catalytic molybdenum atom of xanthine oxidase (IC_{50} 16.9 μM). A subsequent scale-up culture (1000 L) of this new species of *Agrobacterium* did not yield akalone, but rather, 6-hydroxyakalone (**2**) as the sole xanthine oxidase inhibitor (*7*). Its structure was secured by a similar set of spectroscopic data obtained on both the new hydroxy product and its



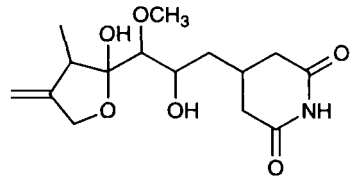
Alkalone (**1**)



6-Hydroxyalkalone (**2**)



Sesbanimide A (**3**)



Sesbanimide C (**4**)

pentamethyl derivative. Hydroxyakalone was essentially equipotent to allopurinol in the xanthine oxidase assay (**2**, IC_{50} 4.6 μM; allopurinol, IC_{50} 4.0 μM).

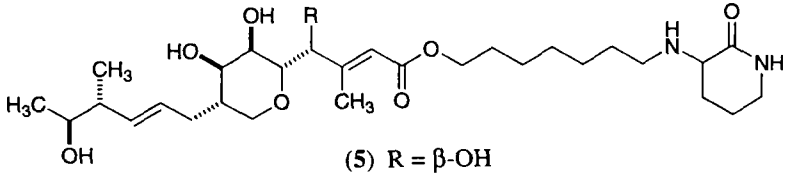
Two strains of *Agrobacterium* sp. were isolated from a Caribbean tunicate, *Ecteinascidia turbinata* (*8*), the source of the very potent antitumor agent ecteinascidin 743 and related natural products (*9,10*), and the Turkish coast tunicate *Polycitonidae* sp. Enriched seawater culture of these bacteria produced excreted lipid soluble natural products with potent antitumor activity, isolated by standard chromatography over normal and reversed phase silica gels. The structures of these compounds, sesbanimide A (**3**) and C (**4**), two metabolites known from previous work with seeds from a higher plant *Sesbania drummondii* and *S. punicea* (*11,12*) were deduced by spectroscopic data in comparison with the published data. This isolation from a microbial source clarifies their probable origin in the earlier studies, and expands the range of their potentially useful properties to include immunosuppressive activity. The sesbanimides appear to derive from a combination of amino acid and polyketide biogenetic subunits.

B. *ALTEROMONAS*

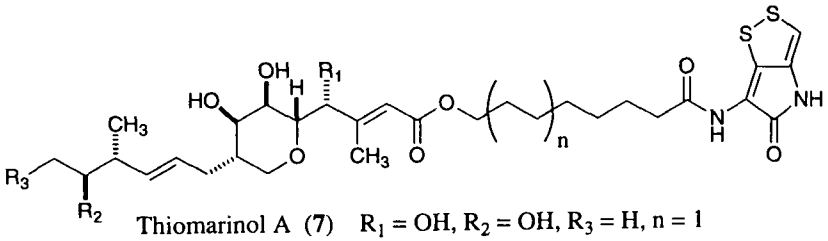
Various marine species of *Alteromonas* have been studied for their natural products, with the major themes in nitrogen-containing metabolites being: 1) invertebrate-associated *Alteromonas* spp. which produce metabolites in pure culture, 2) production of exceptionally efficient siderophores, and 3) pigments of unusual structure.

An *Alteromonas* sp. was obtained from the Bermudian sponge *Darwinella rosacea*, cultured in marine media, and the CH_2Cl_2 solubles of the MeOH extract of the cell mass were found to possess potent antimicrobial activity to *Staphylococcus aureus* (*13*). Two related compounds (**5**, **6**) were isolated as the antimicrobial constituents, and their structures determined by spectroscopic analysis of the natural products, several derivatives, and comparisons with literature compounds. The compounds were new pseudomonic acid derivatives containing an unusual anhydroornithyl end group. Both showed comparable zones of inhibition of *S. aureus* growth to that obtained with tetracycline, penicillin G and streptomycin, using sensitivity test disk methodology.

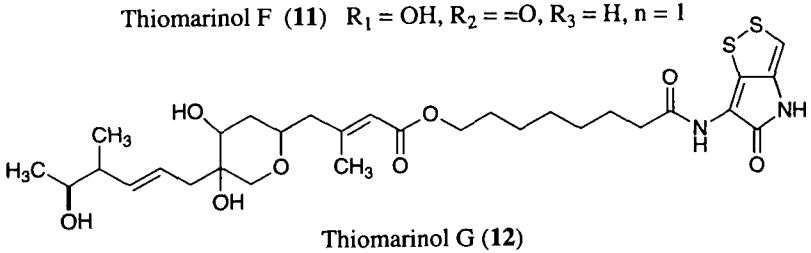
Additional derivatives of pseudomonic acid were obtained from a seawater isolate of *Alteromonas rava* (*14*). Initial work involved the relatively large scale culture of this new species of *Alteromonas* (60 L) and extraction with ethyl acetate. This material was sequentially chromatographed over silica gel, Dianion HP-20 and Sephadex LH-20 to yield thiomarinol A (**7**) in 750 mg quantity. Its structure elucidation relied heavily on HMBC and NOE characterization of the natural product and several key derivatives, and was shown to be a hybrid of two known antibiotics, a pseudomonic acid analog and holothin, a pyrrothine-type antibiotic. The pseudomonic acid portion of thiomarinol was identical to that obtained above from the sponge-derived *Alteromonas* sp. (**6**), and similarly showed very good antimicrobial activity to *S. aureus*. Six additional thiomarinols were subsequently



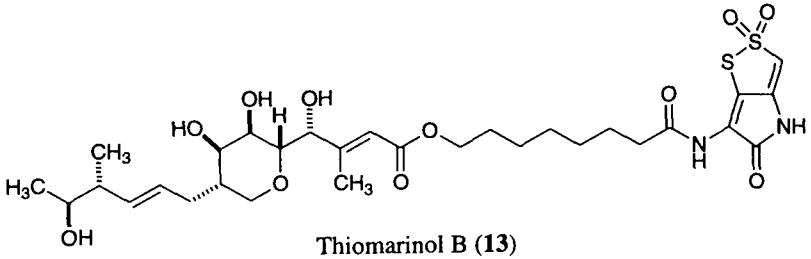
(**5**) R = β-OH
(**6**) R = H



Thiomarinol A (**7**) R_1 = OH, R_2 = OH, R_3 = H, n = 1
Thiomarinol C (**8**) R_1 = H, R_2 = OH, R_3 = H, n = 1
Thiomarinol D (**9**) R_1 = OH, R_2 = OH, R_3 = CH_3, n = 1
Thiomarinol E (**10**) R_1 = OH, R_2 = OH, R_3 = H, n = 2
Thiomarinol F (**11**) R_1 = OH, R_2 = =O, R_3 = H, n = 1

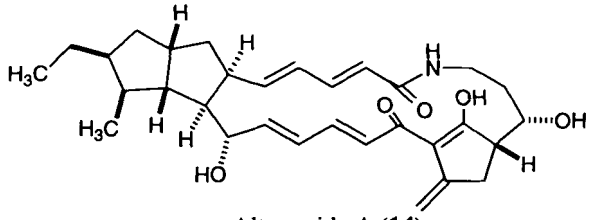


Thiomarinol G (**12**)

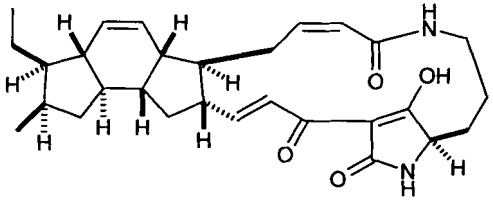


Thiomarinol B (**13**)

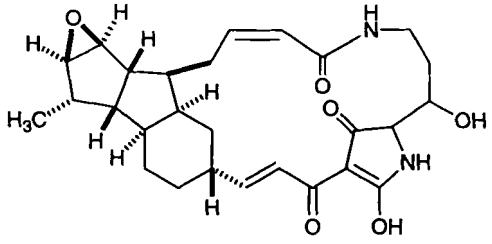
isolated and reported from this organism, and their structures elucidated by various NMR methods applied to the natural products and derivatives, X-ray analysis, and chemical interconversion (*15,16*). Thiomarinols C-G (**8-12**) represent various oxidations of the pseudomonic acid portion, while thiomarinol B (**13**) results from oxidation of the disulfide to an unusual disulfide-sulfone species. All were potently



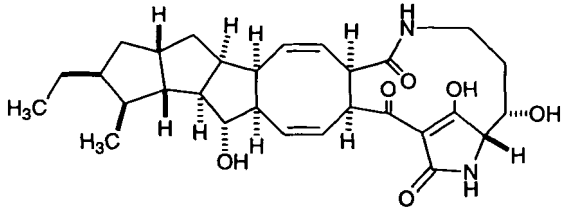
Alteramide A (**14**)



Ikarugamycin (**15**)



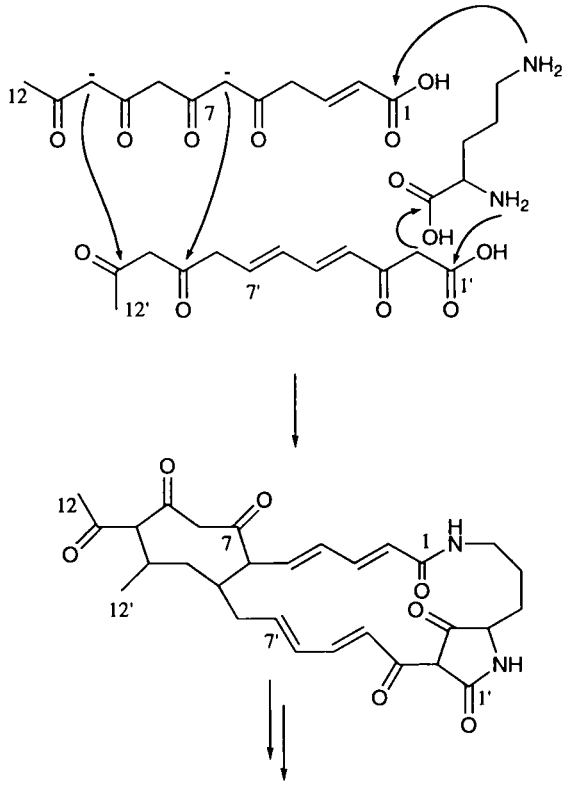
Discodermolide (**16**)



Alteramide A-hexacyclic product (**17**)

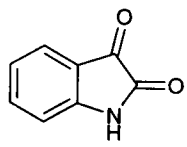
antibiotic, particularly so to *S. aureus*. Relative and absolute stereochemistry in the series was established by NOE analysis and Mosher's methodology (*17*).

The chemically-prolific sponge *Halichondria okadai* from Japan was the source of another isolate of a marine *Alteromonas* sp (*18*). The mycelia of enriched seawater cultures of this isolate were extracted with $CHCl_3$/MeOH and fractionated over Sephadex LH-20 and Sep-Pak ODS to give the new compound, alteramide A (**14**). Extensive 2D-NMR investigation of the natural product and fragments produced by ozonolysis led to the structure of alteramide A (**14**). Similarly, absolute stereochemistry was determined by chiral HPLC characterization of an *L*-erythro-β-hydroxyornithine fragment released by ozonolysis. A structurally-related antibiotic, ikarugamycin (**15**), had previously been isolated from a terrestrial isolate of

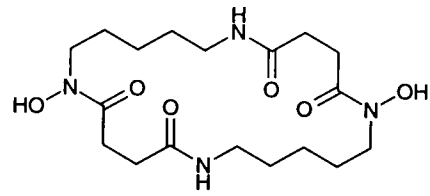


Alteramide A (**14**)

SCHEME 1. Proposed biosynthesis of alteramide A (**14**) based on biosynthetic proposals for discodermolide (**16**)(20).



Isatin (**18**)



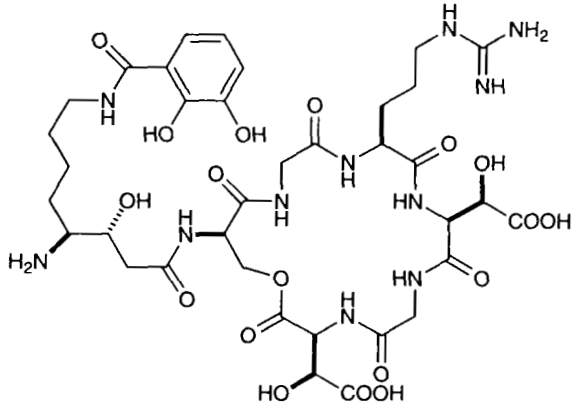
Bisucaberin (**19**)

Streptomyces phaeochromogenes var. *ikaruganensis* (*19*). Isolation of alteramide also gives potential insight into the metabolic source of related compounds (e.g. discodermolide, **16**) previously reported as natural products of the sponge *Discodermia dissoluta* (*20*). Based on the proposed biosynthetic origin of discodermolide (**16**) and ikarugamycin (**15**), a comparable pathway can be proposed for alteramide A (**14**)(Scheme 1). An interesting aspect of the report on alteramide A (**14**) was the finding that it is photochemically converted to a unique hexacyclic product **17** by a presumed [4 + 4] cycloaddition. Alteramide A was cytotoxic to several cancer cell lines *in vitro* (IC_{50} 0.1 - 5.0 μg/mL).

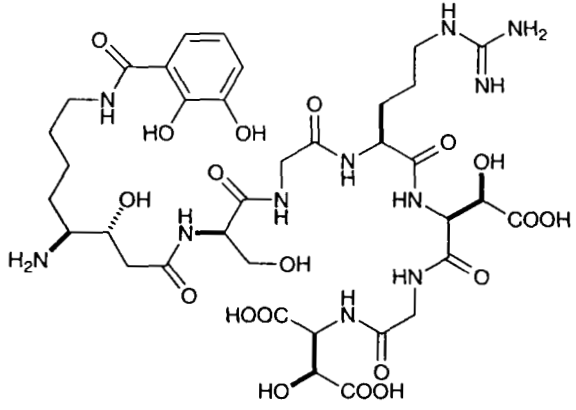
An *Alteromonas* sp. associated with the surfaces of embryos from the shrimp *Palaemon macrodactylus* was found to confer resistance to infection by the pathogenic fungus *Lagenidium callinectes* (*21*). The active antifungal substance produced and released in large quantities by cultures of this bacterium was identified as 2,3-indolinedione (= isatin) (**18**) on the basis of its physical characteristics.

Alteromonas haloplanktis was isolated from a deep sea mud (3,300 m) collected off the Aomori coast of Japan and required seawater for growth in culture (*22*). A crystalline compound was produced in an amount of 0.3 g/L culture media which, when added to a mixed macrophage-tumor cell culture, induced macrophage mediated cytolysis of the tumor cells. By itself, this new substance, named bisucaberin (**19**), had little cytotoxicity. Bisucaberin was also found to have metal chelating properties, and as an excreted low molecular weight substance, fits the definition of a siderophore. Its molecular structure was ultimately characterized by X-ray analysis (*23*), and additionally confirmed through total chemical synthesis (*24*). Its dimeric structure containing two hydroxamate and two amide functionalities is related to that of another siderophore, nocardamine, which is a trimer of the same structural units.

Subsequently, an open-ocean *Alteromonas*, *A. luteoviolaceae* was investigated for its two siderophoric components, alterobactins A (**20**) and B (**21**). The major and most potent siderophore, alterobactin A (**20**), was isolated using an Fe^{+3}/hexadecyltrimethylammonium colorometric assay which allowed direct visualization of the siderophore-containing fractions (*25*). Its molecular structure was determined by extensive NMR study of alterobactin A, as well as of



Alterobactin A (**20**)

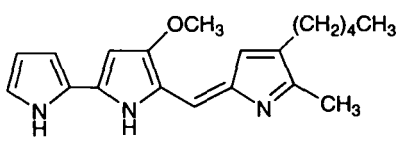


Alterobactin B (**21**)

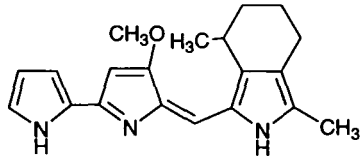
alterobactin B, it linearized equivalent, as that of a cyclic peptide with β-OH-Asp, Gly, Ser, Arg, 4,8-diamino-3-hydroxyoctanoic acid, and dihydroxybenzoic acid (*26*). Molecular modeling supports chelation of iron (III) by the two residues of β-OH-Asp and dihydroxybenzoic acid residues, resulting in an extraordinarily large proton-independent ferric ion stability constant of 10^{49}-10^{53}, making it and enterobactin the highest affinity siderophores known. This likely has substantial consequence to iron availability and cycling in the low-iron, open ocean environment. A stereoselective synthesis of alterobactin has been reported (*27*).

The final trend in the nitrogen-containing chemistry of *Alteromonas* concerns the distinctive red pigments associated with this and related bacteria (*Beneckea gazogenes*). Two red pigments have been described from these sources, prodigiosin (**22**) and cycloprodigiosin (**23**). Prodigiosin was the focus of many decades of structural characterization, and eventually established as a pyrryldipyrrylmethene (**22**) from a combination of biosynthetic and synthetic methods (*28*). Despite considerable experimentation, many features of the biosynthesis of prodigiosin remain uncertain, and it is apparently unrelated to porphyrin metabolism (*29*). Cycloprodigiosin (**23**), detected in *Alteromonas rubra*, was characterized from *B. gazogenes* due to higher yields (*30*). A combination of 1-dimensional ^{1}H and ^{13}C NMR data, in comparison with literature values for prodigiosin, were used to assign its cyclohexyl-fused linear tripyrrole structure. The functions of these pigments in bacteria remain unknown.

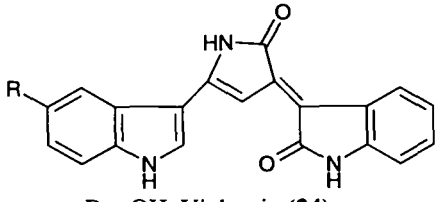
Two additional violet pigments, violacein (**24**) and deoxyviolacein (**25**), were obtained from *A. luteoviolacea* grown in an enriched seawater medium (*31*). These pigments, known since the late 1800's, had been previously characterized from the genus *Chromobacterium* (*32*); however there is some confusion on the taxonomy of members in these two genera (*33*). Structure elucidation of these pigments from *A. luteoviolacea* made use of ^{1}H NMR characterization of synthetic analogs and derivatives, X-ray crystallographic analysis, and various computational methods. These two pigments rationally derive from dimerization of two tryptophan-derived subunits to produce a central pyrrolidone ring. Detailed examination of the biosynthetic pathway to violacein (Scheme 2)(**24**) in *C. violaceum* using stable isotope methods showed tryptophan to undergo a unique indole 1,2 shift (*34*).



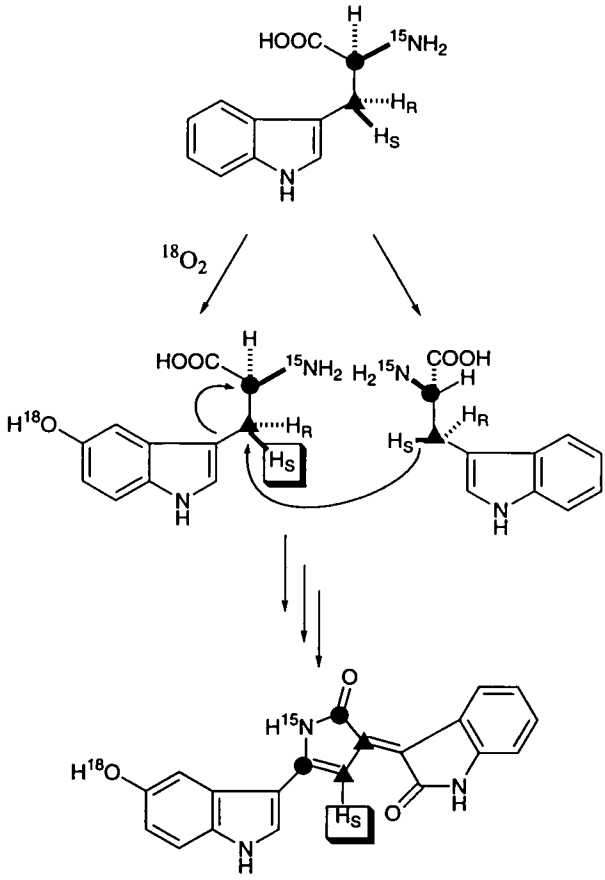
Prodigiosin (**22**)



Cycloprodigiosin (**23**)



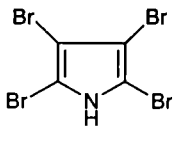
R = OH Violacein (**24**)
R = H Deoxyviolacein (**25**)



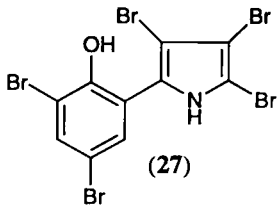
Violacein (**24**)

SCHEME 2. Biosynthesis of violacein (**24**)(*34-36*).

Subsequent studies with the same organism clarified the origin of hydrogen, nitrogen and oxygen atoms of the 2-pyrrolidone nucleus (*35*), and showed the intermediacy of 5-hydroxy-*L*-tryptophan (*36*). The biological function of these pigments is unknown.



(26)



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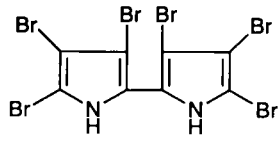

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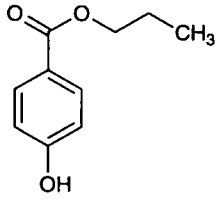

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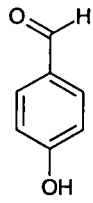


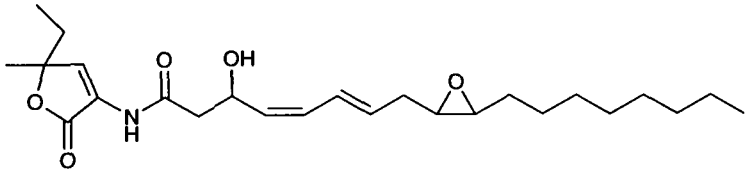
(30)

C. *CHROMOBACTERIUM*

In addition to the above reported isolation of violacein (**24**) and deoxyviolacein (**25**) from this genus, as well as the ensuing detailed biosynthetic work, a series of antibiotic phenols and indoles were isolated from a seawater isolate of *Chromobacterium* (*37*). The organism was cultured both in enriched seawater broth culture and as lawns on agar enriched with NaBr to yield antibiotic EtOAc extracts. The five purified antibiotics (**26-30**) were characterized spectroscopically and subsequently compared with authentic standards. Three of the compounds, tetrabromopyrrole (**26**), 2-(2'-hydroxy-3',5'-dibromophenyl)-3,4,5-tribromopyrrole (**27**) and 4-hydroxybenzaldehyde (**30**) were active to human pathogens and had autoinhibitory activity to the producing strain of *Chromobacterium*. These same five natural products had been previously isolated from *Pseudomonas bromoutilis* (see below)(*38*).

D. *PSEUDOALTEROMONAS*

A screening program for antibiotic properties from cultured marine bacteria identified that a *Pseudoalteromonas sp.* isolated from the tropical green alga *Halimeda* sp. produced a marine bacterium-specific antibiotic (*39*). The bacterium was characterized as requiring salt for growth and was subsequently cultured on large scale in a seawater-based broth. The EtOAc solubles from the EtOH extract of the harvested cells were sequentially fractionated over silica gel and size-exclusion HPLC to give the pure antibiotic, korormicin (**31**). The structure of korormicin was deduced in straightforward fashion from NMR and MS reasoning, and the geometrical features deduced by NOESY and coupling constant analysis. Absolute and relative stereochemistries (except for the *cis*-epoxide orientation) were not examined.

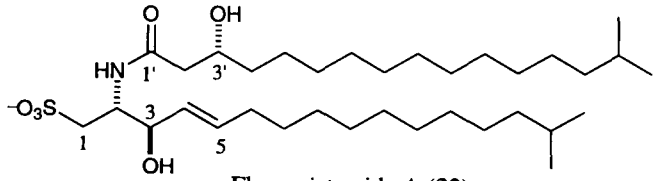


Korormicin (**31**)

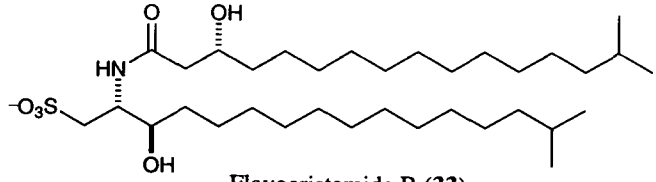
Korormicin (**31**) is an intriguing combination of a peculiarly oxidized fatty acid and an unusual, lactonized amino acid, both of which pose interesting questions for future biosynthetic investigation. The former may derive from an $18{:}3\Delta^{3,6,9}$ fatty acid which is subject to cytochrome P_{450} type oxidation at the 9,10-position and lipoxygenation at the 3-position. The amino acid portion is without biosynthetic precedent. Interestingly, while korormicin (**31**) was inactive to a variety of bacteria derived from terrestrial sources, it was quite potent to several marine Gram-negative bacteria. An intriguing point proposed by these authors is that korormicin may be useful in helping to classify 'true' marine bacteria.

E. *FLAVOBACTERIUM*

A *Flavobacterium* sp. was isolated from the marine bivalve *Cristaria plicata* from Hokkaido and grown in enriched, 90% seawater medium (*40*). The organic extract of the cells was sequentially fractionated by solvent partition, normal phase silica gel chromatography and reversed phase chromatography to yield two new ceramide derivatives, flavocristamide A (**32**) and its 3,4-dihydro-analog, flavocristamide B (**33**). The planar structure of flavocristamide A was deduced from spectroscopic information obtained on the natural product and its acid



Flavocristamide A (**32**)



Flavocristamide B (**33**)

hydrolysis products. Absolute stereochemistry of the β-hydroxy group was determined by optical rotation in comparison with literature values, and ^{1}H NMR coupling constants were indicative of a 2,3-*erythro* geometry. Treatment of **32** with $NaIO_4/KMnO_4$ followed by HCl hydrolysis and chiral HPLC allowed detection of *L*-cysteic acid; hence, flavocristamide A is of the 2*R*,3*R* absolute stereochemistry (erroneously reported as 2*S*,3*R*, but pictured as 2*R*,3*R*). Both flavocristamides were moderately inhibitory to eukaryotic DNA polymerase α (IC_{50} *ca.* 15-20 μg/mL). These are interesting structures for several reasons: 1) the unusual occurrence of the sulfonate in a sphingolipid, and 2) related sphingolipids, which have often been reported as metabolites of sponges, are suggested by this work to possibly be the products of sponge-associated bacteria.

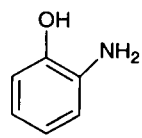
F. *JANTHINOBACTERIUM*

A marine bacterium requiring NaCl for growth, tentatively identified as a *Janthinobacterium* sp., was isolated from an unidentified Japanese species of the sponge *Adocia* (*41*). The broth of cultures of this bacterium were strongly antimicrobial to *Staphylococcus aureus* and *Bacillus subtilis*, and this activity was used to direct an isolation scheme employing solvent partitioning, an Unisil column, and repetitive HPLC. The active substance gave very simple ^{1}H and ^{13}C NMR spectra, and in combination with SIMS and comparison with authentic material, was identified as *o*-aminophenol (**34**). Based on differences in spectrum of antibiotic activity, this substance is apparently not responsible for the antimicrobial activity associated with extracts of the host *Adocia* sp. sponge.

G. *PSEUDOMONAS*

Three major themes exist in the nitrogen-containing metabolites of marine isolates of *Pseudomonas*: 1) generally small molecular size heterocycles deriving from the amino acids tryptophan and/or proline, 2) tetrodotoxin and related toxins, and 3) lipopeptides, both cyclic and acyclic.

The first substance reported from a marine *Pseudomonas* was the antibiotic pentabromopseudilin (**27**) from *P. bromoutilis* (*38*). This bacterium was isolated from leaves of the marine angiosperm *Thalassia* collected in Puerto Rico, and antibiotic production was found to be higher when grown on solid, rather than liquid, enriched seawater media. The structure was defined by X-ray analysis (*42*), and



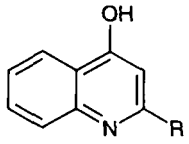
O-aminophenol (**34**)

synthesized by straightforward methodology (*43*). Pentabromopseudilin shows impressive *in vitro* antibiotic activity (complete inhibition of *Staphylococcus aureus*, *Diplococcus pneumoniae*, and *Streptococcus pyrogenes* at 0.0063 μg/mL), but does not seem effective *in vivo* at tolerated doses. A large number of analogs of pentabromopseudilin were synthesized with the aim of providing a set of compounds for establishing SAR in this drug class (*44*). The biosynthesis of pentabromopseudilin (**27**) was examined in another producing bacterium, *Alteromonas luteoviolaceus*, and while it was demonstrated, from ^{13}C-glucose incorporation experiments, that the benzene ring derives from the shikimate pathway *via* *p*-hydroxybenzoic acid, in no case were labeled precursors (acetate, tryptophan, benzoic acid, or glycerol) found to label the pyrrole ring (*45*).

