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ANTHRAQUINONES FROM THE ROOTS OF *ACACIA LEUCOPHLOEA*

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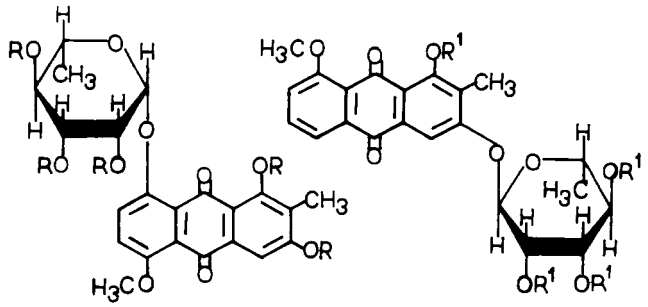
ABSTRACT.—Evidence is presented for the structures of two new anthraquinone glycosides: 1,3-dihydroxy-5-methoxy-2-methylanthraquinone-8-*O*-α-L-rhamnopyranside (**1**) and 1-hydroxy-8-methoxy-2-methylanthraquinone-3-*O*-α-L-rhamnopyranoside (**2**) which occur together with 1,5-dihydroxy-8-methoxy-2-methylanthraquinone-3-*O*-α-L-rhamnopyranoside and galangin-3-*O*-α-L-rhamnopyranoside.

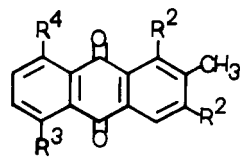
Acacia leucophloea Willd. (Leguminosae) is a medicinal plant employed in the Indian indigenous system of medicine (1,2). This species has previously been found to contain octacosanol and (+)-pinitol (3) and betulinic acid-3-*O*-β-D-maltoside (4), but the literature reveals that no systematic chemical examination has been reported on the roots. We report here the isolation and characterization of two new anthraquinone glycosides as well as a known anthraquinone glycoside and a flavone glycoside. The structures of the new anthraquinone glycosides have been established as 1,3-dihydroxy-5-methoxy-2-methylanthraquinone-8-*O*-α-L-rhamnopyranoside (**1**) and 1-hydroxy-8-methoxy-2-methylanthraquinone-3-*O*-α-L-rhamnopyranoside (**2**) by their color reactions, spectral data, and chemical methods.

Compounds **1** and **2** both gave positive color reactions with $FeCl_3$, the Molisch's test, and the Borntrager reaction (5) for anthraquinone glycosides. The uv-visible spectra are characteristic for anthraquinoids. The principal peaks in the ir spectra indicated the presence of hydroxyl, methoxyl, methyl, unchelated, and chelated carbonyl units. On treatment with Ac_2O-C_5H_5N, **1** and **2** formed the respective pentaacetate (**3**) and the tetraacetate (**4**). The ^{1}H-nmr spectrum of **3** displayed two separate doublets for *ortho*-coupled protons, one methoxyl and one methyl group in the form of separate singlets, five acetate groups, two separate doublets centered at δ 0.78 (J=6 Hz) for rhamnose methyl and δ 4.30 (J=7 Hz) for an anomeric sugar proton and a multiplet for other sugar protons. The ^{1}H nmr of **4** exhibited signals for three *ortho*-coupled protons in the form of three separate doublets (J=8 Hz), assignable to the protons at positions 5, 6, and 7 along with the other usual signals, as observed in **3**, indicative of the presence of one methoxyl and one methyl group both in compounds **1** and **2**, two hydroxyls in **1**, and one hydroxyl in **2** (see Table 1). Acidic hydrolysis of **1** and **2** gave respective aglycones identified as **5** and **6** and L-rhamnose in each case (co-pc and osazone). The aglycones **5** and **6** responded to color reactions characteristic for hydroxy



1 R=H
3 R=Ac

2 R^1=H
4 R^1=Ac



5 R^2=R^4=OH, R^3=OCH_3
6 R^2=OH, R^3=H, R^4=OCH_3
7 R^2=R^4=OAc, R^3=OCH_3
8 R^2=OAc, R^3=H, R^4=OCH_3

TABLE 1. ^{1}H-nmr Spectra of Compound **3-8** (Recorded at 90 MHz)[a]

