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ROXBURGHINOL, A 1,2-DIHYDROANTHRAQUINONE FROM THE LEAVES OF *CASSIA ROXBURGHII*

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Key Word Index—*Cassia roxburghii*; Leguminosae; leaves; 1,2-dihydroanthraquinone; 1,2-dihydro-1,3,8-trihydroxy-2-methyl anthraquinone; roxburghinol.

Abstract—A new 1,2-dihydroanthraquinone, roxburghinol, was isolated from the leaves of *Cassia roxburghii*. Its structure was established as 1,2-dihydro-1,3,8-trihydroxy-2-methyl anthraquinone on the basis of physical and chemical evidence.

INTRODUCTION

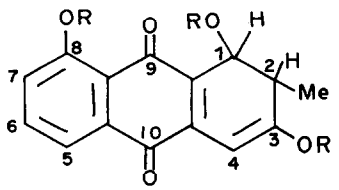
The genus *Cassia* elaborates several types of hydroxyanthraquinones [1]. In the present communication the isolation and structural elucidation of a new anthraquinone designated as roxburghinol (**1**) from the leaves of *C. roxburghii* Benth, is reported.

RESULTS AND DISCUSSION

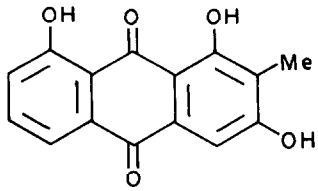
Roxburghinol (**1**), mp 211°, $[\alpha]_D^{25} - 12.2°$, $C_{15}H_{12}O_5$, $[M]^+$ 272, gave a pink colour with alcoholic magnesium acetate indicating it to be a hydroxyanthraquinone [2]. It exhibited reversible oxidation–reduction with aqueous sodium dithionite and air [8], and also gave an intense red colour with aqueous sodium hydroxide indicating the presence of a hydroxyquinone system [3]. Its phenolic nature was indicated by its solubility in aqueous alkali and red colour with alcoholic $FeCl_3$. It formed a triacetate (**2**), mp 170°, $C_{21}H_{18}O_8$, [^{1}H NMR (90 MHz, $CDCl_3$); three OAc at δ2.42, 6H, *s* and 2.15, 3H, *s*] on treatment with acetic anhydride and perchloric acid indicating the presence of one alcoholic and two phenolic hydroxyl groups. Its UV spectrum, $\lambda_{max}^{Dioxane}$ nm (log ε): 240 (4.16), 245 (4.15), 252 (4.13), 258 (4.18), 262 (4.16), 287 (3.9), 424 (4.0), was similar to those of 1,8-dihydroxy-2-methylanthraquinones [4]. In the IR spectrum (KBr) of **1**, absorptions due to phenolic hydroxyl (3320 cm^{-1}), chelated carbonyl (1625 cm^{-1}) and non-chelated carbonyl (1672 cm^{-1}) were readily discernible. The appearance of $[M-2]^+$ (100%) and $[M-H_2O]^+$ (5%) peaks in the mass spectrum of **1** supported a dihydroanthraquinone moiety in **1**. The foregoing indicated that roxburghinol was a trihydroxydihydroanthraquinone containing a chelated and non-chelated carbonyl. The compound in ethanolic solution formed a complex with cupric sulphate showing the presence of at least one hydroxyl group at an α-position [5]. The presence of a β-hydroxyl was indicated by the solubility of the compound in 5% aq. sodium carbonate solution [3, 6].

The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of roxburghinol (**1**) contained five signals in the aromatic region. A singlet at δ7.23 (1H) was assignable to the C-4 proton of the anthraquinone [7]. The spectrum also revealed the presence of two doublets, one at δ7.76 (1H, *d*, J = 8 Hz) and the other at 7.33 (1H, *d*, J = 8 Hz) assignable to H-5 and H-7 respectively [7]. Further a triplet at δ7.65 (1H, *t*, J = 8 Hz) was assignable to H-6 [7]. Signals at δ7.52 (1H, *s*, D_2O exchangeable) and 11.8 (1H, *s*, D_2O exchangeable) were assignable to C-3 and C-8 hydroxyls respectively.

In the aliphatic region of the ^{1}H NMR spectrum of naturally occurring anthraquinones, usually only one signal is observed between δ2.3 and 2.5 due to aromatic =C–Me. The multiplicity of the signals in the aliphatic region of the NMR spectrum of **1** revealed the presence of a $-\overset{1}{C}H(OH)-\overset{2}{C}H(-)-Me$ moiety. The protons of the methyl group resonated as a doublet at δ1.18 (J = 8 Hz). A broad doublet which appeared at δ5.3 (1H, J = 15 Hz) was



1 R=H
2 R=Ac



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attributed to the hydrogen of the C-1 hydroxyl group. Further a multiplet at δ2.5 and an ill defined doublet at 4.58 integrating for one proton each were assigned to H-2 and H-1 respectively.

