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TWO ANTHRAQUINONE GLYCOSIDES FROM *CASSIA MARGINATA* ROOTS

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Key Word Index—*Cassia marginata*; Leguminosea; roots; anthraquinones.

Abstract—From the roots of *Cassia marginata*, 1,3-dihydroxy-6,8-dimethoxy-2-methyl anthraquinone 3-*O*-rhamnosyl-(1 → 6)-glucopyranoside and 1,3,5,8-tetrahydroxy-2-methyl anthraquinone 3-*O*-glucoside have been isolated and their structures elucidated.

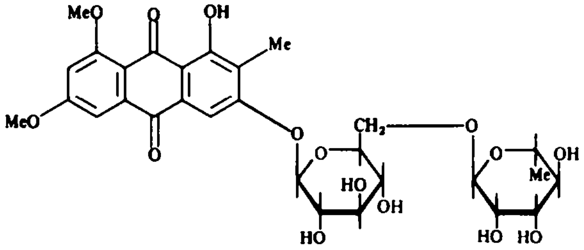
INTRODUCTION

Species of cassia are rich sources of flavonoids [1], anthraquinones [2] and polysaccharides [3]. The plant possesses important medicinal properties [4].

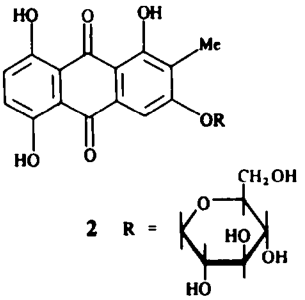
RESULTS AND DISCUSSION

Two anthraquinone glycosides (1 and 2) have been isolated from the roots of *Cassia marginata* Roxb.

The first glycoside, $C_{29}H_{34}O_{16}$ (1), on acid hydrolysis gave glucose, rhamnose and an aglycone, $C_{17}H_{14}O_6$, which was identified as 1,3-dihydroxy-6,8-dimethoxy-2-methyl anthraquinone on the basis of standard colour reactions, spectral data and co-chromatography with the natural product isolated from the seeds of *C. multijuga* [5]. The permethylated glycoside on hydrolysis gave two partially methylated sugars, identified as 2,3,4-tri-*O*-methyl glucose and 2,3,4-tri-*O*-methyl rhamnose by comparison of R_g values using 2,3,4,6-tetra-*O*-methyl-D-glucose as standard (R_g 0.85 and 1.01, respectively; solvent B:A:W, 4:1:5; spray AHP). This established that two sugars, glucose and rhamnose, were present in the form of a 1 → 6-bioside [6, 7]. The 1 → 6-linkage (rutinoside) was also confirmed by the NMR spectrum of the glycoside [8, 9]. The attachment of rutinoside at position 3 was established by colour reactions and UV spectral data [10, 11]. Thus this new glycoside has been identified as 1,3-dihydroxy-6,8-dimethoxy-2-methyl anthraquinone-3-*O*-rhamnosyl-(1 → 6)-glucopyranoside.



1



2 R =

3 R = H

The second glycoside, $C_{21}H_{20}O_{11}$ (2), gave the characteristic colour reactions of an anthraquinone. On acid hydrolysis, it gave glucose and an aglycone, $C_{15}H_{10}O_6$, which on zinc dust distillation gave 2-methyl anthracene. This established that the aglycone was a 2-methyl anthraquinone derivative. The aglycone had one C–Me (δ2.26) and four hydroxyl groups (tetraacetate). The UV absorption maxima and mass fragments at m/z 258 $[M - CO]^+$ and 230 $[M - 2CO]^+$ were typical of an anthraquinone. The ^1H NMR spectrum of the aglycone showed the presence of three aromatic protons, two as an *ortho*-coupled doublet at δ7.20 (2H, J = 9 Hz, H-6, H-7) and the third as a singlet at δ7.90 (1H, H-4). The aglycone was thus a 1,2,3,5,8-penta-substituted anthraquinone having a methyl group at C – 2. On the basis of standard colour reactions [12, 13], IR [14] and UV spectral data [15, 16] it was identified as 1,3,5,8-tetrahydroxy-2-methyl anthraquinone (3).

