Shilajit I: Chemical Constituents

Keyphrases □ Shilajit—chemical constituents determined, compared to extracts of *Euphorbia royleana* plant □ *Euphorbia royleana* plant—chemical constituents determined, compared to extracts of shilajit (Indian rock exudate) □ Triterpenes—isolated from shilajit (Indian rock exudate) and *Euphorbia royleana* plant □ Benzcoumarins—isolated from shilajit (Indian rock exudate) and *Euphorbia royleana* plant □ Ellagic acid—isolated from shilajit (Indian rock exudate) and *Euphorbia royleana* plant

To the Editor:

Shilajit is a blackish-brown exudation from steep rocks of different formations found in the Himalayan regions of Uttar Pradesh, Himachal Pradesh, Jammu, and Kashmir. Aqueous solution of shilajit is used for treating hypertension, chronic bronchitis, asthma, genito-urinary infections, dropsy, and nervous disorders (1). However, the hazards of its collection and the scanty amount available prompt unscrupulous dealers to adulterate it with quercus gall, gums, and cow's urine. Therefore, it is essential to evaluate the chemical constituents of shilajit to assay its quality.

The current information relating to the chemical constituents of shilajit is only fragmentary. It was reported to contain resins, fatty acids, benzoic and hippuric acids, and albuminoids (1). A study of vegetation of the areas of shilajit-exuding rocks indicated (2) that *Euphorbia royleana* Boiss., a latex-bearing plant abundantly growing in the Western Himalayas, could be the source of organic constituents of shilajit. This postulate had not been tested before experimentally. Evidence is now presented to establish that the active principles of shilajit owe their origin primarily to *E. royleana* plants.

Extraction of shilajit with solvents of graded polarity yielded three different classes of organic compounds, (*a*) triterpenes and sterols; (*b*) aromatic carboxylic acids, ellagic acid, and 3,4-benzcoumarins; and (*c*) α-amino acids. The identity of the individual entities was established on the basis of physical and spectral properties of the compounds and their derivatives and, when possible, by direct comparison with authentic samples.

Desiccated and powdered shilajit[1] (200 g) was successively extracted with 500 ml of hot petroleum ether (bp 60–80°), chloroform, and methanol. The extracts were processed separately.

On column chromatography over neutral alumina (activity grade about IV), the petroleum ether extract (148 mg) afforded euphol (8 mg), taraxerol (22 mg), and sitosterol (18 mg) as well as two partially characterized triterpenes and a sterol as minor entities. The petroleum ether extract from the air-dried latex of *E. royleana*[1], collected from the top of shilajit-exuding rocks, yielded the same major constituents in 0.01, 0.08, and 0.03% yields, respectively. Previously, taraxerol, taraxeryl acetate, and glut-5-en-3β-yl acetate were reported (3, 4) in *E. royleana*.

From the chloroform extract (220 mg), benzoic acid (66 mg), *m*-hydroxybenzoic acid (11 mg), and three 3,4-benzcoumarins (I–III) were isolated by solvent extraction and column and preparative layer chromatography. Compound I (80 mg), colorless needles from acetone–hexane, mp 231–232°, $C_{13}H_8O_3$ (M^+, 212), showed UV absorption maxima at λ (ethanol) 232 (*A* 0.74), 248 (0.4), 278 (0.43), 303 (0.34), and 330 (0.23) nm. The IR absorption maxima were at ν (mineral oil) 3280 (OH) and 1698 (lactonic CO) cm^{-1}; the lactonic carbonyl absorption was shifted to ν 1742 cm^{-1} in the corresponding methyl ether. These properties are strikingly similar to those of hydroxycoumarins (5, 6).

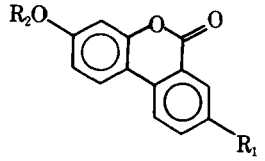
The 60-MHz proton magnetic resonance (PMR) spectrum in trifluoroacetic acid showed a coupled set of three protons in the δ 7.0 region (involving an *ABC* system) and complex multiplets in the δ 8.3–7.5 region associated with four aromatic protons. In the mass spectrum, aside from the molecular ion peak, which is the base peak, significant fragment ion peaks appeared at *m/e* 184 (40%, 212 → 184 transition, m* 160), 156 (8), 155 (14), 128 (27), 127 (18), 126 (8), 115 (7), and 114 (6).

On the basis of these data, I was identified as 7-hydroxy-3,4-benzcoumarin. Hurtley (7) previously accomplished its synthesis while developing a convenient method of synthesis of 3,4-benzcoumarins by the condensation of reactive phenols with *o*-bromobenzoic acid with copper as a catalyst. Its spectral properties, however, were not recorded. Also, this study is the first demonstration of the natural occurrence of I.

