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THREE HYDROXY ELLAGIC ACID METHYL ETHERS, CHRYSOPHANOL AND SCOPOLETIN FROM *SHOREA WORTHINGTONII* AND *VATICA OBSCURA**

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Key Word Index—*Shorea worthingtonii*; *Vatica obscura*; Dipterocarpaceae; hexamethylcoruleoellagic acid; pentamethylflavellagic acid; tetramethylflavellagic acid; tetramethylellagic acid; chrysophanol; scopoletin; triterpenes.

Abstract—Extraction of the bark and timber of *Shorea worthingtonii* and *Vatica obscura* gave three new natural products: hexamethylcoruleoellagic acid, pentamethylflavellagic acid, tetramethylflavellagic acid, together with tetramethylellagic acid, scopoletin and chrysophanol. The above are reported for the first time in the family. Several triterpenes, already described from this family, were also isolated.

INTRODUCTION

Although a large number of sesquiterpenes and triterpenes have been reported from the Dipterocarpaceae [1–14], only a few other types of compound are reported to be present in this plant family. A crystalline phenolic compound called hopeaphenol was first isolated from *Hopea odorata* and *Balanocarpusheimii* in 1951, although its structure could only be established by X-ray methods much later by Coggon *et al.* [15, 16]. It was also later isolated from two other *Shorea* species [17]. Another phenolic compound, bergenin, was first reported in the family by Carruthers *et al.* in 1957 [18]. Bate-Smith and Whitmore [19], who surveyed the leaf extracts of about 28 Malayan Dipterocarp species, detected several flavonoids, ellagic acid and some unidentified fluorescent compounds. Examination of the bark and timber extracts of *Shorea worthingtonii* and *Vatica obscura* by TLC showed the presence of some compounds with bright blue fluorescence in UV light. The main objective of the present investigation was to isolate and characterize these compounds.

RESULTS AND DISCUSSION

Column chromatographic separation of the bark and timber extracts of these two plants gave several triterpenoids and a steroid which were identified by comparison with authentic samples. The bark light petroleum extract of *S. worthingtonii* yielded β-amyrin, dipterocarpol, ursolic acid and sitosterol, while the timber light petrol extract yielded the above compounds except dipterocarpol together with β-amyrin acetate and a red crystalline solid. The UV spectrum of the latter showed an intense benzenoid absorption band at 228 nm and strong quinonoid electronic transition (ET) absorptions at 257 and 277 nm. IR absorption bands at 1685, 1630 and 1610 cm^{-1} and the presence of intense M^+, $[M^+ - CO]$ and $[M^+ - 2CO]$ peaks in the MS of this compound suggested that it was an anthraquinone. The high resolution MS gave the molecular formula as $C_{15}H_{10}O_4$ and it was later identified as chrysophanol (1,8-dihydroxy-3-methylanthraquinone) [20a] by comparison with an authentic sample.

The hot chloroform soluble fraction of the bark methanol extract gave β-amyrin, sitosterol and a mixture of three UV fluorescent compounds which was separated by preparative TLC.

The high resolution MS and elemental analysis of the least polar compound of the three gave the molecular formula as $C_{20}H_{18}O_{10}$ and in the NMR spectrum, all the protons appeared as two singlets at δ 4.25 and 4.91 in the ratio 1:2. Strong IR absorptions at 1745 and 1610 cm^{-1} indicated the presence of C=O and benzenoid C=C bands, respectively. Based on the above, the structure (**1**) 2,3,4,7,8,9-hexamethoxy-[1]benzopyrano[5,4,3-*cde*] [1] benzopyran-5,10-dione was suggested for this compound. The protons of the two methoxyl groups at the 4- and 9-positions appeared low field compared to the others due to a carbonyl effect. In the MS there was no prominent fragmentation except the sequential loss of Me and OMe fragments. $LiAlH_4$ reduction gave a hydroxy compound (IR band at 3340 cm^{-1} which was absent in the parent molecule) with a M^+ ion 8 mu higher than that of the original compound. This indicated the addition of eight protons and confirmed the presence of two lactone groups. The loss of the extended conjugation involved in the transformation from the planar bilactone to the non-planar biphenyl compound was clearly visible in the UV spectrum of the reduced compound. The band at 350 nm in the parent compound disappeared on reduction leaving a single band at 225 nm.

