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Extractives of *Balsamodendron pubescens* : Stocks, Hook. Isolation and a new synthesis of siderin*

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Abstract. β-Sitosterol and cedrelone have been obtained from the hexane extract of the roots of *Balsamodendron pubescens* Stocks, Hook. A dimethoxy methyl coumarin isolated has been characterised as siderin (VIII) (4,7-dimethoxy-5-methyl coumarin). A new synthesis of siderin as well as 6,8-dimethoxy-4-methyl coumarin (II) is also reported.

Keywords. Coumarins; structure; synthesis.

1. Introduction

Balsamodendron pubescens Stocks, Hook. (family : Burseraceae) is a small tree growing in the southern parts of the Western Ghats of India. No previous work appears to have been carried out on the phytochemistry of this plant. During the course of our investigations on the screening of Indian medicinal plants (Desai *et al* 1977) we collected the roots of *B. pubescens* in Peringalkuthu (Kerala) and the present study reports the isolation of some chemical constituents.

2. Results and discussion

2.1. *Isolation of β-sitosterol and cedrelone*

Hexane extract of the powdered roots of *B. pubescens* gave an oily residue which was chromatographed over silica gel. This resulted in the isolation of β-sitosterol and the meliacane type triterpene, cedrelone (Hodges *et al* 1963).

The identity of both these compounds was established by comparison of TLC, mmp, IR and NMR spectra with authentic samples.

It is of interest to note that the isolation of cedrelone has been recorded only in the Meliaceous trees and its occurrence is not too frequent (Govindachari *et al* 1969; Adisogan *et al* 1970). The obtainment of this limonoid from the Bursera-

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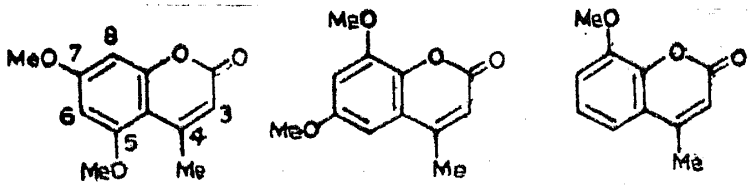
ceae family may be of chemotaxonomic interest (Connolly and Overton 1970; Davon and Scott 1975).

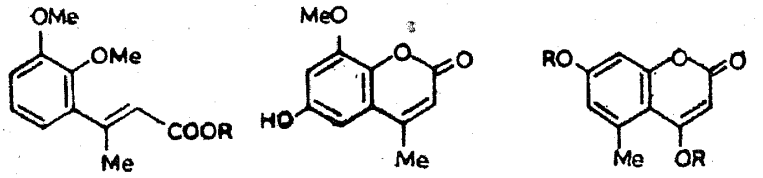
2.2. *Isolation of siderin*

Chromatographic separation of the hexane extract also resulted in the isolation of a colourless compound mp 197–8° analysing for $C_{12}H_{12}O_4$. Its UV and IR spectra indicated it to be a coumarin derivatives and the NMR spectrum showed that the C–3 position of the coumarin was unsubstituted.



I II III

Comparison of the properties of 5,7-dimethoxy-4-methyl coumarin (I) (Canter *et al* 1931) showed that it was not identical with the natural compound. The compound (II) was not known in the literature. However, since (III) was reported to have been isolated from *Ekbergia senegalensis* and also synthesised (Bevan and Ekong 1965) we planned to carry out the Elbs persulphate oxidation of (III). 2,3-Dimethoxy-β-methyl cinnamic acid (V) prepared by alkaline hydrolysis of the corresponding ethyl ester (IV) was cyclized to give the coumarin (III) mp 138–140°. As the natural coumarin as well as the synthetic compound are reported



IV : R = Et VI VII : R=H

V : R = H VIII : R=Me

to have mp 165° (Bevan and Ekong 1965) the structure assigned to the natural coumarin and the reported synthesis appeared to be doubtful. A recent paper has proposed that the structure assigned to the natural product should be revised (Venturella *et al* 1974a). Oxidation of (III) with potassium persulphate gave 6-hydroxy-8-methoxy-4-methyl coumarin (VI) which on methylation afforded (II) mp 181–2°. This also proved to be different from the natural coumarin.

90 MHz NMR spectra of compounds (I), (II), (III) and (VI) showed that in all the compounds, the C–3 proton appeared as a quartet and the C–4 methyl as a doublet due to allylic coupling. This could be of diagnostic value in suggesting the position of the C-methyl group at C–4 in coumarins unsubstituted in the 3-position.

