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Roxburghin—A New Stilbene from the Wood of *Cassia roxburghii*

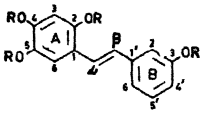
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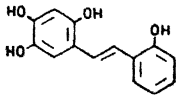
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In addition to β-sitosterol, chrysophanol and roxburghinol, a new stilbene—roxburghin has been isolated from the wood of *Cassia roxburghii* and characterised as 2,3',4,5-tetrahydroxy-*trans*-stilbene on the basis of spectral and chemical evidence.

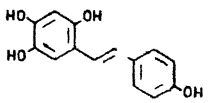
EARLIER, we communicated the isolation and structural elucidation of roxburghinol, a new 1,2-dihydroanthraquinone from the petrol extract of the leaves of *Cassia roxburghii* Benth[1]. In the present communication, the isolation and structural elucidation of a new stilbene designated as roxburghin (1) from the wood of *C. roxburghii* is reported.



1 R=H
2 R=Ac
3 R=Me



4



5

Roxburghin (1), m.p. 224°, $C_{14}H_{12}O_4$, $[M]^{+\cdot}$ 244 was soluble in aqueous alkali and gave initially light green colour, subsequently changed to dark colour with alcoholic ferric chloride indicating its phenolic nature. It formed a tetraacetate (2), m.p. 115°, $C_{22}H_{20}O_8$, $[M]^{+\cdot}$ 412, on treatment with Ac_2O/Py and a tetramethyl ether (3), m.p. 74°, $C_{18}H_{20}O_4$, $[M]^{+\cdot}$ 300 on refluxing with dimethylsulphate in acetone–potassium carbonate medium, indicating the presence of four phenolic hydroxyl groups. Its uv spectrum, λ_{max} (MeOH) (log ε) 220 (4.38), 304 (4.31) (sh) and 325 nm (4.45), was similar to those of *trans*-stilbene tetrols[2]. Further, longer wavelength band at 325 nm suffered a bathochromic shift by 15 nm in the presence of sodium acetate–boric acid, suggesting the presence of *ortho*-dihydroxy system[3]. The ir spectrum (KBr) of 1 showed characteristic bands at 3 500 (free OH), 3 250 (hydrogen bonded OH), 1 600 (C=C), 1 540, 1 440 (aromatic C=C) and 960 cm^{-1} (*trans* HC=CH)[4].

^{1}H nmr (60 MHz, DMSO-d_6) spectrum of 1 showed a singlet at δ 6.38 (2H) assignable to H-α and H-β of stilbenes[5]. A broad signal at δ 5.0 (4H, D_2O exchangeable) was ascribed to four phenolic hydroxyls. The aromatic region of the spectrum showed two singlets at δ 6.1 (1H) and 6.92 (1H) which are assignable to *para*-protons, thus supporting the 2,4,5-trihydroxylation pattern in ring-A. Further, the spectrum also contained a multiplet at δ 6.76 (4H) showing the presence of four aromatic protons. Therefore, it is inferred that ring-B in roxburghin is disubstituted and contains the fourth phenolic hydroxyl group. The foregoing analytical and spectral data permitted three isomeric structures (1,4,5) for roxburghin differing in the orientation of the hydroxyl group in ring-B. Since the ^{1}H nmr spectrum of roxburghin did not have signals arising from A_2B_2 type protons, structure 5 was ruled out.

To determine the correct alternative of the two structures 1 and 4, alkaline hydrogen peroxide oxidation of roxburghin tetramethyl ether and subsequent esterification with CH_2N_2 was carried out. The glc analysis of the products formed in the above reaction was carried out on Silar-10C at 80° and the retention times were compared with those of the authentic samples. On the basis of glc analysis, it was found to be a mixture of methyl-*m*-methoxybenzoate (35%) and methyl-2,4,5-trimethoxybenzoate (63%). On the basis of these findings, 2,3',4,5-tetrahydroxystilbene structure was assigned for roxburghin (1).

The ^{13}C nmr spectrum, (90 MHz, DMSO-d_6) of 1 showed the appropriate absorbances for 12 chemically different carbon atoms, the chemical shifts of which were in accordance with the proposed structure 1 (see Experimental). The signals at δ 128.24 (C-α) and 127.86 (C-β) provided further support for ethylenic carbon[6]. The mass spectrum of roxburghin showed prominent ions at *m/z* 244 $[M]^{+\cdot}$ (100), 229 $[M-CH_3]^+$ (80), 216 [229–CH]$^+$ (25), 151 (30) and 119 (15) supporting the

assigned structure[7]. The appearence of peak at *m/z* 229 (80%) arising due to the loss of a methyl radical from molecular ion by hydrogen scrambling[7] is of diagnostic value as is found in stilbenes.

This is the first report of the isolation and characterisation of 2,3',4,5-tetrahydroxystilbene from natural sources.

Experimental

The wood (3 kg) of *C. roxburghii*, collected locally, was coarsely powdered and extracted successively with petrol (b p. 60–80°), $CHCl_3$ and Me_2CO in a Soxhlet extractor until the extracts were almost colourless. The petrol extract was concentrated under reduced pressure to a small volume (200 ml) and left overnight. The low melting semi-solid (40 g) which separated was filtered off and the filtrate was further concentrated under reduced pressure to furnish a semi-solid (3.5 g) which on elaborate chromatography yielded β-sitosterol (0.66 g), m.p. 138°, $[\alpha]_D$ −36.0° (lit.[8] 139°, −36.0°), and 1,8-dihydroxy-3-methylanthraquinone (chrysophanol) (100 mg), m.p. 196° (lit.[9] 196°). The $CHCl_3$ extract (3.0 g) when chromatographed over silica gel (200 mesh) gave chrysophanol (175 mg) and 1,2-dihydro-1,3,8-trihydroxy-2-methylanthraquinone (roxburghinol) (120 mg), m.p. 211° (lit.[1] 211°).