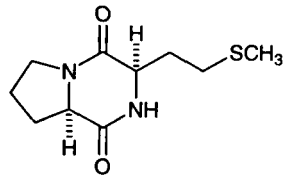
Additional new heterocycles of intriguing structure were subsequently isolated from a partially characterized marine pseudomonad cultured on enriched seawater solid media (*46*). Five excreted products with antibiotic activity to marine *Vibrio* spp. were isolated from the agar support by chromatographic methods, and included two novel compounds (one of which, metabolite **35**, was previously known from *Pseudomonas aeruginosa*) and three other simple and previously known catabolites. These latter three compounds were identified as 4-hydroxybenzaldehyde, indole-3-carboxaldehyde, and 6-bromoindole-3-carboxaldehyde. The more intriguing compounds were 2-*n*-heptyl-4-quinolinol (**35**) and 2-*n*-pentyl-4-quinolinol (**36**). The structure of the new metabolite **36** was confirmed by synthesis using a route analogous to a literature method (*47*). While the most potent antibiotic isolated from this marine pseudomonad was 4-hydroxybenzaldehyde, compound **36** was considered to be the major antibiotic substance formed by these cultures (9% of the EtOAc extract).

Cultures of a marine isolate of *Pseudomonas aeruginosa* were obtained from swabbings of an Antarctic sponge, *Isodictya setifera*, and found to possess Gram positive antimicrobial activity in extracts of the LB Broth fraction (5 g to 10 g NaCl/liter)(*48*). This was traced through several levels of Amberlite XAD-2 chromatography, silica gel column chromatography, and HPLC to six related diketopiperazines (**37-42**) and two phenazine alkaloids (**43**, **44**). The structure of the one new diketopiperazine, *cyclo*-(*L*-proline-*L*-methionine)(**37**), was deduced from spectroscopic features, and confirmed by synthesis. The structures of the two related phenazine metabolites (e.g. **43**, **44**) were determined by comparisons with literature data. None of these metabolites were evident in extracts of the sponge *I. setifera*, leaving somewhat in doubt whether the bacterium is present in low abundance in the sponge, produces these compounds only in enriched media, or is a chance contaminant not actually isolated from sponge tissue.

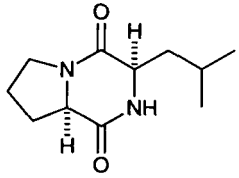
A new species of *Pseudomonas*, *P. magnesiorubra*, was isolated from the surface of the marine alga *Caulerpa peltata* (Chlorophyta) obtained from near Bombay, India (*49*). Culture of this pink bacterium in an enriched broth containing 3% NaCl led to a rich production of a new antibiotic, magnesidin (**45**), which was extractable from the centrifuged cell mass with acetone. The pure compound, 250 mg/l, was isolated by sequential decolorization with charcoal, selective extraction



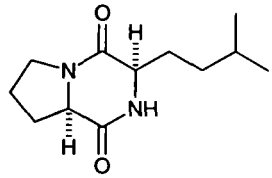
2-*n*-Heptyl-4-quinolinol (**35**) R = C_7H_{15}
2-*n*-Pentyl-4-quinolinol (**36**) R = C_5H_{11}



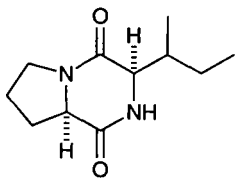
Cyclo-(*L*-proline-*L*-methionine) (**37**)



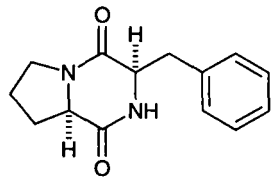
Cyclo-(*L*-proline-*L*-valine) (**38**)



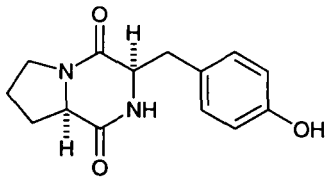
Cyclo-(*L*-proline-*L*-leucine) (**39**)



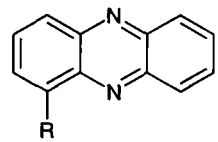
Cyclo-(*L*-proline-*L*-isoleucine) (**40**)



Cyclo-(*L*-proline-*L*-phenylalanine) (**41**)

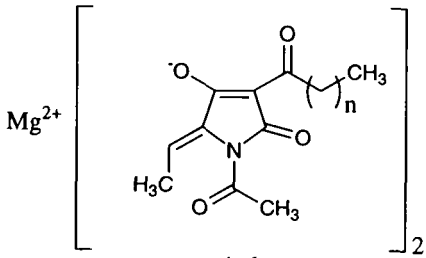


Cyclo-(*L*-proline-*L*-tyrosine) (**42**)



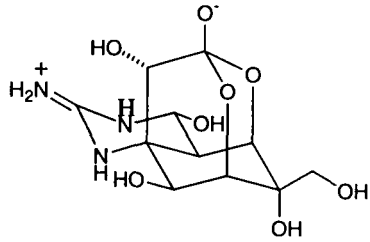
(**43**) R = COOH
(**44**) R = $CONH_2$

with Et_2O, and crystallization from methanol. Structure elucidation was accomplished by extensive chemical degradation in concert with MS and NMR analyses, and proof of the basic structure was established by total chemical synthesis (*50*). Despite this, the geometry of the vinyl methyl group was not determined because of an apparent absence of NOE interactions in this region of the molecule. Magnesidin is a rare magnesium-containing natural product which shows an impressive level of *in vitro* activity to several species of *Bacillus* and *Staphylococcus* and the microalga *Prorocentrum micans*, but is not active *in vivo*. Magnesidin (renamed magnesidin A) was subsequently re-isolated from a marine, mud-derived, bacterium, *Vibrio gazogenes*, and the geometry of the vinyl methyl group was clarified to be *Z* from NOESY analysis (*51*). Biogenetically, magnesidin (**45**) is

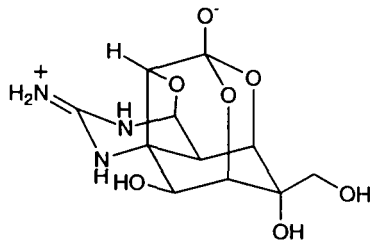


n = 4, 6

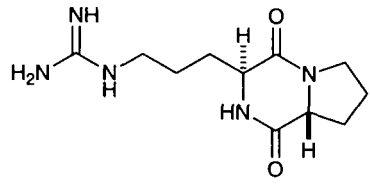
Magnesidin (Magnesidin A) (**45**)



Tetrodotoxin (**46**)



Anhydrotetrodotoxin (**47**)



CI-4 (**48**)

conceivably derived from the Claisen-type condensation of a β-keto fatty acid (or polyketide) and an *N*-acetylated dehydrothreonine residue.

The potent sodium channel blocking neurotoxin, tetrodotoxin (TTX)(**46**), has been isolated from a wide variety of fish, crabs and algae, suggesting that its real origin may be bacterial. In this regard, a *Pseudomonas* sp. bacterium was isolated from the surfaces of a red alga found to "contain" TTX, *Jania* sp., and cultured in an enriched 3% NaCl medium (*52*). The harvested cell mass was broken by sonication in 0.02 N HOAc and then boiled, and cell debris removed by centrifugation. The supernatant was treated through a complex series of solubilizations and other manipulations, and then analyzed versus standards by HPLC, mouse toxicity bioassay, and HPLC and MS following base hydrolysis. Overall, a compelling case was developed for the production of TTX and anhydrotetrodotoxin (**47**) by this marine *Pseudomonas* sp.

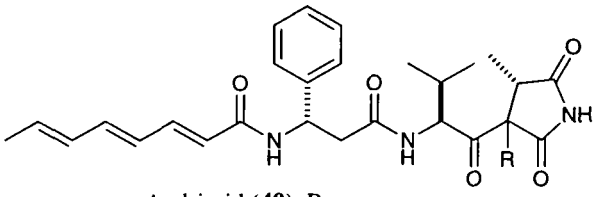
Subsequently, this bacterium was reassigned to the genus *Alteromonas* sp. However, further work in this area led to the isolation of several strains of *Pseudomonas* from the skin of the infamous puffer fish, *Fugu poecilonotus*, which were shown by similar methodologies to those described above to produce TTX in an

enriched seawater medium (see discussion below for *Vibrio* sp.)(*53*).

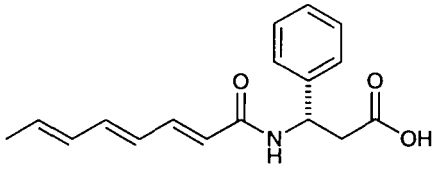
A novel chitinase inhibitor, CI-4 (**48**), was isolated from the enriched, 75% seawater culture broth of a marine bacterium obtained from seawater/sediment samples from several sites in Shizuoka Prefecture, Japan (*54*). The bacterium was identified from Bergey's Manual as a *Pseudomonas* sp. Chitanase activity was used to direct the isolation of a water-soluble, diketopiperazine metabolite from this broth by a variety of chromatographic techniques including a final purification by reversed phase HPLC. Structure elucidation followed from HR FAB-MS and extensive 2D NMR, including ^{1}H-^{1}H COSY, HSQC, and HMBC experiments. A comparable optical rotation and NOE features between the natural product and synthetic cyclo(*L*-Arg-*D*-Pro) were used to assign the stereostructure of CI-4 (**48**). While natural, as well as synthetic, CI-4 showed strong chitinase inhibitory activity at a concentration of 50 μg/disk, various stereoisomers of CI-4 were either weaker or inactive in this assay.

A strain of *Pseudomonas fluorescens* was isolated from an unidentified cold water tunicate from Alaska, cultured for 3 days on enriched solid agar medium containing 1% NaCl at which time the cells were removed, and the solid support repetitively extracted with EtOAc to give an extract highly inhibitory to methicillin resistant *Staphylococcus aureus* (*55*). Bioassay guided fractionation led to the isolation of the known metabolite andrimid (**49**), as well as three new and related structures, moiramides A-C (**50-52**). The new structures were developed from a careful 2D NMR analysis and mass spectrometry, and the absolute stereochemistry proposed based on structural analogy with the known structure andrimid and CD spectroscopy. These intriguing compounds have several features of structural novelty, including β-amino phenylalanine and an acyl succinimide derivative. The biosynthetic origin of this moiety in andrimid (**49**) was probed using various ^{13}C- and ^{15}N-labeled precursors (valine, acetate and glycine). Labeling patterns were detected by NMR spectroscopy that were consistent with its origin from the condensation of two ketide extended amino acids, valine and glycine (Scheme 3). This dipeptide derivative is then envisioned to undergo an aldol-type condensation, followed by decarboxylation and loss of water to yield a five-membered ring heterocycle. Subsequent adjustment of the oxidation state at several positions would allow generation of the succinimide derivative observed in andrimid (**49**). Andrimid (**49**) and moiramide B (**51**) both potently inhibited growth of methicillin resistant *S. aureus* (**49** MIC = 2 μg/mL; **51** MIC = 0.5 μg/mL). Andrimid (**49**) was also isolated from extracts of both a Japanese sponge (*Hyatella* sp.) and from cultures of a *Vibrio* sp. isolated from the sponge sample, providing a superb example of a "sponge natural product" which actually derives from its associated microbial population (*56*). Andrimid isolation in this latter study was accomplished using an anti-*Bacillus* bioassay.

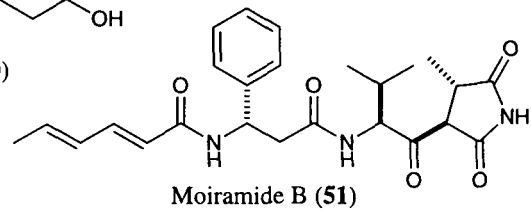
Two isolates of a *Pseudomonas* sp. were obtained from a foliaceous red alga and a tube worm from the Canadian west coast, grown as lawns on enriched solid agar (1.5% NaCl), and extracted with EtOAc to give antitubercular extracts using *Mycobacterium tuberculosis* and *M. avium-intracellulare* (*57*). Fractionation of



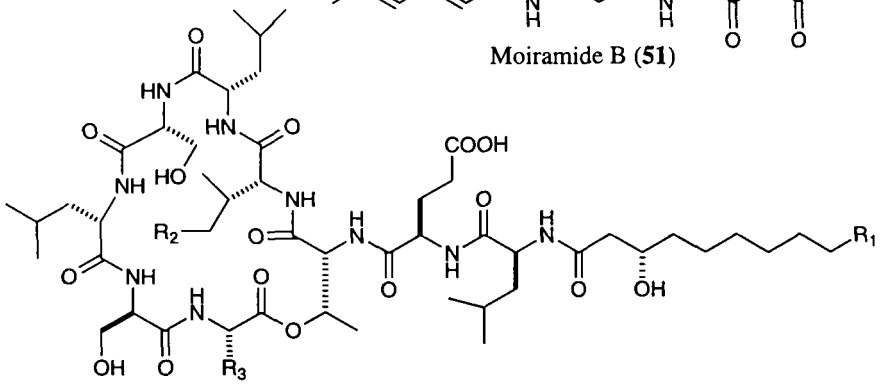
Andrimid (**49**) R = ····H
Moiramide C (**52**) R = —OH



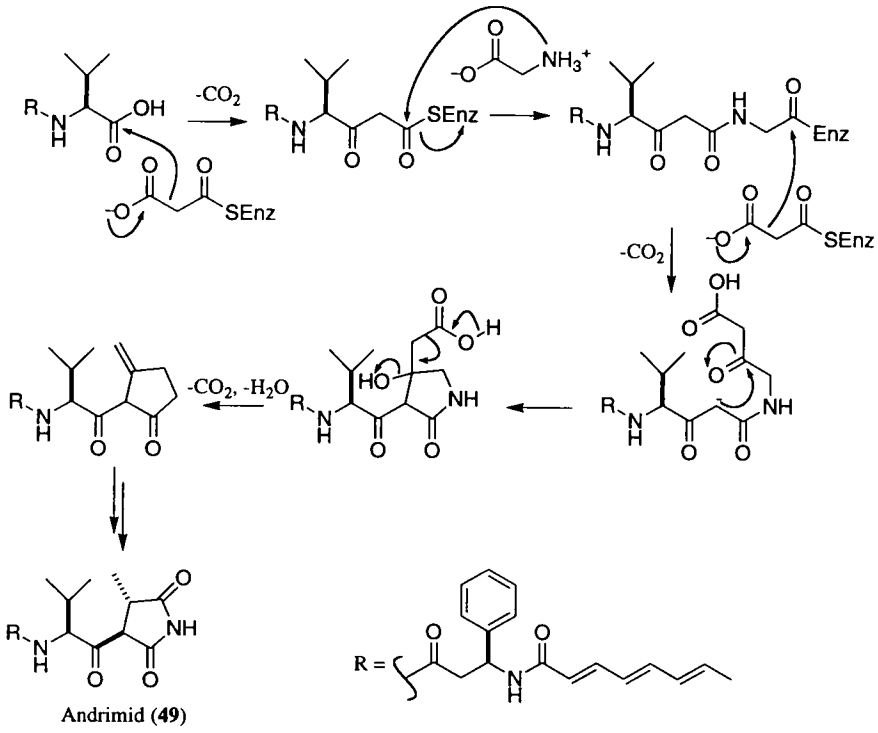
Moiramide A (**50**)



Moiramide B (**51**)



Massetolide A (**53**) $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = CH(CH_3)CH_2CH_3$
Massetolide B (**54**) $R_1 = CH_2CH_3$, $R_2 = CH_3$, $R_3 = CH(CH_3)CH_2CH_3$
Massetolide C (**55**) $R_1 = CH_2CH_2CH_3$, $R_2 = CH_3$, $R_3 = CH(CH_3)CH_2CH_3$
Massetolide D (**56**) $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = CH_2CH(CH_3)_2$
Massetolide E (**57**) $R_1 = CH_3$, $R_2 = H$, $R_3 = CH(CH_3)_2$
Massetolide F (**58**) $R_1 = CH_3$, $R_2 = H$, $R_3 = CH_2CH(CH_3)_2$
Massetolide G (**59**) $R_1 = CH_2CH_3$, $R_2 = H$, $R_3 = CH(CH_3)CH_2CH_3$
Massetolide H (**60**) $R_1 = CH_2CH_2CH_3$, $R_2 = H$, $R_3 = CH(CH_3)CH_2CH_3$
Viscosin (**61**) $R_1 = CH_3$, $R_2 = H$, $R_3 = CH(CH_3)CH_2CH_3$



SCHEME 3. Biosynthesis of andrimid (**49**)(*55*).

these extracts by Sephadex LH-20, RP flash CC, and RP-HPLC gave a series of new lipopeptides, massetolides A-H (**53-60**) as well as one known compound, viscosin (**61**). The structures of the new lipopeptides were deduced by extensive 2D NMR analyses, HR FABMS, FABMS fragmentations, and comparisons with the known compound viscosin (**61**), with most of the stereochemical features being deduced by chiral GC of suitable derivatives. Massetolide A (**53**) was 2-4 times more potent than viscosin in its *in vitro* inhibition of *M. tuberculosis* (MIC = 5-10 μg/mL) and *M. avium-intracellulare* (MIC = 2.5-5.0 μg/mL), and was non-toxic to mice at 10 mg/kg (intraperitoneal injection). Culture of the producing *Pseudomonas* sp. in the presence of various leucine analogs (*L*-butyrine, *L*-norvaline, *L*-cyclopropylalanine and others) led to the `precursor directed production' of several intriguing analogs of the massetolides; however, the small quantities produced precluded their biological evaluation.

H. *VIBRIO*

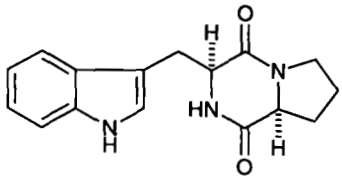
A *Vibrio* sp. isolated from the Okinawan sponge *Hyrtios altum* was grown in seawater medium to produce an antibiotic EtOAc extract (*58*). Bioassay guided isolation yielded two antibiotic substances, the diketopiperazine brevianamide F (**62**) and a new compound, trisindoline (**63**). Its structure was formulated from various spectrochemical data, with HMBC data providing the critical link between the indole subunits.

Marine *Vibrio* sp. are producers of the well-known neurotoxin, tetrodotoxin (TTX, **46**), first obtained from the internal organs of puffer fish, and subsequently isolated from a variety of vertebrates and invertebrates. This was first demonstrated with an isolate from the intestines of the xanthid crab, *Atergatis floridus* (*59*). Culture of a suspension of intestinal material in enriched ½-strength seawater yielded a *Vibrio* sp. as one of the dominant bacteria. Extracts of both the cell mass, as well as the culture material, were analyzed by HPLC and showed peaks corresponding to TTX and anhydro-TTX. Alkaline hydrolysis of these collected materials, conversion to the corresponding trimethylsilyl derivative, and analysis by GCMS confirmed the identities of these two toxins. Subsequently, a *Vibrio alginolyticus* was cultured from the intestines of the puffer fish, *Fugu vermicularis vermicularis*, the traditional source of TTX, and was shown by methods similar to those described above to produce both TTX and anhydro-TTX, as well as a possible epimeric substance, in a ½-strength seawater-based culture medium (*60*).

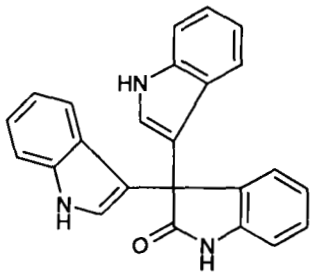
Additional isolates of TTX-producing bacteria have been made from a starfish, the 'blue-ringed octopus', and the 'lined moon snail'. Strains of *V. alginolyticus*, *V. damsela*, and a *Staphylococcus* sp. isolated from intestines of the starfish *Astropecten polyacanthus* were found to produce TTX (*61*). Similarly, TTX production was demonstrated from 2 strains of *Alteromonas*, 2 strains of *Bacillus*, and 1 strain each of *Vibrio* and *Pseudomonas* isolated from various tissues of Philippine and Japanese specimens of the 'blue-ringed octopus', *Octopus maculosus* (*62*). More recently, *V. alginolyticus* and *Aeromonas* spp. were isolated from the digestive gland and muscle of the Taiwanese 'lined moon snail', *Natica lineata*, and shown to produce TTX or related substances (*63*).

Vibrio parahaemolyticus was cultured from dilutions of the stress-induced ichthyotoxic mucus of the box fish *Ostracion cubicus* (*64*). The EtOAc extract of the enriched NaCl-containing medium (2.5 g/L) was chromatographed over silica gel to give two indolic compounds, the known natural product 2,2-di(3-indolyl)-3-indolone (**64**), and a new indole dimer named 'vibrindole' (**65**). The structure of the new substance was developed from 1D and 2D NMR arguments in combination with MS information. Both compounds gave appreciable zones of inhibition to Gram positive bacteria at 100 μg/disk.

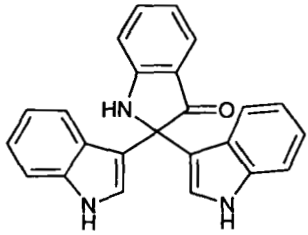
The fish pathogen *Vibrio anguillarum* was the source of a fundamentally new class of siderophore, anguibactin (**66**)(*65*). Production of this substance is closely tied to virulence of this bacterium. Culture of the organism was accomplished in a minimal medium containing a non-assimilable iron chelator (*66*). Isolation of



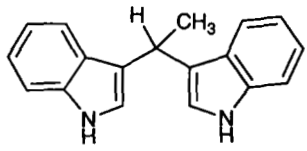
Brevianamide F (**62**)



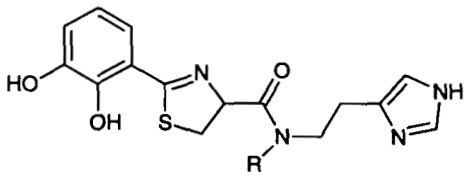
Trisindoline (**63**)



2,2-di(3-indolyl)-3-indolone (**64**)

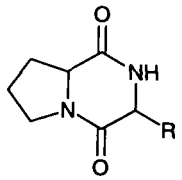


Vibrindole (**65**)

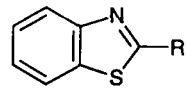


Anguibactin (**66**) R = OH
Anhydroanguibactin (**67**) R = H

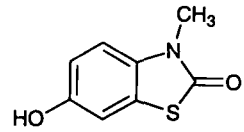
anguibactin from culture medium was by absorption onto XAD-7 resin, elution with MeOH, and then partial purification on Sephadex LH-20 (*67*). This was followed by repetitive silica gel chromatography and an additional Sephadex LH-20 step to yield pure anguibactin (**66**). A derivative, anhydroanguibactin (**67**), was produced via acetylation of anguibactin, and formed crystals from EtOH/Et_2O suitable for X-ray analysis. This analysis, in concert with NMR and MS analyses of anguibactin itself, allowed formulation of its unique, modified peptide, structure. It appears to derive from histidine, cysteine, and 2,3-dihydroxybenzoic acid subunits, with basic atoms in each subunit (*o*-hydroxy group, thiazoline N, hydroxamate, deprotonated N of imidazole) contributing to metal ligation.



(68) R = CH_3
(69) R = $CH(CH_3)_2$
(70) R = $CH_2CH(CH_3)_2$



(71) R = SH
(72) R = CH_3
(73) R = OH



(74)

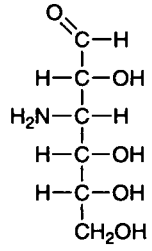
I. *MICROCOCCUS*

The first experimental examination of the hypothesis that some of the natural products chemistry of marine sponges actually derives from associated microorganism metabolism was performed with a *Micrococcus* sp. isolated from the Caribbean sponge *Tedania ignis* (*68*). Repetitive isolation of a bright orange species of *Micrococcus* sp. was made from several collections of the sponge taken over a two year period. The bacterium was grown in enriched seawater broth and the organic material sequentially extracted from the lyophilized material. Sephadex LH-20, Bio-Beads S-X8 and Counter Current Chromatography were used to isolate the same three diketopiperazines (**68-70**) which had been previously isolated from the source sponge (*69*). However, no chiroptical data for the bacterial metabolites were reported (note, two of these diketopiperazines, **68** and **70**, are reported in this review with stereochemistry from *Pseudomonas aeruginosa* as metabolites **38** and **39**). While many bacterial cultures are known to produce diketopiperazines, the repetitive isolation of this *Micrococcus* sp. from *T. ignis*, coupled to these two isolation studies, makes a strong case for the correctness of the hypothesis.

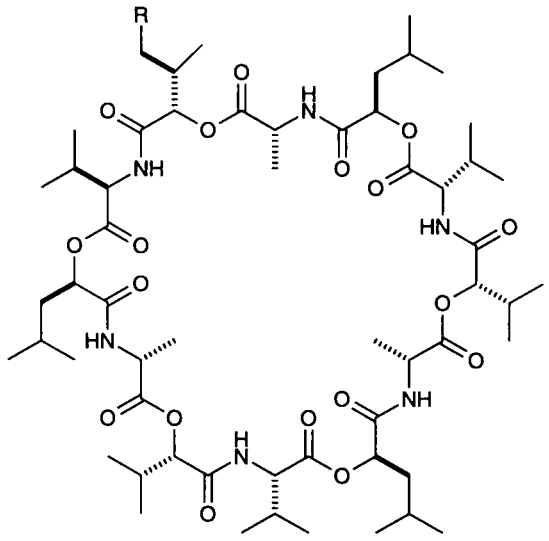
Subsequently, four benzothiazoles of known, yet intriguing, structure (**71-74**) were isolated from the cell mass and growth medium of this sponge-derived *Micrococcus* sp. (*70*).

J. *BACILLUS*

A deep sea mud sample yielded a *Bacillus* sp. which produced a potent antibiotic. Scale-up culture in enriched media containing 0.5% NaCl yielded an antibiotic broth. Adjustment of the pH followed by ion exchange and CM-Sephadex C-25 (NH_4^+ form) chromatography gave 200 mg of the antibiotic (*71*). Its structure was deduced as a known amino-sugar by various spectroscopic data, and the absolute stereochemistry was determined by comparing the rotations of derivatives of the α- and β-anomers. The antibiotic, 3-amino-3-deoxy-D-glucose (**75**), had previously



3-Amino-3-deoxy-D-glucose (**75**)

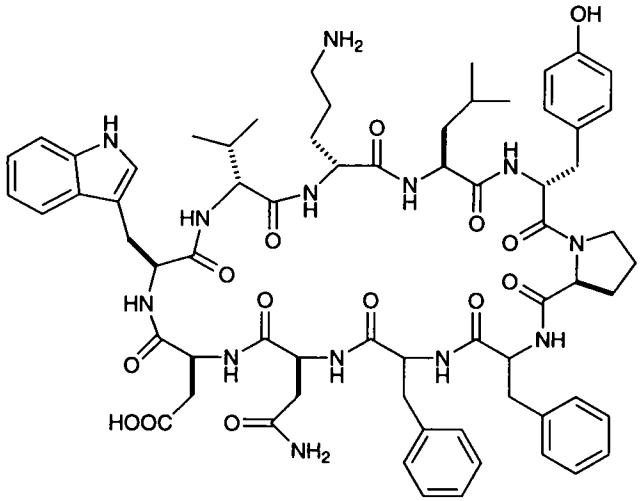


Homocereulide (**76**) R = CH_3
Cereulide (**77**) R = H

been characterized from various terrestrial bacteria.

Marine *Bacillus* species have yielded several types of cyclic peptides, including depsipeptides and lipopeptides. Homocereulide (**76**), an analog of the known terrestrial *Bacillus* product cereulide (**77**), was isolated, along with cereulide, from a marine isolate of *Bacillus, B. cereus* (obtained from the surface of an intertidal snail)(*72*). The lipid extract of cultures of *B. cereus* was highly cytotoxic to cancer cells *in vitro*, and this activity was used to track the isolation of **76** and **77** using NP, RP, and HP liquid chromatography. Comparison of the extensive 1D and 2D NMR data sets for the two compounds allowed formulation of the homocereulide structure. Hydrolysis of **76** and **77** gave a series of peptides which were used to substantiate the homocereulide structure, as well as establish its stereochemistry. Both compounds were extraordinarily cytotoxic showing IC_{50} values between 1 and 35 pg/mL to P388 and Colon 26 cell lines.

