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3,5,7,3′,5′-PENTAHYDROXYFLAVAN AND 3α-METHOXYFRIEDELAN FROM *HUMBOLDTIA LAURIFOLIA*

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Key Word Index—*Humboldtia laurifolia*; Leguminosae; *O*-acetyloleanolic aldehyde; lupeol; 5,7,4′-trihydroxyflavone; (2*R*,3*R*)-3,5,7,3′,5′-pentahydroxyflavan; 3α-methoxyfriedelan.

Abstract—The leaf, bark and timber extractives of *Humboldtia laurifolia* were investigated and the following compounds have been isolated: *O*-acetyloleanolic aldehyde, a sitosteryl ester, lupeol, sitosterol, a fatty acid, 5,7,4′-trihydroxyflavone (apigenin), (2*R*,3*R*)-3,5,7,3′,5′-pentahydroxyflavan and 3α-methoxyfriedelan. The latter two compounds are new natural products.

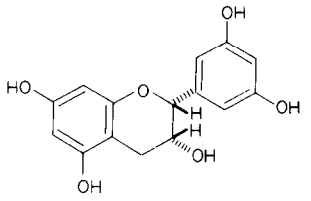
INTRODUCTION

Humboldtia laurifolia belongs to the subfamily Caesalpinoideae of the Leguminosae. The genus *Humboldtia* has not been chemically investigated and we report now the chemical investigation of the leaf, bark and timber of *H. laurifolia*.

RESULTS AND DISCUSSION

The leaf benzene extract on cooling gave a triterpenoid. The ^{1}H NMR spectrum showed the triterpenoid to be a methoxy compound (δ 3.33). High resolution mass spectrometry gave a molecular formula of $C_{31}H_{54}O$ (M^+ at *m/z* 442.4172, requires 442.4174). Demethylation by hydrogen bromide followed by acetylation gave an acetate. Hydrolysis of the acetate yielded a triterpene alcohol, $C_{30}H_{52}O$ (M^+ at *m/z* 428.4013, requires 428.4017) which was identified as friedelan-3α-ol. The natural product is thus 3α-methoxyfriedelan (**2**). This compound was synthesized from friedelin. Friedelin was reduced with sodium–*n*-propanol to give friedelan-3α-ol which on treatment with potassium–methyl iodide gave 3α-methoxyfriedelan (**2**) which was identical (mmp, IR and co-TLC) with the natural product. This is the first report of the occurrence of 3α-methoxyfriedelan as a natural product.

The timber extracts on CC over Si gel gave *O*-acetyloleanolic aldehyde and lupeol. The bark extracts on similar separation gave a sitosteryl ester, *O*-acetyloleanolic aldehyde, lupeol, sitosterol, a fatty acid, 5,7,4′-trihydroxyflavone (apigenin) and a pentahydroxyflavan, mp 240°, $[\alpha]_D = -39.7°$ (MeOH). The latter compound was tentatively identified to be (2*R*,3*R*)-3,5,7,3′,5′-pentahydroxyflavan (**1**) as follows. It had a molecular formula of $C_{15}H_{14}O_6$ (M^+ at *m/z* 290.0787, requires 290.0790). The IR spectrum showed the presence of absorptions at 3545 cm^{-1} (OH) and 1620 cm^{-1} (C=C) but had no absorptions for any type of carbonyl group.



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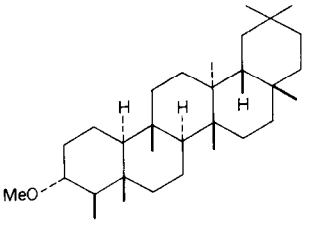