As the natural compound was found to be different from (I) and (II), we considered structures having a methoxyl group in the C–4 position of the coumarin

nucleus. A survey of the recent literature subsequent to the year 1974 (Davon and Scott 1975) indicated the reported isolation of siderin, a constituent of *Sideritis canariensis* Ait., (family : Labiatae) originally thought to be 6,7-dimethoxy-4-methyl coumarin (Gonzàles *et al* 1972) and later formulated correctly as 4,7-dimethoxy-5-methylcoumarin (Venturella *et al* 1974b). Siderin has also been isolated as a metabolite of *Aspergillus variecolour* (Chexal *et al* 1975). The physical and spectral properties of siderin appeared to be similar to the compound isolated by us. As no specimen of siderin was available for comparison, we have synthesized this by a new route.

2.3. *Synthesis of siderin*

Orcinol was condensed with ethyl cyanoacetate in the presence of zinc chloride and dry hydrogen chloride (Spencer *et al* 1956) and the imide formed hydrolysed to give 4,7-dihydroxy-5-methyl coumarin (VII) mp 283°. Methylation of (VII) with dimethylsulphate, potassium carbonate in acetone afforded the dimethyl ether (siderin) (VIII) mp 194–5°, identical in all respects with the compound isolated from *B. pubescens*.

3. Experimental

All melting points are uncorrected. UV and IR spectra were determined on Beckmann DK–2A and Perkin–Elmer infracord or Model 421 spectrophotometers. NMR spectra were taken on Varian A–60 or Bruker, WH–90 spectrophotometers with TMS as internal reference standard. Chemical shifts δ are given in ppm downfield from Me_4Si. Mass spectra were determined on Atlas CH–7 instrument.

3.1. *Extraction of Balsamodendron pubescens*

The dried and milled roots (3·1 kg) were extracted with hot hexane (20 litres) and the solvent removed under vacuum to give a viscous residue (32 g). This was dissolved in benzene (50 ml) and chromatographed over silical gel (80 g). The column was gradient eluted with increasing amounts of benzene, benzene chloroform and chloroform-methanol. Fractions (75 ml) were collected and the chromatographic separation monitored by TLC. Fractions 5–9 (benzene) (5·2 g) gave on crystallisation from ethanol colourless plates (2 g) m p 203–4°. (Found : C, 73·7; H, 7·3. $C_{26}H_{30}O_5$ requires C, 73·9; H, 7·2%) M^+, m/e 422. It was found to be identical with cedrelone in its mmp, IR and NMR spectra.

Fractions 12–20 (benzene) (1·6 g) on crystallisation from ethanol gave β-sitosterol (260 mg) mp 138°.

Fractions 43–54 (benzene : chloroform 1 : 1) (210 mg) on crystallisation from ethanol afforded colourless plates (VIII; 45 mg) mp 197–8°. UV λ_{max} (ethanol) 220 (infl.), 285 (infl.), 304 and 312 nm (log ϵ, 4·3, 4·04, 4·18 and 4·13). IR ν_{max} (nujol) 1720, 1700, 1620, 1600 cm^{-1}. NMR ($CDCl_3$): δ 6·65, 6·4 (d, J = 2·3 Hz, 2H, H–6, H–8), 5·53 (s, 1H, H–3), 3·93 (s, 3H, OMe), 3·83 (s, 3H, OMe), 2·6 (s, 3H, Me). MS m/e 220 (M^+, 100%), 205 (4), 192 (75), 177 (60), 163 (15), 162 (20), 149(35), 134(20), 121(15), 119(15). (Found: C, 65·6; H, 6·0. Calc. for $C_{12}H_{12}O_4$; C, 65·4; H, 5·5%).

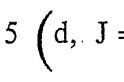
3.2. *5,7-Dimethoxy-4-methylcoumarin (I)*

A solution of 5,7-dihydroxy-4-methylcoumarin (Canter *et al* 1931) (2·7 g) in acetone was refluxed with anhyd. potassium carbonate (10 g) and methyl iodide (16 ml). Usual work up gave the dimethyl ether (I; 1·7 g) mp 172°. UV λ_{max} (ethanol) 242 (infl.) 252 and 318 nm (log ϵ 3·72, 3·70 and 4·14). IR ν_{max} (KBr) 1720, 1700, 1620 cm^{-1}. NMR ($CDCl_3$): δ 6·31, 6·2 (d, J = 2·5 Hz, 2H, H–6, H–8), 5·85 (d, J = 1 Hz; 1H, H–3), 3·8 (s, 6H, OMe), 2·49 (d, J = 1 Hz, 3H, Me). MS m/e 220 (M^+, 90%), 192 (100), 177 (92), 163 (10), 148 (25), 133 (10). (Found: C, 65·3; H, 5·7; Calc. for $C_{12}H_{12}O_4$: C, 65·4; H, 5·5%).