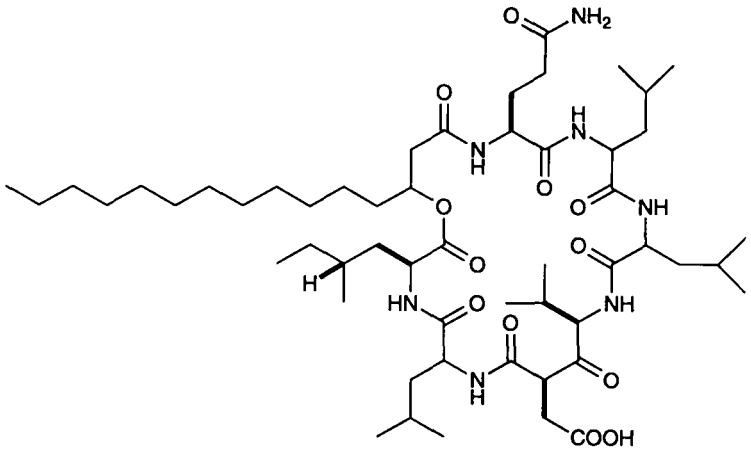
A *Bacillus* sp. isolated from a sub-tidal tube worm from Papua New Guinea was cultured on enriched agar containing 1% NaCl (*73*). The cell extract was antimicrobial to several antibiotic resistant strains of bacteria, and was sequentially fractionated via Sephadex LH-20, RP CC and RP HPLC to give pure loloatin B (**78**) as the active antibiotic. Acid hydrolysis of **78**, followed by chiral GC analysis, identified ten amino acid residues which accounted for all of the atoms in loloatin B. HMBC and ROESY effectively allowed sequencing of the peptide; this was confirmed by HR FABMS and MS/MS which showed peptide sequence ions following initial opening of the macrocycle at the Tyr-CO/Pro-N bond.



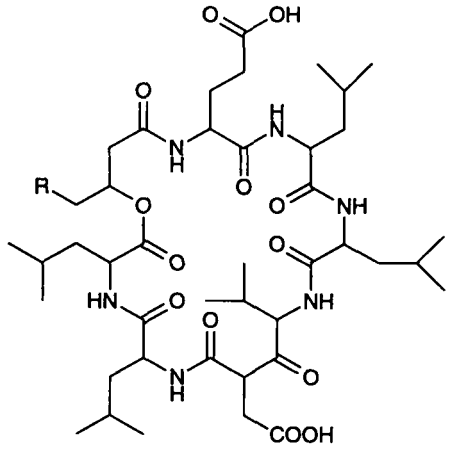
Lololactin B (**78**)

Two groups of closely related cyclic lipopeptides have been isolated from marine species of *Bacillus*, both of which are similar in structure to that of the surfactins and iturins, terrestrial *B. subtilis* metabolites (*74*). The first one, halobacillin (**79**), was obtained only from a seawater-based liquid culture of a *Bacillus* sp. isolated from a deep-sea sediment core (*75*). A combination of LH-20, RP-vacuum flash, and RP-HPLC were used to isolate halobacillin as 7.3% of the extract. As the methyl ester of **79** gave better-defined NMR spectra, a host of experiments, including TOCSY, HMBC, and ROESY, were run on this derivative, leading to formulation of the structure of halobacillin (**79**). Absolute stereochemistry of all amino acids were determined by chiral GC-MS; however, it was not possible to place the two *D*- and one *L*-leucine residues in the peptide. Despite its close structural similarity to surfactin, halobacillin shows inhibitory activity only to cancer cells (HCT-116 IC_{50} 0.98 μg/mL) and no antibacterial activity.

The second group of surfactin-type lipopeptides, the bacircines (**80-83**), were obtained from *B. pumilus* isolated from the sponge *Ircina* sp. (*76*), and were close in structure to those previously obtained from a terrestrial isolate of *B. pumilus* (*77*). The EtOAc extract of an enriched seawater culture of the marine *B. pumilus* was fractionated by NP CC and HPLC to give five, closely related, metabolites. These differed in their length and methyl substitution (*iso* and *antiso* fatty acyl groups). Remarkably, the peptide portion of the bacircines (**80-83**) differ from the comparable portion of halobacillin only in the replacement of a glutamine with a glutamic acid, and an isoleucine with a leucine. Unfortunately, because a complete NMR data set was not reported for the bacircines, it is not possible to make a meaningful comparison of the spectral properties of these two families of peptides. Absolute



Halobacillin (**79**)

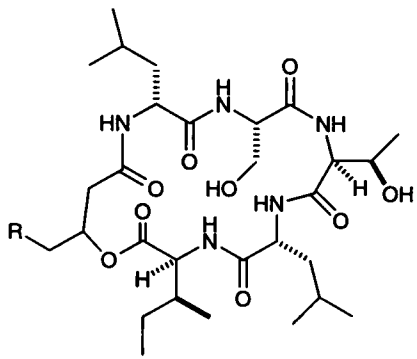


Bacircine-1 (**80**) R = $(CH_2)_5CH(CH_3)CH_2CH_3$
Bacircine-2 (**81**) R = $(CH_2)_7CH(CH_3)_2$
Bacircine-3 (**82**) R = $(CH_2)_7CH(CH_3)CH_2CH_3$
Bacircine-4 (**83**) R = $(CH_2)_8CH(CH_3)_2$

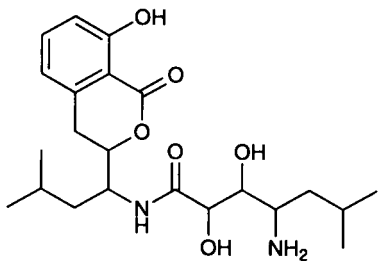
stereochemistry in the bacircines series was not investigated. The bacircines are noted to "cause anomalies in the development of ova of echinus and stop blastomere fission" at concentrations of 2.5-10 μg/mL.

While possibly taxonomically out of place, for reasons of structural parallelism it is appropriate to discuss here the kailuins (**84-87**), a series of related cyclic lipopeptides isolated from an unidentified Gram-negative bacterium obtained from a piece of driftwood in Hawaii (Oahu)(*78*). The bacterium was cultured in enriched seawater medium which was extracted with EtOAc and subjected to Sephadex LH-20 and RP HPLC to give four related lipopeptides. The structure of the major compound, kailuin A (**84**), was assembled from 2D NMR strategies, with HMBC being used to sequence the amino acid and acyl subunits. Marfey's analysis was used to assign absolute stereochemistries to the amino acid subunits. The stereochemistry of the β-carbinol position of the acyl chain was not determined. However, it may be proposed to be of the *D* configuration based on structural parallel with other β-hydroxydecanoyl residues (e.g. massetolide A **53** from *Pseudomonas* sp.). The four kailuins showed GI_{50}'s = 2-4 μg/mL to several cancer cell lines (A-549 lung, MCF-7 breast, and HT-29 colon). Structurally, they have an overall similarity to halobacillin (**79**) and the bacircines (**80-83**), being truncated in the number of amino acids (2 less), and having Glu or Gln and Asp residues being replaced by Ser and Thr residues at slightly different locations in the peptide chain. Parallels in the nature of the amino acid residues and their sequence also exist between the kailuins (**84-87**) and the massetolides (**53-60**).

A marine sediment-derived *Bacillus* sp. was cultured in enriched SW medium and its EtOAc extract was shown to be cytotoxic to several cancer cell lines (*79*). Repetitive fractionation using NP and RP silica gel chromatography gave the pure



Kailuin A (**84**) R = $(CH_2)_5CH_3$
Kailuin B (**85**) R = $(CH_2)_7CH_3$
Kailuin C (**86**) R = $(CH_2)_5CHCH(CH_3)_2$
Kailuin D (**87**) R = $(CH_2)_2CH$=^{trans}$CH(CH_2)_5CH_3$

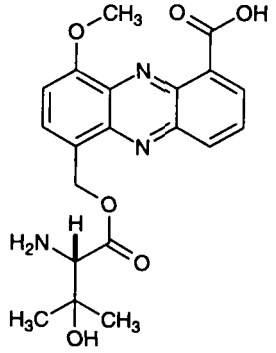


PM-94128 (**88**)

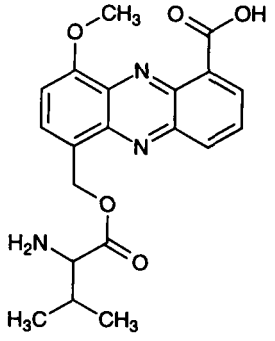
cytotoxin. Comparison of IR and UV data with known substances suggested a dihydroisocoumarin derivative; this was supported by 1-dimensional ^{1}H and ^{13}C NMR data and a ^{1}H-^{1}H COSY. The remaining features of this structure, named `PM-94128' (**88**), were a prenyl group substituted with what may be a ketide extended leucine residue. While no heteronuclear NMR correlation experiments were run to confirm this structure, the occurrence of a related molecule in the literature, `Y-05460-A', provides some conceptual support. This new substance was quite potently cytotoxic to several cancer cell lines (IC_{50} 0.05 μM to P-388, A-549, HT-29 and MEL-28), and may act *via* inhibition of protein synthesis.

K. *PELAGIOBACTER*

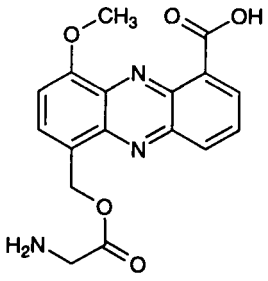
A new marine bacterium *Pelagiobacter variabilis* was found in the course of an anticancer screening program to make three new phenazine antibiotics, pelagiomicins A-C (**89-91**), related in structure to the chromophore portion of actinomycin D (*80*). The bacterium was isolated from blades of the tropical brown alga *Pocockiella variegata* and cultured in enriched seawater medium to give a bioactive $CHCl_3$ extract. Preparative HPLC was used to isolate one known compound, griseoluteic acid (**92**), and the three new pelagiomicins, all of which formed crystalline or amorphous solids. Structures of the three new compounds were assembled from various NMR and MS data, and confirmed, including stereochemical features, by chemical synthesis. Pelagiomicin A (**89**) was strongly antimicrobial to several Gram-positive and negative bacteria, and showed ID_{50} values between 0.04-0.2 μg/mL to several cancer cell lines. However, its *in vivo* antitumor effects to P388 leukemia were described as weak.



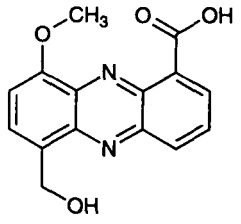
Pelagiomicin A (**89**)



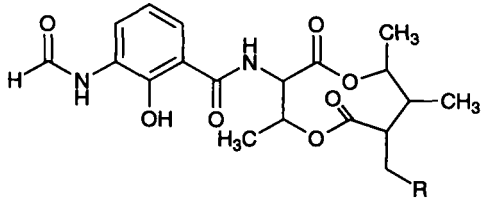
Pelagiomicin B (**90**)



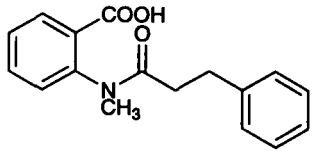
Pelagiomicin C (**91**)



Griseoluteic acid (**92**)



Urauchimycin A (**93**) R = $CH(CH_3)CH_2CH_3$
Urauchimycin B (**94**) R = $CH_2CH(CH_3)_2$



(**95**)

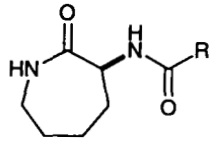
L. *STREPTOMYCES*

Two interesting metabolites containing "*m*-C_7N" units were obtained from cultures of a marine *Streptomyces* sp. isolated from an unidentified Japanese sponge (*81*). The microorganism was cultured in an enriched 50% seawater liquid medium and the new compounds, urauchimycins A (**93**) and B (**94**) isolated in low titer (0.1 to 0.5 mg/L) from the EtOAc extract of the broth by flash silica gel chromatography and RP HPLC. However, as production of the urauchimycins was unaffected by changes in salinity of the culture medium (0% - 100%), it is uncertain if this *Streptomyces* represents a true marine isolate. Structures were assembled from FABMS, spectrophotometric data, and 2D NMR, principally ^{1}H-^{1}H COSY, NOESY, and HMBC. Stereochemical aspects at the 5 (urauchimycin B) or 6 (urauchimycin A) stereocenters in these metabolites were not addressed. These new compounds are related to the antimycin group of antibiotics produced by terrestrial isolates of *Streptomyces* sp. (*82*), although they have branched and odd-numbered side chains.

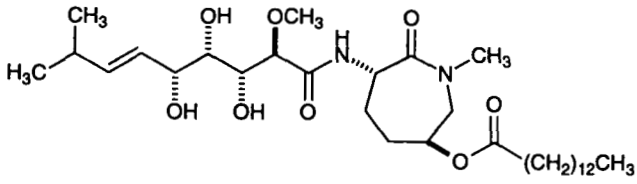
A sediment-derived *Streptomyces* sp. from the Gulf of Mexico was fermented in enriched 50% seawater and found to produce a new anti-algal anthranilamide derivative (**95**)(*83*). The EtOAc extract was fractionated over Sephadex LH-20 and silica gel CC with the isolation of anti-algal metabolites monitored by testing to several *Chlorella* spp. and *Scenedesmus subspicatus*. The new compound isolated in this study was shown to be an *N*-methyl anthranilamide derivative of phenylpropionic acid (**95**) by MS and ^{1}H and ^{13}C NMR, and confirmed by chemical synthesis. A series of related substances were synthesized and their anti-algal properties examined, with the general finding that methyl esters of these anthranilamides were more active than the corresponding free acids.

A *Streptomyces* sp. obtained from a deep-sea sediment sample was cultured in enriched marine broth to yield, following Sephadex LH-20 and RP HPLC of the EtOAc extract, two new caprolactams, caprolactins A (**96**) and B (**97**)(*84*). The structures of the two compounds in this unresolvable mixture were solved using a combination of MS and NMR methods, degradation to *L*-lysine, and total synthesis with ensuing spectroscopic comparison with the natural products. Stereochemistry at C-14 in caprolactin B (**97**) was not examined. These caprolactam natural products were mildly cytotoxic to KB and LoVo cells (MIC's 5-10 μg/mL) and displayed antiviral activity to *Herpes simplex* type II at 100 μg/mL. It is interesting to note the structural similarity to the bengamides [e.g. bengamide B (**98**)], sponge-derived caprolactams with oxidized acyl side-chains (*85*), a fact which may also indicate a bacterial origin for these latter compounds.

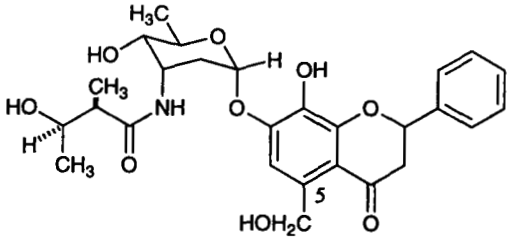
A novel flavanoid-like compound, present as a glycoside, was obtained from a *Streptomyces* sp. acquired from intertidal mud collected in New Zealand (*86*). Extracts of seawater-based cultures of this *Streptomyces* were modestly antimicrobial to *Bacillus subtilis*. The active compound, actinoflavoside (**99**), was isolated by repeated RP-HPLC and characterized by extensive multinuclear NMR experiments. Relative stereochemistries in the different portions of actinoflavoside were deduced from ^{1}H-^{1}H coupling constants and comparisons with model compounds.



Caprolactin A (**96**) R = $(CH_2)_5CH(CH_3)_2$
Caprolactin B (**97**) R = $(CH_2)_4CH(CH_3)CH_2CH_3$



Bengamide B (**98**)

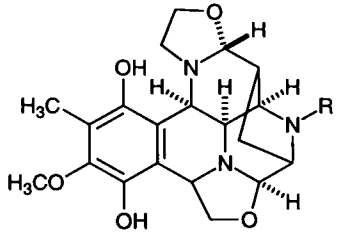


Actinoflavoside (**99**)

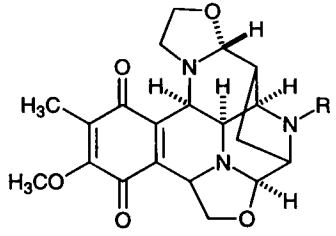
Actinoflavoside was only weakly inhibitory to several Gram positive bacteria (64 μg/mL). Actinoflavoside is biosynthetically intriguing for two reasons: 1) flavanoids are generally unknown in prokaryotes, and 2) compound **99** possesses a hydroxymethyl group at C5, an unprecedented carbon appendage in any other flavanoid. It seems likely that a novel pathway, unrelated to flavonoid biosynthesis in higher plants, is being used to construct this skeleton in *Streptomyces*.

Fermentation of a intertidal marine sediment-derived *Streptomyces virdostaticus* spp. *litoralis* from Florida gave a potently Gram positive antimicrobial extract (*87*). Subsequently, it was found that the antimicrobial compounds were also

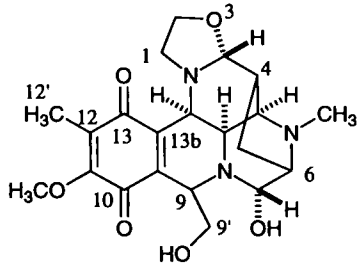
active to a panel of neoplastic cell lines. Although isolated from a marine habitat, scale-up cultivation occurred in an enriched tap water-based medium. It was noted, however, that an increase in biomass and antimicrobial activity was observed when this strain was grown in medium containing 2% NaCl. Purification of the active materials, four related bioxalomycins (**100-103**), was accomplished by preparative HPLC, and structures deduced from NMR and MS methods, synthetic chemical manipulations, and comparisons to the related known compound naphthyridinomycin (**104**). Bioxalomycin α2 was the most potent antibiotic of the group showing MIC values between 0.002 - 0.25 μg/ml. Owing to the close structural relationship of the bioxalomycins to naphthyridinomycin (actually, the structure of naphthyridinomycin has been corrected to be identical to bioxalomycin β_2)(*88*), it is likely that they have a similar biogenesis. In the case of the latter metabolite, extensive radioactive and stable isotope feeding experiments have delineated the origins of most of the biosynthetic units, which include all of the atoms of tyrosine, an ornithine residue, and a glycine residue which is converted into serine, decarboxylated, and incorporated into C-1 and C-2 of naphthyridinomycin (Scheme 4). Three methyl groups, the O-Me, the N-Me and the C-12 methyl, derive from the methyl group of methionine. The origin of C-9 and C-9' remain unknown (*89,90*). It has recently been shown that, following metabolic reduction of the quinone, bioxalomycin α2 cross-links DNA through alkylation of guanine residues in the minor groove (*91*).



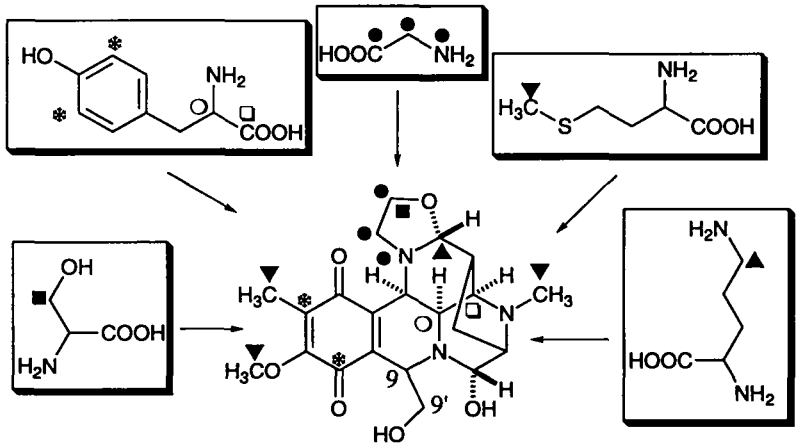
Bioxalomycin α1 (**100**) R = H
Bioxalomycin α2 (**101**) R = CH_3



Bioxalomycin β1 (**102**) R = H
Bioxalomycin β2 (**103**) R = CH_3



Naphthyridinomycin (**104**)



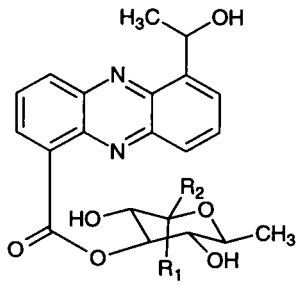
Naphthyridinomycin (**104**)

SCHEME 4. Biosynthetic precursors to naphthyridinomycin (**104**)

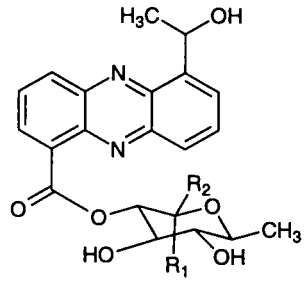
Two new classes of phenazines have been isolated from different strains of marine-derived *Streptomyces*. A shallow sediment from Bodega Bay, California, was the source of a new species of *Streptomyces* (*92*). Fermentation of this organism in an enriched seawater-based medium gave an EtOAc extract with broad spectrum antibacterial activity. Four new alkaloid esters, **105-108**, were isolated from this extract by HPLC. Their structures were deduced by various NMR methods, and the identity of the sugar portion as quinovose was determined by coupling constant analysis. Absolute stereoconfiguration of the sugar was determined as *L* by CD analysis of the isolated quinovose, following hydrolysis of **105**, in comparison with the authentic *D* sugar. The isolated phenazine derivatives were only modestly antimicrobial to Gram positive and Gram negative bacteria. Biosynthetically, these phenazine derivatives appear to derive from an unsymmetrical dimerization of *m*-C7N units and *m*-C8N units, and the unusual *L*-form of quinovose.

A second set of phenazine derivatives, closely related to phencomycin (**109**) which was also isolated from this organism, were obtained from a *Streptomyces* cultured from a sediment sample taken in the Gulf of Mexico (*93*). Cultivation occurred in enriched 50% seawater medium and gave an antimicrobial extract to several test organisms. Column chromatography gave several compounds, including one new compound that was identified on the basis of its NMR characteristics, including HMBC. The new substance, 5,10-dihydrophencomycin dimethyl ester (**110**), was less antimicrobial than phencomycin itself. In this case, dimerization of identical *m*-C7N units explains the likely origin of **110**.

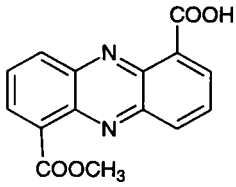
Streptomyces sioyaensis was collected from a marine mud in Japan and cultured in enriched 50% seawater to give, following extensive chromatography using



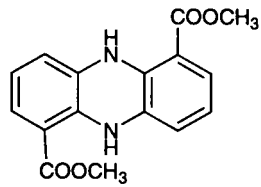
(**105**) $R_1 = OH, R_2 = H$
(**108**) $R_1 = H, R_2 = OH$



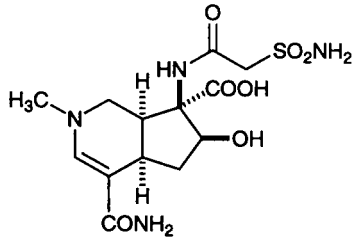
(**106**) $R_1 = OH, R_2 = H$
(**107**) $R_1 = H, R_2 = OH$



Phencomycin (**109**)



5,10-Dihydrophencomycin methyl ester (**110**)

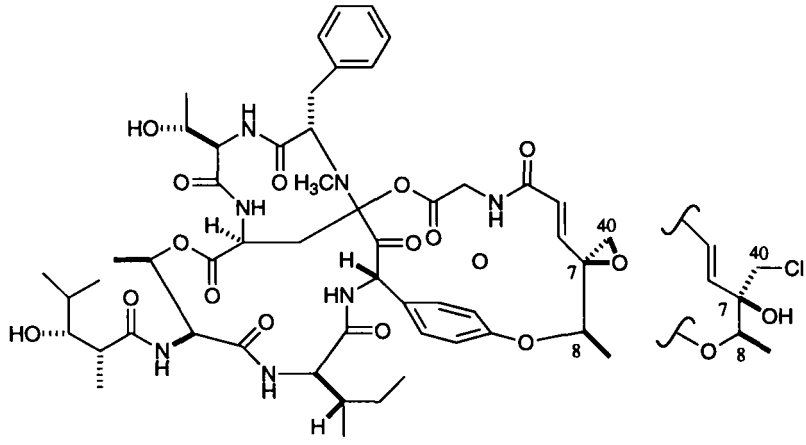


Altemicidin (**111**)

activated charcoal, Dianion CHP-20P, and Sephadex LH-20, a novel brine shrimp toxic metabolite, altemicidin (**111**)(*94*). Its structure was deduced using various 2D NMR, MS and other spectroscopic techniques in combination with the formation of key derivatives (*95*). Confirmation of structure was accomplished through X-ray diffraction analysis of a crystalline xanthenyl derivative. Absolute stereochemistry of this light atom structure was determined from an analysis of Friedel pairs in the

crystallographic data set. Altemicidin (**111**) is a unique bacterial alkaloid which incorporates a carbon structural unit consistent with a monoterpene origin. Altemicidin (**111**) was not only brine shrimp toxic (LC_{50} 3.0 μg/mL), but also prevented two-spotted spider mite infection of kidney bean leaves (ED_{50} 10 μg/mL), displayed cytotoxicity to cancer cells *in vitro* (LD_{50} 0.84 μg/mL to L1210) and possessed a limited spectrum of antimicrobial activity (MIC 6.25 μg/mL to *Xanthomonas oryzae*).

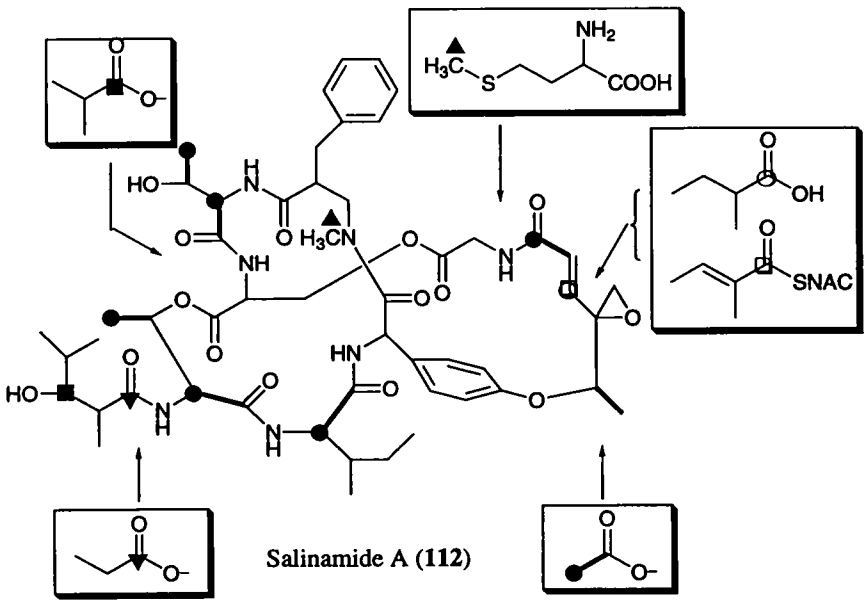
Two of the most exciting substances isolated to date from a marine bacterium, from the perspective of unusual chemical structure and biological properties, are the exceptionally complex depsipeptides, salinamides A (**112**) and B (**113**)(*96*). The salinamide producing *Streptomyces* sp. was isolated from the surface of a tropical jellyfish, *Cassiopeia xamachana*, and cultured in an enriched seawater-based medium. The EtOAc extract of the entire culture was fractionated over silica gel and RP-HPLC to give salinamide A as a major component. Salinamide B (**113**), a crystalline chlorohydrin derivative of A, was isolated from a subsequent re-fermentation and used for X-ray crystallographic studies. Chemical conversion of salinamide A into B provided a firm structure proof of the former substance. These are remarkable for their bicyclic peptidic constitution with resulting densely packed molecular core. While both salinamide A and B showed modest Gram positive antimicrobial activity, the more intriguing biological property was the potent antiinflammatory effects of salinamide A in the inhibition of phorbol ester-induced edema in the mouse ear model (84% inhibition at 50 μg/ear). Subsequently, salinamide A was isolated from another *Streptomyces* sp. and characterized as possessing potent inhibitory activity against bacterial RNA polymerases (*97*). The biosynthetic origins of several of the unusual structural units making up salinamide A have been probed by stable isotope incorporation studies (Scheme 5)(*98*).



Salinamide A (**112**) Salinamide B (**113**)

By feeding a variety of ^{13}C-labeled precursors, including acetate, propionate, methionine, isobutyrate and 2-methyl-but-2-enoyl NAC thioester, it was discovered that the two 7-carbon non-amino acid residues were formed from acetate (malonyl CoA) or propionate (methyl malonyl CoA) extension of a fragment deriving from branched-chain aliphatic amino acids. In the one case, a valine unit is converted into isobutyrate and then extended into (2*S*,3*S*)-3-hydroxy-2,4-dimethylpentanoic acid, and in the other, an isoleucine fragment is condensed with acetate (malonyl CoA) to yield the epoxide-containing residue. Insight into the timing of formation of the phenolic linkage that forms one of the two macrocycles was gained through isolation of two naturally occurring methyl ethers at the phenolic position. These latter metabolites are presumed dead-end shunt metabolites in the salinamide A/B pathway. It has been reported that a peptide synthetase, possibly involved in salinamide biosynthesis, has been cloned and sequenced from this marine *Streptomyces* sp (*98*).