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Methylation with dimethyl sulphate gave a tetramethoxylated compound. The ^{1}H NMR spectrum of the natural product had a one-proton multiplet at δ 4.21 typical of a –CHOH signal. Two types of benzylic protons appeared at δ 2.78 (2H, *dd*, *J* = 2 and 4 Hz) and at 4.9 (1H, *d*, *J* = 2 Hz). The presence of two types of aromatic system was discerned (a) from the presence of a *meta*-coupled proton system at δ 5.93 (1H, *d*, *J* = 2 Hz) and 6.05 (1H, *d*, *J* = 2 Hz) in one system, and (b) from the two-proton doublet at 6.84 (*J* = 1.2 Hz) and a one-proton doublet at 7.05 (*J* = 1.2 Hz). These protons, which were present in the second aromatic ring, were also *meta*-coupled. These data and the mass spectral fragmentations (Scheme 1) were in keeping with the structure **1** for the pentahydroxyflavan. The configurations at C-2 and C-3 were obtained as follows. The two benzylic protons centred at δ 2.78 in the ^{1}H NMR spectrum were coupled to each other and to the C-3 proton and appeared as a double doublet (*J* = 4 and 2 Hz) due to their being chemically non-equivalent. This situation is created by the C-3 hydroxy

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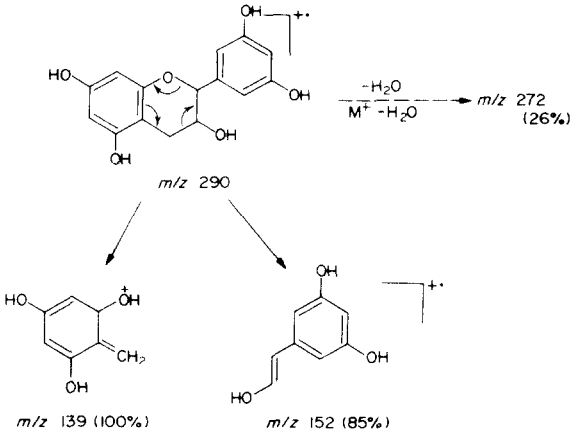

Scheme 1. Mass spectral fragmentation of compound 1.

group which takes up an axial conformation. The C-2 and C-3 hydrogen atoms should be in an (a,e) *cis*-orientation or (e,e) *trans*-orientation to give the relatively small coupling effect ($J = 2$ Hz) noticed for the C-2 proton which appeared at δ 4.9 in the ^{1}H NMR spectrum. The (a,e) *cis*-orientation is more likely since the C-2 aryl ring can then be in an equatorial configuration. An axial aryl arrangement would influence the chemical shift of the C-4 axial proton which was found to have almost the same δ_H value as that of the C-4 equatorial proton. The above information gives the relative stereochemistry at C-2 and C-3 but the absolute configurations at C-2 and C-3 remain to be established and at present they are only tentatively assigned as (2*R*,3*R*).

EXPERIMENTAL

Plant materials were collected from the Kanneliya and Ratnapura forests of Sri Lanka. The leaf, bark and timber of *Humboldtia laurifolia* Vahl. were separately dried and powdered. They were separately extracted with C_6H_6, Me_2CO and finally with MeOH. TLC and prep. TLC were carried out using Si gel. Mp's are uncorr. and were obtained using a Kolfer Hot Stage Reichert model apparatus.

Isolation of 3α-methoxyfriedelan. The C_6H_6 extract (145 g) of the leaves (2.5 kg) on cooling deposited white crystals of 3α-methoxyfriedelan (0.52%); mp 301°, $[\alpha]_D - 25°$ ($CHCl_3$); $[M]^+$ at *m/z* 442.4172, $C_{31}H_{54}O$ requires 442.4174; ^{1}H NMR (CCl_4): δ 3.33 (3H, *s*, –OMe) 1.26, 1.16, 2 × 1.0, 0.93, 0.83, 0.75 (21 H, *s*, 7 × Me), 0.86 (3H, *d*, $J = 7$ Hz); MS *m/z* (rel. int.): 442(34), 428(43), 410(42), 395(50), 269(49), 257(75), 247(54), 231(79), 205(64), 202(69), 189(81), 177(84), 149(81), 148(86), 125(100).