3.3. *2,3-Dimethoxyacetophenone*

This was prepared according to the procedure of Bruce (Bruce 1960). Distillation under vacuum gave the major fraction bp 113–5°/4–5 mm. IR ν_{max} (thin film) 1680, 1580 cm^{-1}. NMR ($CDCl_3$): δ 7·0 (m, 3H, Arm–H), 3·87, 3·83 (singlets, 3H each, OMe), 2·6 (s, 3H, COMe).

3.4. *2,3-Dimethoxy-β-methyl-ethyl cinnamate (IV)*

A solution of 2,3-dimethoxyacetophenone (15 g) in dimethyl formamide (100 ml) was added under stirring at 25° to a solution of triethyl phosphonoacetate (20 g); sodium ethoxide (7 g) in dimethylformamide (100 ml). After complete addition, the solution was stirred for one hr and 10% HCl was added to make the solution acidic. It was extracted with methylenechloride, then with water and dried over sodium sulphate. Removal of the solvent gave an oil (19 g). Chromatography over silica gel and elution with hexane gave a viscous oil (IV; 7·5 g). IR (thin film) ν_{max} 1710, 1635, 1585, 1570 cm^{-1}. NMR ($CDCl_3$) δ : 6·8 (m, 3H, Arm–H), 5·85 (d, J = 1·5 H, 1H, ), 4·2 (q, J = 7 Hz, $-CH_2-Me$), 3·8, 3·75 (singlets, 3H each, OMe), 2·5 (d, J = 1·5 Hz, ), 1·28 (t, J = 7 Hz 3H, $-CH_2-Me$). MS m/e 250 (M^+, 10%), 219 (55), 205 (20), 191 (100), 175 (10), 162 (20), 161 (20), 147 (14). (Found: C, 67·1; H, 7·7. $C_{14}H_{18}O_4$ requires: C, 67·2; H, 7·3%).

3.5. *2,3-Dimethoxy-β-methylcinnamic acid (V)*

The ester (IV; 6·6 g), 20% sodium hydroxide (100 ml) and ethanol (5 ml) were refluxed for 16 hr. The solution was acidified with dil. hydrochloric acid, extracted with ether, dried over anhyd. sodium sulphate and evaporated to give a crude product (5·1 g). Crystallisation from hexane afforded (V; 3·5 g) mp 127–8°. IR (KBr) ν_{max} 1680, 1622, 1580 cm^{-1}. NMR ($CDCl_3$) : δ 10·7 (br, 1H, COOH), 6·9 (m, 3H, Arm–H), 5·91 (d, J = 1·5 Hz, 1H, ), 3·85, 3·75 (singlets, 3H each, OMe), 2·52 (d, J = 1·5 Hz, 3H, Me), MS m/e 222 (M^+, 35%), 205 (2), 191 (100), 175 (7), 162 (20), 161 (25), 148 (22), 147 (18). (Found : C, 64·6; H, 6·5. $C_{12}H_{14}O_4$ requires: C, 64·9; H, 6·4%).

3.6. 8-*Methoxy*-4-*methylcoumarin* (*III*)

The carboxylic acid (V; 5·5 g) in dry ether (100 ml) and 33% HBr in acetic acid (225 ml) were kept stirring at r.t. for 3 hr. The solution was warmed to 50° for 15 min and poured into crushed ice. It was extracted with ether, the ether layer washed with aq. sodium bicarbonate, water and dried over sodium sulphate. Removal of the solvent gave a residue (3·3 g) which on crystallisation from methanol afforded (III; 2·2 g) mp 138–140°. UV λ_{max} (ethanol) 249, 285 nm (log ϵ 4·03, 4·04). IR ν_{max} (KBr) 1720, 1620, 1565 cm^{-1}. NMR ($CDCl_3$) δ: 7·2 (m, 3H, Arm–H), 6·31 (s, 1H, H–3), 3·95 (s, 3H, OMe), 2·42 (s, 3H, –Me). MS m/e 190 (M^+ 100%), 176 (8), 175 (8), 162 (50), 161 (40), 147 (40), 131 (18), 119 (50), 105 (10), 103 (15). (Found: C, 69·5; H, 5·6. $C_{11}H_{10}O_3$ requires: C, 69·5; H, 5·3%).

3.7. 6-*Hydroxy*-8-*methoxy*-4-*methylcoumarin* (*VI*)

To a solution of the coumarin (III; 1 g), in 10% sodium hydroxide (12 ml) and pyridine (10 ml) was added during 3·5 hr, an aq. saturated solution of potassium persulphate (1·5 g) under stirring maintaining the temp. below 20°. The solution was kept for 16 hr and acidified to congo red with hydrochloric acid. It was extracted with ether, the ether layer washed with water, dried over sodium sulphate and the ether evaporated to give the unreacted material (0·32 g).