The hexahydroxy compound corresponding to (**1**), i.e. coruleoellagic acid (**2**), has been identified as one of the

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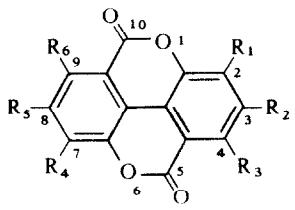

1 $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OMe$
2 $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OH$
3 $R_1 = R_2 = R_3 = R_4 = R_5 = OMe; R_6 = H$
4 $R_1 = R_3 = R_4 = OMe; R_2 = R_5 = OH; R_6 = H$
5 $R_1 = R_3 = R_5 = OMe; R_2 = R_4 = OH; R_6 = H$
6 $R_1 = R_2 = R_4 = R_5 = OMe; R_3 = R_6 = H$
7 $R_1 = R_2 = R_3 = R_4 = R_5 = OH; R_6 = H$

oxidation products of gallic acid [21]. Coruleoellagic acid, prepared according to the method described by Perkin, was methylated using Me_2SO_4 and K_2CO_3, and the resultant completely methylated hexamethylcoruleoellagic acid was found to be identical with the isolated natural product **1** (mmp, co-TLC and IR). This is the first report of a coruleoellagic acid derivative from a natural source.

The next polar compound, which had a molecular formula $C_{19}H_{16}O_9$ (high resolution MS and elemental analysis), was identified as pentamethylflavellagic acid, 2,3,4,7,8-pentamethoxy-[1] benzopyran[5,4,3-*cde*] [1] benzopyran-5,10-dione (**3**). In the NMR spectrum of the compound, the protons of five methoxy groups appeared as five singlets at δ 4.26, 4.15, 4.14, 4.03 and 3.99 while the aromatic proton appeared as a singlet at δ 7.84. The identity was confirmed by comparison with a synthetic sample prepared according to the method described by Perkin [22a]. Although two tri-*O*-methyl derivatives of flavellagic acid (**4** and **5**) have been reported [22b], the pentamethyl ether has not been reported before from a natural source. The most polar compound was identified as tetramethylellagic acid, 2,3,7,8-tetramethoxy[1]benzopyran[5,4,3-*cde*] [1] benzopyran-5,10-dione (**6**) by comparison with an authentic sample [22a].

The bark extract of *Vatica obscura* yielded β-amyrin acetate, β-amyrin, sitosterol, hexamethylcoruleoellagic acid, pentamethylflavellagic acid and tetramethylellagic acid.

The timber extract gave two more UV fluorescent compounds in addition to the above compounds. The less polar compound of the two had a UV absorption pattern similar to that of a flavellagic acid (**7**) derivative and was shown to be a hydroxy compound (IR absorption at 3400 cm^{-1}) which gave a greenish-yellow colour with neutral alcoholic $FeCl_3$. Methylation of this compound with Me_2SO_4 and K_2CO_3 gave a pentamethylflavellagic acid. The high resolution MS gave the molecular formula as $C_{18}H_{14}O_9$ and the compound was identified as a tetramethyl ether of flavellagic acid, but the lack of material did not permit the determination of the methylation pattern. The other compound was identified as scopoletin and this is the first coumarin reported from this family.

EXPERIMENTAL

Plant materials were collected from the Kanneliya forest reserve in Sri Lanka and voucher specimens are deposited in the herbarium at the Department of Chemistry, Peradeniya Campus.