In the ^{1}H NMR spectrum (90 MHz, $CDCl_3$) of acetate **2** the following signals were observed: δ1.3 (3H, *d*, J = 8 Hz, Me), 1.64 (1H, *m*, H-2), 2.15 (3H, *s*, C-1 OAc), 2.42 (6H, *s*, C-3 and C-8 OAc), 5.18 (1H, ill defined doublet, H-1), 7.32 (1H, *s*, H-4), 7.34 (1H, *dd*, J = 8, 2.5 Hz, H-7), 7.72 (1H, *t*, J = 8 Hz, H-6), 8.16 (1H, *dd*, J = 8, 2.2 Hz, H-5). The foregoing data permitted two alternative isomeric structures (**1** and **4**) for roxburghinol.

DDQ oxidation of **4** would yield 1,3,5-trihydroxy-2-methylanthraquinone mp 280° (lit. [9]), whereas **1** would afford 1,3,8-trihydroxy-2-methylanthraquinone mp 230–232° (lit. [10, 11]). DDQ oxidation of roxburghinol (**1**) in benzene afforded 1,3,8-trihydroxy-2-methylanthraquinone (**3**), mp 229°, $C_{15}H_{10}O_5$, $[M]^+$ 270. The IR spectrum (KBr) exhibited absorptions due to phenolic hydroxyls (3350 cm^{-1}), and chelated (1625 cm^{-1}) and non-chelated (1675 cm^{-1}) carbonyl groups. Its UV spectrum, λ_{max}^{MeOH} nm: 224, 252, 285 and 427, was in close agreement with that reported for 1,3,8-trihydroxy-2-methylanthraquinone (λ_{max}^{MeOH} nm: 220, 255, 285 and 427) [10, 11], as were the ^{1}H NMR data and the mass spectral fragmentation pattern [10] (see Experimental). However, a direct comparison could not be made for want of an authentic sample of 1,3,8-trihydroxy-2-methylanthraquinone. On the basis of these findings the 1,2-dihydro-1,3,8-trihydroxy-2-methylanthraquinone structure was assigned to roxburghinol and the other isomeric structure (**4**) was discarded. The mass spectral fragmentation of roxburghinol (see Experimental) was in good agreement with the assigned structure (**1**). The *m/z* peak at 270 (100%) arising by loss of two mass units from the parent ion (272) is of diagnostic value in distinguishing 1,2-dihydroanthraquinones from the corresponding fully aromatic anthraquinones. The ^{13}C NMR spectrum of **1** showed the appropriate absorbances for 15 chemically different carbon atoms, the chemical shifts of which were in accordance with the proposed structure (**1**). The signals at δ62.25 (C-1), 32.25 (C-2), 18.67 (Me) provided further support for a –CH(OH)–CH–Me moiety in **1**. The stereochemistry at C-1 and C-2 could not be settled, since the spectra of reference compounds are not available.

This is the first report of the isolation and characterization of a 1,2-dihydroanthraquinone from natural sources.

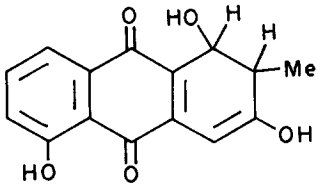
EXPERIMENTAL

The air dried leaves (1.5 kg) of *C. roxburghii*, collected locally, were coarsely powdered and extracted successively with petrol (60–80°), $CHCl_3$ and Me_2CO in a Soxhlet extractor until the extracts were almost colourless. The petrol extract was concd under red. pres. to a small vol. (250 ml) and left overnight. The waxy material (50 g) which separated was filtered off and the filtrate further concd under red. pres. to furnish a green semi-solid (5 g) which was subjected to CC on silica gel (200 mesh). Fractions of 100 ml each were collected. The column was successively eluted with petrol (fractions 1–8), C_6H_6 (fractions 9–18), C_6H_6–$CHCl_3$ (1:1) (fractions 19–24), C_6H_6–$CHCl_3$ (2:8) (fractions 25–29), $CHCl_3$ (fractions 30–43), $CHCl_3$–EtOAc (8:2) (fractions 44–53), $CHCl_3$–EtOAc (1:1) (fractions 54–87), EtOAc (fractions 88–124), EtOAc–Me_2CO (1:1) (fractions 125–138), and Me_2CO (fractions 139–160). Each fraction was monitored by TLC and similar fractions were combined. Fractions 1–8 yielded aliphatic esters (1.2 g). Fractions 9–42 on rechromatography over silica gel (200 mesh) gave β-sitosterol (1.5 g) (mp 137°, $[\alpha]_D$ – 36.0°, lit. [12], mp 139° $[\alpha]_D$ – 36.0°). Fractions 44–53 gave an anthraquinone-containing orange semi-solid material (250 mg). Fractions 54–124 yielded a mixture of quinonoid compounds (450 mg) whose separation is in progress. Fractions 125–160 yielded a red resinous material (1.1 g).