As this aglycone had not been reported earlier, its structure was confirmed by synthesis. The synthetic anthraquinone, synthesized by the cyclization of 3,6,2′,4′,-tetrahydroxy-3′-methyl-*O*-benzoyl benzoic acid with sulphuric acid containing boric acid [17], was found to be identical in every respect with the aglycone isolated from the roots of *C. marginata*.

The attachment of the sugar moiety at position 3 was established by colour reactions and UV spectral data. The occurrence of glucose in the pyranose form was confirmed by periodate oxidation, and the β-nature of the sugar linkage was confirmed by β-glucosidase hydrolysis.

Thus the new glycoside is 1,3,5,8-tetrahydroxy-2-methyl anthraquinone 3-*O*-β-D-(+)-glucopyranoside. This structure was confirmed by synthesis of the glycoside by the glycosidation of **3** in acetone in the presence of potassium hydroxide [18].

EXPERIMENTAL

The roots, purchased from United Chemicals and Allied Products, Calcutta, were extracted with boiling EtOH (7 l.). The conc extract (800 ml) was poured in H_2O, to give an aq. soln (fraction I) and a coloured residue (20 g fraction II).

Glycoside **2**. Fraction I was concentrated (300 ml) and extracted successively with petrol, C_6H_6, Et_2O and EtOAc. The Et_2O extract (three spots on a silica gel TLC plate) was chromatographed over a silica gel column and eluted with hexane, C_6H_6 and EtOAc. The C_6H_6–EtOAc (1:1) eluate (single spot on TLC) gave compound **2** (700 mg), crystallized from EtOAc–petrol (3:1), mp 190° (d). (Found: C, 56.20; H, 4.40. Calc. for $C_{21}H_{20}O_{11}$: C, 56.25; H, 4.46%.) UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 275, 320, 410, 480, 520; IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3450, 3272, 1610, 1455, 1360, 1230, 1080, 840, 730, 720; ^{1}H NMR (250 MHz, $CDCl_3$): δ3.85 (6H, sugar protons), 5.02 (H-1′ glucosyl), 2.26 (3H, *s*, Me), 7.88 (1H, *s*, H-4) and 7.20 (2H, *d*, *J* = 8.5 Hz, H-6, H-7). *Acetate* (Ac_2O–C_5H_5N), mp 165°. Acid hydrolysis (7% HCl) of glycoside **2** gave an aglycone and glucose. The aglycone was crystallized from C_6H_6–EtOAc (4:1), mp 220°. (Found: C, 62.80; H, 3.54. Calc. for $C_{15}H_{10}O_6$: C, 62.90; H, 3.49%.) UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 275, 320, 410, 480, 525; IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3450, 3275, 1610, 1505, 1455, 1360, 1230, 1075; ^{1}H NMR (250 MHz, $CDCl_3$): δ2.26 (*s*, 3H, Me), 7.90 (*s*, 1H, H-4), 7.20 (*d*, *J* = 8.5 Hz, H-6, H-7); MS (70 eV) *m/z*: 286 $[M]^+$, 268, 258, 240, 230, 212, 183, 110, 108. *Acetate* (Ac_2O–C_5H_5N), mp 185°.

Synthesis of 1,3,5,8-*tetrahydroxy-2-methyl anthraquinone*. 3,6-Dihydroxy phthalic anhydride (3.5 g), 2,6-dihydroxy toluene (2.4 g) and dry $AlCl_3$ (9 g) were heated at 40–60° until copious evolution of HCl gas took place. More dry $AlCl_3$ (1 g) was added and the reaction mixture was heated at 140–160° for 30 min, cooled and iced H_2O (200 ml) added slowly. A dark mass was obtained, which was decomposed with conc HCl. To the residue containing benzoyl benzoic acid, a warm soln of dry Na_2CO_3 (3 g in 50 ml H_2O) was added and steam was passed into the mixture. The hot soln was filtered and the filtrate was acidified with conc HCl (5–7 ml) to liberate the acid as a brown gum.