A *Streptomyces* sp. was isolated from the surface of a mollusc collected in Kanagawa Prefecture, Japan, cultured in 100 L quantity, and extracted (supernatant plus mycelia) with EtOAc to yield 19 g of oil (*99*). This was sequentially fractionated

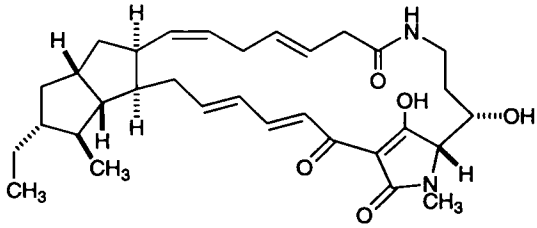


Salinamide A (112)

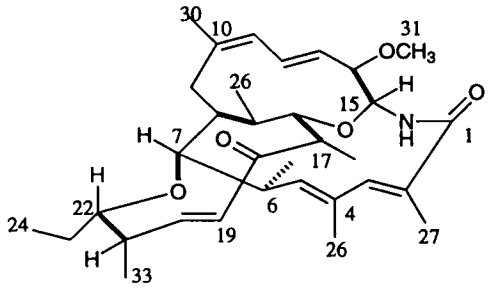
SCHEME 5. Biosynthesis of salinamide A (112)(*98*).

over silica gel, Sephadex LH-20, and ODS to yield a new inhibitor of superoxide anion generation in cultured human neutrophils. The inhibitor, aburatubolactam A (**114**), was a complex crystalline substance that was analyzed by various spectroscopic techniques, including X-ray crystallography. Absolute stereochemistry was not determined. Aburatubolactam A is an intriguing structure that appears to derive from two C_{12} carboxylic acids, 3-hydroxyornithine, and methionine, and is related to several terrestrial Actinomycete products as well as alteramide A (**14**), a product of the marine bacterium *Alteromonas* (*18*). It was noted that aburatubolactam C, a structure not pictured, but apparently related to aburatubolactam A, is the most potent inhibitor of superoxide anion generation in this structural series (IC_{50} 2.7 μg/mL).

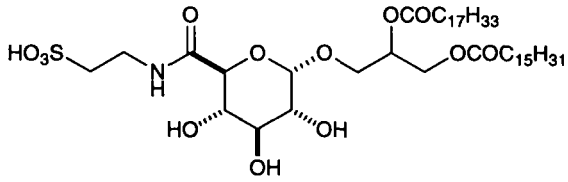
Streptomyces hygroscopicus was obtained from the gastrointestinal tract of a marine fish and cultured in an enriched seawater medium to give a cytotoxic extract (*100*). Bioassay-guided isolation of the active compound, halichomycin (**115**), was achieved with a combination of Sephadex LH-20, silica gel CC and RP HPLC. A structure for halichomycin was proposed on the basis of a normal set of spectroscopic information, including COSY, HMBC, and NOESY experiments. An unusually high field shift of δ75.8 was recorded for a methine carbon (C-15) in halichomycin which



Aburatubolactam A (**114**)



Halichomycin (**115**)



(116)

possesses both ether oxygen and amide nitrogen substituents. As formulated, the biogenetic subunits of halichomycin are not apparent. Halichomycin showed potent cytotoxicity to cultured P-388 lymphocytic leukemia cells (0.13 μg/mL).

M. *Hyphomonas*

An examination of the lipids of the budding seawater bacterium *Hyphomonas jannaschiana* revealed that it is nearly devoid of phospholipid and has a preponderance of glycolipid. Moreover, the nature of a major glycolipid molecular species was found to be quite unique in that it incorporates a unit of the amino acid taurine in its structure (**116**)(*101*). The organism, *H. jannaschiana*, was obtained from the ATCC (33883) and grown in enriched seawater medium in shake flasks. The cells were harvested by centrifugation, extracted with 2:1 $CHCl_3$/MeOH, and the extract was subjected to chromatography over Sephadex G-25, DEAE cellulose, and silica gel to give the new glycolipid **116**. Its structure was established from extensive chemical degradations, as well as formation of derivatives, coupled with mass spectrometry and other spectroscopic characterization. This type of tauroglycolipid is unique in nature, and the timing of its assembly, specifically the connection between the taurine and glucuronic acid components relative to that of the diacylglycerol and glucuronic acid, remains unknown.

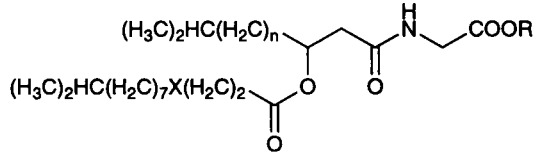
N. *Cytophaga*

A *Cytophaga* sp. from a seawater sample taken in Shizuoka Prefecture, Japan was cultured in 300 L of nutrient-enriched artificial seawater (*102*). The acetone extract from the cells was dried and then extracted with EtOAc to give an oil that was active at inhibiting the binding of ω-conotoxin to N-type calcium channels. The extract was subjected to initial chromatography using silica gel column chromatography, and then, because of difficulty in following the isolation of the compounds, derivatized to UV-absorbing esters using diphenyldiazomethane. These derivatives were effectively separated using HPLC to give a series of three crystalline

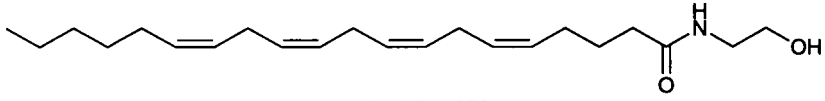
derivatives, **117-119**. The active natural products could easily be reformed by mild acid hydrolysis using trifluoroacetic acid. Structure elucidation of the natural products isolated in this manner utilized NMR and FABMS, as well as hydrolysis to their basic components. Confirmation of structure was obtained by chemical synthesis of the natural products as well as of a homologous series which were used to probe structure-activity relationships. As it was found that the racemate at the single chiral center was as active as the natural products, stereochemistry was concluded to not be important to drug binding. However, a double bond in the fatty acyl group attached to this hydroxy group appears to potentiate the activity of the series. These *N*-(3-acyloxy)acylglycines show good selectivity for N-type verses L-type calcium channels. Biosynthetically, these are quite simple ester derivatives of a β-hydroxy fatty acid which are further modified as glycine derivatives. In this latter respect, they join a growing number of biologically-active fatty acid amides [e.g. anandamide (**120**) and oleamide (**121**)].

O. UNIDENTIFIED MARINE BACTERIA

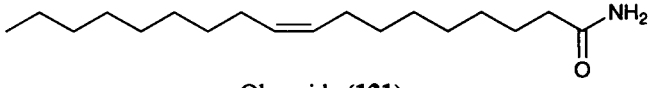
The exquisitely potent, nicotine receptor antagonists neosurugatoxin (**122**) and prosurugatoxin (**123**), as well as the less potent decomposition product surugatoxin (**124**), were originally isolated from the Japanese ivory shell, *Babylonia japonica*



(**117**) R = H, X = *cis*CH=CH, n = 11
(**118**) R = H, X = *cis*CH=CH, n = 10
(**119**) R = H, X = CH_2CH_2, n = 11

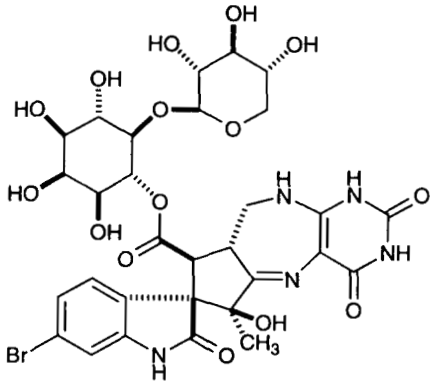


Anandamide (**120**)

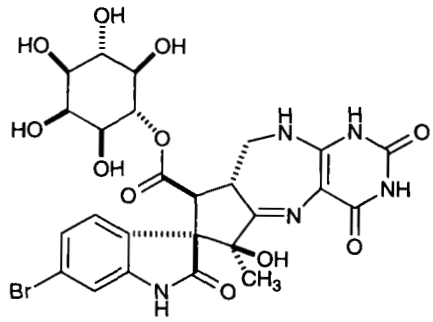


Oleamide (**121**)

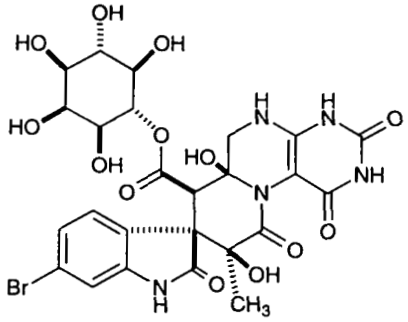
(*103*). Sporadic outbreaks of food poisoning resulting from ingestion of this snail between 1965 and 1978 were attributable to these toxic alkaloids. Because of the periodic nature of the intoxications, and the ability to render toxic snails non-toxic after growth in a new environment for as little as 1 month, it was reasoned that a microorganism was the likely source of the toxins (*104*). While a screen of more than 100 strains of Suruga Bay sediment-derived bacteria failed to yield the toxins, a Coryneform bacterium isolated from the digestive gland of the ivory snail was shown to produce all three compounds in culture. Yields of neosurugatoxin and prosurugatoxin were too low to detect by chemical means. However, HPLC retention times in concert with biochemical and pharmacological assays were able to



Neosurugatoxin (**122**)



Prosurugatoxin (**123**)



Surugatoxin (**124**)

conclusively show their presence. Sufficiently high yields of the breakdown product surugatoxin were obtained such that its presence was confirmed by MS and ^{1}H NMR.

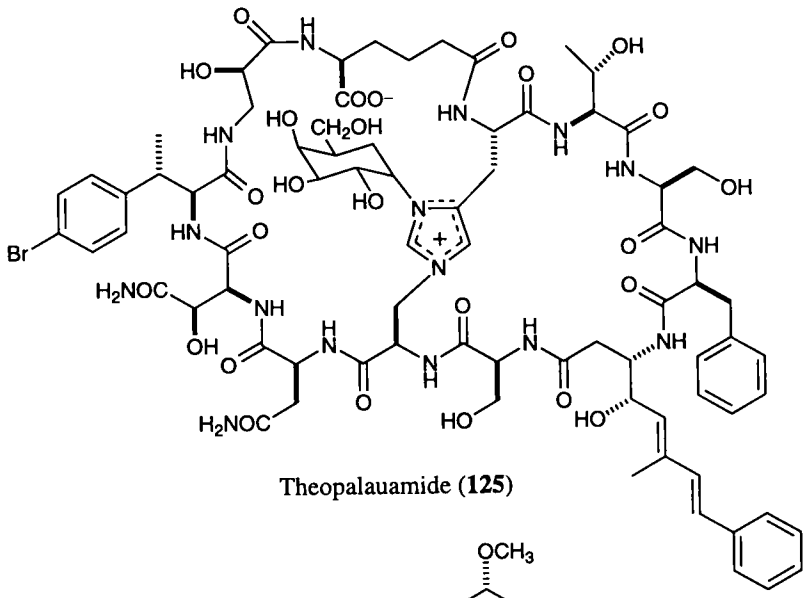
An interesting insight into the relative contribution of bacteria, cyanobacteria, and sponge cells to the mixture of natural products isolated from the chemically-productive sponge *Theonella swinhoei* was gained by separate chemical analysis of these various cell populations isolated from the intact sponge (*105*). Specimens of *T. swinhoei* were obtained by SCUBA in Palau and processed by excision of particular tissues, dissociation of cells using a "juicer", and purified by sequential filtrations and centrifugations. Four cell populations were obtained in relatively high purity; sponge cells, unicellular cyanobacteria, filamentous heterotrophic bacteria, and unicellular heterotrophic bacteria. These were separately extracted and analyzed by gradient HPLC with diode-array detection. No major metabolites were found in extracts of either the sponge or cyanobacterial cells. Rather, antifungal cyclic peptides, such as theopalauamide (**125**)(*106*), were found only in the filamentous bacteria whereas the unicellular heterotrophic bacteria contained the macrolide swinolide A (**126**). These results are surprising for several reasons as each of these two types of metabolites have structural precedent in the chemistry of cyanobacteria. One possible explanation for these findings is that these substances are excreted by cyanobacterial populations in the sponge and are being selectively absorbed by these other two microbial populations. Alternatively, horizontal gene transfer from cyanobacteria to these bacterial populations is conceivable. In the case of the heterotrophic filamentous bacteria which closely resemble cyanobacteria but lack thylakoids by transmission electron microscopy, it is possible that this population has evolved from a cyanobacterial ancestor. Whatever the case, this work appropriately illustrates the complexity that exists in the ecology and biochemistry of these assemblages of organisms we call "sponges".

III. Conclusions

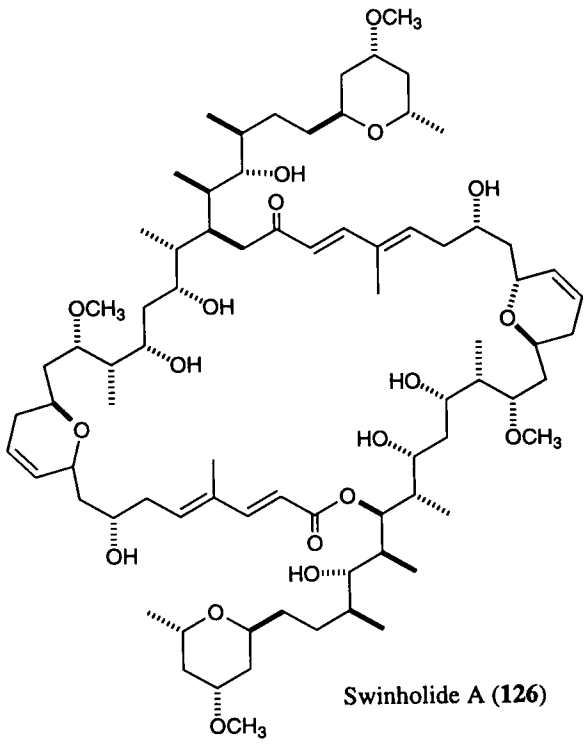
As noted in the introduction, the assemblage of nitrogen-containing natural products reported to date from marine bacteria and covered in this review represent a fascinating and diverse group. No single biogenetic theme dominates. Of course, with the alkaloid-theme, most although not all of these metabolites contain residues recognizable as components of amino acids. The dominant non-amino acid theme is the occurrence of simple amide functionalities.

A surprising uniformity of scientific approach was noted in assembling this review. Mechanism-based bioassays are increasingly forming the motivation for in-depth natural product investigations of marine bacteria. Most workers in this field are culturing marine bacteria in enriched medium containing added salt, or are made with a seawater base. Extraction is usually effected with EtOAc, and initial chromatography is by Sephadex LH20, with eventual final purification using RP HPLC. Structure elucidations in recent years have relied heavily on the cadre of very powerful inverse-detected 2D NMR techniques, such as HMBC and HSQC.

Many of the natural products discussed in this review have precedent in those



Theopalauamide (**125**)



Swinholide A (**126**)

isolated from terrestrial bacteria. This accentuates many questions concerning whether these prokaryotes are truly marine in origin, or if they possibly exist in the ocean only transiently following inoculation from terrestrial environments. Are sustained populations of these natural product-rich bacteria maintained in the sea? What really defines a terrestrial or marine bacterium? Do "exclusively" marine and "exclusively" terrestrial bacteria represent extremes of what is a continuum in nature? These and other questions, such as the role that microorganisms play in the production of metabolites isolated from various marine invertebrates, are compelling areas of investigation for the new millennium.

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CHAPTER 5

THE PHARMACOLOGY AND THERAPEUTIC ASPECTS OF COLCHICINE

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THE ALKALOIDS, VOL. 53
0099-9598/00 $30.00

I. History of Colchicine

Colchicine is one of those medications that is as 'old as the world' and whose use is derived from long-forgotten empirical tradition. It's long and obscure history has accompanied humanity from the deadly colchicum, dear to the heart of the poisoner Medea, to colchicine, the reigning medical remedy for gout (*1*). Over the centuries, different substances have been considered as one, situating the agent somewhere between poison and medication (*2*). In his volume, *De Materia Medica*, written in 77 AD, Dioscorides, the Greek physician and botanist, distinguished between the consumable small colchicum and the wild bulb. The small colchicum was nourishing, had aphrodisiac and therapeutic properties, and was found in Africa. On the other hand, the wild bulb or *colchicos* was dangerous and grew in the ancient country of Colchis bordering the Black Sea south of the Caucasus Mountains. This latter corresponds to our *Colchicum autumnale* and was considered to be a poison at that time. It also bore the names of colchicum, ephemeral and hermodactylus or Hermes' finger. The small colchicum used in Greek and Egyptian cooking was also called hermodactylus in Alexandria, but had no known therapeutic attributes, thus explaining the subsequent confusion that persisted for several centuries. The therapeutic properties of *Colchicum autumnale* were finally identified by Byzantine physicians during the 5th and 6th centuries. In 400 AD, Jacques Le Psychriste cured burning inflammations of the feet with Asian *colchicos*, *i.e.*, with *Colchicum autumnale*. Avicenne, the 10th century

Iranian physician and philosopher, called the autumn crocus the 'panacea of the articulations' and clearly distinguished between two varieties. Because the Humanists of the Renaissance doubted these observations, colchicum once again became a poison and remained so for several centuries, and was even proscribed by the French School of Medicine from the 16th to the 19th century. It then continued to prosper as an amulet or mystical therapy and its rehabilitation was initiated in 1764 by the Viennese physician Anton von Störk (*3*). It was introduced into the United States in 1798 by Benjamin Franklin. As of 1820, many medical texts were written about it, and that same year, two chemists, Pelletier and Caventou, isolated an alkaloid substance. In 1833, Geiger and Hesse were able to separate colchicine. In 1857 Oberlin isolated colchiceine and, four years later, Hubler identified an isomer of it. For the first time, in 1884, Laborde and Houdé were able to reproducibly obtain crystallized colchicine. However, its chemical formula was only established in 1945 by Dewar (*2*).

II. Chemistry

The alkaloid has several isomers and analogues have been obtained by substituting one or several functional groups: deacetylmethylcolchicine, deacetylcolchicine, trimethylcolchinic acid, colchicoside, colchiceine... Except for the latter, their efficacy is comparable to that of colchicine, and they are less toxic. Deacetylcolchicine is used as an anti-mitotic drug in cancerology (*4*). Although colchicine can be synthesized, it is currently extracted from *Colchicum autumnale* and *Gloriosa superba*.

III. Pharmacokinetics

Colchicine pharmacokinetics and metabolism have not been known for long (*5, 6*), and determination of plasma concentrations using a radioimmunoassay is a recent development. Two proteins can modify its pharmacokinetics: tubulin and P-glycoprotein. Tubulin, the specific intracellular receptor for colchicine, conditions the elimination half-life of colchicine from the blood. P-glycoprotein, the cellular detoxication pump, regulates the tissue distribution of colchicine and its biliary and renal eliminations (*7*). The therapeutic level is difficult to quantify because it is low; however, a sensitive and specific radioimmunoassay with a detection threshold of 0.25 ng/ml is now available. Because the biological effects do not reflect the plasma concentration but are associated with the intraleukocytic concentration, the plasma concentration is not routinely measured. The serum peak occurs between 30 and 90 min after oral administration. After a single administration, the absorption rate is of 0 order proportional to the dose. Interindividual variations are broad and unexplained. The bioavailability of orally administered colchicine varies from 24 to 88% of the dose given. Approximately half of the dose binds to protein, predominantly albumin. The concentration in leukocytes is 5–10 times higher than that in plasma. Colchicine is primarily metabolized in the liver by oxidative demethylation. Molecules entering into competition with cytochrome *P*-450 (cimetidine, tolbutamide, erythromycin, cyclosporine) or inhibiting P-glycoprotein (cyclosporine) can also interfere with

colchicine by decreasing its hepatic and/or renal clearance. For the most part, colchicine elimination is biliary in the form of inactive metabolites. By blocking P-glycoprotein in the proximal tubules, cyclosporine is able to reduce renal clearance of colchicine by 50% (*8*). Cyclosporine significantly decreases biliary clearance of colchicine and thereby increases its plasma concentration (*9*). Colchicine, taken orally or injected intravenously, has a short distribution half-life (1.8 h). The elimination half-life varies as a function of the technique used to evaluate it and the timing of sampling ($t_{1/2}$ = 50.7 h for colchicine and its metabolites, 20 h for non-metabolized colchicine, 1 h for metabolized colchicine as assessed by radioimmunoassay); it has been estimated to be between 14 and 30 h in healthy volunteers (*6*). After stopping treatment, colchicine can persist for 7–10 days in urine and 10 days in leukocytes. The volume of distribution is very high (500–900 l) and reflects the strong tissue binding of this molecule. Total clearance is 37.8 l/h and renal clearance is 4 l/h, demonstrating the predominance of extrarenal elimination. The dose should be lowered in the case of liver disease or renal insufficiency (prescribing half a dose when renal clearance is comprised between 10 and 50 ml/min) and in the aged patient. When renal clearance is <10 ml/min, an alternative drug should be considered.

IV. Mechanisms of Action

Over the years, colchicine has been found to have multiple properties. Many are the consequence of its capacity to block the assembly of tubulin into microtubules, which are in a perpetual state of dynamic equilibrium. Colchicine binds tubulin in an equimolar manner. All cell types, except erythrocytes, contain microtubules, which are essential for cell function and division. An increasing number of non-assembled microtubules brakes the synthesis of tubulin mRNA, but does not affect already formed microtubules or the disassembly of tubulin. This anti-mitotic action was first described by the Sicilian pathologist Pernice in 1889 (*10*).

The properties inherent in tubulin inhibition are numerous (*11*): modification of the form and ultrastructure of the cell; inhibition of the movement of cells, stimulated or not; movement of cilia, flagella and pseudopods; modification of mitochondrial structure; inhibition of spindle formation during cell division; perturbations of chromosome distribution; inhibition of receptor mobility on leukocytes and macrophages; inhibition of pinocytosis; diminished mobility of lysosomes and thus diminution of their degranulation and release of chemotactic factor(s); decreased mobility of secretion granules with concomitant diminution of secretion; inhibition of collagen synthesis and stimulation of collagenases; increased cyclic AMP; inhibition of platelet secretion; stimulation of prostaglandin secretion; decreased mobility and chemotactism of polymorphonuclear leukocytes and monocytes and thus less phagocytosis (*12*); inhibition of the Arthus reaction (*13*); and inhibition of histamine release by mastocytes (*14*). These abilities to block lysosome degranulation and inhibit the Arthus reaction endow colchicine with anti-inflammatory properties.

Other properties of colchicine are independent of its impact on microtubules. Included among them are (*11*): inhibition of axonal transport, modification of synapse

morphology, inhibition of neurotransmission; inhibition of DNA synthesis, perhaps by altering calcium and amino-acid transport; diminished proliferation of lymphoblasts and non-lymphoid cells in the presence of mitogen; inhibition of cell fusion by specific binding or not to membrane proteins; decreased platelet aggregation by altering calcium flow; modification of the lateral mobility of membrane components and diminished membrane fluidity (*15*); *in vitro* inhibition of insulin and parathormone secretion at high concentrations (*14*); immunoregulatory properties attributable to the restoration of the suppressor T-cell function that is defective in familial Mediterranean fever (*16-18*) and primary biliary cirrhosis (*19*); and immunosuppressive properties (*20*).

It should be noted that colchicine is active in two experimental models of autoimmune disease in animals: without modifying renal histology, it lowers proteinuria resulting from Heymann's nephritis in the rat which simulates membranous glomerulonephritis in man (*21*); and it attenuates murine experimental allergic encephalomyelitis which mimics multiple sclerosis (*22*). In addition, colchicine is able to prevent experimental immunoglobulin A nephropathy (*23*).

Thus, colchicine has many mechanisms of action; they are complex and are *a priori* partially known. Colchicine has been used successfully to treat diverse diseases in which its efficacy cannot always be explained objectively.

V. Side Effects and Toxicity

Side effects are rare when colchicine is used correctly. Lévy *et al.* (*15*) exhaustively reviewed the adverse effects reported for this drug: they are the same, but less severe, than the different manifestations of acute intoxication. The most common side effects are digestive, neuromuscular, hematological and cutaneous. Severe events and even deaths attributed to colchicine are usually the consequence of an overdose, independently of the administration route. Indeed, intoxication was reported after intraurethral administration of 50 mg of colchicine (*24*). Adverse events occur more frequently after intravenous injection or when the patient suffers from hepatic and/or renal insufficiency.

A. Digestive Side Effects

With an adapted oral dose (≈ 1 mg/day for an adult with normal renal and hepatic functions), eight patients out of ten have undesirable gastrointestinal side effects, usually minor: diarrhea initially, followed by nausea, vomiting and abdominal pain. These warning signals precede the development of intoxication. These premonitory signs are absent or only weak after intravenous injection. Steatorrhea, malabsorption and intestinal enzyme deficits have been described after long-term administration and are the direct results of mucosal cell toxicity (*15*). Comparison of small intestine biopsies from nine patients receiving colchicine for familial Mediterranean fever and 14 untreated patients suffering from minor gastrointestinal disorders showed accelerated cell turnover in colchicine-treated specimens (*25*). In familial Mediterranean fever, colchicine increases intestinal permeability (*26*) and induces significant malabsorption

of lactose (*27*). The number of patients who must discontinue treatment because of diarrhea is low, even for long-term therapy (*28*), or when hepatic cirrhosis is present (*29*).

B. Muscle and Neurological Side Effects

These events are common, but often poorly understood. They classically occur during treatment with a supposedly adapted dose lasting several months or during acute intoxication (*15*). They are usually favored by the existence of slight renal insufficiency, as demonstrated by the only published prospective and retrospective series of twelve patients (*30*). All the patients presented the same symptoms: marked proximal muscle (pelvic and shoulder girdles, thighs, arms) weakness, some minor distal sensory deficits, osteotendinous areflexia and blatant elevation of muscle enzymes. Electromyography demonstrated proximal myopathy and axonal polyneuropathy. For six patients, histological examination of muscle biopsies found typical vacuolar myopathy with accumulation of autophagic vacuoles, lysosomal debris, heterogeneous membrane debris and perinuclear aggregates of filamentous material. Other than the distal electromyographic signs, the overall picture was reversed 3–4 weeks after the withdrawal of colchicine. Similar observations have been reported for renal transplant recipients who were treated with colchicine and cyclosporine, which interacted (*31-38*). Myoneuropathy can be associated with electromyographic and clinical manifestations of myotonia (*39, 40*). The clinical picture can also include myalgias (*36, 37, 41, 42*) and muscle involvement can be isolated (*43, 44*). Normal renal function (*42, 44*) or muscle enzyme levels (*35, 45*) do not exclude either their involvement in short-term colchicine therapy (*36, 44-46*). In every case, the symptoms regressed progressively after discontinuation of colchicine.

C. Hematological Side Effects

Usually, these adverse events are preceded by digestive, muscle and/or neurological symptoms. The risk of agranulocytosis or abnormal differential blood counts is generally low under long-term adapted colchicine therapy. However, interindividual variations remain unexplained (*15*). An epidemiological case–control study showed a higher frequency of aplastic anemia in patients who had taken colchicine or allopurinol during the preceding 5 years (*47*). Nevertheless, it must be kept in mind that a short-term treatment with a supposedly adapted dose can be complicated by agranulocytosis (*48*) or pancytopenia, whereas a toxic dose of 30 mg might not cause any hematological side effects (*15*).

D. Cutaneous Side Effects

Allergic reactions, most commonly urticarial, are estimated to occur in 3/1,100 patients (*15*). Other manifestations have also been described: eczema, dermatitis, bullae, epidermolysis, purpura, cutaneous vasculitis (*49*) and fixed drug eruption (*50*).

Reversible alopecia, a classical reaction to intoxication, can exceptionally occur during long-term treatment with an adapted dose (*15*).

E. Effects on Reproduction

Investigations on spermatogenesis, male and female fertility, and the fetus have not been controlled studies and have generated divergent data (*51*). The major studies were conducted on patients with familial Mediterranean fever (see the corresponding section), which itself affects fertility, thereby complicating the interpretation of the findings (*15*).

Several cases of azoospermia have been published (*52-54*). The spermograms of six patients suffering from familial Mediterranean fever (*55*), six patients with Behçet's disease (*56*), and seven healthy volunteers (*57*) receiving long-term colchicine therapy were normal. In contrast, 23 of the 62 men treated with colchicine for Behçet's disease had oligospermia and two were azoospermic (*58*). It has also been reported that colchicine might modify sperm penetration (*53*). Fertility is considered to be normal in the majority of men treated with colchicine for familial Mediterranean fever (*59*) or gout (review of 540 patients over 20 years who were receiving 0.5–1 mg/day) (*60*).