The anthraquinone-containing material (250 mg) was rechromatographed over silica gel (200 mesh). Fractions of 100 ml each were collected. The column was eluted with C_6H_6 (fractions 1–3), C_6H_6–EtOAc (1:1) (fractions 4–12) and EtOAc (fractions 13–20). Fractions 1–3 yielded negligible amounts of material and were not further investigated. Fractions 4–12 were combined, on the basis of identical TLC behaviour, and on concn furnished an orange solid (205 mg) which was crystallized from $CHCl_3$ to yield roxburghinol as orange crystals (200 mg), mp 211°, $[\alpha]_D^{25}$ – 12.2° (MeOH; *c* 0.54) (Found: C, 66.15; H, 4.42. $C_{15}H_{12}O_5$ requires: C, 66.17; H, 4.41%); EIMS (probe) 70 eV, *m/z* (rel. int.): 272 $[M]^+$ (25), 270 $[M-2H]^+$ (100), 254 $[M-H_2O]^+$ (5), 242 $[270-CO]^+$ (20), 241 $[270-CHO]^+$ (94), 214 $[242-CO]^+$ (8), 213 $[241-CO]^+$ (16), 186 $[214-CO]^+$ (8), 185 $[213-CO]^+$ (8), 158 $[186-CO]^+$ (5), 157 $[185-CO]^+$ (8), 129 $[158-CHO]^+$ (10), 128 $[157-CHO]^+$ (12), 127 $[128-H]^+$ (23), 121 [242/2 doubly charged]$^+$ (25), 114 $[129-Me]^+$ (6), 107 [214/2 doubly charged]$^+$ (6), 63 $[114-C_4H_3]^+$ (18); ^{13}C NMR (100 MHz, DMSO-d_6): δ191.52 (C-9), 181.24 (C-10), 153.71 (C-8), 152.7 (C-3), 137.27 (C-6), 133.24 (C-5a), 133.14 (C-4a), 124.35 (C-7), 120.78 (C-4), 119.37 (C-5), 116.67 (8a), 114.27 (C-1a), 62.25 (C-1), 32.25 (C-2), 18.67 (Me). The $CHCl_3$ extract (5.5 g) when chromatographed over silica gel (200 mesh) also gave roxburghinol (150 mg). The Me_2CO extract yielded a water-soluble phenolic compound whose structure elucidation is in progress.

Roxburghinol triacetate (**2**). To roxburghinol (40 mg) dissolved in 1 ml of Ac_2O, 4–5 drops of $HClO_4$ was added, and the mixture left at room temp. for 48 hr. The triacetate was purified by prep. TLC (silica gel G), using C_6H_6–EtOAc (9:1). The band at R_f 0.34 was located under UV, scraped off and extracted with $CHCl_3$. The product crystallized from C_6H_6 as yellow crystals (35 mg), mp 170°. (Found: C, 63.33; H, 4.53; $C_{21}H_{18}O_8$ requires C, 63.31; H, 4.52%.) MS *m/z* (rel. int.): 398 $[M]^+$ (< 1) 356 $[M-CH_2=C=O]^+$ (8), 354 $[356-2H]^+$ (31), 312 $[354-CH_2=C=O]^+$ (31), 270 $[312-CH_2=C=O]^+$ (100), 242 $[270-CO]^+$ (6), 241 $[270-CHO]^+$ (18).

DDQ oxidation of roxburghinol (**1**). Roxburghinol (40 mg) in dry C_6H_6 (100 ml) and DDQ (0.1 g) was heated on a water bath for 4 hr. The clear soln was subjected to CC on silica gel (200 mesh). Fractions 25 ml each were collected. The column eluted with C_6H_6 (fractions 1–5), $CHCl_3$–EtOAc (1:1) (fractions 6–15), EtOAc (fractions 16–25), and Me_2CO (fractions 26–30). Fractions 16–25 were combined, on the basis of identical TLC behaviour, and on concn furnished an orange solid, which was crystallized from Me_2CO to give 1,3,8-trihydroxy-2-methyl anthraquinone (**3**) as orange crystals, mp 229°, lit. [10, 11], mp



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230–232°. (Found: C, 66.67; H, 3.68. $C_{15}H_{10}O_5$ requires C, 66.66; H, 3.70%.) $[M]^+$ 270. IR ν_{max}^{KBr} cm^{-1}: 3350, 1675, 1625, 1575, 1570, 1545, 1460, 1420, 1390, 1295, 1280, 1210, 1175, 1163, 1095, 1065, 1045, 1020, 915, 875, 842, 829 and 760; ^1H NMR (90 MHz; DMSO-d_6): δ6.9–7.2 (2H, H-4 and H-7), 7.3–7.6 (2H, H-5 and H-6) and 2.3 (3H, *s*, Me); EIMS (probe) 70 eV, *m/z* (rel. int.): 270 $[M]^+$ (100), 242 $[M-CO]^+$ (24), 241 $[M-CHO]^+$ (95), 214 $[242-CO]^+$ (10), 213 $[241-CO]^+$ (16), 186 $[214-CO]^+$ (5), 185 $[213-CO]^+$ (8), 158 $[186-CO]^+$ (5), 157 $[185-CO]^+$ (9), 129 $[158-CHO]^+$ (10), 128 $[157-CHO]^+$ (12), 127 $[128-H]^+$ (26), 121 (242/2 doubly charged]$^+$ (25), 114 $[129-Me]^+$ (7), 107 [214/2 doubly charged]$^+$ (10), 63 $[114-C_4H_3]^+$ (15).

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