Proton	Compounds					
	3	**4**	**5**	**6**	**7**	**8**
H-4	7.90 s	7.88 s	7.90 s	7.88 s	7.90 s	7.88 s
H-5	—	7.60 d (8)	—	7.60 d (8)	—	7.60 d (8)
H-6	7.62 d	7.50 d (8)	7.62 d (8)	7.50 d (8)	7.60 d	7.50 d (8)
H-7	6.90 d (8)[b]	7.00 d (8)	6.90 d (8)	7.00 d (8)	6.90 d	7.00 d (8)
OMe	4.00 s	4.00 s	4.00 s	4.00 s	3.95 s	4.00 s
C-Me	2.40 s	2.40 s	2.40 s	2.40 s	2.40 s	2.40 s
OAc	2.10 s 2.08 s 2.04 s 2.00 s 1.98 s	2.12 s 2.10 s 2.05 s 2.00 s	—	—	2.00 s 2.05 s 2.10 s	2.00 s 2.10 s
OH	—	—	12.00 s 13.00 s	12.10 s	—	—
Rhamnose-Me	0.78 d (6)	0.78 d (6)	—	—	—	—
Anomeric sugar proton	4.30 d (7)	4.30 d (7)	—	—	—	—
Other sugar protons	3.80-3.95 m	3.80-3.95 m	—	—	—	—

[a]s=singlet; d=doublet; m=multiplet.

[b]Numbers in parentheses represent the coupling constants in Hz.

anthraquinones, and this observation was also supported by their uv-visible and ir spectra. Both **5** and **6** furnished 2-methylanthracene on zinc dust distillation.

The aglycone **5** formed a tetramethylether (Me_2SO_4-K_2CO_3), and a triacetate (Ac_2O-C_5H_5N), **7**. The ^{1}H-nmr spectrum of **7** displayed three singlets at δ 2.00, 2.05, and 2.10 for three acetate groups and one singlet at δ 4.00 for one methoxyl group, confirming the presence of three hydroxyl and one methoxyl groups in **5**. The aglycone **6** formed a trimethylether (Me_2SO_4-K_2CO_3), and a diacetate (Ac_2O-C_5H_5N), **8**. The ^{1}H nmr of **8** exhibited two singlets at δ 2.00 and 2.10 for two acetate groups and one singlet at δ 4.00 for one methoxyl group, confirming the presence of two hydroxyl and one methoxyl groups in **6**.

The aglycones **5** and **6** exhibited no absorption in the region 480-520 nm, indicating the absence of two α-hydroxyl groups in a 1,4 or 5,8 relationship (6). The aglycones **5** and **6** formed a complex with ethanolic $CuSO_4$, showing the presence of an α-OH (7) and also gave an orange-red color with 5% methanolic magnesium acetate, showing the presence of an hydroxyl group at position C-3 (8) in both the structures. The aglycones **5** and **6** further gave a deep red color with H_2SO_4, indicating the presence of a methoxyl group at an α-position to a carbonyl group (9). Compound **5** gave a positive test with zirconium nitrate (1,8-dihydroxy system) (10), while compound **6** gave the above test after demethylation (HI-P), which confirmed a hydroxyl at C-8 in **5** and a methoxyl at C-8 in **6**. Thus, the new aglycones **5** and **6** were confirmed as 1,3,8-trihydroxy-5-methoxy-2-methylanthraquinone and 1,3-dihydroxy-8-methoxy-2-methylanthraquinone, respectively.

The attachment of L-rhamnose in compounds **1** and **2** was shown to be at the 8-OH and 3-OH, respectively, by comparative studies of the color reactions of their glycosides with their aglycones and also by methylation studies of the glycosides. Com-

pound **5** gave positive tests for a 3-OH (8) and an 8-OH (10), while **1** gave only a positive test for a 3-OH (8), showing the rhamnosidation at 8-OH in compound **1**. Compound **2** could not be methylated with CH_2N_2 (11), showing that the rhamnose was attached to the β-OH at position-3 in **2**. In addition, **6** gave a positive test for a 3-OH (8), while the same was not observed by compound **2**, thus further confirming the rhamnosidation at 3-OH in compound **2**. Compounds **1** and **2** could be hydrolyzed with takadiastase solution to give **5** and **6** (mmp and co-tlc) and L-rhamnose (in each case, co-pc), indicating the presence of α-linkages. Thus, on the basis of the above results, the new anthraquinone glycosides were assigned the structures **1** and **2** and named 1,3-dihydroxy-5-methoxy-2-methylanthraquinone-8-*O*-α-L-rhamnopyranoside and 1-hydroxy-8-methoxy-2-methylanthraquinone-3-*O*-α-L-rhamnopyranoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were taken on a melting point apparatus (Toshniwal, India) and are corrected. Ir spectra in KBr were recorded on a Perkin-Elmer 175 spectrophotometer (ν max in cm^{-1}). ^{1}H-nmr spectra were obtained with $CDCl_3$ at 90 MHz on a varian EM 360L instrument using TMS as an internal standard. Chemical shifts are given in δ ppm. Uv visible spectra were recorded on an Hitachi 320 instrument using EtOH as a solvent (λ max in nm).