The aq. layer was acidified with excess conc. hydrochloric acid, heated on the water bath at 70° for 1 hr, cooled and extracted with ether to give the crude hydroxy coumarin (0·22 g). It was purified by passing through a short column of silica gel. Crystallisation from methanol gave colourless plates (VI; 0·175 g) mp 230–2°. UV λ_{max} (ethanol) 254, 286 and 351 nm (log ϵ 3·89, 3·95, 3·36). IR ν_{max} (KBr) 1710, 1660, 1580 cm^{-1}. NMR (CD_3COCD_3) δ: 6·8, 6·69 (each doublet; J = 2·8 Hz; each 1H, H–5, H–7), 6·28 (q, J = 1 Hz, 1H, H–3), 3·9 (s, 3H, OMe), 2·4 (d, J = 1 Hz, 3H, –Me). MS m/e 206 (M^+, 10%), 178 (60), 177 (50), 163 (15), 149 (80), 135 (100). (Found: C, 64·0; H, 5·2. $C_{11}H_{10}O_4$ requires: C, 64·1; H, 4·9%).

3.8. 6,8-*Dimethoxy*-4-*methylcoumarin* (*II*)

The hydroxycoumarin (VI; 135 mg), anhyd. potassium carbonate (500 mg) was refluxed with acetone (15 ml) and dimethyl sulphate (100 mg) for 2·5 hr. The mixture was filtered, the solvent removed under vacuum and the residue crystallized from methanol to afford colourless needles (II; 90 mg) mp 181–2°. UV λ_{max} (ethanol), 253, 285 and 345 nm (log ϵ 3·99, 4·0 and 3·38). IR ν_{max} (KBr), 1740, 1720, 1600, 1580 cm^{-1}. NMR (CD_3COCD_3) δ: 6·87, 6·76 (each doublet, J = 2·7 Hz, 1H each, H–5, H–7), 6·3 (q, J = 1·2 Hz, 1H, H–3), 3·93, 3·9 (singlets, 3H each, OMe), 2·47 (d, J = 1·2 Hz, 3H, –Me). MS m/e 220 (M^+ 100%), 205 (5), 192 (25), 177 (55), 163 (5), 149 (75), 134 (10), 121 (15). (Found: C, 64·9; H, 5·9. $C_{12}H_{12}O_4$ requires: C, 65·4; H, 5·5%).

3.9. 4,7-*Dihydroxy*-5-*methylcoumarin* (*VII*)

In a stirred solution of orcinol (12·4 g), ethylcyanoacetate (12 g), anhyd. zinc chloride (10 g) in dry ether (150 ml) maintained at 0°, dry hydrogen chloride gas

was passed until saturation (2·5 hr). The solution was kept for 18 hr and the ether decanted off. The residue was washed twice with dry ether and the solids dissolved in 1 litre of water and the solution refluxed for 1 hr. It was cooled to 0° and filtered. The crude solids (3·2 g) on two crystallisations from methanol afforded (VII; 0·6 g) mp 283°. UV λ_{max} (ethanol) 310, 320 (infl.) nm (log ϵ 4·2, 4·14). IR ν_{max} (nujol) 1660, 1600, 1540 cm^{-1}. NMR (CD_3COCD_3 + CD_3SOCD_3) δ: 6·55 (s, 2H, H–6, H–8), 5·42 (s, 1H, H–3), 2·6 (s, 3H, –Me). MS m/e 192 (M^+ 65%), 165 (12), 150 (100), 135 (10), 122 (70). (Found: C, 62·5; H, 4·5. $C_{10}H_8O_4$ requires: C, 62·5; H, 4·2%).

3.10. *4,7-Dimethoxy-5-methylcoumarin (Siderin) (VIII)*

The dihydroxycoumarin (VII; 125 mg), anhydr. potassium carbonate (0·5 g), acetone (20 ml) and dimethyl sulphate (0·2 ml) were refluxed for 2·5 hr. The solution was poured into crushed ice (25 g), the acetone removed and the precipitate filtered and dried (87 mg). Crystallisation from methanol afforded colourless crystals (VIII; 60 mg) mp 194–5°. The mmp with the natural sample was undepressed and it showed identity in its TLC, IR, NMR and mass spectral comparison. (Found: C, 65·7; H, 5·8. Calc. for $C_{12}H_{12}O_4$: C, 65·4; H, 5·5%).

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