Air-dried powdered bark and timber were extracted with boiling petrol (bp 60–80°) and MeOH. Separation was by column chromatography over Si gel (Merck) and preparative TLC on Si gel (Merck G) when necessary. Mps (uncorr.) were recorded on a Kofler hot stage apparatus. All known compounds were identified by comparison with authentic samples (co-TLC, mp, mmp and IR spectra).

Shorea worthingtonic Ashton bark (4.0 kg) gave (a) petrol extract (27.0 g, 0.68 %), (b) MeOH extract (325 g, 8.03 %). Timber (3.5 kg) gave (a) petrol extract (13.0 g, 0.34 %), (b) MeOH extract (270 g, 8 %).

Bark extracts. The petrol extract (2.0 g) on chromatography over Si gel (160 g) gave (a) with C_6H_6, β-amyrin (0.820 g) mp 198° (petrol), $[\alpha]_D^{27}$ +85° ($CHCl_3$) (lit. [23] mp 200°, $[\alpha]_D^{26}$ +88°); (b) with $CHCl_3$–C_6H_6 (1:9), dipterocarpol (0.250 g) mp 134° (from petrol), $[\alpha]_D^{27}$ +66.5° ($CHCl_3$) (lit. [24] mp 134° $[\alpha]_D^{26}$ +66°]; (c) with $CHCl_3$–C_6H_6 (1:3), sitosterol (0.320 g) 136–7°, $[\alpha]_D^{26}$ −36.5° ($CHCl_3$) (lit. [20b] 136–7°, $[\alpha]_D^{26}$ −36°); (d) with $CHCl_3$, ursolic acid (0.110 g) mp 280–3°, $[\alpha]_D^{27}$ +60° ($CHCl_3$) (lit. [25] mp 283°, $[\alpha]_D^{26}$ +65.9°).

The MeOH extract (200 g) was re-extracted with boiling $CHCl_3$ and EtOAc successively in a Soxhlet. The $CHCl_3$ extract (1.12 g) on Si gel (40 g) gave (a) with C_6H_6, β-amyrin (0.080 g) mp 200°; (b) with $CHCl_3$–C_6H_6 (1:3), sitosterol (0.095 g) mp 137°; (c) with $CHCl_3$–C_6H_6 (1:3), a green-white solid (0.050 g, 0.002 %). The above on PLC after repeated elution for 3 × with $CHCl_3$ gave 3 compounds. The bands were visualized under UV light, separated and eluted with $CHCl_3$.

Isolation of hexamethylcoruleoellagic acid. The least polar band gave needles from EtOH (0.015 g, 0.006 %) mp 229° (Found: C, 57.40; H, 4.30; M^+ 418.0900. $C_{20}H_{18}O_{10}$ requires: C, 57.41; H, 4.30 %; M^+ 418.0900). λ_{max} nm: 345 (log ε 4.01), 284 (3.22), 356 (3.39) and 360 (3.46); ν_{max}^{KBr} cm^{-1}: 2900, 2840, 1745 (⟩C=O), 1590, 1560, 1465, 1400, 1350, 1290, 1275, 1200, 1145, 1100, 1060, 990, 960, 930, 840, 760 and 715; *m/e* 418 M^+ (100 %), 403 (M − Me, 67), 389 (50), 375 (33), 358 (21), 345 (17), 326 (18), 317 (21), 261 (16), 231 (12), 209 (14), 206 (10), 192 (10), 190 (7), 175 (13), 147 (14), 131 (15), 119 (11), 116 (12), 104 (13), 101 (14), 91 (80), 83 (10), 77 (11).