Other than for familial Mediterranean fever, the fertility of women taking colchicine has rarely been evaluated: germinal cells are destroyed by colchicine in animals, but at levels equal to 30–50 times higher than those prescribed to humans (*57*). The molecule is potentially teratogenetic in animals, but again at a very high dose. Most children fathered by men taking colchicine are normal (*53, 55, 61, 62*). Several cases of trisomy 21 potentially secondary to colchicine treatment have been reported (*59, 63*). Most children born to women taking colchicine at the time of conception or during pregnancy were normal (*54, 64-67*). The frequency of chromosomal anomalies is difficult to calculate, but seems to be below 2% (*65, 66, 68*). Because the patient's age was missing from some reports of trisomy 21, no clear-cut conclusions can be drawn. Therefore, when colchicine is indicated, it seems preferable not to interrupt treatment during pregnancy, but amniocentesis should be performed for fetal karyotyping.

Finally, it is still too early to evaluate the fertility of those children whose fetal development proceeded while the mother was receiving colchicine. This point will be addressed by a study on the offspring of parents suffering from familial Mediterranean fever, which has been routinely treated with colchicine since 1972.

F. Renal Side Effects

Such events have only been described in intoxication when acute renal insufficiency was multifactorial (hypotension, mitotic arrestation in the pyramides, crystal precipitation ...). Colchicine also inhibits the division of the transitional epithelia of the urinary bladder (*15*).

G. Other Side Effects

Other adverse events are more anecdotal: hypothermia, diabetes, blood lipid abnormalities, diminished libido, phlebitis at the injection site (*15*). One case has been reported of vertebral malformation (hemi-vertebra D8 and the absence of the contralateral rib) with thoracic scoliosis in the child of a 25-year-old woman who had been taking 1 mg/day of colchicine at the time of conception and during the first trimester of pregnancy (*69*). It is more likely that this type of isolated malformation would be the consequence of a factor modifying the fetal environment: thus, it is difficult to establish a cause-and-effect relationship in this case. Several patients taking Colchimax™ (Laboratoire Houdé, Paris–La Défense, France) developed thyroid dysfunction (*70-72*), which was attributed to the tiemonium iodide contained in this formulation. It should be noted that, for the past several years, Colchimax™ has no longer contained tiemonium iodide. The four reported corneal ulcers resistant to conventional local therapy (two of unknown etiology and two postsurgical) that healed within several days after the discontinuation of colchicine prescribed for another pathology, which could suggest that colchicine can inhibit the healing of this tissue (*73, 74*).

VI. Intoxication

Poisoning can be intentional in an attempted suicide or accidental, and in most cases is due to a dose poorly adapted to the situation. Colchicine has a low safety margin. Fewer than 50 such incidents were reported between 1947 and 1991 in the United States, but more events have occurred in France since 1977 (*75*). The dose responsible for death from intoxication varies widely: roughly, when a single oral dose was less than 0.5 mg/kg, the patients survived; when the dose was higher that 0.8 mg/kg, death occurred within 72 h (*75*). According to the more recent review of the literature by Putterman *et al.*, published in 1991, when administered orally, toxicity developed with doses ranging from 16 to 60 mg of colchicine (*63*).

Three successive clinical stages can be noted (*15*). Digestive signs can start within the first 6 h following administration: profuse diarrhea, abdominal pain, repeated vomiting leading to hypovolemia. Multivisceral dysfunction follows associated with hypothermia, rhabdomyolysis, medullary insufficiency, disseminated intravascular coagulation, metabolic acidosis, electrolyte imbalances (hyponatremia, -phosphatemia, -magnesemia, -calcemia) and convulsions. Sepsis of digestive tract origin can also develop. Colchicine is directly toxic to the heart, especially during the first 36–54 h; the prognosis of cardiogenic shock is poor. Respiratory failure is multifactorial: hypoperfusion, paralysis of respiratory muscles, interstitial and alveolar edema. Renal insufficiency is caused by hypoperfusion, necrosis of the pyramides and rhabdomyolysis. Medullary aplasia is usually of short duration (*75*). Inappropriate secretion of anti-diuretic hormone has been described (*75, 76*). A fatal Waterhouse–Friderichsen syndrome occurred after administration of 10 mg of colchicine (*77*).

Most accidental intoxications are due to inadapted dosing: a too high intravenous dose combined with an oral dose (*78*), the presence of renal (*30, 45*) and/or hepatic insufficiency, advanced age, interaction with another drug (*63*). However, despite the

lack of dose adaptation, no severe side effects were observed in patients treated for renal amyloidosis secondary to familial Mediterranean fever (*79*) or in a study of 30 patients with primary biliary cirrhosis (*80*). As mentioned above, the interference between cyclosporine and colchicine has been responsible for intoxications. Interactions with cytochrome *P*-450 inhibitors have also been reported (*63, 81*).

The treatment of intoxication is symptomatic. The mortality rate is high. Because the volume of distribution is high, hemodialysis is not very effective. Recently, immunotoxicotherapy has brought new hope (*63*). The intracellular binding of colchicine is reversible *in vitro*: administration of anti-colchicine antibodies before or after that of a toxic dose lowered mortality in animals, and efficacy was dependent upon the antibody/colchicine concentration ratio. Along the same line of thinking, polyploidies and chromosomal aberrations regressed after *in vitro* administration of specific antibodies (*82*). Anti-colchicine-specific Fab fragments (480 mg) were successfully injected intravenously to combat resistant cardiogenic shock in one patient (*83*): the classical phases of intoxication did not develop, free colchicine was undetectable as of the 7th h, the volume of distribution decreased 4.4-fold and the urinary excretion rate was multiplied by 6. Because these Fab fragments are not available in routine practice, this approach remains experimental. Nevertheless, a second case has been reported (*84*). Use of hematopoietic growth factor (granulocyte–colony stimulating factor (G-CSF)) was able to limit the intensity and the duration of neutropenia (*85, 86*).

VII. Indications of Colchicine in Internal Medicine

The efficacy of colchicine in the treatment of gout has been known at least since the time of Diocles, the Greek physician who lived in the 3rd century AD. For several decades, colchicine has been prescribed for numerous other systemic pathologies: familial Mediterranean fever, amyloidosis, Behçet's disease, progressive systemic sclerosis, dermatopolymyositis, leukocytoclastic vasculitis, primary biliary cirrhosis, primary sclerosing cholangitis, atrophic polychondritis, pulmonary fibrosis, sarcoidosis... Numerous other clinical situations might benefit from also colchicine (*87*).

A. GOUT

Since antiquity, colchicine has been the treatment of choice, even though its mechanism of action has not been clearly elucidated: it decreases the leukocyte influx and the resulting phagocytosis. However, some doubt remains because trimethylcolchicinic acid does not interact with microtubules and is as effective as colchicine in this pathology (*88*). The therapeutic efficacy of colchicine in the treatment of gout was objectively proven for the first time in 1987 in a double-blind, controlled trial *versus* placebo that included 43 patients. In addition, colchicine generated fewer side effects than anti-inflammatory drugs (*89*).

B. Familial Mediterranean Fever

The pathogenesis of periodic polyseritis or familial Mediterranean fever is unknown. This hereditary disease is characterized by recurrent polyseritis whose main clinical manifestation is a relapsing febrile syndrome with acute abdominal pain. Its prognosis is poor because of the inevitable occurrence, at more-or-less long term, of (AA form) amyloidosis in the kidneys (proteinuria, nephrotic syndrome, renal insufficiency) and/or elsewhere. The interval between proteinuria and end-stage renal insufficiency can vary from 2 to 10 years, and renal insufficiency can last from 6 months to 6 years (*90*). The frequency of amyloidosis is high in Sephardic Jews, intermediate in Turks and Arabs, and low in Armenians: 60 of the 83 Sephardic Jews died before the age of 30 years prior to the era of colchicine use for this indication and kidney transplantation (*91*). Colchicine is able to prevent the episodes of abdominal pain of familial Mediterranean fever and is the only drug able to block the overproduction of amyloid. Its efficacy in familial Mediterranean fever is associated with the reduced chemotaxis of polymorpho-nuclear leukocytes, the decreased release of chemotactic factors and the restoration of suppressor T-lymphocyte function (*16-18*). The various steps in the demonstration of the therapeutic efficacy of colchicine in familial Mediterranean fever are reported in detail below, followed by descriptions of its interference with cyclosporine, its impact on reproduction, and its effectiveness and consequences in children.

1. Stepwise Demonstration of Colchicine Efficacy

Colchicine prevents the episodes of abdominal pain, stops the accumulation of amyloid deposits and, for those already present, can induce their regression, and can prevent the development of amyloidosis in a transplanted kidney.

a. The Prophylactic Treatment of Episodes of Abdominal Pain. A new era for familial Mediterranean fever patients began in 1972, when Goldfinger (*92*) showed that colchicine, given continuously at a dose of 0.6–1.8 mg/day, was able to prevent painful crises in three out of five patients. Benmussa had already suggested such a possibility at the North African Pediatrics Meeting of 1954 held in Tunis (*93*). About 20 molecules were tested unsuccessfully against familial Mediterranean fever from 1950 to 1967 (*90*). Three prospective, double-blind, controlled studies confirmed the efficacy of colchicine in preventing painful episodes. Using a cross-over protocol, Goldstein and Schwabe (*94*) evaluated ten patients taking 1.8 mg/day per os for 3 months, then a placebo for 3 months or *vice versa* and found a significant reduction of the number of episodes in the colchicine-treated group ($P < 0.002$): 80% of the treated patients had not experienced a painful episode *versus* 10% of those taking the placebo. The attacks were significantly less frequent under colchicine ($P < 0.01$), as of the first month of therapy, in the cross-over study, conducted by Zemer *et al.* (*95*), which included 22 patients who took 1 mg of colchicine/day orally for 2 months, then a placebo for 2 months and *vice versa*. Dinarello *et al.* (*96*) prescribed, for an acute episode of abdominal pain, 1.8 mg of colchicine/day for 28 days to eleven patients who were then

assigned to receive either colchicine or a placebo for a period of 11 months; patients taking colchicine had significantly fewer attacks ($P < 0.001$): seven episodes for 60 on colchicine as opposed to 38 for 60 taking the placebo. In addition, the crises were less severe under colchicine ($P < 0.02$) and less frequent with a daily dose of 1.8 mg, as compared to 1.2 and 0.6 mg. A fourth controlled double-blind study (*97*), which included nine patients over a period of 10 months, demonstrated that this molecule can decrease the intensity of, or eliminate, 75% of the painful episodes, while the placebo had similar effects on only 10%.

These observations were confirmed for prolonged treatment durations (1–4 years, i.e., 60 patient-years): 46 of the 47 patients receiving 0.5–1.5 mg/day of colchicine were asymptomatic (*98*). In contrast, once the acute episode had started, colchicine was not effective and, in the case of non-compliance, pain reappeared rapidly (*98*). The drug was well tolerated and no escape or acquired-resistance phenomena were recorded. In addition, the growth of five affected children which had been retarded until the introduction of colchicine became normalized after at least 6 months of colchicine therapy (*98*). The conclusions drawn by Zemer *et al.* (*61*) confirmed the efficacy of prophylactic therapy lasting 1–3 years (prevention of painful attacks for 50 of the 84 patients and decreased frequency and intensity for 28 of them), the normalization of growth in children, and the good evolution of pregnancies without interruption of treatment during the period surrounding conception and the first trimester. Additional elements came to light: no proteinuria, which would indicate the development of renal amyloidosis, became worse or appeared during the course of therapy; and the initial proteinuria levels, evaluated as ++ or +++ (13 patients), decreased or became negative at successive determinations (*61*). At this stage in the analysis of colchicine, we are in 1976. It can already be perceived that this drug can prolong life expectancy by preventing amyloidosis or causing it to regress.

b. Prevention of Amyloidosis. Indeed, colchicine blocks the development of casein-induced experimental amyloidosis in mice, perhaps by inhibiting the secretion of serum amyloid A (SAA) protein by hepatocytes (*99*). In 1986, after 4–11 years of follow-up, Zemer *et al.* (*79*) were able to demonstrate that continuous administration of colchicine at a dose of 1 or 2 mg/day prevented the development of amyloidosis. Their prospective study of 1070 patients, among whom 960 had no amyloid deposits at inclusion, was initially non-controlled, but became so because of the emergence of a group of non-compliant patients. No renal puncture biopsy was performed. Proteinuria appeared within 9 years in 49% of the 54 non-compliant patients as opposed to 1.7% of the 906 treatment-adherent patients over 11 years ($P < 0.0001$). Among the latter, two had non-insulin-dependent diabetes with no signs of amyloidosis on their rectal biopsies, thereby suggesting that the nephropathy was of diabetic origin and not associated with familial Mediterranean fever. It should be mentioned that amyloidosis, regardless of its localization, can be diagnosed by rectal biopsy. Four percent of the compliant patients continued to have abdominal pain independently of colchicine efficacy against amyloidosis. The 110 patients with signs of amyloid nephropathy before the initiation of colchicine evolved differently according to the stage of their renal

involvement: proteinuria remained stable in 68 of the 86 positive patients while it became negative in five, and became more severe with the development of renal insufficiency in 13; renal function deteriorated in conjunction with a nephrotic syndrome in nine patients and/or renal insufficiency in 15. In the retrospective study of 45 patients conducted by Ben-Chetrit and Lévy (*54*), the majority of the patients took 1 or 1.5 mg of colchicine/day for at least 15 years; the four subjects with proteinuria at entry remained stable with no change in renal function. It should also be noted that only 2.3% of the 435 children taking colchicine developed amyloidosis (*100*).

Thus, colchicine prevents the development of amyloidosis in familial Mediterranean fever.

c. Regression of Amyloidosis. In 1992, Zemer *et al.* (*101*) reported that daily doses of 2 mg (2 patients) or 1 mg (1 patient) of colchicine taken for 3–9 years were able to reverse nephrotic syndromes that were present at the onset of therapy. Despite the absence of a controlled study, the recommended dose for patients with renal involvement, but normal renal clearance, is 2 mg/day. Indeed, in their 1997 report, Ravid *et al.* (*102*) came to the same conclusion after 30 months of follow-up of three patients whose nephrotic syndromes regressed, and whose renal function improved, under daily colchicine doses of 1.5 mg. In all three cases, the rectal biopsies taken 2–3 years after the initiation of colchicine demonstrated the clear-cut diminution of the amyloid deposits. The reversal of nephrotic syndromes was also described for two similar isolated cases: one patient took 1 mg of colchicine/day for 21 months (*103*), whereas the other received 1.5 mg/day for 48 months (*104*). In the former patient, the renal amyloid deposits regressed by 60%.

d. Prevention of Amyloidosis after Kidney Transplantation. Prophylaxis with at least 1.5 mg of colchicine/day should be prescribed in conjunction with immunosuppressants to avoid the development of familial Mediterranean fever-associated amyloid deposits in the grafted organ, or its progression to extrarenal sites (*105*). In a retrospective study of 21 individuals who received renal transplants because of amyloid nephropathy, proteinuria was again detected after 3 years in eleven patients and was directly attributable to amyloidosis in eight of them: the rate of disease recurrence was significantly higher when a daily colchicine dose of 0.5 mg was prescribed as opposed to 1.5 mg ($P = 0.0002$). As a corollary, those patients taking < 1 mg of colchicine/day developed proteinuria more rapidly (2.8 years) than those taking > 1 mg/day (8.8 years) ($P = 0.004$) (*105*).

e. Unusual Clinical Forms of Familial Mediterranean Fever. Colchicine was also proven to be effective in three patients with severe pyoderma occurring during episodes of abdominal pain; the cutaneous symptoms did not recur under maintenance therapy (*106*). In addition, this molecule seems to have been active against recurrent benign meningitis presenting as lymphocytic meningitis or transient aseptic mixed leukocytic pleocytosis, and spontaneously regressing in a patient with familial Mediterranean fever. This clinical association is rare: approximately five published cases among the 2000

with familial Mediterranean fever reported in the literature (*107*), and, in a cohort of 101 children, one of the 13 with neurological symptoms (*108*). Despite having been resistant to anticonvulsant drugs, the latter group of children stopped having convulsions after starting colchicine. Colchicine also appeared to be effective in another patient presenting this clinical form of familial Mediterranean fever (*109*).

2. Interference Between Colchicine and Cyclosporine

Colchicine prevents, and sometimes can even cause, the regression of familial Mediterranean fever amyloidosis. However, a drawback exists because the combination of oral cyclosporine, at a dose giving a non-toxic plasma concentration (100–250 ng/ml), and oral colchicine (1 mg/day) is poorly tolerated. Cyclosporine inhibits the biliary excretion of colchicine in the rat (*110*). Through its inhibitory effect on glycoprotein P, cyclosporine decreases the biliary and renal clearances of colchicine (*8,9*). Despite the good tolerance of colchicine prior to renal transplantation, gastrointestinal and/or muscle side effects developed early in seven patients followed by Cohen *et al.* (*111*). These undesirable effects were reversed by the withdrawal of cyclosporine (5/5 cases), attenuated by lowering its dose (1/2 cases), unaffected by the discontinuation of colchicine (1/1 case), and recurred upon the reintroduction of cyclosporine (2/2 cases). These adverse events were not attributed to cyclosporine because its serum concentrations were within the desired therapeutic range considered to be not toxic. In contrast, the colchicine level was never determined. Siegal *et al.* reported similar findings concerning three subjects with the same regimen of immunosuppressive agents. The side effects were reversible for all three, with a lowering of the cyclosporine dose for one and its withdrawal for the other two in whom this approach was unsuccessful (*112*). These observations necessitate a re-evaluation of the immunosuppression protocol prescribed for familial Mediterranean fever. Indeed, the outcome of renal transplantation for all pathologies combined is not optimal when cyclosporine is not given. Thus, Siegal *et al.* proposed using cyclosporine immediately after transplantation and then decreasing its dose or changing the immunosuppressant at the first signs indicating interference with colchicine (*112*). This scheme would have the advantage of decreasing the total dose of corticosteroids required and lowering the mortality and morbidity inherent in immunosuppressive therapy. The ideal would be to adjust the doses of colchicine and cyclosporine as a function of their serum concentrations.

3. Colchicine and Reproduction

Despite the absence of prospective and controlled studies, several conclusions have been drawn concerning the effects of colchicine on male fertility and female fecundity, and its teratogenic risk.

a. Male Fertility. The princeps report described azoospermia that could be reversed by stopping therapy, but reappeared with the reintroduction of the drug (*52*), in agreement with other observations (*62*). Ben-Chetrit and Lévy (*54*) recorded only one case of irreversible azoospermia upon withdrawal of colchicine in their 15-year study of 45 patients, 23 of whom were men. Also, it should be noted that the three children

sired by three treated fathers were normal (*61*). The semen analyses, blood testosterone, follicle-stimulating hormone, luteinizing hormone and prolactin levels were normal for six men who had been treated for 7–31 months. In addition, three children of three of these fathers who had been taking colchicine at the time of conception were normal (*55*). Ghozlan *et al.* (*59*) did not describe any fertility disorders and reported several normal pregnancies attributed to the treated fathers. Peter *et al.* (*28*) told of only one case of disturbed libido in a series of 62 patients, including women, who had been taking colchicine for more than 3 years. It should be noted that, in this study, it was recommended that colchicine be discontinued 3 months prior to attempting to conceive a child. Ehrenfeld *et al.* (*53*) divided the 39 pregnancies issued from 18 men, treated with colchicine (0.5–2 mg/day) and followed for 11 years, into three groups: 14 normal infants were born to six men before any colchicine therapy; among the 12 documented conceptions attributed to eight treated men, three ended in miscarriages; 13 normal children were fathered by nine treated men who had discontinued colchicine 3 months prior to conception. In this series, three young men produced sperm with penetration abnormalities which prevented conception while they were taking colchicine, but which were reversed by drug withdrawal (*53*). However, prudence is warranted before blaming azoopermia on colchicine because testicular amyloidosis can cause azoospermia (*113*). A clear conclusion would be to advise semen analysis for all men prior to initiation of colchicine.

b. Female Fecundity. Familial Mediterranean fever itself can lower fertility. Primary infertility, for the most part attributable to ovulatory disorders, was evaluated to be at least 50% for 15 untreated women aged 20–35 years in a non-selected population with familial Mediterranean fever (*114*). This figure was much higher than the 10–15% infertility rate of 665 supposedly healthy Israeli couples (*115*). One of the hypothetical etiologies for female infertility in this pathology is the existence of peritoneal adhesions secondary to episodes of abdominal pain. Despite promising experimental data from animals, where the number of induced abdominal adhesions diminished significantly under colchicine, fertility does not seem to be improved from this point of view in humans (*64, 116*). Indeed, 36% of the 36 women treated for 3–12 months were sterile (*64*), a percentage lower that than for untreated women, but higher than that for supposedly healthy couples. However, twelve women, for whom hormone treatment had been unsuccessful in nine, carried a pregnancy to term after 1–15 months of colchicine therapy (*59*). In parallel, the miscarriage rate decreased significantly to 20.2% for 94% of the untreated women and to 12.2% for 131 treated women (*66*). Similarly, the frequency of spontaneous abortions sometimes increased when colchicine was stopped, attributable, at least in part, to the enhanced number of episodes of abdominal pain (*117*).

c. Teratogenicity. Although colchicine was detected in the umbilical cord of a fetus whose mother was taking the drug (*118*), the possible teratogenic role of this molecule has not been demonstrated. The frequencies of mitoses, and the percentages of tetraploid cells and chromosome breaks in cultured lymphocytes from 22 patients treated with colchicine (0.5–1.5 mg/day) for 1 week to 36 months were normal compared to ten controls (*62*). Almost 300 pregnancies, for which one of the parents

was on long-term colchicine therapy including during the period of conception, gave rise to numerous normal children and several miscarriages (*54, 55, 59, 61, 62, 64-66, 68, 98, 117, 119*). Among 256 newborns, five cases of trisomy 21 were reported, but without any details concerning the ages or karyotypes of the parents (*59, 66, 68, 117*). For one of these children, the father's semen analysis made prior to colchicine administration showed 85% abnormal forms (*59*). Among 131 pregnancies, two such cases concerned women under 30 years of age (*66*). In another series, the findings differed as a function of the time at which colchicine was stopped: five miscarriages, five abortions and seven normal infants for the 17 withdrawals after conception; 22 normal babies and two miscarriages for the 24 discontinuations before conception; nine normal children and two miscarriages when colchicine was maintained (*64*). The different authors agree that colchicine should be maintained during the period of conception and pregnancy, and recommend that amniocentesis be performed early to karyotype the fetus (*54, 64, 68, 117*). Pregnancy can even be considered after kidney transplantation. A normal child was born to a mother who had received a renal graft 17 months earlier for amyloidosis secondary to familial Mediterranean fever and was on a 3-drug immunosuppression regimen (cyclosporine, azathioprine, prednisolone) in combination with colchicine (*120*).

4. Efficacy and Consequences of Colchicine in Children

The literature includes several large-scale studies on this subject. Colchicine is as effective as in adults at preventing episodes of abdominal pain (*121-123*) and preventing amyloidosis even when the painful syndrome persists (*100, 123*). It can also attenuate proteinuria (9/17 patients) (*123*). The recommended daily dose is 1–2 mg, independent of weight (*61, 121, 123*), without the development of side effects (*122, 123*).

Colchicine is excreted in human milk at variable concentrations (*124-126*). The quantity ingested by the suckling infant would be low (*125, 126*), especially when the baby is nursed 8 hours after the mother has taken the drug (*125*).

Overall, colchicine does not stop the child from growing normally, or almost normally, compared to untreated children with familial Mediterranean fever. No growth or developmental anomaly was reported for 184 children whose conceptions and gestations progressed under colchicine therapy (*66*). No conclusions concerning growth could be drawn from a prospective study of 14 children treated for 29 months (*121*). The growth of five children who had been retarded until treatment initiation became normal after 6 months of colchicine therapy (*98*). According to another study (*54*), seven treated children grew normally. Zemer *et al.* reported that, at 17 years of age, the height of the 350 children they followed fell within the normal range and the mean height of a treated child was greater than that of an untreated child (*123*).

To date, only Zemer *et al.* have studied the reproduction of patients chronically treated with colchicine during their childhood (*123*). However, this cohort of 350 subjects is still young. Nineteen of the 24 married men have fathered normal children. This rate of primary infertility is lower than that of untreated women. Among the 95 married women, 31 were able to conceive 48 offspring while taking colchicine: 44

normal children were born at term and four spontaneous abortions occurred during the first trimester (every woman who miscarried had previously had at least one normal pregnancy while taking colchicine). The gestational periods and the babies' weights were within their respective normal ranges. No fetal anomaly has been reported. However, it is still too early to evaluate the consequences on the offspring of long-term colchicine administration during gestation.

C. MOLLARET'S MENINGITIS

The pathogenesis of this meningitis, characterized by cerebrospinal fluid containing a mixture of cells, including giant 'endothelial-like' cells or Mollaret's cells, is unknown. Although the distinction between the relapsing meningitis of familial Mediterranean fever and Mollaret's meningitis is unclear, the latter is not associated with polyseritis or amyloidosis, and is usually self-limiting. Few therapeutic options are available. In several patients, disabling disease could be attenuated or even cured with 1–1.5 mg/day of colchicine; corticosteroids had been ineffective for two out of four cases (*127-130*). Despite good compliance, colchicine also failed in one case (*131*). These observations must be interpreted with caution because of the small number of cases reported.

D. AMYLOIDOSIS

Because colchicine has been shown to be effective in preventing the secondary AA amyloidosis of familial Mediterranean fever, it has been prescribed for primary or secondary AL amyloidosis and AA amyloidosis secondary to a pathology other than familial Mediterranean fever (*132*). No drug has been proven to be curative in these poor-prognosis diseases. For AL amyloidosis, for which no animal model exists, colchicine provides no objective benefit. Secondary AL amyloidosis will not be addressed because its treatment is based on that of the underlying hemopathy. Chemotherapy is often prescribed for primary AL amyloidosis because of the relationship existing between myeloma and primary AL amyloidosis. Colchicine has been given in combination with chemotherapy and sometimes as monotherapy.

1. Colchicine and Primary AL Amyloidosis

In 1983, the Mayo Clinic reported a median duration of survival of 12 months for 229 patients with primary AL amyloidosis (*133*). Also described (*134*) was the exceptional case of a patient still alive after 19 years of evolution of primary AL amyloidosis with digestive, cardiac and pulmonary localizations, but without monoclonal protein or medullary plasmocytosis. Without being able to formulate any definitive conclusion, this woman received the following treatment: after 4 years of evolution, the combination of melphalan and prednisone for 30 months; then, after 13 years of evolution, melphalan–colchicine (1.2 mg/day) for 84 months. According to another report, the nephrotic syndrome of a patient with primary AL amyloidosis was attenuated at the end of 1 year under 2 mg of colchicine/day; this finding persisted at

30 months of follow-up (*102*).

Although several failures have been reported (*132, 135*), colchicine does not seem to lack activity, and the results obtained with it were compared to those observed under chemotherapy. In an open study, median survival of 53 patients seen between 1976 and 1983 and prescribed 0.5–1 mg of colchicine/day as monotherapy was 17 months, whereas that of a retrospectively constituted group of 29 patients seen between 1961 and 1973, and not given colchicine, was 6 months (*136*). In a double-blind, randomized trial including 55 patients, six of the 13 patients treated with the melphalan–prednisone combination for > 12 months were clinically improved, as compared to the placebo group, suggesting that treatment with this combination was beneficial. Concerning 24 patients with a nephrotic syndrome, its manifestations disappeared in two and proteinuria decreased by > 50% for eight. However, survival remained unchanged (*137*).

Subsequently, colchicine's efficacy was compared directly to that of melphalan-prednisone: in a randomized trial of 101 patients, some of whom participated in a cross-over protocol, the median survival durations observed for the combination and colchicine (1.2 mg/day) after 12 months of treatment were not significantly different (25.2 *versus* 18 months, respectively). Nevertheless, the median survival of the 41 patients who had received only the combination regimen was 16 months while that of the 17 patients who had received only colchicine was 3 months (*138*). In a small series of seven patients, five were still alive after 17–60 months of treatment combining melphalan, prednisone and colchicine, the nephrotic syndrome had regressed in two, and two had improved cardiac function (*139*). Two recent studies enabled better comparison and evaluation of three therapeutic regimens: melphalan–prednisone–colchicine (1.2 mg/day), melphalan–prednisone, or colchicine alone (1.2 mg/day) (*140, 141*). A randomized trial of 100 patients receiving either colchicine alone (1.2 mg/day) or melphalan–prednisone–colchicine (1.2 mg/day) showed longer median survival in the group given melphalan (6.7 *versus* 12.2 months, $P = 0.087$) (*140*). In a randomized trial including 220 patients stratified according to their clinical characteristics, median survival was 8.5 months for patients treated with colchicine alone (at least 1.2 mg/day) as opposed to 18 months for those receiving melphalan–prednisone and 17 months for those assigned to receive melphalan–prednisone–colchicine ($P < 0.001$). Thus, the melphalan–prednisone combination prolonged survival in comparison to colchicine alone, with comparable median durations of survival for patients taking melphalan–prednisone or melphalan–prednisone–colchicine (*141*). Adjunction of colchicine to the melphalan–prednisone regimen does not seem to provide any additional benefit.