Demethylation and acetylation of 3α-methoxyfriedelan. 3α-Methoxyfriedelan (200 mg) in $CHCl_3$ (2.5 ml) was refluxed with PhOH (2.0 ml), Ac_2O (2.5 ml) and HBr (47%) (6 ml) for 7 hr. The reaction mixture was evaporated and washed with H_2O to give 180 mg of a crude product. This, on purification by prep. TLC, gave pure 3α-acetoxyfriedelan (105 mg) mp 302°, lit. [1] 300°, $[\alpha]_D - 12°$ ($CHCl_3$), lit. [1] – 12°

Hydrolysis of 3α-acetoxyfriedelan. 3α-Acetoxyfriedelan (55 mg) in MeOH (1.0 ml) was heated with 10% methanolic KOH (0.6 ml) for 6 hr. The hydrolysed product was worked-up in the usual manner to give friedelan-3α-ol (35 mg) mp 301°, lit. [1] 308°, $[\alpha]_D + 16°$ ($CHCl_3$), lit. [1] + 13°; $[M]^+$ at *m/z* 428.4013 ($C_{30}H_{52}O$ requires 428.4017).

Synthesis of 3α-methoxyfriedelan. Friedelin (250 mg) in *n*-PrOH (2.0 ml) was refluxed with Na (0.5 g) for 1 hr. The solvent was evaporated, the crude product acidified and extracted with $CHCl_3$. Prep. TLC of the residue yielded pure friedelan-3α-ol (98 mg) mp 301°, lit. [1] 308°; [identified by comparison with an authentic sample (mmp, co-TLC)].

Friedelan-3α-ol (300 mg) was dissolved in dry toluene (3.0 ml) and K (1 g) was added to the boiling soln. Reaction was continued for 2 hr and then MeI (5 ml) was added. After another 2 hr the mixture was cooled, excess K was destroyed using MeOH and the solvent evaporated. The residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer on drying and evaporation gave a residue (170 mg) which was purified by prep. TLC to yield pure 3α-methoxyfriedelan (80 mg) mp 295°, $[\alpha]_D = -24°$ ($CHCl_3$). It was found to be identical with the natural product isolated from the leaf (mmp, co-TLC).

Isolation of O-acetyloleanolic aldehyde. The petrol extract (16 g) of the timber (4.2 kg) was separated by CC over Si gel. Elution with C_6H_6 yielded *O*-acetyloleanolic aldehyde (0.533 g) mp 225°, lit. [2] 225–228°, $[\alpha]_D + 59°$ ($CHCl_3$). In the absence of an authentic sample to compare, the following data were obtained: $[M]^+$ at *m/z* 482.3761 ($C_{32}H_{50}O_3$ requires 482.3759); ^{1}H NMR (CCl_4): δ 9.25 (1H, *s*, CHO), 5.33 (1H, *t*, $J = 5$ Hz, $-CH_2-\underline{CH}=C$), 4.43 (1H, AcO–$\underline{CH}$–CH_2–), 1.93 (3H, *s*, Me–CO–O), 1.06 (3H, *s*, Me), 0.91 (9H, *s*, 3Me), 0.83 (6H, *s*, 2Me), 0.70 (3H, *s*, Me).

Hydrolysis of *O*-acetyloleanolic aldehyde by alcoholic KOH (10%) gave oleanolic aldehyde mp 171°, lit. [3] 170°, $[\alpha]_D + 76°$ (lit. [3] + 72°).

Isolation of sitosterol. Continued elution of the column with C_6H_6 gave sitosterol (0.25 g), mp 133–134°, lit. [1] 140°, $[\alpha]_D - 35°$. It was identical with an authentic sample (mmp, co-TLC).

Isolation of lupeol. Further elution with C_6H_6 gave lupeol (380 mg) mp 214°, lit. [4] 212°, $[\alpha]_D + 33°$. It was identical with an authentic sample.

Bark petrol extractives. The dried powdered bark (3.5 kg) gave 36 g of petrol extract. CC over Si gel gave an ester of sitosterol (1.5 g), lupeol (0.75 g), *O*-acetyloleanolic aldehyde (0.75 g), sitosterol (2.09 g) and an aliphatic acid (2.0 g). Known natural products were identified by comparison with authentic samples (mmp, co-TLC).