The acetone extract on concentration under reduced pressure gave a dark brown syrupy mass (8.0 g), which was macerated with excess water and the clear aqueous solution was repeatedly extracted with EtOAc. The EtOAc extract on concentration furnished a brown semi-solid (4.0 g) which was subjected to column chromatography on silica gel (200 mesh). Fractions of 100 ml each were collected. The column was successively eluted with petrol (fractions 1–10), petrol-C_6H_6 (1 : 1) (fractions 11–15), C_6H_6 (fractions 16–21), C_6H_6-$CHCl_3$ (1 : 1) (fractions 22–34), $CHCl_3$ (fractions 35–46), $CHCl_3$-EtOAc (8 : 2) (fractions 47–58), EtOAc (fractions 59–65) and MeOH (fractions 66–83). Each fraction was monitored by tlc and similar fractions were combined. Fractions 1–15 yielded negligible quantity of substances. Fractions 16–34 furnished 1,8-dihydroxy-3-methylanthraquinone (50 mg), m.p. 196°. Fractions 35–46 gave 1,2-dihydro-1,3,8-trihydroxy-2-methylanthraquinone (roxburghinol) (60 mg), m.p. 211°. Fractions 47–58 yielded pale yellow solid (1.0 g). Fractions 59–83 gave resinous material (1.2 g). The pale yellow solid was rechromatographed over silica gel (200 mesh). Fractions of 50 ml each were collected. The column was eluted with C_6H_6 (fractions 1–10), $CHCl_3$-EtOAc (8 : 2) (fractions 11–20), EtOAc (fractions 21–32) and Me_2CO (fractions 33–40). Fractions 1–10 yielded negligible amounts of material. Fractions 11–20 were combined on the basis of identical tlc behaviour and on concentration furnished a cream coloured solid (250 mg) which was crystallised from $CHCl_3$-Me_2CO (9 : 1) to yield roxburghin (1) as cream coloured fluffy substance (200 mg), m p. 224° (Found : C, 68.82 ; H, 4.93. $C_{14}H_{12}O_4$ requires : C, 68 85 ; H, 4.91%) ; EIMS (probe 70 eV) *m/z* (rel. int) 244 $[M]^+$ (100), 229 $[M-CH_3]^+$ (80), 216 $[229-CH]^+$ (25), 199 $[216-OH]^+$ (30), 197 $[199-2H]^+$ (95), 151 (30), 125 (45), 119 (15), 93 (20) ; ^{13}C nmr (90 MHz, DMSO-d_6) δ 158.04 (C-3',5), 145.14 (C-2) 144.98 (C-4), 138.86 (C-1'), 128.24 (C-α), 127.86 (C-β), 125.15 (C-5'), 118.16 (C-6'), 115.34 (C-1), 112.91 (C-4'), 103.97 (C-2',6) and 101.42 (C-3).

Roxburghin tetraacetate (2): Roxburghin (40 mg) was refluxed with Ac_2O (10 ml) and pyridine (3 drops) for 4 h to yield a tetraacetate which was crystallised from alcohol as colourless needles (30 mg), m.p. 115° (Found : C, 64.08 ; H, 4.86. $C_{22}H_{20}O_8$ requires : C, 64.07 ; H, 4.85%) ; *m/z* (rel. int.) 412 $[M]^{+\cdot}$ (35), 370 $[M-CH_2=C=O]^+$ (40), 328 $[370-CH_2=C=O]^+$ (100), 286 $[328-CH_2=C=O]^+$ (60), 244 $[286-CH_2=C=O]^+$ (70), 229 $[244-CH_3]^+$ (5) and 216 $[229-CH]^+$ (6).

Roxburghin tetramethylether (3): Roxburghin (25 mg) taken in dry acetone (100 ml) was refluxed with Me_2SO_4 (1 ml) and anhydrous K_2CO_3 (1.1 g). The product was crystallised from chloroform-methanol as pale yellow crystalline solid (20 mg), m.p. 74° (Found : C, 72.01 ; H, 6.65. $C_{18}H_{20}O_4$ requires : C, 72 00 ; H, 6 66%) ; *m/z* (rel. int.) 300 $[M]^+$ (45), 285 $[M-CH_3]^+$ (100), 270 $[M-CH_2O]^+$ (75), 269 $[M-OCH_3]^+$ (60) and 258 $[M-CH_3CO+H]^+$ (45)

Alkaline hydrogen peroxide oxidation of roxburghin tetramethylether (3) : Roxburghin tetramethylether (3 ; 60 mg) in aqueous potassium hydroxide solution (40%, 15 ml) was oxidised with H_2O_2 (30%, 2×5 ml). When the reaction ceased, the unchanged material was filtered and the filtrate acidified with dilute HCl and extracted with ether (4×25 ml). The ethereal layer was extracted with sodium bicarbonate solution (5×10 ml). The product obtained on neutralisation was dissolved in ether and excess of ethereal solution of CH_2N_2 added. The reaction mixture was kept at 0° for 10 min and the solvent was evaporated carefully. The glc analysis of the products indicated the presence of methyl-*m*-methoxybenzoate (r.t.=205 s) and methyl-2,4,5-trimethoxybenzoate (r. t. 163 s), on comparison with authentic samples under identical column conditions and temperature.

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