Pure & Appl. Chem., Vol. 62, No. 7, pp. 1285–1288, 1990.
Printed in Great Britain.

Chemistry of shilajit, an immunomodulatory Ayurvedic *rasayan*

Shibnath Ghosal

Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India

Abstract - The chemical polemics in the reported literature on shilajit are resolved. This study shows that humification of latex and resin-bearing plants is responsible for the major organic mass (80-85%) of shilajit. The low mol. wt. chemical markers (8-10%), viz. aucuparins, oxygenated dibenzo-α-pyrones and triterpenic acids of the tirucallane type (free and conjugated), occurring in the core structure of shilajit humus, are the major active constituents of Himalayan shilajit. The therapeutic effects of shilajit are the consequences of hormonal control and regulation of immunity.

INTRODUCTION

Shilajit is a blackish-brown exudation, from steep rocks of different formations, commonly found in the Himalayas, at altitudes between 1000-5000 m, from Arunachal Pradesh in the East to Kashmir in the West. It is also found in other countries, e.g. Afganisthan (Hindukush), Bhutan, China, Nepal, Pakistan, Tibet (Himalayan belt) and the USSR (Tien Shan,Ural). Shilajit is believed to arrest aging and produce rejuvenation (ref. 1), - two important attributes of a rasayan (refs. 2,3).

Considerable controversy had existed in the reported literature (ref. 3) when we initiated our study on the nature and chemical constituents of shilajit about fourteen years ago. It was variously described, as a bitumen or mineral resin varying greatly in consistency from a free-flowing liquid to a hard brittle solid; a plant fossil exposed by a elevation of the Himalayas; a substance of mixed animal and plant origin (refs. 3,4). Twelve years after the publication of the circumstantial evidence for the contribution of plants in shilajit formation (ref.5), we obtained further direct evidence regarding the chemical character of shilajit (refs. 6,7). It would now require summation of our earlier findings for resolving the chemical polemics (refs. 3,4) on this subject and to report our recent findings, from analyses of shilajit from different regions, to show the generality of our conclusion.

The first major advance in our understanding of the chemical character of shilajit was the observation that shilajit, from different regions, contained a large variety of organic compounds that can be broadly grouped into humic and non-humic substances (refs. 6,7). The non-humic substances, in soil-sediment humus (ref. 8), are low mol. wt. organic compounds that are characterizable by chemical and spectroscopic methods. The humic substances, by contrast, do not exhibit any specific physical and chemical characteristics (e.g. sharp m.p., consistent elemental composition, consistent pH, well-defined IR and NMR spectra), normally exhibited by characterizable organic compounds. Humic substances are produced by interaction of plants, algae, mosses, and microorganisms. The phytochemistry of vegetation around shilajit-bearing rocks, therefore, constituted an important part of our investigation.

The common plant sources of humus, in mountain soils, are the perennial grasses and legumes, which possess finely branched root systems capable of regeneration. Other important sources of humus are the litter and latex of plants. Variation in the quality of shilajit humus (both chemical and biological) is, therefore, conceivable. The other factors that cause variations in shilajit humus are: (i) altitude and the nature of shilajit-bearing rocks; (ii) atmospheric conditions (e.g. alternate wetting and drying); (iii) pH and moisture content of the rock source; and (iv) activity of the rhizospheric microorganisms and their exo-enzymes. The stability of the humus reserve depends on one or more of these factors. Shilajit samples collected from different places, as expected, exhibit variations in chemical characteristics and bioactivities. Furthermore, the hazards of collection of shilajit and the scanty amount generally available in any one locale prompt unscrupulous traders to adulterate it with rock soil, plant debris and quercus gums. It was, therefore, thought imperative to determine certain standards of shilajit on the basis of bioactivity-directed investigation of its chemical constituents.

CHEMISTRY OF SOURCE MATERIALS OF SHILAJIT

During our bioactivity-directed investigation of shilajit samples, from different countries, some striking similarities were observed in respect of their contained low mol. wt. bioactive compounds. Several phenylpropanoid-acetate-derived aucuparins, oxygenated biphenylcarboxylates, isolated and characterized as their permethylated derivatives (1-3) (ref. 7), and oxygenated dibenzo-α-pyrones (4-6) (refs. 6,7) were found to occur ubiquitously, albeit in different amounts, in all authentic samples of shilajit. We also located some of the living plant ancestors of these compounds.