2. Secondary AA Amyloidosis Outside the Context of Familial Mediterranean Fever

The principal treatment of AA amyloidosis is that of its cause. However, based on its beneficial effect on familial Mediterranean fever and several cases of AL amyloidosis, colchicine could be added to this etiological therapy. Several isolated successes have been reported. An amyloid nephrotic syndrome secondary to granulomatous colitis regressed in 7 months under 1 mg of colchicine daily and remained stable over 61 months of follow-up (*103*). Two nephrotic syndromes caused

by amyloidosis secondary to ulcerative colitis disappeared after 108 and 8 months of treatment with colchicine (1.2 mg/day), intermittently combined with corticosteroids, independently of the evolution of the causal disease (*142*). Another patient's nephrotic syndrome regressed after intestinal resection for Crohn's disease and 5 years of colchicine (1.5 mg/day) (*143*). After 2 years of colchicine (1 mg/day) and sulfasalazine, a nephrotic syndrome secondary to ankylosing spondylarthritis disappeared (*144*). A nephrotic syndrome that persisted after excision of a tumoral mass of Castleman's disease was attenuated after 9 months of treatment with colchicine (1.5 mg/day) and was immediately followed by a pregnancy with a favorable evolution (*145*).

The outcomes of two patients with cystic fibrosis complicated by AA amyloid nephropathy were less evident and should be seen in light of the fact that, at this stage, the prognosis for this pathology is dismal with a mean survival of < 1 year. Colchicine was able to stabilize the nephrotic syndrome for one patient and delay the appearance of renal insufficiency for the other, but the latter patient died, nonetheless, shortly thereafter (*146*).

Several failures of colchicine have been published, but their real frequency is probably underestimated. The drug was unable to attenuate the amyloid nephropathy of Muckle–Wells syndrome (*147*), a genetic disorder characterized by recurrent urticarial rashes, arthralgias, and progressive sensorineural deafness, and complicated by amyloidosis. Likewise, colchicine does not seem to prevent the development of the b_2-microglobulin amyloidosis in hemodialysis patients (*148*),and did not modify the serum b_2-microglobulin concentrations in a group of 15 hemodialyzed patients (*149*).

These observations should be interpreted with caution because of the short duration of treatment and the small number of patients studied. Nevertheless, generally speaking, adding colchicine to any therapeutic regimen for secondary amyloidosis should be considered.

E. BEHÇET'S DISEASE

Behçet's disease, a systemic vasculitis more commonly encountered in the Middle East and Japan, is characterized by buccal and genital aphthous ulcers, diverse cutaneous lesions, uveitis able to cause blindness, arthritis, thrombophlebitis, neurological and gastrointestinal manifestations, and arterial aneurisms. All organs can be involved. Its pathogenesis remains unknown: hyperactivity of polymorphonuclear leukocytes and platelets has been advanced as has the enhanced release of cytokines by monocytes. Colchicine diminishes or normalizes the chemotaxis of polymorphonuclear leukocytes (*150-152*) and blocks phagocytosis, thereby decreasing the secretion of chemotactic factors (*153*). It has been widely used in Japan and has been shown to be effective against aphthae and uveitis. At present, immunosuppressive agents in combination with corticosteroids are the preferred treatment for severe lesions, be they ocular (posterior uveitis, panuveitis, retinal vasculitis, treatment-resistant lesions), neurological, gastrointestinal, articular...(*154, 155*). Other molecules, for example, levamisole, thalidomide or dapsone, have also been tested and, more recently, penicillin G benzathine and the interferons.

The first double-blind controlled study to evaluate colchicine (1 mg/day for 6 months) *versus* placebo did not demonstrate any significant difference, as far as cutaneous, articular or ocular signs are concerned, between the two groups of 14 patients each. Nevertheless, a difference seems to exist in favor of colchicine for erythema nodosum-like cutaneous lesions and arthralgias (*156*). Matsumura and Mizushima (*150*) prescribed 1 mg of colchicine/day to twelve patients with Behçet's disease evolving for approximately 2 years: after 8–9 months, ocular signs disappeared in seven of them and, for the most part, aphthae and arthralgias were attenuated. Ocular lesions regressed for 83% and mucocutaneous manifestations diminished for 58% of the 24 patients participating in a non-controlled prospective trial who received a daily dose of 1 mg of colchicine for > 1 year (*151*). Among the 157 patients enrolled in a non-controlled study testing the efficacy of 1 mg of colchicine/day for 1 year, 104 showed ocular improvement (*157*). The same authors had to stop their parallel controlled investigation of colchicine *versus* non-steroidal anti-inflammatory drugs because of the lack of improvement of severe ocular lesions in the latter group (*157*). Other isolated case reports have evoked the same trends. Following 10 months of failure under diverse therapeutics including corticosteroids, the anterior uveitis, cutaneous aphthae, arthralgias, and gastrointestinal signs regressed after 5 weeks of colchicine (1.2 mg/day) in one patient and recurred 2 weeks after its withdrawal (*158*). Another similar case was also reported (*159*). In addition, colchicine was effective against the oral aphthosis and genital erosions of ten patients (*160-164*); the arthropathies of five (*163-165*); the ocular lesions of five (*160, 166*), one of whom was a 14-year-old adolescent who had anterior uveitis associated with panuveitis (*166*); and thrombosis of the superior vena cava for which treatment was combined with corticosteroids (*167*). Symptoms recurred, sometimes rapidly, after the discontinuation of colchicine (*158, 159, 161, 166*), even when this drug had been given in combination with an immunosuppressant (*168*).

Sometimes, colchicine has been effective after corticosteroids or immunosuppressants failed (*158, 160, 163*). On the other hand, several publications described failures, whose frequency is probably underestimated: short-term treatment (1 mg/day for < 1 month) of the oral aphthosis and genital erosions of one patient with cutaneous lesions and arthropathy (*169*), short-term therapy (< 1 month) of arthropathy (*170*), five failures and seven intermediate responses for 13 patients with cutaneous–articular forms of the disease (*171*).

The superiority of immunosuppressive agents over colchicine has been difficult to elucidate: the former are sometimes preferred in combination with colchicine, especially for very severe uveitis. The first agent studied was cyclophosphamide. Ninety-one patients were divided into four groups: 50–100 mg of oral cyclophosphamide/day; 0.5–1 mg of colchicine/day; 50 mg of oral cyclophosphamide/day and 0.5 mg of colchicine/day; and the fourth group was comprised of previously recruited patients who had received either corticosteroids, non-steroidal anti-inflammatory drugs or antibiotics, or undergone desensitization. With a mean treatment duration of 1 year, compared to the fourth group, the number of acute ocular episodes was significantly lower for the first three groups ($P < 0.01$), and visual acuity was improved or had

stabilized. It should be noted that the fewest ocular episodes per year occurred in those patients on colchicine monotherapy (*172*). The clinical criteria – frequency of ocular attacks and visual acuity – used to evaluate efficacy during and after treatment with oral cyclophosphamide (100–150 mg/day) or colchicine (1–1.5 mg/day) were similar for 64 patients also taking corticosteroids during exacerbations (*173*). The efficacy of pulse cyclophosphamide against severe uveitis was demonstrated and was associated with improved visual acuity in 54% of the cases, and patients with central nervous system involvement experienced attenuation of their symptoms; some of these patients had not benefited from any improvement under previous immunosuppressive therapy (*168*). Although other such agents (cyclophosphamide, chlorambucil or methotrexate) have been evaluated, these studies were not controlled and were unable to discern an advantage of one over the others (*154, 168, 174*).

The only double-blind, randomized, prospective trial *versus* placebo was conducted with azathioprine (2.5 mg/kg/day). Significant attenuation and prevention of ocular exacerbations were obtained in the azathioprine group, regardless of whether the patients had previously experienced ocular involvement or not. Concomitantly, oral aphthosis and genital erosions and arthritis also regressed significantly (*175*). The beneficial effect of cyclosporine was confirmed in a double-blind, randomized trial including 96 patients and comparing cyclosporine (10 mg/kg/day) to colchicine (1 mg/day). Despite numerous severe side effects, cyclosporine was significantly more effective than colchicine at lowering the frequency ($P < 0.001$) and intensity ($P < 0.001$) of ocular episodes, improving visual acuity and attenuating cutaneous ($P < 0.01$) and mucosal lesions ($P < 0.001$) (*176*). In a retrospective study of 22 patients, for nine of the twelve patients with uveitis who took azathioprine or cyclosporine, these positive results were confirmed (*155*). The azathioprine–cyclosporine–corticosteroids combination can be effective against resistant cutaneous, articular and/or ocular lesions (*155*).

Levamisole, thalidomide and dapsone have been used, but not compared to colchicine. No controlled study has been conducted for levamisole, which can be useful in treating mucosal and/or ocular lesions (*177-179*). Thalidomide has been reported to be effective against mucocutaneous ulcers, arthritis and, sometimes, ocular lesions (*180*). But, in another study, it seemed to be ineffective against these latter (*181*). In addition, it is responsible for numerous side effects (*181*). Dapsone proved to be effective against the aphthae, erythema nodosum-like lesions and pathergy of seven patients without any reported adverse events (*182, 183*).

Penicillin G benzathine has been evaluated for the prevention of arthritis in a randomized trial including 120 patients taking either this molecule alone (1.2 MU intramuscularly every 3 weeks) or in combination with colchicine (1.5 mg/day) for 24 months. The duration and severity of inflammatory exacerbations of arthritis were similar in the two groups compared to the preceding year, which served as the reference. Nevertheless, the frequency of arthritis exacerbations was significantly lower, and the crisis-free interval was significantly longer in the group receiving combined colchicine–penicillin G benzathine therapy. Thus, penicillin G benzathine can effectively prevent arthritis episodes, provided that it be given in conjunction with colchicine

(*184*). Similarly, in a prospective study comparing colchicine alone (60 patients taking 1–1.5 mg/day) to colchicine–penicillin G benzathine (94 patients taking, respectively, 1–1.5 mg/day, and receiving 1.2 MU intramuscularly every 3 weeks), the frequencies and durations of buccal aphthae and cutaneous lesions, and the frequency of genital aphthae were significantly reduced in both groups and even more so in the group receiving combination therapy (*185*). Interferon-γ was effective against mucocutaneous lesions and arthritis (60% complete remissions and 20% partial remissions among ten treated patients), but not against ocular lesions (80% failures among five patients) in a retrospective series (*155*). Interferon-α was reported to be effective against mucocutaneous manifestations and arthritis (*186, 187*), and also several isolated cases of ocular involvement (*188, 189*).

Thus, colchicine appears to be effective in treating Behçet's disease, especially mucosal lesions and anterior uveitis, and its long-term administration seems to be justified, even though this has not been demonstrated. Colchicine seems to be the drug that has the best efficacy/toxicity ratio for long-term treatment of signs other than severe ocular or visceral involvement. These latter manifestations and resistant forms require the adjunction of an immunosuppressive agent. Ideally, all these observations should be confirmed by controlled studies.

F. Progressive Systemic Sclerosis

Progressive systemic sclerosis is a systemic pathology caused by the excessive accumulation of collagen that can infiltrate all organs, especially the lungs and the digestive tract. Its evolution is unpredictable and its objective evaluation difficult.

Despite diverse investigations, no specific, effective therapeutic agent has been identified. Because it is able to block the synthesis of collagen and stimulates the *in vitro* activity of collagenases, colchicine was prescribed to counter the functional anomalies of fibroblasts and collagen synthesis suspected in this disease. Other pathophysiological mechanisms are at play (autoimmunity, endothelial cell involvement). Findings have differed according to the study considered, but all had low enrollment, various stages of evolution, and short follow-up periods. In light of the complex pathogenesis of this disease, numerous other molecules have been tested, sometimes successfully: penicillamine D, platelet-aggregation inhibitors, immunosuppressants, interferon-γ...

The first publication dates from 1967. Using trimethyl-colchicinic acid, Housset (*190*) described the clear attenuation of skin infiltration in three patients with sclerodermal plaques as of the 15th day of treatment and in nine subjects with recent-onset acrosclerosis at the end of the first month of therapy. This attenuation and healing of cutaneous ulcers occurred in five of the 13 individuals with advanced acrosclerosis and in two of the nine patients with generalized progressive systemic sclerosis. This analogue allowed the administration of higher doses, compared to those tolerated for colchicine.

In a small, double-blind, randomized, cross-over trial, including 14 patients for 6 months, then open for 6 months, that tested colchicine (1 mg, 6/7 days) *versus* placebo,

nine patients improved under treatment as assessed by clinical criteria, and continued to do so during the open period. The skin collagen content, evaluated histologically, decreased in six patients. Serum proline concentrations decreased, and those of hydroxyproline increased, in eight cases, findings suggesting the enhanced degradation of collagen (*191*). The long-term outcome of these patients, with a mean treatment duration of 39 months, was reported in 1979. Based on clinical criteria of skin elasticity, improvement was noted for 17 of the 19 with generalized progressive systemic sclerosis, and three of the four with localized forms. The results were significantly better when the progressive systemic sclerosis had been evolving for < 5 years, and when the mean colchicine dose was > 1.43 mg/day (*192*). Diffuse cutaneous scleroderma regressed in 2 months in a young girl taking 1 mg of colchicine daily after the failure of penicillamine D that had been administered for 5 months. A relapse occurred rapidly after the discontinuation of colchicine which was successfully reintroduced (*193*). This drug can also be active against the subcutaneous calcifications secondary to progressive systemic sclerosis (*194*). Thus, this molecule might slow the progression of systemic scleroderma before the appearance of visceral localizations and improve the quality of life of a certain number of patients.

Colchicine was not effective in other patients. With treatment durations varying from 2 to 12 months, no significant effect on skin elasticity, as assessed with a Petechiometer, was observed for four non-treated and seven treated (daily dose of 1 mg of colchicine) patients. It should be kept in mind that the daily dose was only 1 mg, and the time since the onset of the disease was not mentioned (*195*). No improvement was recorded for ten subjects with advanced progressive systemic sclerosis (including six with esophageal involvement and five with pulmonary fibrosis), a mean of 9 years of evolution since onset and a mean weekly dose of 10.6 mg for 1 year. The disease continued to progress in some patients: two of them already had pulmonary fibrosis and disease progressing for < 5 years, and the third received a low weekly dose of colchicine (6.4 mg) (*196*). No clinical improvement was observed in eight patients treated with 2 mg/day for 8–24 months (*197*). A double-blind, cross-over study of twelve patients that lasted 12 months and compared colchicine (1.2 mg/day) to a placebo did not detect any difference in skin elasticity, as assessed clinically (*198*).

Among the other molecules evaluated, penicillamine D gave the best results with a prolongation of survival (*199*). In a retrospective series of 118 patients with progressive systemic sclerosis evolving for < 3 years, the 5-year cumulative survival time of patients taking penicillamine D for a mean duration of 24 months was longer than that of those who were not taking the drug ($P < 0.05$) (*200*). Furthermore, the speed with which visceral localization(s) appeared was significantly slower ($P = 0.01$) in this group. None of these improvements was observed under immunosuppressive therapy (*200*). Carbon monoxide diffusion was retrospectively found to be significantly improved in the 44 patients on penicillamine D, as compared to the 48 not taking the drug (*201*), with a mean treatment duration of 2.3 years. This significant improvement ($P < 0.005$) of carbon monoxide diffusion was subsequently confirmed in another retrospective study of 17 patients and ten controls, and a mean treatment duration of 2.5 ± 1.6 years (*202*). Penicillamine D is thus preferred over colchicine, despite its

frequent side effects that can necessitate its discontinuation.

The hope invested in platelet-aggregation inhibitors dissipated quickly. The combination of dipyridamole (225 mg/day) and salicylic acid (975 mg/day) given for 1–2 years to 28 patients with progressive systemic sclerosis of recent onset failed in a double-blind, randomized trial *versus* placebo (*203*). Other than isolated successes reportedly obtained with methotrexate (*204*), immunosuppressants seem to have little impact (*199, 205, 206*). Cyclosporine, after initial promising results, is being investigated (*199, 206-208*). The same can be said for interferon-γ, for which a first open, non-controlled trial showed significant attenuation of skin involvement, but no improvement of pulmonary function, after 6 months of treatment in ten patients (*209*).

Colchicine can be used for patients with skin lesions, but not systemic involvement, before prescribing penicillamine D, which is responsible for a certain number of side effects. However, prospective randomized trials including patients in the early stages of progressive systemic sclerosis are needed to determine the factors of disease progression, and to compare these different therapeutic agents.

G. DERMATOMYOSITIS CALCINOSIS

Dermatomyositis, an inflammatory pathology of skin and muscle, can be complicated in children by extensive calcinosis (universalis), that is to say subcutaneous then periarticular deposits of calcium hydroxyapatite. This series of events is independent of the evolution of the dermatomyositis and is often a source of sequelae. Colchicine has proven effective, probably because of its anti-inflammatory properties, without hindering the healing of future potential surgical débridement. The local inflammatory signs and general symptoms regressed in several days under 1.2–1.8 mg of colchicine/day in two children with stabilized dermatomyositis, but their hydroxyapatite deposits remained unchanged (*210*). A similar case was reported (*211*), as was the healing of the cutaneous ulcers that developed secondary to the calcifications in yet another (*194*). However, another case report described the failure of colchicine in this context (*211*).

H. LEUKOCYTOCLASTIC VASCULITIS

This type of vasculitis is usually localized in the skin and is histologically characterized by the presence of abnormal polymorphonuclear leukocytes infiltrating the walls of small vessels. Manifestations include purpura, superficial ulcerations and ulcers. Colchicine acts by modifying the chemotaxis of polymorphonuclear leukocytes and their lysosomal degranulation, and by blocking the release of kinins. Before the publication of Hazen and Michel's report (*159*), diverse and poorly effective therapeutic agents, including corticosteroids and immunosuppressants, had been proposed without the benefit of a controlled study (*212*).

Hazen and Michel described, for the first time, the successful administration of colchicine in an open study of four patients with idiopathic leukocytoclastic vasculitis and normal complement levels. Three of them relapsed when the drug was withdrawn

and responded favorably every time it was reintroduced. Prednisone and immunosuppressants had previously failed for two of them (*159*). Comparison of the different studies must be approached prudently because of the small numbers of patients, the different variables evaluated, and the existence, or not, of an underlying systemic disease. The results of an open, prospective trial including 13 patients with leukocytoclastic vasculitis, six of which were idiopathic and seven secondary to another systemic disease, were encouraging: nine complete responses with the possibility of stopping prior treatment (most frequently corticosteroids, non-steroidal anti-inflammatory drugs, hydroxychloroquine, dapsone) and three partial responses were obtained with 1.2 mg/day at a mean follow-up of 11.7 months. Among the ten patients followed, seven relapsed when colchicine was discontinued, but reintroduction of the molecule was always successful (*213*). Expanding this series to 26 patients, among whom 15 had idiopathic leukocytoclastic vasculitis, 17 complete responses and four partial responses were obtained at a mean follow-up of 4.8 years. It should be noted that 16 relapses occurred when colchicine was stopped (*214*). The cutaneous leukocytoclastic vasculitic lesions of a 14-year-old girl with a homozygous deficit in complement component C3 disappeared under colchicine and recurred twice when the drug was withdrawn (*215*). The skin and joint manifestations of a female patient were attenuated after 4 days of colchicine following the failures of corticosteroids and methotrexate (*216*).

The only prospective, randomized trial is relatively recent: 41 leukocytoclastic vasculitis patients were treated for 1–3 months with either oral (1 mg/day) or topical colchicine. The number of complete and/or partial remissions was the same in the two groups after 1 month (8 *versus* 10) and persisted at 3 months (8 *versus* 10) of treatment. Although no objective benefit in favor of colchicine seems to exist, for three patients in complete remission who relapsed when it was withdrawn, their manifestations regressed again when the drug was reintroduced. The conclusions drawn based on this study should be tempered because half the patients had leukocytoclastic vasculitis secondary to another pathology and the double-blind protocol was not applied (*217*). Colchicine has been reported to be effective against pustular cutaneous vasculitis secondary to rheumatoid arthritis, with the lesions becoming attenuated after 2 weeks of therapy and healing after 4 weeks (*218*). A retrospective study concluded that colchicine was ineffective against the idiopathic leukocytoclastic vasculitis of 13 patients who had responded poorly to corticosteroids and immunosuppressants (*219*). The results obtained with other medications (corticosteroids, immunosuppressants, dapsone) are a mixed bag (*219, 220*). Anti-malarial drugs exhibited poor efficacy (*220*).

Thus, colchicine can be effective in leukocytoclastic vasculitis, and usually in the absence, rather than the presence, of an underlying systemic pathology. It warrants being prescribed as first-line therapy before corticosteroids and immunosuppressants, which are more toxic.

I. URTICARIAL VASCULITIS

This localized vasculitis of the skin is characterized by an urticarial eruption. It can

be primary or secondary to a viral infection, systemic disease (systemic lupus erythematosus, Sjögren's syndrome) or serum sickness. Colchicine efficacy has only been observed in several cases of urticarial vasculitis and particularly after the failure of classical therapeutic approaches (corticosteroids, chloroquine, dapsone, antihistamines, immunosuppressants, non-steroidal anti-inflammatory drugs). Colchicine was effective in two patients with the initial hypocomplementemia persisting in one. Corticotherapy could be stopped after 3 months of colchicine (1.2–1.8 mg/day), with the latter being continued as maintenance therapy. It should be noted that both patients relapsed when colchicine was discontinued, but they responded well to the reintroduction of the drug (*221*). Colchicine (1.5 mg/day) was rapidly effective against the urticarial lesions, arthralgias and abdominal pain of a patient who had previously been corticodependent. Maintenance therapy required 1 mg/day and symptoms reappeared under 0.5 mg/day (*222*). The same pattern concerning the cutaneous lesions was seen after 30 months of follow-up of a patient whose pathology had been evolving for 15 years under unsuccessful immunosuppressive therapy (*223*). Corticosteroid doses could be lowered for two patients with idiopathic urticarial vasculitis (*213, 220*). Colchicine was transiently effective against urticarial vasculitis associated with hypocomplementemia secondary to antibodies to the C3 convertase of the alternative pathway (*224*).

The literature concerning urticarial vasculitis is scarce and does not enable us to advance an objective conclusion. Nevertheless, regardless of whether the other therapeutic agents, including immunosuppressants, failed, were poorly tolerated or were contraindicated, colchicine merits being tried.

J. Primary Biliary Cirrhosis

Primary biliary cirrhosis is characterized by a T lymphocyte-dependent attack against the interlobular bile ducts that leads to their destruction and the appearance of fibrosis of the portal tracts and the hepatic parenchyma which, in turn, causes cirrhosis after several decades of evolution. Treatment aims at preventing the destruction of the bile ducts and the ensuing cholestasis. In this pathology, colchicine acts by normalizing monocyte and lymphocyte functions (*225*), restoring the defective stimulation of suppressor T lymphocytes (*19*), and significantly decreasing the synthesis and secretion of interleukin 2 and tumor necrosis factor-a which are elevated in this disease, but it remains unknown whether these latter are a cause or a consequence (*226*). Therapeutic evaluation is difficult, and especially actuarial studies because they require large cohorts of patients at comparable clinical stages, and their long-term follow-up. The side effects of treatment should be kept to a minimum, as for all chronic diseases. The ultimate therapeutic recourse is a liver transplant. Colchicine has been widely prescribed alone and, more recently, in combination with ursodeoxycholic acid, which seems to hold some promise. Penicillamine D, corticosteroids, immunosuppressants and cyclosporine have also be evaluated.

Four prospective, randomized, double-blind trials tested colchicine *versus* placebo and colchicine was accorded a slight advantage (*80, 227-229*). The main criteria of

evaluation of primary biliary cirrhosis were hepatic function, gamma globulin levels, liver histology and survival. Each study included about 60 patients, and the clinical and demographic characteristics of the colchicine and placebo groups were comparable. For patients receiving colchicine, serum albumin was significantly elevated (*80, 228*) and gamma globulin concentrations were decreased (*80, 228, 229*), especially after 33 months of follow-up (*229*). In contrast, neither the slowing of histological progression nor diminution of fibrosis was significant after 1 year (*227, 228*), 2 years (*80*), or 4 years of therapy (*229*). Survival was similar for patients taking colchicine and those given the placebo after 18 months (*227, 228*) or 2 years (*80*). In the study conducted by Kaplan *et al.*, all the patients received colchicine in an open trial from the second to the fourth year: survival was significantly prolonged at 4 years for those who had taken colchicine from the beginning (*80*). The fourth study (*229*) did not evaluate survival, but six patients receiving the placebo died or had a transplant, as opposed to two in the colchicine group. The survivors (12 taking colchicine and 14 the placebo) were treated openly with 1.2 mg of colchicine/day, and followed for an additional 4 years: although hepatic function was significantly improved for all of them, no influence on survival could be demonstrated at 8 years. The number of patients with histological disease progression was the same in the two groups (*230*). Side effects were rare and moderate in the four studies.

Thus, despite improved hepatic function, colchicine alone had little impact on histological evolution and survival. Another molecule, ursodeoxycholic acid, was evaluated. It was prescribed because primary biliary cirrhosis lesions can be secondary, in part, to the accumulation in the liver of endogenous, cytotoxic bile acids. Experimentally, ursodeoxycholic acid can prevent, at least partially, fibrosis secondary to cellular necrosis induced by cholestasis, and diminish bile duct proliferation and portal inflammation. It was prescribed alone initially and then combined with colchicine. The promising results obtained in a non-controlled trial (*231*), led to two prospective, controlled studies, which compared ursodeoxycholic acid to a placebo and showed, as of the early 1990s, that ursodeoxycholic acid improved clinical status, hepatic function and liver histology, and lowered gamma globulin levels (*232, 233*). However, progression towards fibrosis was not modified (*232, 233*).

In mid-1994, the results of three prospective, randomized, double-blind trials (*234-236*) were published and demonstrated the benefit provided by ursodeoxycholic acid on histological evolution (*234, 236*), prolonged survival and in the need for fewer transplants (*234*). Ursodeoxycholic acid was also compared to colchicine and a placebo in a randomized, controlled trial. Ursodeoxycholic acid was associated with significantly improved hepatic function and liver histology after 2 years, and improvement was more pronounced with colchicine than the placebo. Despite the small number of patients, fewer deaths occurred and fewer transplants were performed in the ursodeoxycholic acid group (*237*). The next step was to compare the effect of combined colchicine–ursodeoxycholic acid *versus* ursodeoxycholic acid alone. The adjunction of colchicine to ursodeoxycholic acid led to significant biological improvement in a prospective, randomized, double-blind study that enrolled 22 patients but did not include histological evaluation (*238*). Similarly, a non-significant difference

favoring the colchicine–ursodeoxycholic acid combination was observed for biological parameters and histological stage in a prospective, randomized, double-blind trial conducted on 74 patients (*239*). The combination of these two drugs, which have different mechanisms of action, might have an additive or synergistic effect that merits very long-term assessment.

All the other treatments, except methotrexate, prescribed for primary biliary cirrhosis had no proven efficacy or were too toxic. Older studies using prednisone showed that this drug did not modify liver histology, but significantly accelerated the osteoporosis that is already frequent in this pathology; these observations were confirmed by a controlled study (*240*). Penicillamine D was widely assessed in large-scale, randomized trials (*241-246*). The more recent ones did not confirm the improvements reported earlier. In 1997, Kaplan (*247*) published a review of immunosuppressants in this setting: azathioprine has limited efficacy, chlorambucil is too toxic, and the slight efficacy of cyclosporine is not sufficient to counter its too frequent side effects. The results obtained with methotrexate are promising because of the improved clinical status and hepatic function, similar to those noted under ursodeoxycholic acid; significant histological improvement was also observed. In addition, it seems that the effects of methotrexate and ursodeoxycholic acid are additive. Thus, at present, the recommended primary biliary cirrhosis therapy is based on ursodeoxycholic acid, colchicine and methotrexate (*247*).