EXTRACTION OF ANTHRAQUINONE GLYCOSIDES.—Air-dried and powdered roots of *A. leucophloea* (3 kg) procured from the United Chemicals and Allied Products, Calcutta, India, and authenticated by the Botanical Survey of India, Allahabad Circle, U.P., India, were extracted with EtOH under reflux conditions on a water bath for 25 days each for five times (using fresh EtOH). The EtOH extract (50 liters) was concentrated under reduced pressure (500 ml) and segregated (1 liter) into H_2O soluble and insoluble fractions. The H_2O soluble portion was concentrated to remove the excess H_2O in a porcelain dish on a steam bath to yield a syrupy mass (6 g). The resulting mass was then successively extracted with petroleum ether, C_6H_6, $CHCl_3$, and EtOAC.

The EtOAC extract (800 ml) was concentrated under reduced pressure (200 ml) and kept in a refrigerator for 5 days which deposited compound **1**. Compound **1** was removed by filtration, purified over a column of silica gel, eluted with an EtOAC/Me_2CO mixture, and the eluate crystallized as a brown amorphous substance (yield 1.3 g), mp 156-160°. The mother liquor was found by tlc examination to be a mixture of two substances. The mother liquor was concentrated and re-chromatographed over silica gel. The column was eluted successfully with Me_2CO and MeOH to afford compound **2** and galangin-3-rhamnoside (mp 228-230°, yield 1.45 g, mmp, and co-tlc) (12). Compound **2** was crystallized from MeOH as a reddish brown crystalline substance, mp 218-220° (yield 1.40 g). The H_2O insoluble fraction was extracted successively with petroleum ether, C_6H_6, $CHCl_3$, EtOAc, Me_2CO, and MeOH. The MeOH extract was concentrated under reduced pressure. The resulting solid was purified over silica gel, eluted with MeOH, crystallized as a brown colored crystalline substance (MeOH) and was found to be identical with 1,5-dihydroxy-8-methoxy-2-methylanthraquinone-3-*O*-α-L-rhamnopyranoside [mp 338-340° (dec.), yield 1.28, mmp, and co-tlc] (13).

CHARACTERIZATION OF COMPOUND **1**.—λ max: 235, 280, and 430; ν max 3430-3400 (br, OH), 2930 and 1460 (C-Me), 2870 and 1170 (OMe), 1675, 1625, 1580, 1285, 1120, 1090, 860 (anthraquinone skeleton), and 825 (glycoside) [Found: C, 59.20; H, 4.90; $C_{22}H_{22}O_{10}$ required: C, 59.30; H, 5.0%].

ACETYLATION OF COMPOUND **1**.—The glycoside (100 mg) was acetylated with Ac_2O (5 ml) and C_5H_5N (5 ml) as usual under reflux conditions for 5 h. The product, **3**, was crystallized from Et_2O as light brown colored needles, mp 130-132° (yield 85 mg) [Found: C, 58.20; H, 4.84; $C_{32}H_{32}O_{75}$ required C, 58.50; H, 4.87%].

ACID HYDROLYSIS OF COMPOUND **1**.—A solution of **1** (800 mg) in EtOH (25 ml) and 7% H_2SO_4 (40 ml) was refluxed for 5 h, poured into ice-cooled H_2O (100 ml) and kept at room temperature for 24 h. The precipitated aglycone **5** was filtered off. The filtrate was neutralized ($BaCO_3$) and was found to contain L-rhamnose (co-pc and osazone).

AGLYCONE **5**.—The aglycone **5** was chromatographed over silica gel and eluted with $CHCl_3$. Attempted crystallization of the $CHCl_3$ eluate from EtOAC/petroleum ether mixture furnished yellowish brown colored needles, mp 198-200° (yield 400 mg); λ max 235, 285, and 430; ν max 3430-3400 (br, OH), 2930 and 1455 (C-Me), 2875 and 1175 (OMe), 1675, 1630, 1580, 1280, 1125, 1085, and 860;

[Found; C, 64.10; H, 4.00; OMe, 10.26 (Zeisel's method); $C_{16}H_{12}O_6$ required; C, 64.00; H, 4.00; 1×OMe 10.30%].