Over eighty different plant species were reported (ref. 9) in and around the shilajit rocks in Kumaon itself. One species which was consistently found to be present in shilajit-bearing rocks, throughout the Eastern and the Western Himalayas, was a rich latex producing plant, Euphorbia royleana Boiss. (Euphorbiaceae). Some other latex and resin producing common species, in these regions, are the legumes, e.g. Trifolium, Rhus (family, Anacardiaceae), Ficus (Moraceae), and Juniperus (Cuprassaceae).

T. ripens (Leguminosae), collected from different places in the Himalayan belt, yielded several phenylpropanoid-acetate-derived metabolites including (1) to (3). E. royleana (latex and debris), putrefied by shilajit rhizospheric microorganisms, yielded the three other important shilajit marker compounds (4-6) along with several other equivalent metabolites. A conceptual model for the genesis of (1) to (6), involving an intermediate (7), was envisaged (ref.10). Another key intermediate (8), isolated from a free-flowing (premature) sample of shilajit, provided strong circumstantial evidence in support of the aforesaid biogenetic route (ref. 10). The reactive intermediate (8) was autoxidized, in presence of light and air, to give a mixture of (4) and (6), presumably via the endo-peroxide (9).

Continuing the phytochemical investigation, we have now isolated and characterized from R. cotinus and R. succedanea (Anacardiaceae), several phenolic lipids of the type (10) and triterpenoids (both free and conjugated,- oligoglycosides) of the tirucallane types (11-12). Enzymatic hydrolysis of a major triterpenoid saponin fraction, with hesperidinase, followed by column chromatography (Si gel using n-BuOH saturated with water) of the sapogenin fraction afforded a mixture of 24(Z)-3β-hydroxytirucalla- 7,24-dien-26-oic acid (11a) and 24(Z)-3β-hydroxytirucalla-8,24-dien-26-oic acid (12a). From the aqueous hydrolysate, L-arabinose, L-rhamnose, D-xylose and D-glucose were isolated, as their alditol acetates, and identified by GLC. In case of shilajit, from different regions, both E- and Z-isomers of the triterpenoid sapogenins (11a-b) and (12a-b) and the phenolic constituents were isolated and characterized. The structures of these compounds were established by comprehensive spectroscopic analyses, crucial chemical transformations and synthesis, where possible. Pharmacological and immunological screening of these compounds, individually and in combination, established their significant contribution to the therapeutic efficacy of shilajit. Among the other organic compounds contributing to the bioactivity of shilajit, humic and fulvic acids, from shilajit humus, are noteworthy. However, the main task that confronts researchers in this field (study of humus) today is to decipher the complexity of the building units of humus and their allignments, after polycondensation, in the core structures of humic substances.

HUMIC SUBSTANCES FROM SHILAJIT

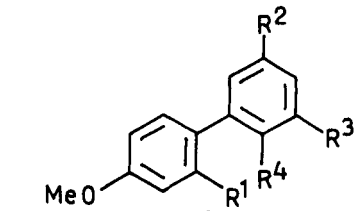
Scanning electron microscopy and viscosity measurements of humic acids (HAs) and fulvic acids (FAs), from shilajit, suggested for the FAs a relatively open, flexible structure punctured by voids (micropores) of different diameters, at different pH. FAs from biologically equiactive shilajit samples exhibited a number of similarities in respect of: (i) elemental and micronutrient (trace metal ions) compositions; (ii) aromatic and aliphatic carbon ratio; (iii) absorbance ratio at 465/665 nm (E-4/E-6); (iv) viscosity and particle size; and (v) PMR and CMR spectra.

Relatively mild degradation of shilajit-HAs, by boiling with water, yielded several aliphatic (C-16 to C-20) and phenolic acids together with common sugars, glucose, arabinose, rhamnose and xylose. These compounds were, presumably, loosely held in the core structures of shilajit HAs either by weak bonding or by adsorption/intercalation in their internal voids. The plant secondary metabolites which are trapped in the internal voids of humic substances are spared from and resistant to common chemical and biological decomposition. This is consistent with the observed ubiquitous occurrence of the aucuparins and dibenzo-α-pyrones in the core of shilajit from different regions. During systemic administration of shilajit, these constituents, even if, present as minor entities, elicit their potent biological effects and are, therefore, regarded as markers of shilajit.