$LiAlH_4$ *reduction of hexamethylcoruleoellagic acid.* A mixture of the above compound (0.010 g) and $LiAlH_4$ (0.020 g) in dry Et_2O (25 ml) was heated under reflux for 2 hr, diluted with H_2O (100 ml) and extracted with Et_2O (25 × 4 ml). The Et_2O extract was dried and on evapn gave a yellow solid (0.008 g). It was purified from EtOH as a powder, mp 275°, M^+ (MS) 426. $\lambda_{max}^{95\%\ EtOH}$ nm: 245 (log ε 3.8); ν_{max}^{nujol} cm^{-1}: 3340(OH), 2900, 1560, 1460, 1410, 1375, 1105 and 715; *m/e* 426 (100 %), 411 (60), 396 (45), 382 (15), 368 (12), 261 (14), 231 (10), 212 (20), 206 (11), 192 (18), 180 (5), 104 (6), 77 (23).

Synthesis of hexamethylcoruleoellagic acid. To a mixture of arsenic acid syrup (0.8 ml) in 96 % H_2SO_4 (9.2 ml) finely powdered gallic acid (1.0 g) was added gradually at 110°. The mixture was heated at 120° on an oil bath for 6 hr, cooled and poured into H_2O (50 ml). The dark brown solid was filtered, dried and recrystallized from Py. Coruleoellagic acid (0.250 g) thus obtained was refluxed for 12 hr with dry K_2CO_3 (3.5 g) and Me_2SO_4 (1 ml) in dry Me_2CO (100 ml), filtered and concd. On cooling, the methyl ether which separated as needles was filtered and recrystallized from EtOH (0.130 g), mp 229°; M^+ (MS) 418.0900; $\lambda_{max}^{95\%\ EtOH}$ nm: 245 (log ε 4.01), 284 (3.22), 356 (3.39) and 360 (3.46); ν_{max}^{KBr} cm^{-1}: 2900, 2840, 1745 and 1590.

Isolation of pentamethylflavellagic acid. The next polar band gave colourless needles from EtOH (0.020 g), mp 245° (lit. [22b] 242°). (Found: C, 58.72; H, 4.09. $C_{19}H_{16}O_9$ requires: C, 58.72;

H, 4.09%). $\lambda_{max}^{95\%\ EtOH}$ nm: 248 (log ε 3.76), 350 (3.24) and 359 (3.26); ν_{max}^{KBr} cm^{-1}: 2900, 2840, 1745 (C=O), 1660, 1490, 1465, 1400, 1350, 1320, 1245, 1160, 1140, 985, 902, 752 and 740; ^1H NMR (d_6-Py 100 MHz): δ 7.84 (1H, *s*, ArH), 4.26 (3H, *s*, OMe), 4.15 (3H, *s*, OMe), 4.14 (3H, *s*, OMe), 4.03 (3H, *s*, OMe), 3.99 (3H, *s*, OMe); *m/e* 388 (100%, M^+), 373 (60), 358 (53), 345 (30), 230 (12), 315 (18), 300 (10), 287 (9), 259 (7), 231 (8), 117 (13), 77 (10). It was identified as pentamethylflavellagic acid by comparison with a synthetic sample, prepared according to published procedure [22a].

Isolation of tetramethylellagic acid. The highest polar band gave a yellow solid (0.020 g) which was sparingly soluble in organic solvents. Crystal nature transformed from cubes to needles at 275° and 300° (dec.) (lit. [22b] 270–271°). (Found: M^+ (MS) 358.0571. $C_{18}H_{14}O_6$ requires: 358.0687). $\lambda_{max}^{95\%\ EtOH}$ nm: 242 (log ε 3.41) and 354 (3.12); ν_{max}^{KBr} cm^{-1}; 2900, 2840, 1740, 1610; *m/e* 358 (100%), 342 (78), 328 (12), 314 (60), 312 (70), 298 (40), 286 (38), 282 (30), 271 (28), 254 (29), 243 (14), 229 (17), 178 (13), 144 (18), 122 (30), 117 (8), 85 (20), 77 (57). It was identified as tetramethylflavellagic acid by comparison with an authentic sample.