K. Primary Sclerosing Cholangitis

This chronic cholestatic hepatic disease is characterized by inflammation and fibrosis of the intra- and extrahepatic bile ducts that lead to cirrhosis. The cause is unknown. At present, no therapeutic approach has been established (*248*). However, colchicine might have a beneficial effect in this pathology. Twelve patients treated openly received 10 mg of prednisone and 1.2 mg of colchicine daily and were compared to twelve patients taken from a historical control group that had be given a placebo. Although hepatic function and histology were unchanged at the end of the 2-year study, portal hypertension complications were less frequent in the treated group. However, these findings are only indicative because of the small number of patients, the non-randomized distribution and the failure to verify histology in all cases (*249*). A prospective, controlled, double-blind 3-year trial that included 84 patients who received either 1 mg of colchicine/day or a placebo showed no difference in terms of clinical status, hepatic function, histology or survival (*250*). Similarly, a randomized, controlled study of 59 patients receiving ursodeoxycholic acid (600 mg/day), colchicine (1.2 mg/day) or no treatment showed no difference after 2 years (*251*). The other treatments tested have been disappointing. Corticosteroids were evaluated in isolated cases and over the short term. Penicillamine D, tested in a prospective, randomized, double-blind study including 70 patients, was ineffective as far as hepatic function, histology and survival at 36 months are concerned (*252*). Methotrexate has been effective in some cases (*253*). With a mean follow-up of 2.2 years, ursodeoxycholic acid provided no clinical benefit in a randomized, controlled, double-blind study *versus* placebo that

enrolled 105 patients (*254*). Additional investigations are need to clarify the situation.

L. Retractile Mesenteritis

Two published case reports have indicated that retractile mesenteritis evolved favorably with the combination of prednisone (0.5–1 mg/kg/day) and colchicine (1 mg/day) (*255*). Based on these observations, no formal conclusion can be drawn because the natural history of this entity is not known and because the two molecules were combined from the start. Nevertheless, this combined therapy proved to be effective.

M. Relapsing Polychondritis

The etiology of this inflammatory necrosis of cartilage (ears, nose, trachea, articulations), sometimes accompanied by ocular involvement, remains undetermined. Colchicine inhibits the chemotaxis of leukocytes and lysosomal degradation that occurs in this pathology. Colchicine (1 or 1.2 mg/day) has proven effective in several cases. Indeed, three episodes of chondritis and one of episcleritis regressed rapidly under this therapy. Despite the more rapid healing obtained with colchicine, a spontaneous favorable evolution cannot be excluded (*256*). Similar disappearances of cutaneous lesions and chondritis within 2 months were also reported (*257*). In contrast, colchicine did not induce any attenuation for a third patient, whose treatment lasted only 6 days (*257*).

To conclude, comparisons should be made between corticosteroids, prescribed for acute exacerbations, and dapsone, given as long-term therapy. Because of its innocuousness, colchicine perhaps merits being used at first intention in the absence of severe manifestations.

N. Pulmonary Fibrosis

Because no treatment has been validated for idiopathic pulmonary fibroses or those secondary to another systemic pathology, therapy remains empirical. Colchicine was prescribed and then tested because of its anti-fibrosing properties. In secondary forms, etiological therapy is needed to treat the underlying disease.

In a Chinese study, colchicine significantly decreased renal fibrosis in the rat (*258*). Although colchicine was also reported to reduce bleomycin-induced pulmonary fibrosis in rats (*259*), it did not do so in hamsters (*260*). However, at doses that can be given *in vivo*, it significantly inhibited *in vitro* release by alveolar macrophages of fibronectin and growth factor, both mediators associated with the development of pulmonary fibrosis (*261*). A retrospective study of 23 patients with idiopathic pulmonary fibrosis treated with colchicine (mean dose of 0.6 mg/day) demonstrated, after 22 months of follow-up, improvement in 22%, stabilization in 39%, and progression in 39%, based on clinical criteria of evaluation and pulmonary function tests. Eighteen patients had previously received corticosteroids alone or in combination with an

immunosuppressant. Clinical improvement was noted between the 3rd and 12th months of treatment with vital capacity being improved by 18% and the diffusion of carbon monoxide by 37%. Finally, three of the five responders had received only colchicine (*262*). In another retrospective study of 44 patients given either colchicine or corticosteroids, no significant difference was established in terms of pulmonary function (*263*). A prospective, non-randomized study of 56 patients, prescribed prednisone alone (1 mg/kg/day) or in combination with colchicine (1 mg/day) or penicillamine D (600 mg/day), or all three molecules, showed no difference as assessed by pulmonary function tests (*264*). A prospective, randomized study of 26 patients with symptomatic idiopathic pulmonary fibrosis demonstrated that colchicine (0.6–1.2 mg/day) generated as good results as high-dose prednisone, but without the latter's inherent side effects, and similar to those observed without therapy (*265*).

The other molecules seem poorly effective. In the absence of a prospective trial, the number of responders to corticosteroids was < 20% on the average and was associated with enhanced morbidity attributable to iatrogenic side effects (*266, 267*). After 10 years of follow-up of 222 patients, it was concluded that untreated patients fared better than those on steroids (*268*). Azathioprine had a beneficial effect according to one prospective study of 20 patients (*269*). Its combination with prednisone was more effective than prednisone alone according to a prospective, randomized, double-blind trial that included 27 patients (*270*). The combination of cyclophosphamide and corticosteroids seemed to prolong survival, but not significantly so, as compared to the latter alone, in a prospective, randomized study (*271*). Anti-fibrosing agents, like interferon-gamma, have been prescribed, but trials are needed (*272*). Thus, the place of colchicine and the other anti-fibrosing drugs in this setting remains to be determined.

O. FIBROMATOSES

Fibromatoses are a heterogeneous group of disorders of proliferating fibroblasts that infiltrate tissues and produce large amounts of collagen. Surgery, radiotherapy, chemotherapy and hormonal therapy have been tried, and were found to be ineffective and toxic. The size of a musculoaponeurotic fibromatosis (desmoid tumor) decreased by 40%, as well as the pain it caused, over 3 weeks under 3 mg of colchicine/day (*273*). Similarly, two cases of Dupuytren's palmar fibromatosis (*273, 274*) and one of penile fibromatosis or La Peyronie's disease (*273*) regressed. For the latter pathology, a pilot study concluded that the plaques regressed or disappeared for twelve out of 24 patients in association with a significant attenuation of painful erections for seven out of nine patients under the maximum tolerated dose and for a mean treatment duration of 3–5 months (*275*). These promising results merit further investigation.

P. SARCOIDOSIS

This inflammatory, potentially systemic, pathology can affect diverse organs, including articulations. The efficacy of colchicine might be explained by its anti-inflammatory properties. Other molecules have been shown to be active: non-steroidal

anti-inflammatory drugs and corticosteroids, each having its own contraindications and side effects. Colchicine can be used for acute and chronic arthropathy. Maintenance treatment sometimes requires long-term administration of a dose lower than that given for induction. The pain of two patients suffering from acute sarcoidal arthritis was relieved within 24 h by high doses of intravenous colchicine. Although such acute episodes recurred several times, they were always colchicine-sensitive (*276*). Three similar successes were reported, with one of these patients having had symptoms for 4 years. No relapse occurred for two of them with a daily maintenance dose of 1 or 2 mg/day (*277*). Other comparable successes, as well as several failures, have been reported for oral or intravenous colchicine, sometimes after the failure of non-steroidal anti-inflammatory drugs, salicylated compounds or phenylbutazone (*278-282*). An isolated case of pseudotumoral cutaneous and hepatosplenic sarcoidosis evolved favorably under the combination of hydroxychloroquine (400 mg/day) and colchicine (1 mg/day) (*283*). Therefore, it seems warranted to explore the possible efficacy of colchicine on the articular manifestations of sarcoidosis.

Q. PORPHYRIAS

These are hereditary diseases caused by a deficit of an enzyme intervening in heme synthesis. Acute porphyria is characterized by more-or-less severe recurrent abdominal pain, neuropathies and multineuritides. Other than the suppression of known triggering factors, several treatments, despite not having been validated, have been prescribed (high glucose intake, hematin perfusion, β-blocker). The discovery of the efficacy of colchicine was a mere coincidence, but merits prospective evaluation. A patient whose porphyria was misdiagnosed as familial Mediterranean fever, for which colchicine was prescribed, again suffered from abdominal pain when the drug was discontinued. This pain attributable to porphyria again subsided when colchicine was reintroduced. These authors then demonstrated that colchicine inhibits the experimental induction of porphyria in the chick embryo *in vitro* and in the rat *in vivo* (*284*). Four other cases of abdominal pain due to porphyria evolved favorably under colchicine (2 mg/day maximum dose), whereas other non-specified medications failed. Taken at the first premonitory symptom of disease, colchicine prevents the development of the acute episode. Ingested once the first signs are present, it attenuates the intensity and shortens the duration of the attack (*285*). Nothing else has been published on this subject. Thus, colchicine should be prescribed for the acute abdominal pain associated with porphyria.

R. RELAPSING MULTIFOCAL PERIOSTOSIS

This rare disease, inherited in an autosomal dominant manner, is characterized by cortical hyperostosis, which is responsible for intermittent migrating arthralgias or arthritides. Under colchicine, pain disappeared and the general condition improved for a 10-year-old boy, whose illness had started at 9 months of age, and had rapidly become corticodependent and associated with major side effects. Hydroxychloroquine sulfate and penicillamine D had failed. After adjustment, the long-term therapy

combined corticosteroids (3 mg every other day) and colchicine (1 mg daily). At each diminution of the colchicine dose, the symptoms recurred (*286*).

Although this case report does not allow us to draw any conclusions, it suggests that colchicine can be used in this context because it is potentially effective and has much fewer side effects than corticosteroids, especially on growth.

S. CRYOGLOBULINEMIA

This pathology is characterized by an abnormal production of circulating protein(s) that precipitate when blood/plasma is cooled, and is manifested by purpura, cutaneous ulcers, arthralgias, and/or renal and/or peripheral neurological involvement. As a general rule, concentrations of complement components (C3 and C4) are low. This cryoglobulin synthesis can be primary or secondary (usually to an infection with hepatitis C virus). In the absence of a known etiology, its treatment is difficult and poorly defined (corticosteroids, interferon-α, immunosuppressants...). Colchicine actions would include, among others, preventing the secretion of immunoglobulins, blocking the cellular immune response, and inhibiting the chemotaxis of polymorphonuclear leukocytes.

The clinical signs (purpura, cutaneous ulcers, arthralgias, hematuria) regressed, and plasma cryoglobulin levels decreased, in an open trial that included 17 patients with cryoglobulinemia (eight primary and nine secondary) who took 1 mg of colchicine/day for 6–48 months. After 6–12 months of follow-up, purpura was attenuated for 15 of the 17, and arthralgias for nine out of 17, while cutaneous ulcers healed for only three of the five concerned and hematuria regressed for only six of the 14 affected; cryoglobulin levels diminished for 13 of the 17 patients and complement concentrations normalized for three of the 17. After a longer follow-up of 18–48 months, the only parameter that continued to improve was the lowering of the cryoglobulin concentration (*287*). The successful introduction of colchicine in several isolated cases has suggested the possibility of stopping or lowering the dose of corticosteroids (*213, 216*) or immunosuppressants (*216*). Colchicine might be active against urticaria (*221, 222*) or abdominal pain (*222*). However, it failed in a patient with leukocytoclastic vasculitis associated with hypocomplementemia and cryoglobulinemia (*159*).

At present, interpreting these findings is difficult, but an objective conclusion could be obtained with a controlled trial. Spontaneous evolution may explain all of the improvements seen, as cryoglobulin levels fluctuate spontaneously, and, in turn, their dependent clinical manifestations.

T. HYPER-IG D SYNDROME

This syndrome associates oversecretion of immunoglobulin D, recurrent febrile episodes, headaches and bilateral cervical adenopathies. Colchicine proved to be effective against the manifestations of one patient (*288*) with concomitant lowering of the immunoglobulin D level.

U. ERYTHEMA NODOSUM LEPROSUM

Certain infections, among them leprosy, can manifest themselves as cutaneous lesions of immune origin that resemble erythema nodosum, that is to say, inflammatory dermal–subdermal nodules. Classically, the treatment is that of the etiological cause. Colchicine acts by inhibiting the Arthus phenomenon, blocking the mobilization of polymorphonuclear leukocytes and normalizing the number of suppressor T lymphocytes (*13, 16*). Its first use in this setting dates from 1832. It was later compared to acetylsalicylic acid. Most of the publications refer to the classically accepted drugs used to treat the erythematous nodules of leprosy: thalidomide, clofazimine, non-steroidal anti-inflammatory drugs, chloroquine and systemic corticosteroids. Colchicine was successfully prescribed in 1967 for erythema nodosum with arthralgias of unspecified origin (*289*). In a non-controlled study on ten men with either recurrent or chronic erythema nodosum, leprous erythema nodosum lesions and fever disappeared within 2 days (1.5–2 mg of colchicine/day). The only relapse recorded was attributed to the premature discontinuation of long-term therapy and was reversed by the reintroduction of colchicine. Maintenance therapy (1 mg/day) was able to prevent relapses for all ten subjects and side effects due to long-term therapy were infrequent (*290*). In an open study of 15 patients with lepromatous leprosy evolving for a mean of 6.26 years with erythema nodosum present for a mean of 1.73 years, colchicine led to attenuation of > 75% for ten and 50% for three others in < 1 week. Because several patients also took dapsone or the combination dapsone–rifampicin, interpretation is difficult. No new skin lesions appeared after 7 days of treatment, and this held true for 1–2 months under 1 mg of colchicine/day. In contrast, other signs of leprosy (neurological and ocular) were not modified (*291*). Unfortunately, colchicine did not allow the corticosteroid dose to be lowered for five patients taking dapsone and clofazimine for severe erythema nodosum leprosum, perhaps because the steroids perpetuated the lesions (*292*).

Colchicine (1.5 mg/day for 4 days) was compared to aspirin (1.8 g/day for 4 days) in a controlled, double-blind trial that included 30 adults with 68 histologically proven erythema nodosum leprosum lesions. All were taking dapsone, sometimes in combination with rifampicin or prothionamide. Colchicine was found to be more active against mild lesions, attenuating 74% of them, than acetylsalicylic acid which caused the regression of only 29%. The two molecules were equally effective against some lesions of moderate intensity and both were ineffective against severe nodules (*293*).

Thus, colchicine seems to be active against mild erythema nodosum lesions. Controlled studies are needed to determine precisely the indications of the different drugs prescribed and whether or not maintenance therapy is beneficial.

VIII. Colchicine Indications in Other Specialties

In addition to these pathologies primarily seen in internal medicine, colchicine has also been used in many other fields: dermatology, rheumatology, cardiology, hepatogastroenterology, hematology, ENT, stomatology, and pneumology.

A. IN DERMATOLOGY

1. Palmoplantar Pustulosis

The etiology of this disease, manifested by pustules on the palms and soles, is unknown, and the frontier between it and pustular psoriasis is poorly defined. The roofs and squamae of the pustules contain chemotactic factors that enhance the chemotaxis of polymorphonuclear leukocytes and, as discussed above, colchicine inhibits this chemotaxis. On the other hand, it has little or no effect on vesicle formation, which is mediated by mononucleated cells (*294*). The diverse other medications prescribed gave rise to variable and unsatisfactory responses: tetracyclines, clofazimine, methotrexate, hydroxyurea, dapsone, sulfapyridine, corticosteroids, psoralen, long-wave ultraviolet radiation. At present, retinoid is the preferred first-line therapy, but it has many side effects. Although the results obtained with colchicine have been divergent, it can act on the articular and cutaneous symptoms. Prescribed at a daily dose of 1–2 mg, colchicine was shown to be effective in an open series of 32 patients without articular signs and for whom local or systemic corticotherapy had failed. The lesions regressed as of the first week of therapy for 90% of the patients, completely disappearing for 13 of them within 2–8 weeks and clearly regressing for 14, with no new lesions appearing in any of these responders; lesions stabilized in one subject and four patients exhibited drug-intolerance. Eight relapses occurred during the 3 months following the end of therapy (*294*). Colchicine maintained the attenuation of articular symptoms obtained with a retinoid and was more effective against the joint pain associated with aseptic rheumatic osteitis of the episternum of a patient for whom non-steroidal anti-inflammatory drugs had failed. The arthralgias recurred at each attempt to stop colchicine (*295*). This favorable effect was not confirmed by three subsequent studies, but two of them were difficult to analyze. Among the 27 patients enrolled in a double-blind, crossover study who took 1.5–2 mg of colchicine/day for 4 weeks, then a placebo for 1 month or *vice versa*, the number of pustules decreased for ten patients and increased for seven. Under the placebo, three patients improved and 19 deteriorated (*296*). None of the twelve patients in a group receiving 1 mg of colchicine/day or a placebo for 6 weeks and inversely in a double-blind protocol showed improvement (*297*). A similar trial that included ten patients and consisted of two 3-month periods did not demonstrate any difference between colchicine and the placebo (*298*).

Despite these diverging observations, it seems that colchicine might be effective against palmoplantar pustulosis.

2. Psoriasis

Psoriasis is the result of an accelerated turnover of the epidermal cells of the Malpighian layer. Clinically, it presents as silvery scaling plaques, primarily on the extensor surface of the elbows and knees. Colchicine acts by inhibiting the mitosis and chemotaxis of polymorphonuclear leukocytes. Successful treatment has been reported for thin psoriatic plaques and the pustular and chronic extensive forms of the disease.

Classical therapies include tar, keratolytic agents and ultraviolet radiation.

In 1959, colchicine was applied under a pressure bandage: the psoriatic lesions regressed in 4–15 days (*299*). Results were similar in another study on twelve patients with psoriatic plaques resistant to topical corticotherapy; their untreated lesions showed no attenuation (*300*). Local colchicine therapy was quickly forgotten because of the local irritation and the unquantified transcutaneous absorption of the drug. Then, several years later, 22 patients were given colchicine orally (0.02 mg/kg/day for 2–4 months) in combination with emollients as the only local treatment. The cutaneous lesions were significantly attenuated for eleven patients including five who had generalized psoriasis. However, colchicine was only slightly or not at all effective against chronic psoriasis or thick squamous plaques. In the only reported case of pustular psoriasis, no new lesions appeared after 1 week of treatment. In addition, among five other patients with extensive, chronic psoriasis, the molecule was effective in four with maintenance therapy for 8–9 months after the disappearance of cutaneous lesions obtained using Goeckerman's method (topical tar and ultraviolet B radiation) or methotrexate (*301*). Similarly, a total regression was obtained within 2 weeks for three of the four patients with acute generalized pustular psoriasis with erythroderma previously resistant to immunosuppressants and ultraviolet radiation (*302*). Although methotrexate had failed after 10 weeks of administration, the adjunction of colchicine (2 then 1 mg/day) led to a satisfactory and durable response (> 6 months) and allowed the methotrexate dose to be lowered for a patient with generalized pustular psoriasis. However, the lesions reappeared when methotrexate was stopped, *i.e.*, when colchicine was prescribed as monotherapy (*303*). No difference was noted between colchicine and placebo in two controlled, double-blind crossover studies (15 patients treated for 16 weeks and 25 patients for 23 weeks) (*304, 305*).

Although few data are available, colchicine can nonetheless be effective against psoriasis, especially the thin lesions and severe pustular forms, including when immunosuppressants have failed.

3. Vesicular and Bullous Diseases

Colchicine has been prescribed for herpetiform dermatitis, acquired bullous epidermolysis, the bullous eruption of systemic lupus erythematosus, linear IgA dermatitis or chronic bullous dermatitis of children. These are diseases with autoantibodies directed against skin components. Classical treatments include dapsone, sulfapyridine, corticosteroids and immunosuppressants (*306*). In light of the pathophysiology of these disorders, colchicine is a logical, attractive and well-tolerated therapy. However, it is obviously dangerous to draw conclusions based on the isolated case reports published. Colchicine would act by blocking autoantibody synthesis and secretion (*307*), inhibiting the migration of polymorphonuclear leukocytes, and modulating lymphocyte functions and the formation of circulating immune complexes (*13, 308*). It might alter the structure of the carboxyterminal group of type VII procollagen, the etiological antigen in acquired bullous epidermolysis and the bullous eruption of systemic lupus erythematosus (*309*).

Colchicine (1.8 mg/day) was effective for three of the four patients with herpetiform dermatitis for whom dapsone and sulfapyridine were contraindicated for three and ineffective for the last. At each dose reduction, the blisters reappeared (*310*). None of the numerous treatments tried for acquired bullous epidermolysis, including cyclosporine and other immunosuppressive agents, showed any real efficacy. Mucosal and cutaneous lesions disappeared, respectively, within 1 and 2 weeks, under 2 mg of colchicine daily administered to a patient whose lesions had been resistant to the combination of prednisone and methotrexate; all of these lesions reappeared 3 days after its discontinuation (*306*). Among the five similar cases reported (*311, 312*), it should be noted that, for two of them, corticosteroids and immunosuppressants had failed previously (*312*). Maintenance therapy is sometimes necessary (*295, 306, 312*). The prednisone–colchicine combination led to the attenuation of the general, articular and cutaneous symptoms of the bullous eruption of systemic lupus erythematosus, whereas prednisone–dapsone had only been active against cutaneous lesions and was complicated by the development of methemoglobulinemia (*313*).

Colchicine (1.5 mg/day) was reported to be successful in linear IgA dermatitis, characterized by IgA deposits in the basement membrane, after the failure of prednisone alone and in combination with dapsone. A subsequent relapse responded to colchicine (*314*). In addition, its efficacy within 2 weeks in a child allowed the withdrawal of corticosteroids, which had been effective, but caused Cushing's syndrome medicamentous, retarded growth and corticodependence (*315*). Among eight children with chronic bullous dermatosis or linear IgA dermatitis, colchicine alone was effective for five of them within 4–6 weeks and 3 times in combination with low-dose steroids. Long-term therapy (colchicine alone or in combination with steroids) with colchicine at a dose of 0.5 mg/day (for 4–14 months), was well tolerated (*316*). After the failure of sulfones, intraepidermal neutrophilic IgA dermatitis was controlled following 2 weeks of colchicine (1 mg/day) (*317*). Thus, colchicine indeed has a place among the other molecules in the treatment of bullous diseases.

4. Sweet's Syndrome

This febrile acute neutrophilic dermatosis of unknown etiology is characterized by an inflammatory patch with infiltration of the dermis by polymorphonuclear leukocytes whose chemotaxis is enhanced. Corticosteroids are often prescribed in this context. Because the enhanced chemotaxis of polymorphonuclear leukocytes, colchicine can be active, as demonstrated by several case reports. A second episode of Sweet's syndrome, which developed after a first regressed under prednisone, disappeared within 8 days under 1.5 mg of colchicine/day (*318*). In addition, four cases, including a relapse (*319*), evolved favorably within several days after the administration of colchicine (*319, 320*). Thus, colchicine can be effective in this disease.

5. Aphthae

The numerous therapies proposed for aphthae reflect the poorly defined indications

in this setting. By analogy with its efficacy against the oral aphthosis and genital erosions of Behçet's disease, colchicine was prescribed for banal buccal aphthae that can become debilitating by the frequency of the exacerbations and the intensity of the lesions. Maintenance therapy appears to be necessary because colchicine is not curative. Whereas other molecules failed, a prospective, non-randomized analysis showed that colchicine, at a daily dose of 1 mg, was completely successful within 7–30 days for 60%, and partially effective for 15% of the 20 patients evaluated (*321*). Several similar cases have been described (*322, 323*). Furthermore, colchicine significantly attenuated relapsing banal aphthosis in 20 patients included in a prospective open study that lasted 4 months, with each patient serving as his own control ($P < 0.001$) (*324*).

Colchicine should be prescribed before corticosteroids, levamisole and thalidomide which are effective, but non-curative and more toxic. Systemic corticotherapy is effective but rarely used; local steroids are able to lower the number and shorten the duration of aphthae provided they are applied as of the first signs (*325*). Based on two controlled trials evaluating levamisole (47 and 124 patients), it was concluded that this drug can lower the number of aphthae, diminish their duration and prevent their appearance (*326, 327*). In an open trial that enrolled 40 patients to assess the efficacy of thalidomide, 35% remission and 40% marked attenuation were observed (*328*). This molecule was also tested alone and in combination with colchicine in an open study on 25 patients, 13 of whom had Behçet's disease. The two arms were equally effective. However, because of the small number of patients included, the superiority of one drug over the other could not be determined (*329*). Thus, colchicine is a well-tolerated treatment of banal and debilitating buccal aphthae.

6. Keloid Scars

This hyperplastic scar tissue does not conform to the original scar, and sprouts a pediculated mass, like a mushroom, at surface of the skin at the site of some type of trauma (surgery, burn, severe skin disease...). It is caused by excess collagen synthesis and diminished collagenase activity. Colchicine effectively counters these two mechanisms, especially when a lathyrogenic agent has been used previously. For ten patients who had already undergone surgery and received *in situ* corticosteroids without success, excision of their keloid scars and skin grafting were followed, after re-epithelialization of the donor zone, by prescription of colchicine at a dose of 1.8 mg/day for 4 months; with follow-up ranging from 18 to 60 months, all the scars were hypertrophic and stable, and no longer keloid (*330*). No significant difference was observed between 27 keloids treated with surgery and *in situ* corticosteroids and 28 subjected to surgery and oral colchicine (1.2 mg/day) in a prospective, randomized trial with a mean follow-up of 24 months (*331*).

More studies are needed to confirm a possible positive effect and to determine the optimal doses and durations of colchicine therapy.

7. Condylomata

This sexually transmitted disease is caused by a virus. Colchicine was prescribed because of its anti-mitotic properties, but was abandoned because of its local toxicity. It has been replaced by the combination of local liquid nitrogen and podophyllin, or CO_2-laser therapy. The urethral condylomata of four patients disappeared, with no side effects, after 2–3 months of twice weekly local applications of a 0.5% colchicine solution in water; these lesions had resisted excision, podophyllin and thiotepa (*332*). In a prospective, non-randomized study on 227 patients divided into three groups, the efficacies of colchicine, podophyllin and podophyllotoxin were comparable, but corrosive local side effects were more frequent with colchicine (*333, 334*).

8. Acne Conglobata

Three drugs (isotretinoin, colchicine and cyclosporine) were administered successively to a male patient with acne conglobata, a clinical form of acne that is difficult to treat. Although the lesions regressed under each of the molecules, they recurred every time therapy was discontinued (*335*). Thus, colchicine can be used to treat acne conglobata.

9. Pyoderma Gangrenosum

These deep necrotic cutaneous ulcers usually occur in patients with inflammatory bowel disease. The treatments prescribed include corticotherapy, dapsone, clofazimine and cyclosporine. As reported above under familial Mediterranean fever (*106*), colchicine proved effective for two patients with pyoderma gangrenosum associated with Crohn's disease: the cutaneous lesions disappeared within 2 months for one of them for whom it was possible to stop corticotherapy without relapse after 22 months of treatment, and the lesions regressed within 3 months for the other patient (*336*). Three years later, no relapse had occurred under maintenance therapy of 1 mg of colchicine/day (*337*).

10. Delayed Pressure Urticaria

No significant difference was observed for these lesions in a small, randomized, double-blind trial that included 13 patients who took 1 mg of colchicine/day in a crossover protocol *versus* placebo. It should be noted that treatment was prescribed for only 1 week (*338*).

11. Anetoderma

These cutaneous lesions, initially inflammatory or not, become atrophic. The results obtained with the diverse agents tested have been disappointing. A patient's inflammatory lesions, corresponding to neutrophilic and lymphocytic perivascular

infiltrates between collagen fibers, disappeared after 2 weeks of colchicine therapy (1 mg/day). No new lesions appeared during the 7 months of treatment. However, lesions recurred when colchicine was stopped, thereby providing another argument supporting the efficacy of this molecule in this setting (*339*).

B. IN RHEUMATOLOGY

1. Chondrocalcinosis

The calcium pyrophosphate that precipitates in the articulations is phagocytosed by polymorphonuclear leukocytes, thereby provoking the release of factor(s) chemotactic for polymorphonuclear leukocytes and monocytes. Colchicine acts on this major mediator of inflammation. It is effective in the acute forms of the disease, but also over the long term to reduce the number of relapses. Colchicine can be given intravenously for rheumatological disorders provided that certain specific precautions are taken (*340*).

Arthralgias disappeared for five patients and regressed for one out of seven who had received 1 or 2 mg of colchicine intravenously in an open study (*341*). A similar case was also reported (*342*). Intravenous doses of 1 and 2 mg were compared in 17 patients: the 2-mg dose was significantly more effective ($P < 0.05$) (*343*). In another open study, the arthralgias and inflammatory signs of seven patients given up to 4–6 mg of colchicine intravenously were attenuated in 2 days (*344*). Oral colchicine was able to prevent relapses. After the success of the combination colchicine–non-steroidal anti-inflammatory drug, a maintenance therapy of 0.5 mg of colchicine/day lowered the frequency of relapses for one patient, but an attempt to discontinue treatment led to reappearance of the pain (*345*). The annual incidence of painful episodes decreased from 9.3 to 2.4 in a study of twelve patients taking 1 mg of oral colchicine daily for 1 year ($P < 0.05$) (*346*). This finding was confirmed by a prospective study that included ten patients followed for 1 year before and after taking colchicine. The number of exacerbations decreased significantly from 3.2/patient/year before colchicine to one during the year of treatment with 1.2 mg/day ($P < 0.001$) (*347*).