Bark Me_2CO extract. Defatted bark was extracted with Me_2CO to give 310 g of extract. This extract (70 g) was separated on a Si gel column and elution with 40% EtOAc–petrol gave sitosterol (0.50 g); 5,7,4′-trihydroxyflavone (apigenin) (0.53 g). Apigenin was characterized by its mp 348° (lit. [5] 347°) and by its characteristic ^{1}H NMR spectrum: δ (DMSO-d_6) 12.96 (1H, *s*, –OH), 7.94 (2H, *d*, $J = 8$ Hz), 6.94 (2H, *d*, $J = 8$ Hz), 6.77 (1H, *s*, =CH), 6.50 (1H, *d*, $J = 2$ Hz), 6.21 (1H, *d*, $J = 2$ Hz). Apigenin was acetylated to give a diacetate (mp 185°). The diacetate had $[M]^+$ at *m/z* 354.0739. $C_{19}H_{14}O_7$ requires 354.0790.

Bark MeOH extract. The bark MeOH extract (10 g) was re-extracted with EtOAc to give 5 g of EtOAc-soluble fraction. Prep. TLC separation of 1 g of this material gave 120 mg of 3,5,7,3′,5′-pentahydroxyflavan, mp 240°, $[\alpha]_D - 39.7°$ (MeOH); $[M]^+$ at *m/z* 290.0787, $C_{15}H_{14}O_6$ requires 290.0790; IR ν_{max}^{KBr} cm^{-1}: 3545, 1620, 1510, 1458, 1440, 1250, 1189, 1140, 1090, 1015, 810, 792; ^{1}H NMR (Me_2CO-d_6): δ 7.05 (1H, *d*, $J = 1.2$ Hz), 6.84 (2H, *d*, $J = 1.2$ Hz), 6.05 (1H, *d*, $J = 2$ Hz), 5.93 (1H, *d*, $J = 2$ Hz), 4.9 (1H, *d*, $J = 2$ Hz), 4.21 (1H, *m*), 2.78 (2H, *dd*, $J = 2$ and 4 Hz); MS *m/z* (rel. int.): 290(71), 286(45), 272(26), 175(46), 167(46), 163(37), 152(85), 141(54), 140(57), 139(100), 124(68), 123(81), 110(51); UV λ_{max}^{MeOH} nm (log ε): 214(4.47), 225(4.46), 281(3.76), 394(3.16).

Methylation of 3,5,7,3′,5′-pentahydroxyflavan. The phenol (0.075 g) in dry Me_2CO (5 ml) was treated with Me_2SO_4 (0.5 ml)

and dry K_2CO_3 (0.20 g). The product, a tetramethyl ether (0.70 g), mp 160° was isolated in the usual manner. It had $[\alpha]_D$ −29° ($CHCl_3$); ^1H NMR (CCl_4): δ6.95 (1H, *d*, *J* = 2 Hz), 6.84(2H, *d*, *J* = 2 Hz), 6.07(1H, *d*, *J* = 2 Hz), 5.95(1H, *d*, *J* = 2 Hz), 4.80(1H, *d*, *J* = 2 Hz), 4.14(1H, *m*-CHOH), 3.85(3H, *s*, OMe), 3.83(3H, *s*, OMe), 3.80(3H, *s*, OMe), 3.74(3H, *s*, OMe), 2.77(2H, *dd*, *J* = 2 and 4 Hz).

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REFERENCES

1. Hui, W. H. and Ho, C. T. (1968) *Aust. J. Chem.* **21**, 1675.
2. Eyton, W. B., Ollis, W. D., Fineberg, M., Gottlieb, O. R., de Souza Guinaraes, I. and Taveira Magalhaes, M. (1965) *Tetrahedron* **21**, 2697.
3. Corsano, S. and Mincione, E., (1967) *Chem. Abstr.* **67**, 116 988.
4. Ames, T. R., Halsall, T. G. and Jones, E. R. H. (1951) *J. Chem. Soc.* 450.
5. Nakano, M. (1932) *J. Pharm. Soc. Jpn.* **52**, 341.