Compound II (4 mg), mp 140–141°, $C_{14}H_{10}O_3$ (M^+, 226), was readily identified as the methyl ether of I. Compound III (7 mg), yellow needles from acetone, mp >360°, $C_{13}H_8O_4$ (M^+, 228, 100%), showed significant fragment ions at *m/e* 200 (35), 172 (5), 171 (12), and 144 (22). In the PMR spectrum, two coupled sets of three protons each appeared in the δ 7.5 and 7.0 regions and were assigned to two *ABC* systems. Compound III gave a diacetate, mp 208–209° (M^+, 312). The compound was assigned 2′,7-dihydroxy-3,4-benzcoumarin structure and was previously reported in castoreum (secretions from the scent glands of beaver) (8).

Benzoic and *m*-hydroxybenzoic acid and the three benzcoumarins (I–III) were also isolated from the latex of *E. royleana*. Additionally, the presence of a number of benzcoumarins, in the form of amino acid conjugates, was detected in the latex.

The methanol extract (2.8 g) was separated into two fractions by trituration with a moist solvent ether. In the ethereal extract, ellagic acid was detected readily by inspection of a paper chromatogram in Forestal solvent (9). Two minor, less polar, phenolic components also were detected in this fraction. The same compounds were detected as the major constituents in the



I: $R_1 = R_2 = H$
II: $R_1 = H, R_2 = CH_3$
III: $R_1 = OH, R_2 = H$

[1] Shilajit and the latex samples were collected, with the assistance of members of the Amalgamated Units, Central Council of Research in Indian Medicine and Homeopathy, Tarikhet, from Jorassi village, Almorah, India. A voucher specimen of each has been preserved at the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University.

corresponding extract of the latex of *E. royleana*. Ellagic acid and its 3,3′-dimethyl ether were previously reported in two other *Euphorbia* species (5).

The ether-insoluble fraction of the methanol extract showed a number of ninhydrin-positive spots, suggesting the presence of α-amino acids. It was dissolved in methanol and chromatographed[2]. Elution was carried out with increasing proportions of triethylamine in methanol. The identity of the amino acids was established by paper chromatography using authentic markers according to the method of Hardy *et al.* (10). Among the 18 free amino acids detected in the effluents, seven were identified. These were, in order of abundance, glycine, aspartic acid, methionine, asparagine, glutamic acid, tyrosine, and phenylalanine. The major amino acid composition in the latex of *E. royleana* was similar to that of shilajit.

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[2] Dowex-50-X8.

Failure of Prednisone to Alter Plasma Salicylate Concentrations in Dogs

Keyphrases □ Prednisone—effect on plasma salicylate concentrations, dogs □ Salicylate—plasma concentrations, effect of prednisone, dogs □ Interactions—prednisone and salicylate, dogs

To the Editor:

Drug interactions are important considerations in modern therapy. Among the important drug interactions are those affecting disposition of salicylates. Plasma salicylate concentrations are altered by agents such as ammonium chloride, ascorbic acid (1), aminobenzoic acid (2), and "nonsystemic" antacids (3).

An interaction of corticosteroids and salicylates was reported by Klinenberg and Miller (4). They observed a rise in plasma salicylate concentrations in four patients with rheumatoid arthritis as corticosteroid dosage was tapered and salicylate dosage remained constant. The magnitude of increased salicylate concentration was sufficient in one patient for the development of salicylate intoxication. These authors cited the tapering of corticosteroid as the mechanism responsible for the rise in plasma salicylate. This interaction has important implications, because corticosteroids and salicylates frequently are administered simultaneously in the treatment of diseases other than rheumatoid arthritis such as acute rheumatic fever. Because, to our knowledge, the interaction has not been confirmed, we studied the effect of prednisone treatment on plasma salicylate concentration in dogs.

Six mongrel dogs, 13–20 kg, were administered aspirin tablets orally (900 mg/dose, except Dog 6 which received 600 mg/dose) on an 8 am–2 pm–8 pm–2 am schedule. The dosage schedule resulted in average plasma salicylate concentrations between 10 and 20 mg %. Heparinized blood was drawn from each dog at 1 pm daily to monitor plasma salicylate concentrations. Once salicylate concentrations were stable, 10 mg of prednisone was administered orally once daily at 8 am to each dog for 6 days. The aspirin dosage schedule remained unchanged throughout prednisone administration. Plasma salicylate concentrations were determined by a reported method (5).

The salicylate concentrations on the 3 days prior to prednisone administration and on the last 3 days of prednisone administration were averaged for each dog and analyzed by the Student *t* test (paired comparison). Thus, each dog served as its own control for the comparison of aspirin treatment alone *versus* aspirin plus prednisone treatment.

Table I shows the average plasma salicylate concentrations of each dog before and during prednisone treatment. Prior to prednisone treatment, the mean salicylate concentration was 16.1 mg %. During prednisone therapy, the mean salicylate concentration was 17.3 mg %. Comparison of each dog's average plasma salicylate level before and during prednisone treatment by the paired *t* test showed no significant change ($p > 0.05$).

Table I—Average Plasma Salicylate Concentration (Milligrams Percent)

Dog	Before Prednisone	During Prednisone	Difference
1	19	18	−1
2	15	21	+6
3	17	13	−4
4	14	13	−1
5	18	23	+5
6	14	16	+2
Mean	16.1	17.3	1.2 ± 1.5