The brown gum (3 g) was heated with conc H_2SO_4 (60 ml) and boric acid (10 g) on a water bath for 80 min with occasional stirring, cooled and poured into iced H_2O (200 g). The orange-yellow solid obtained was extracted with $NaHCO_3$ soln to remove the unchanged acid, washed with H_2O and extracted with MeOH. The MeOH extract was purified by CC to give the anthraquinone (3 g), mp 220°. TLC: R_f 0.75 (MeOH–EtOAc, 4:1).

Synthesis of 1,3,5,8-*tetrahydroxy-2-methyl*-3-O-*glucopyranoside*. To a soln of the above-synthesized anthraquinone (200 mg) and acetobromoglucose (600 mg) in Me_2CO (50 ml), a soln of KOH (150 mg) in H_2O (17 ml) was added dropwise. After stirring for 2 hr, the Me_2CO was evaporated and the residue extracted with $CHCl_3$. The $CHCl_3$ soln was washed with H_2O, dried and the solvent evaporated. The acetylated product in MeOH (10 ml) was treated with M NaOMe (10.5 ml) at room temp. for 24 hr and subsequently acidified (HOAc) to pH 6. The product was purified by CC to yield light-brown crystals from HOAc, 120 mg (28%), mp 188° (lit. 190°).

Glycoside **1**. The EtOAc extract of the roots of *C. marginata* was chromatographed over silica gel and eluted successively with hexane, C_6H_6 and EtOAc. The C_6H_6–EtOAc eluate (single spot on TLC) gave a yellow-coloured compound (**1**), 800 mg, crystallized from EtOAc–petrol (1:5), mp 120° (d). (Found: C, 47.08; H, 4.70. Calc. for $C_{29}H_{34}O_{16}$: C, 47.15; H, 4.61%.) UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 222, 250, 373, 440; IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3340, 2945, 2892, 1765, 1725, 1650, 1185, 730, 720; ^{1}H NMR (250 MHz, $CDCl_3$): δ0.98 (*br*, rhamnose methyl), 2.10 (*s*, Me), 3.85 (*s*, 6H-OMe), 3.40–3.85 (*br*, sugar protons), 5.00 (H-1″ rhamnosyl), 5.25 (H-1″ glucosyl), 6.85 (*d*, *J* = 2.5 Hz, 1H, H-7), 7.68 (*d*, *J* = 2.5 Hz, 1H, H-5), 7.85 (*s*, 1H, H-4). Acetate (Ac_2O–C_5H_5N) mp 260°. *Permethylation* of **1** was carried out by the method described in ref. [19]. The characterization of the permethylated sugars is described in the Results and Discussion. Acid hydrolysis of glycoside **1** (400 mg) with 7% HCl (3 ml) was performed for 4 hr. The mixture was then diluted with H_2O and the soln extracted with Et_2O (100 ml). The aq. layer was chromatographed on Whatman No. 1 paper in (a) EtOAc–C_5H_5N–H_2O (12:5:4) and (b) EtOAc–*i*-PrOH–H_2O (3:1:1) using glucose, rhamnose, arabinose, and xylose as standards. The chromatogram was developed with *p*-anisidine HCl (1 g) and NaHS (0.1 g) in MeOH (10 ml) diluted to 100 ml with *n*-BuOH and aniline hydrogen phthalate spray at 120–130° for 10–15 min. The chromatogram showed the presence of glucose and rhamnose (R_f 0.18, 0.34). The Et_2O extract was evaporated to yield the aglycone, crystallized from EtOAc–petrol (3:1) mp 272°. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 220, 250, 285, 375, 440; MS (70 eV) *m/z*: 314 $[M]^+$, 296, 286, 268, 258, 240, 229, 136 and 122. *Acetate* (Ac_2O–C_5H_5N), mp 230°.

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