ACETYLATION OF AGLYCONE **5**.—It formed an acetyl derivative **7** (100 mg **5**, 5 ml Ac_2O, and 5 ml C_5H_5N; 5 h; on a water bath) which crystallized from $CHCl_3/C_6H_6$ as buff colored needles, compound **7**, mp 130-132° (yield 58 mg), ν max 1720 (acetate) [Found; C, 61.96; H, 4.12; OAc, 30.26; $C_{22}H_{18}O_9$ required C, 61.67; H, 4.22; 3×OAc 30.28%].

METHYLATION OF **5**.—The aglycone **5** (100 mg) was methylated with Me_2SO_4 (4 ml) and K_2CO_3 (2 g) in dry Me_2CO (15 ml) by refluxing on a water bath for 8 h and worked up as usual. The product was crystallized from Et_2O as light brown colored prisms, mp 160-163° (dec.) (yield 70 mg) [Found; C, 66.64; H, 6.12; OMe, 36.24; $C_{19}H_{18}O_6$ required C, 66.66; H, 6.14; 4×OMe 36.25%].

CHARACTERIZATION OF COMPOUND **2**.—λ max 230, 285, and 430; ν max 3420-3400 (br, OH), 2930 and 1460 (C-Me), 2870 and 1175 (OMe), 1870, 1630, 1575, 1280, 1125, 1085, 860 (anthraquinone skeleton), and 820 (glycoside) [Found; C, 61.10; H, 5.00; $C_{22}H_{22}O_9$ required C, 61.30; H, 5.1%].

ACETYLATION OF COMPOUND **2**.—The compound **2** (100 mg) was acetylated with Ac_2O (5 ml) and C_5H_5N (5 ml) under reflux condition and worked up as usual. The product, **4**, was crystallized from Et_2O as reddish colored needles, mp 140-142° (yield 80 mg) [Found; C, 60.19; H, 5.00; $C_{30}H_{35}O_{13}$ required C, 60.20; H, 5.01%].

ACID HYDROLYSIS OF COMPOUND **2**.—The glycoside (800 mg) in EtOH (25 ml) was refluxed with 7% H_2SO_4 (40 ml) for 5 h on a steam bath and worked up as usual. The aglycone **6** was separated by filtration and sugar was identified as L-rhamnose (co-pc and osazone).

AGLYCONE **6**.—The aglycone **6** was chromatographed over silica gel and eluted with $CHCl_3$. The $CHCl_3$ eluate was concentrated and the product was crystallized from EtOAc/petroleum ether mixture as yellowish brown colored needles, mp 238-240° (yield 430 mg); λ max 240, 285, and 430; ν max 3400-3380 (br-OH), 2930 and 1450 (C-Me), 2870 and 1170 (OMe), 1670, 1630, 1580, 1280, 1120, 1085, and 855 [Found; C, 67.40; H, 4.00; OMe, 10.78 (Zeisel's method); $C_{16}H_{12}O_5$ required C, 67.60; H, 4.22; 1×OMe, 10.90%].

ACETYLATION OF AGLYCONE **6**.—The aglycone (100 mg) was acetylated with Ac_2O (5 ml) and C_5H_5N (5 ml) on a water bath for 5 h and worked up as usual. The product, **8**, was crystallized from Et_2O as buff colored needles, mp 140-144° (yield 80 mg); ν max 1715 (OAC); [Found; C, 65.20; H, 4.33; OAc, 23.34; $C_{20}H_{16}O_7$ required; C, 65.21; H, 4.34; 2×OAc, 23.36%].

METHYLATION OF AGLYCONE **6**.—The aglycone **6** (100 mg) was methylated with Me_2SO_4 (4 ml) and K_2CO_3 (2 g) in dry Me_2CO (20 ml) as done earlier. The product was crystallized from Et_2O as yellowish brown colored needles, mp 247-248° (yield 75 mg) [Found: C, 67.26; H, 5.11; OMe, 29.76 (Zeisel's method); $C_{18}H_{16}O_5$ required C, 67.28; H, 5.12; 3×OMe, 29.80%].

DEMETHYLATION OF AGLYCONE **6**.—The aglycone **6** (50 mg) was treated with HI (1 ml) in the presence of phosphorous (50 mg) on a steam bath for 4 h. The resulting product was extracted with Et_2O, mp 230-231° (mmp and co-tlc) (14); [Found; C, 66.52; H, 3.68; $C_{15}H_{10}O_5$ required; C, 66.60; H, 3.70%].

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