Timber extracts. The petrol extract (5.0 g) was extracted with aq. Na_2CO_3 and on usual work-up gave neutral fraction (3.9 g) and acidic fraction (0.650 g). The neutral fraction on chromatography over Si gel (200 g) gave (a) with petrol, chrysophanol (0.012 g), mp 196° (from EtOH) (lit. [20a] 196°), (M^+ (MS) 254.1758. $C_{15}H_{10}O_4$ requires: 254.1651). UV and IR spectral props. as in lit.; (b) with C_6H_6–petrol (9:11), β-amyrin acetate (0.650 g) mp 240° (from EtOH), $[\alpha]_D^{26}$ +76° ($CHCl_3$) (lit. [23] 241°, $[\alpha]_D^{26}$ +80° ($CHCl_3$)); (c) with C_6H_6, β-amyrin (0.050 g) mp 198°; (d) with $CHCl_3$–C_6H_6 (1:17), sitosterol (1.20 g) mp 136–7°. The acidic fraction, on recrystallization from EtOH, gave ursolic acid (0.570 g), mp 280°.

Vatica obscura *Trimen.* Bark (2.7 kg) gave (a) petrol extract (12.0 g, 0.44%); (b) C_6H_6 extract (8.5 g, 0.32%). Timber (4.0 kg) gave (a) petrol extract (15.0 g, 0.35%); (b) C_6H_6 extract (12.0 g, 0.3%).

Bark extracts. The petrol extract and the C_6H_6 extract were found to be very much similar on TLC and the latter (4.0 g), on chromatography over Si gel (150 g), gave (a) with C_6H_6–petrol (1:49), β-amyrin acetate (0.759 g); (b) with C_6H_6–petrol (3:7), β-amyrin (0.130 g); (c) with C_6H_6–petrol (3:17), sitosterol.

The greenish white solid (A) was shown to be a mixture of 3 UV fluorescent compounds and on PLC gave (a) hexamethylcoruleoellagic acid (0.013 g) mp 229°; (b) pentamethylflavellagic acid (0.011 g) mp 245°; (c) tetramethylellagic acid (0.010 g) 300° dec.

Timber extracts. The petrol extract (5.0 g) on chromatography over Si gel (200 g) gave (a) with C_6H_6–petrol (1:3), β-amyrin acetate mp 241°; (b) with C_6H_6–petrol (3:17), β-amyrin mp 200°; (c) with C_6H_6–petrol (1:1), greenish-white solid (B) (0.016 g); (d) with C_6H_6, a white solid (C) (0.060 g) and (e) with $CHCl_3$, ursolic acid (0.210 g) mp 280°. The solid B on PLC gave (a) hexamethylcoruleoellagic acid (0.008 g) mp 229°; (b) pentamethylflavellagic acid (0.006 g) mp 245°. The solid C on PLC gave (a) tetramethylflavellagic acid (0.015 g), mp 256° (from EtOH) (M^+ (mass spectrometry) 374.2671. $C_{18}H_{14}O_9$ requires 374.2669). $\lambda_{max}^{95\%EtOH}$ nm: 244 (log ε 3.80), 306 (3.11), 357 (3.16) and 371 (3.14); ν_{max}^{nujol} cm^{-1}: 3400 (OH), 1735 (>C=O), 1600 (C=C), 1475, 1400, 1360, 1160, 1115, 1090, 1040 and 980; *m/e* 374 (100%), 360 (45), 359 (80), 344 (23), 331 (60), 313 (22), 288 (18), 260 (15), 245 (18), 218 (18), 204 (20), 187 (26), 116 (25), 101 (11); with alcoholic $FeCl_3$ it gave a green colour; Me_2SO_4 (0.2 ml) methylation of the compound (0.010 g) with K_2CO_3 (1.5 g) in dry Me_2CO (50 ml) gave needles of pentamethylflavellagic acid, mp 245° undepressed by an authentic sample; (b) scopoletin (0.024 g), mp 200° (from *n*-hexane; lit. [26] mp 204°). UV and IR spectra as in lit.

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