It should be noted that colchicine can be as effective in acute and chronic forms as non-steroidal anti-inflammatory drugs, and it has fewer contraindications and is less toxic.

2. Hydroxyapatite Rheumatic Disease

The classical treatments are non-steroidal anti-inflammatory drugs and corticosteroids. The only publication on this subject reported that six out of eight patients responded to colchicine therapy: five completely and one only partially (*348*).

3. Paget's Disease

This benign pathology is caused by anarchic bone remodeling in a gross distortion of the physiological mechanisms of bone resorption and formation. This remodeling

leads to a disorganization of bone structure and is characterized by pain and bone deformations. Calcitonins and diphosphonates are currently prescribed as first-line therapy: they are effective, but certain precautions must be taken for their use and they generate undesirable side effects.

Results obtained with colchicine have been promising, but, unfortunately, did not issue from controlled trials. This molecule would have an anti-mitotic effect on bone stem cells, and would bind to osteoblasts and osteoclasts. Five patients took 1.8 mg/day and were followed for 8–28 weeks (mean 20 weeks). Pain disappeared for two and regressed rapidly by > 50% for the other three. The antalgic activity was positively correlated with serum alkaline phosphatase levels (indicators of bone formation), and hydroxyprolinuria (a reflection of bone resorption); the former decreased 18–38% and the latter diminished 26–53% in four of the five patients. The positive clinical and biological effects occurred early and lasted throughout the duration of treatment. Two relapses developed when colchicine was withdrawn, but were reversed by its reintroduction (*349*). In another poorly detailed report, it was effective for five of the seven patients (*350*). The combination of colchicine (1.5 mg/day) and prednisone (20 mg/day) was tested openly for various durations in six patients: the data are heterogeneous because the treatment did not follow a precise protocol; pain decreased for two patients, alkaline phosphatases for three. This combination appeared to be active in several cases even after the failure of calcitonins and diphosphonate (etidronate disodium). However, the exact or potentiating role of the corticosteroids remains uncertain (*351*).

Although they are difficult to compare, these findings seem to be equivalent to those obtained with etidronate disodium (*i.e.*, clinical improvement for 60%, and normalization of hydroxyprolinuria for 68% and alkaline phosphatases for 45% of the 109 patients studied) (*352*) or calcitonins (*i.e.*, clinical improvement for 80%, diminution of hydroxyprolinuria for 50%) (*353*).

4. Palindromic Rheumatism

The etiology of these acute, recurrent, inflammatory mono- or oligoarthritides is unknown. Some of the cases develop into rheumatoid arthritis. Colchicine would act, *via* its anti-inflammatory properties and its ability to inhibit polymorphonuclear leukocyte chemotaxis. Little information is available. Among five patients, two responded very well, and three partially, to 0.6–1.2 mg of colchicine/day for 12–18 months of prophylactic treatment against relapses. The frequency and intensity of the exacerbations decreased over the months of treatment, while spontaneous remissions are rarely described for this disease. None of the patients fulfilled the diagnostic criteria of rheumatoid arthritis (*354*). In contrast, when prescribed prophylactically to eight patients in a retrospective series of 34, colchicine failed. Although four of these patients were rheumatoid factor-positive, no case of rheumatoid arthritis was diagnosed during the 9.3 years of follow-up (*355*). These observations are too limited to propose any precise role for colchicine in this pathology.

5. Psoriatic Rheumatism

The reported results have been mixed for the joint involvement that can complicate psoriasis. The arthralgias diminished for eight patients, among the 22 included in an open trial who received 0.02 mg of colchicine/kg/day for 2–4 months (*301*). The Ritchie score, which quantifies joint pain, was significantly improved ($P < 0.01$) for the twelve patients enrolled in a double-blind and crossover, controlled study testing 1.5 mg of colchicine daily *versus* placebo for 16 weeks (*304*). In contrast, arthralgias were not significantly affected in another double-blind and crossover protocol that evaluated 25 patients who took 0.6–1.8 mg of colchicine/day for 23 weeks (*305*).

No constructive conclusions can be drawn based on these data. Investigations evaluating the different known therapeutic agents (methotrexate, salazopyrin, azathioprine, retinoid) (*356*) and also colchicine need to be pursued.

6. Pachydermoperiostosis or Hypertrophic Osteoarthropathy

This clinical picture, either primary or paraneoplastic, associates clubbing of the fingers, thickening of the skin, seborrhea and periosteosis. Although the score grading pain, including that of the articulations, was significantly improved ($P < 0.05$) for 14 patients taking 0.5 mg of colchicine/day for 1 month as compared to those taking a placebo, their other clinical manifestations (finger clubbing, pachyderma) and radiological signs (periosteitis) persisted (*357*). Finger clubbing, arthralgias, pachyderma and folliculitis regressed in another patient whose skin symptoms recurred 15 days after discontinuation of colchicine (*358*).

Thus, colchicine has a place in the treatment arsenal of this pathology.

7. Disk disorders

Colchicine acts by inhibiting the release of lysosomal enzymes by polymorphonuclear leukocytes. Small series of patients, diverse pathologies and follow-up of sometimes short duration limit the ability to interpret the observations reported and might explain the different findings obtained in the various studies that administered colchicine either intravenously or orally. For the acute forms, results were very good for 63% and good for 13% of the 50 patients treated with intravenous ($\approx$ 1 mg) then oral colchicine (at least 0.6 mg/day). The outcome was good or excellent for only 55% of the chronic forms (*359*). Similarly, colchicine was effective for a cohort of 3,000 patients with painful, disk or vertebral anomalies, even after the failure of classical treatments, which included *in situ* corticosteroid infiltration, chemonucleolysis and surgery (*360*), and in a double-blind trial *versus* placebo that applied the same protocol to 38 patients (*361*). In contrast, a prospective, double-blind, randomized trial that enrolled 27 patients did not demonstrate any significant difference after 12 weeks of treatment with oral colchicine or placebo (*362*).

Thus, in this setting, colchicine can be effective, and with fewer contraindications and side effects than non-steroidal anti-inflammatory drugs.

C. IN CARDIOLOGY AND PHLEBOLOGY

1. Venous Thromboses and Lymphedemas

The publications in this area are quite old and open to criticism. The efficacy of colchicine can be explained, in part, by its anti-inflammatory properties. Local and systemic inflammatory signs diminished under 3 then 1 mg of colchicine/day for 40 patients, 31 with superficial and nine with deep vein thromboses; these latter had not received anticoagulant therapy. Several patients had been given the drug intravenously (*363*). Among the 20 patients with superficial vein thromboses, provoked vein endothelium inflammation (*e.g.*, after sclerotherapy or chemotherapy) or post-thrombotic edema, results were good for 14 and intermediate for four patients (*364*).

Because colchicine decreases the release of proteolytic lysosomal enzymes and thereby minimizes the production of lymph, its efficacy against lymphedema was examined. Among 33 patients with lymphedema of recent onset, 13 of the 16 with minor valve disease were controlled, as opposed to only one of the 17 suffering from complete valvular insufficiency (*365*). Five of the seven patients with scleroderma-like lesions secondary to varicose veins showed improvement (*363*).

2. Acute and Recurrent Pericarditides

Relapses of inflammatory pericarditides are frequent and their treatment is difficult. Various etiologies are recognized: idiopathic, postsurgical, postinfarctus, systemic disease, etc., and their incidence has certainly decreased since salicylated compounds replaced corticosteroids. The positive effect of colchicine was suspected and the necessity of its continuous utilization to prevent relapses has since been demonstrated. The other treatments prescribed are poorly effective and more toxic (azathioprine, chlorambucil, interferon-α).

Colchicine's efficacy at 1 mg/day was spectacular for three patients, two with idiopathic pericarditis and one with systemic lupus erythematosus. Although these patients had become corticodependent, the steroids could be stopped within 2 months and no relapse occurred after 15–36 months of treatment with 0.5 mg/day of colchicine (*366*). Nine patients, who had experienced more than three episodes of pericarditis, despite aspirin, indomethacin (non-steroidal anti-inflammatory drug), prednisone or combination therapy, received 1 mg of colchicine/day in a prospective, open trial in which each patient served as his own control. Cortisone could be withdrawn within 2–6 weeks and no relapse was observed after a mean follow-up of 24.3 months. The duration of the asymptomatic interval was significantly prolonged ($P < 0.002$). One relapse occurred after colchicine discontinuation, but was sensitive to its reintroduction (*367*). The relapse frequency for eight patients with corticodependent pericarditis was significantly lowered under long-term colchicine (0.5–1.5 mg/day), as assessed after a mean follow-up of 26 months ($P < 0.0001$). Corticosteroids could be stopped within 2–6 months, but four pericarditides recurred when colchicine was withdrawn (*368*).

Colchicine was effective for a series of 19 acute pericarditis patients, among

whom only two relapsed in 5 months, and also in a series of eleven chronic pericarditis patients, none of whom relapsed during a 6–24 month interval (*369*). Expanding this series, four relapses were recorded for 100 patients (*70*). In a prospective, open trial that included 19 patients with relapsing pericarditis (at least two episodes), 14 did not relapse during the 32–44 months of follow-up. The duration of the between-episode interval was significantly longer after treatment with colchicine ($P < 0.0001$) (*71*). The treatment schedule devised for the first episode of pericarditis was 3 mg the first day, 2 mg the second and third days, then 1 mg/day for 3 months. In the case of relapses, the treatment was continued beyond this termination date: the dose was lowered to 0.5 mg/day and prescribed for 6–12 months (*369*). The largest multicenter study enrolled 51 patients with recurrent pericarditis who had been followed for 6 months–10 years. The number of relapses decreased under colchicine with a significant prolongation of the interval between exacerbations (3.1 ± 3.3 *versus* 43.0 ± 35.0 months; $P < 0.0001$); 60.7% of the patients did not suffer a relapse (*370*). Colchicine can even be effective against the voluminous fluid accumulations of pericarditis (*371*). It can also be prescribed as first-line therapy, as suggested by: the absence of relapse for 74% of the 19 patients included in a prospective, randomized study and followed for a mean of 32.5 months (*368*); the three successes, four failures and one case of intolerance among 15 patients described in a poorly detailed report (*372*); and an isolated case report (*373*). After 6 months of treatment, colchicine was shown to be effective, without any side effect, in three children in whom anti-inflammatory agents had failed. In addition, no relapse was reported between 11 and 18 months after the end of therapy (*374*).

At present, colchicine is the only treatment known to prevent relapses of pericarditis.

3. Atherosclerosis

The property to inhibit the *in vitro* formation of atheromatous plaques was not confirmed in humans at a usable dose. Colchicine should be effective because it inhibits the secretion of diverse atherogenic substances (low-density lipoproteins, fibrinogen, collagen, catecholamines, serine), activates adenylate cyclase and inhibits guanylate cyclase (*375*). It is able to reduce the collagen content and can increase the number of elastic fibers in the artery walls. One can easily imagine a beneficial effect on atheromas in humans (*376*). Lastly, colchicine diminished the mobility of the human smooth muscle cells of atheromatous plaques in culture (*377*). Combining colchicine with a cholesterol-lowering diet diminished the formation of fibrous intimal tissue in the rabbit (*378*). At a high dose, corresponding to 14 mg/day for man, colchicine moderately lowered the frequency of restenoses, after balloon angioplasty, of the iliac artery in the rabbit (*379*).

For 51 hypertensive patients, colchicine (1 mg/day for 3–4 months) led to improved peripheral resistance index ($P < 0.01$) and arterial elasticity ($P < 0.05$), and thus the microcirculation. In contrast, the serum lipid concentration and arterial blood pressure were not modified (*380*). When a similar dose was given to patients with atherosclerosis, colchicine decreased glycosaminoglycans and the syntheses of collagen

and non-collagen proteins, all of which would normally be enhanced in the endothelial cells of the capillaries and the smooth muscle cells of the large-sized vessels with atheromatous involvement (*381*). However, according to a controlled, double-blind study on 253 patients comparing colchicine (1 mg/day for 1 month as of angioplasty with reassessment at 3 and 6 months) *versus* placebo, the drug proved to be ineffective in preventing restenoses after coronary endoluminal angioplasty, based on clinical evaluation criteria and myocardial scintigraphy (*382*). The same conclusions held true for another prospective, double-blind, randomized study (1.2 mg of colchicine/day *versus* placebo for 6 months) that included 197 patients with 393 dilated lesions, angiographically reevaluated 6 months postintervention (*383, 384*). Among the numerous molecules tested (*385*), anti-platelet aggregating agents (*385*), and angiotensin-converting enzyme inhibitors (*386*) seem to be the most effective. Nevertheless, the combination of an anti-hypercholesterolemic of the statin family (*e.g.*, lovastatin), an inhibitor of the angiotensin-converting enzyme (enalapril) and colchicine (1.2 mg/day), started 5–15 days before angioplasty, proved unable to prevent restenosis in an open, pilot trial that enrolled 50 patients with 69 lesions evaluated by a control angioplasty 6 months after the first intervention (*387*).

Despite promising experimental data, colchicine does not appear to be active against atheromas in humans.

D. IN HEPATOGASTROENTEROLOGY

1. Alcoholic Cirrhosis

Other than symptomatic treatment of complications, colchicine has been used over the long term as an etiological therapy because of its anti-fibrosing properties. It proved effective during the early stages of cirrhosis without deleterious side effects. However, its clearance is diminished in alcoholic cirrhosis (*388*). In the experimental model of the cirrhotic rat, colchicine reduced liver fibrosis and portal hypertension, increased albuminemia, and lowered bilirubinemia, and the incorporation of proline in the diseased liver (*389*). Based on its efficacy against primary biliary cirrhosis, the combination of colchicine and ursodeoxycholic acid seemed to merit testing.

For seven patients with advanced cirrhosis, colchicine (1–2 mg/day) was able to almost normalize the conjugated bilirubin levels for five out of six and the transaminase concentrations for four out of four (*390*). The evolution of the disease seemed slower under colchicine (1 mg/day) in an open study on 91 patients (*391*). In a prospective, randomized, double-blind study that compared 43 patients taking colchicine over the long term (5 mg/week) to a placebo, the evaluation after 4 years showed significant clinical improvement and stabilization of albuminemia *versus* the placebo group. Fibrosis, evaluated by repeated biopsies, was significantly attenuated for three patients taking colchicine. In addition, the mortality rate was lower in the colchicine group (24.9 *versus* 41.3%) (*392*). The cumulative 5- and 10-year survival rates and liver histology were significantly improved by colchicine (5 mg/week) in a randomized, double-blind, controlled study on 100 patients with cirrhoses of various etiologies, comprised of 73%

stage Child–Turcotte class A and 26% class B. Under colchicine therapy, the median survival was 11 years, as opposed to 3.5 years for the placebo group ($P < 0.001$). The cumulative 5- and 10-year survival rates were, respectively, 75 and 56% for the colchicine group and 34 and 20% for the placebo group ($P < 0.001$) (*29*). This study was broadly criticized because, among others, the nutritional status was not assessed and the survival rates of compliant *versus* non-compliant patients were not examined separately (*393-395*). Similarly, a randomized, double-blind study, testing colchicine (5 mg/week) *versus* placebo in 52 patients, showed significantly more regression of the ascites ($P < 0.01$) and the median survival at 11 years was 42 months for the colchicine group *versus* 15 months for the placebo group ($P < 0.001$) (*396*).

Thus, colchicine is potentially useful in this pathology.

2. Alcoholic Hepatitis

Despite its anti-inflammatory properties, colchicine appears to be ineffective against alcoholic hepatitis, a pathology for which the number of patients lost-to-follow-up is high. For the 74 patients with acute severe alcoholic hepatitis taking colchicine (1 mg/day for 4 weeks), or a placebo, the short-term mortality rates did not differ (*397*). Similarly, the biological parameters, histology and survival rates at 3 and 6 months for 67 patients included in a randomized, double-blind trial comparing colchicine (1 mg/day for 6 months) to a placebo were equivalent, with 52% of them having been lost-to-follow-up at 6 months (*398*). Penicillamine D, an anti-fibrosing agent with major side effects, proved ineffective, as assessed using clinical and biological parameters, in a prospective, controlled, double-blind protocol that enrolled 40 patients and lasted 8 weeks (*399*). Based on the results obtained in 13 studies, only corticosteroids have a positive effect on short-term mortality in the severe forms (effectiveness demonstrated by four controlled studies and three meta-analyses), but not those without criteria of severity (*400*).

3. Chronic Hepatitis B Virus Infection

Various treatments have been tried (corticosteroids, lathyrogen, penicillamine D, and interferon-α), but are not routinely applied. A pilot study on nine patients with biopsy-proven chronic hepatitis B virus infection, evolving for 2–21 years and confirmed by serum DNA detection, prescribed colchicine at 5 mg/week for 6 months. Serum HBe antigen disappeared in two patients and DNA became undetectable in six, both findings supporting the control of infectivity. Thus, colchicine seems to have an antiviral effect (*401*). According to a randomized, open trial that included 66 patients, after 4 years of follow-up, it appears that colchicine alone (5 mg/week) prevented the development of cirrhosis in patients infected with the hepatitis B virus by significantly lowering the number of acute episodes of hepatitis ($P < 0.005$) (*402*).

These positive observations merit further investigation in a large-scale trial or a study combining colchicine and an antiviral agent.

4. Hepatic Fibrosis

Colchicine afforded no clinical, biological or histological benefit in two open trials conducted on children with hepatic fibrosis. The first included 20 youngsters who took 5 mg of colchicine/week for 1 year, and the second enrolled 45 children who received 7.5 mg/week for 24–48 months (*403*).

5. Chronic Constipation

Colchicine was prescribed (1.8 mg/day for 8 weeks) in the framework of an open, pilot trial including seven patients suffering from chronic constipation resistant to multiple medical treatments. Colchicine was indeed able to stimulate peristalsis. The mean number of spontaneous bowel movements increased significantly ($P < 0.05$), mean colonic transit time, calculated by using radiopaque markers, decreased significantly ($P < 0.05$) and symptoms were significantly attenuated (*404*). These promising results served as the basis of a large-scale, randomized, controlled trial *versus* placebo that is currently being conducted.

E. In Hematology

1. Hodgkin's Disease

Colchicine was prescribed because it is an anti-mitotic agent, and, in addition, it possesses antalgic and antipyretic properties. Two publications, dating from 1952 and 1955, are instructive. For two patients with advanced disease, their general conditions improved, adenopathies regressed transiently, and fever and pain disappeared under 3 mg of colchicine given intravenously every 3 days, after unsuccessful treatment attempts (radiotherapy, nitrogen mustards, cortisone). Similar results were obtained by two patients with limited disease (*405*). Responses were also favorable for ten patients with early or late stage Hodgkin's disease, even after the failure of prior therapies; moreover, side effects were rare (*406*). One author cited a report describing the transient efficacy of colchicine in a patient with malignant lymphogranulomatosis (*405*).

2. Chronic Lymphoid Leukemia

In vitro, colchicine at a low concentration (0.1 mg/ml) can destroy malignant lymphocytes without affecting normal lymphocytes, a finding that can serve as a diagnostic tool (*308, 407-409*). Fourteen patients with refractory chronic lymphoid leukemia were given colchicine intravenously every week, starting at 2–3 mg, and increasing the dose by 1 mg/week until the appearance of tumor regression or side effects, or, in the absence of one of these, disease progression. Adenopathies and splenomegaly decreased for two patients, and the disease stabilized for seven. Disparate results and frequent side effects have limited the use of colchicine in this context. Nonetheless, this drug was effective in several patients (*360, 410*).

Because colchicine is not completely devoid of activity against this malignant hemopathy, its incorporation into polychemotherapy protocols merits consideration (*411*).

3. Lymphomas

Small and large lymphomatous cells are destroyed experimentally by colchicine, while lymphocytes from reactive lymph nodes survive. Thus, colchicine was able to serve as a tool for the histological diagnosis of lymphoma. Using as a criterion a sensitivity to colchicine of ≥ 30%, only one false-negative and two false-positives were obtained compared to the standard histological examination of 31 lymphomatous and 30 reactive adenopathies (*412*).

4. Myeloma

In the rat, colchicine lowers urinary excretion of Tamm–Horsfall protein and modifies its structure by blocking its complexation with Bence–Jones protein (*413*). This aggregation is responsible for the acute renal insufficiency seen in myeloma patients. Despite these promising experimental results, the serum and urinary excretion levels of Tamm–Horsfall protein of six healthy volunteers were not affected by 6 days of colchicine administration (*414*). Nonetheless, this potential capacity merits further investigation.

5. Idiopathic Thrombopenic Purpura

Classical therapeutic approaches include corticosteroids, immunosuppressive agents, vincristine and/or splenectomy. Cyclophosphamide and/or danazol gave variable, and sometimes non-sustained, results over time. By inhibiting the phagocytosis of opsonized platelets, colchicine was effective against some refractory cases of idiopathic thrombopenic purpura. It was also effective in several cases after the failure of corticosteroids and/or splenectomy and/or vincristine: after 1.2 mg of colchicine daily for 2 weeks, thrombopenia had completely regressed for three and was partially attenuated for one of the 14 patients suffering from idiopathic thrombopenic purpura that had been evolving for a mean of 3.7 years. Responses were optimal at 4–6 weeks and efficacy was maintained for 14–16 months, at a daily dose of 0.6 mg, for three of these patients. This response rate is not negligible for refractory disease. In addition, three patients insensitive to vincristine responded to colchicine, even though the mechanism of action of these two molecules is thought to be the same. Unlike other therapeutic agents, colchicine can be used over the long term because of its low toxicity (*415*). Other similar successes have been reported (*416, 417*). Thrombopenia regressed under the colchicine–steroid combination for eight out of eleven patients and under colchicine alone for four out of nine of the 14 children suffering from corticoresistant idiopathic thrombopenic purpura (*418*). It is possible that synergy exists between corticosteroids and colchicine (*415, 418*).

Thus, colchicine has been shown to be well tolerated in the case of resistance to other known therapies prescribed for idiopathic thrombopenic purpura.

F. IN CANCEROLOGY

A positive effect was reported long ago for patients with gout who underwent radiotherapy (*11*), but required high doses which caused severe toxicity. In a study on 90 women with advanced breast cancer who had undergone surgery and radiotherapy, after 5 years of follow-up, the cure rate was better for the 55 women who had received colchicine derivatives before and after surgery and maintenance treatment with a colchicine preparation for 1 month every 6 months compared to those who had not. However, comparison of the data was not clear-cut (*419*). Trimethylcolchinic acid or *N*-deacetylcolchicine, a colchicine derivative, seems to hold some promise because it is less toxic and thus can be used at higher doses (*408, 420*). A colchicine analogue that binds to tubulin at the colchicine-binding site was poorly effective and toxic in a Phase II clinical trial in ovarian cancer refractory to platinum (*421*).

Thus, the anti-mitotic effect of colchicine seems difficult to master and apply *in vivo*.

G. IN OTORHINOLARYNGOLOGY AND STOMATOLOGY

In these fields, colchicine is effective because of its anti-inflammatory properties. The data reported based on two series of 167 and 50 patients, including inflammatory ear, nose and throat pathologies of diverse etiologies, showed 97.5 and 90% good and moderate results, respectively, under 2 mg of colchicine/day. This efficacy is similar to that obtained with non-steroidal anti-inflammatory drugs but without their contraindications or toxic side effects (*422, 423*). For 27 patients suffering from temporomandibular joint pathologies, resistant to known therapeutic agents and attributable to diverse etiologies grouping together acute and chronic causes, the results were excellent for 44% and good for 30% (*424*).

A double-blind trial is warranted to confirm these potentially promising observations.

H. IN PNEUMOLOGY

1. Asthma

Asthmatic patients have a deficit of concanavalin A-dependent suppressor T-cell activity. The experimental demonstration that exposing peripheral blood leukocytes from asthmatics taking oral theophylline to colchicine and concanavalin A *in vitro* corrected this defect prompted the adjunction of oral colchicine to the therapeutic regimen of these patients (*425*). Indeed, these two molecules are synergistic *in vitro* and increase the intracellular cyclic adenosine monophosphate concentration (*426*).

In a prospective, randomized, double-blind and crossover protocol that included

ten pauci-symptomatic atopic patients, each serving as his own control, colchicine (1 mg/day) afforded significantly attenuated clinical signs ($P < 0.05$), decreased use of albuterol ($P < 0.02$), lowered serum IgE levels ($P < 0.05$) and normalized the suppressive function induced by concanavalin A ($P < 0.05$). In contrast, pulmonary function tests were not modified. The impact of these results is limited by the small number of patients evaluated (*426*). Controlled, double-blind trials of colchicine *versus* placebo, with the objective of lowering the inhalation of corticosteroids, revealed no benefit for the 20 patients studied in one (*427*) and the 71 patients enrolled in another (*428*).

Promising experimental results encourage further investigation of colchicine in this pathology. In addition, randomized, large-scale studies are needed to evaluate the precise clinical contribution of colchicine (*429*).

2. Emphysema

Colchicine, because of its anti-elastase activity, can alleviate emphysema caused by elastase hyperactivity. Sixteen former smokers with chronic obstructive bronchopneumopathies took colchicine in a prospective, randomized, controlled, double-blind protocol *versus* placebo. The bronchial elastase concentration was significantly reduced after 15 days of colchicine (1.8 mg/day) ($P < 0.006$) with a mean decrease of 51.5% for the colchicine group (*430*). These observations evoke a potential positive effect of colchicine in this setting.

3. Cystic Fibrosis

The world's first Phase II controlled study of colchicine *versus* placebo for the treatment of cystic fibrosis was started in September, 1998 at Necker Enfants- Malades Hospital in Paris, France. As described above for other organs, colchicine would act in the lung as well *via* its anti-inflammatory and anti-fibrosing properties (*265*), and by its capacity to stimulate multidrug-resistance (MDR) and multidrug-resistance-associated proteins (MRP). These latter share a marked "resemblance" with the protein that is defective (*i.e.*, the cystic fibrosis transmembrane-conductance regulator (CFTR) protein) and is the cause of this disease. Thus, the resistance proteins stimulated by colchicine assume the role of the defective CFTR (*431*), thereby justifying the clinical evaluation of this molecule in cystic fibrosis.

I. IN OPHTHALMOLOGY

Although colchicine inhibits the *in vitro* proliferation and migration of astrocytes, fibroblasts and pigmented retinal cells (*432*), and decreases the incidence and severity of retinal detachment in an experimental animal model (*433*), it had no such effect against proliferative vitreoretinopathy of any origin in a prospective, controlled study of 22 patients given 1.2 mg/day (*434*).

J. IN DIABETOLOGY

Activation of the polyol degradation pathway produces an aldose reductase that shunts glucose metabolism towards sorbitol, which is transformed into fructose. The latter accumulates in Schwann cells and induces the segmental demyelinization that is at the origin of diabetic neuropathy. Colchicine could be beneficial because it inhibits the action of aldose reductase, thereby preventing the activation of the polyol pathway.

In the first phase of an open, non-controlled study, none of the 50 patients with diabetic neuropathy whose diabetes had been evolving for a mean of 8 years who had been treated with insulin and diet for 1 month showed any improvement. During the second phase, half of them continued the initial regimen and the other 25 took colchicine alone (1 mg/day for 1 month and then 1 mg every other day for 5 months). After 6 months, the subjective signs of the latter had disappeared for 17 of them and were attenuated for the remaining 8, and the conduction speeds along the median and peroneal nerves were significantly faster. No improvement was noted for the patients in the diet and insulin group (*435*). These promising findings warrant further investigation and histochemical confirmation of the extent of demyelinization by examination of peripheral nerve biopsies. According to an open study on twelve patients with non-insulin-dependent diabetes of recent onset, glycemia was significantly lowered under 1.5 mg of colchicine given daily for 15 days after 15 days of placebo; no particular diet was imposed.

These observations might be explained by the restoration of the insulin response as a consequence of reduction of the prostaglandin levels and perhaps modulation of the immune system (*436*), but in-depth studies are needed because colchicine also decreases insulin secretion.

IX. Conclusion

Colchicine has thus dramatically altered the prognosis of familial Mediterranean fever because it is able to prevent amyloidosis or cause it to regress. This molecule has been prescribed to treat many pathologies, primarily because of its numerous properties. Some, unfortunately, like its capacity to reduce atheromatous plaques experimentally, have not proven applicable *in vivo*. It merits being used even after the failure of corticosteroids and/or immunosuppressants in some diseases. Some of the observations reported herein have not yet been confirmed by controlled studies, but the undertaking of such trials is heartily justified to reach objective conclusions, for example, concerning the long-term treatment of Behçet's disease. After reading this review, one is tempted to think that this 1,000-year-old drug has not yet finished surprising us.

Acknowledgment

I want to thank Janet Jacobson for her diligence, patience and contributions to the realization of this translation.

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ISBN 0-12-469553-1

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