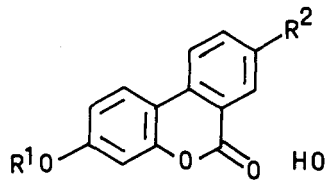
According to accepted tenets, biogenesis of humus (ref. 8) involves a two-stage process: (i) fragmentation of plant and microbial constituents into small molecules (monomers), and (ii) heteropolycondensation of the monomers into high mol. wt. humus. Our results of

investigation of shilajit HAs, however, suggested participation, at least in part, of some plant-derived intact phenolic metabolites, viz. biphenyl carboxylates (and equivalents), in their core structure. These phenolic moieties were transformed into polynuclear aromatic hydrocarbons, phenanthrene, 2-methylphenanthrene and 2,3-benzofluorene, on Zn dust distillation of shilajit HAs. The same degradation products were also obtained when soil-sediment HAs were subjected to Zn dust distillation. Thus, some inherent structural similarities were indicated for shilajit and other common HAs.

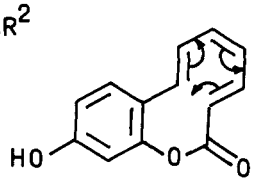
Variations in chemical characteristics and biological actions were observed in the humic substances of shilajit itself having different consistencies, e.g. dark brittle (over exposed), brown viscous (mature), and free-flowing liquid (premature shilajit). This may be due to the fact that humus reserve is a complex mass whose complexity is determined by the intensity of several factors: (a) the rate of formation of fresh humus; (b) adsorption of plant root exudates and leached materials from debris of plants and microorganisms to humus reserve; (c) the rate of decomposition of the caged and free low mol. wt. compounds; and (d) the rate of decomposition of HAs and FAs into humin and other intractable products. Hence the quality of humus, which primarily acts as the liposomic carrier of low mol. wt. bioactive compounds of shilajit, would constitute an important determinant to the therapeutic potential of shilajit. It was, therefore, thought necessary to determine the biological activity profiles of the low mol. wt. organic compounds and the HAs and FAs of shilajit, individually and in combinations, to evaluate their additive and synergistic potential.



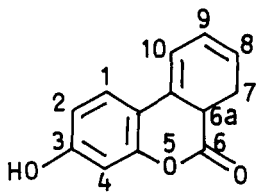
(1) $R^1=R^4=H$, $R^2=R^3=OMe$
(2) $R^1=R^2=R^3=H$, $R^4=CO_2Me$
(3) $R^1=OMe$, $R^2=R^3=H$, $R^4=CO_2Me$



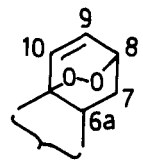
(4) $R^1=R^2=H$
(5) $R^1=Me$, $R^2=H$
(6) $R^1=H$, $R^2=OH$



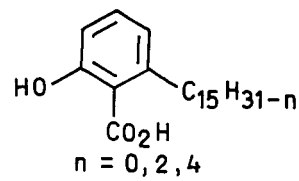
(7)



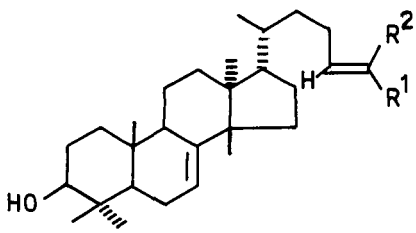
(8)



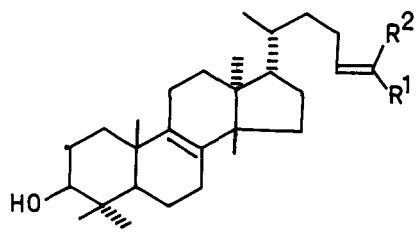
(9)



(10)
n = 0, 2, 4



(11a) $R^1=CO_2H$, $R^2=Me$
(11b) $R^1=Me$, $R^2=CO_2H$



(12a) $R^1=CO_2H$, $R^2=Me$
(12b) $R^1=Me$, $R^2=CO_2H$

BIOACTIVITY OF SHILAJIT AND ITS CONSTITUENTS

Clinical applications of shilajit in Ayurveda, as a rasayan, are well documented (refs. 1,3). However, no modern scientific study was carried out before on the mode of action of shilajit. The effects of shilajit, as reported in the Ayurvedic literature, seem to suggest its influence on endocrine, autonomic, and brain functional changes. The discovery that these changes can be mediated by cytokines, released by activated immunologic cells (ref. 11), has opened up possibilities for similar mechanism of action

of shilajit. Certain combinations of the phenolic and triterpenoid constituents, and the FAs of shilajit produced significant effects against restraint stress-induced ulcers (ref. 6). The mechanism of anti-ulcerogenic actions of shilajit and its constituents was also evaluated (ref.6). This was based on their effects on mucin contents, and on the concentrations of DNA and protein in the gastric juice. The combinations provided significant resistance to mucosa against the effects of ulcerogens and also prevented the shedding of mucosal cells. The anti-allergic action of these compounds was successfully tested against antigen- and compound 48/80 (histamine releaser)- induced degranulation of mast cells (ref. 12). The anti-stress activity of these compounds was suggested by their augmentation of murine swimming endurance exercises. Shilajit and its combined constituents also elicited and activated, in different degrees, murine peritoneal macrophages and activated splenocytes of tumour-bearing animals at early and later stages (unresponsive) of tumour growth (tested according to ref.13). Shilajit from USSR, and its corresponding combined fractions, acted essentially as cell-growth factors in both normal and tumour cells by maintaining membrane integrity. The results obtained till now are sufficiently impressive to warrant expectation that more extensive and comprehensive studies on shilajit and its constituents would validate the Ayurvedic rasayan, shilajit, as more effective than several currently available clinically efficacious immunomodulators (refs. 14, 15).

REFERENCES

1. U.C.Datta and G. King, Materia Medica of the Hindus, p.33-37, Machine Press, Calcutta, India (1877).

2. P.V. Sharma, Introduction to Dravyaguna, p.63, 4th Edn., Chaukhamba, Orientalia, India (1978).

3. V.P. Tiwari, K.C. Tiwari and P. Joshi, J. Res. Ind. Med., 8, 53-60 (1973).

4. Y.C. Kong, P.P.H. But, K.H. Ng, K.F. Cheng, R.C.Cambie and S.B. Malla, Int. J. Crude Drug Res., 25, 179-182 (1987).

5. S. Ghosal, J.P. Reddy and V.K. Lal, J. Pharm. Sci., 65, 772-773 (1976).

6. S. Ghosal, S.K. Singh, Y. Kumar, R.S. Srivastava, R.K. Goel, R. Dey and S.K. Bhattacharya, Phytother. Res., 2, 187-191 (1988).

7. S. Ghosal, S.K. Singh and R.S. Srivastava, J. Chem. Res. (S) 196-197 (1988).

8. M. Schnitzer, Soil Organic Matter (M. Schnitzer and S.U. Khan, Eds.) Ch.3, Elsevier, New York (1978).

9. H.C. Pandey and V.P. Tiwari, J. Res. Ind. Med., 12, 113-115 (1977).

10. S. Ghosal, J. Lal, S.K. Singh, Y. Kumar and F. Soti, J. Chem. Res. (S) (1989).

11. H.O. Besedovsky,A.E. Dei-Rey and E. Sorkin, J. Immunol., 135, 750-754s (1985).

12. S. Ghosal, J. Lal, S.K. Singh, G. Dasgupta, J. Bhaduri, M. Mukhopadhyay and S.K. Bhattacharya, Phytother. Res., 3, (1989).

13. U. Chattopadhyay, S. Das, S. Guha and S. Ghosal, Cancer Lett., 37, 293-299 (1987).

14. H. Wagner and A. Proksch, Economic and Medicinal Plant Research, p. 113-153 (H. Wagner, H. Hikino and N.R. Farnsworth, Eds.), Academic, New York (1985).

15. R. Bomford, Phytother Res., 2, 159-164 (1988).