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ROXBURGHIADIOL A AND ROXBURGHIADIOL B, TWO 14α-METHYLSTEROLS FROM *AGLAIA ROXBURGHIANA*[1]

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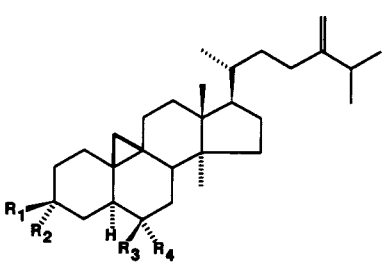
ABSTRACT.—Two new 14α-methylsterols, roxburghiadiol A and roxburghiadiol B, were isolated from the leaves and fruits of *Aglaia roxburghiana*. Previously their structures were tentatively assigned as 4-bisnormethyl-24-methylene-cycloarta-3β,7α-diol and 4-bisnormethyl-24-methylene-cycloarta-3β,6α-diol, respectively. A reinvestigation using 2D-nmr technique has confirmed the structure **2** for roxburghiadiol B as previously reported, and roxburghiadiol A is now found to be the corresponding 6β epimer **1**.

Aglaia roxburghiana Miq. (Meliaceae) is one of the source drugs for "Priyangu" used in the Ayurvedic system of medicine in India. It has several medicinal properties (1,2). Two new 14α-methylsterols, roxburghiadiol A and roxburghiadiol B, from the leaves and fruits of this plant were reported earlier from this laboratory (3). Their structures were tentatively designated as 4-bisnormethyl-24-methylenecycloarta-3β,7α-diol and the corresponding 3β,6α-diol, respectively, based on the data available at that time. Their structures have now been reinvestigated using both homonuclear and heteronuclear 2D-nmr techniques. The structure of roxburghiadiol B [**2**] proposed earlier has been confirmed, while roxburghiadiol A is now found to be the 6β-epimer **1** of compound **2** and does not have the 3β,7α-dihydroxy structure as previously suggested.



1 $R_1=R_3=OH$, $R_2=R_4=H$
2 $R_1=R_4=OH$, $R_2=R_3=H$
3 $R_1=R_3=OAc$, $R_2=R_4=H$
4 R_1, $R_2=O$; R_3, $R_4=O$
5 $R_1=R_4=OAc$, $R_2=R_3=H$

Roxburghiadiol A [**1**], mp 173°, $[\alpha]D +51°$ ($c=2$, $CHCl_3$) analyzed for $C_{29}H_{48}O_2$. The ir spectrum showed the presence of hydroxyl (3325, 1050 cm^{-1}), cyclopropane (3030 cm^{-1}), and methylene (1635, 887 cm^{-1}) functions. The presence of two hydroxyl groups was confirmed by the formation of a diacetate **3**. The presence of a C-24 methylene group in the side chain was shown by a strong peak at *m/z* 83 in the mass spectrum due to allylic cleavage and also by comparing the ^{13}C-nmr spectrum of **1** with that of cycloeucalenol (4). The ^1H-nmr as well as the 2D-nmr spectra showed the presence of cyclopropane protons. Both appeared as doublets ($J=4$ Hz) at δ 0.20 and 0.90 and were attached to the same carbon appearing at δ 30.2 (Table 1). The presence of two secondary hydroxyls (δ c 71.6 and 69.6) was shown by two one-proton multiplets at δ 3.75 ($W½=25$ Hz) and 3.90 ($W½=9$ Hz). The former is due to the axial hydrogen at C-3. The broad band with a half band width of 25 Hz showed that C-4 is devoid of methyl groups. The narrow multiplet corresponding to the *CH*OH of the second hydroxyl group showed that the hydrogen must be equatorial. Because the side chain is

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TABLE 1. 2D nmr ^{1}H-^{13}C Heteronuclear Chemical Shift Correlations of Roxburghiadiol A [**1**] and Roxburghiadiol B [**2**].

Position	Compound			
	1		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
C-1	31.6	1.18, 1.26	30.9	1.26, 1.56
C-2	35.5	1.28, 1.28	35.2	1.32, 1.32
C-3	71.6	3.75	70.9	3.70
C-4	38.4	1.55, 1.88	37.2	1.10, 2.36
C-5	41.0	1.52	45.1	1.38
C-6	69.6	3.90	71.0	3.10
C-7	32.2	1.34, 1.50	35.1	1.20, 1.50
C-8	40.2	2.04	46.9	1.84
C-9	22.7		23.2	
C-10	27.6		31.6	
C-11	26.3	1.34, 1.92	26.5	1.24, 1.94
C-12	35.1	1.38, 1.98	34.4	1.35, 1.98
C-13	45.6		45.3	
C-14	48.3		48.8	
C-15	32.8	1.66, 1.66	32.6	1.65, 1.65
C-16	28.0	1.34, 1.94	27.9	1.34, 1.96
C-17	52.3	1.60	52.2	1.62
C-18	19.4	0.94	19.1	0.93
C-19	30.2	0.20, 0.90	27.8	0.14, 0.30
C-20	36.0	1.40	36.0	1.44
C-21	18.3	0.90	18.3	0.90
C-22	35.0	1.15, 1.60	35.0	1.16, 1.58
C-23	31.2	1.90, 2.10	31.3	1.94, 2.10
C-24	156.8		156.7	
C-25	33.7	2.24	33.8	2.29
C-26	21.9	1.02	21.9	1.02
C-27	21.8	1.03	21.8	1.03
C-28	105.9	4.66, 4.72	106.0	4.65, 4.71
C-29	18.3	1.04	17.5	0.96

similar to that of cycloeucalenol, as shown by the spectroscopic data, the second hydroxyl group could not be in the side chain. The diketone **4** obtained on oxidation showed no absorption for cyclopentanone in the ir spectrum; hence the hydroxyl group could not be in ring D. The diketone did not show characteristic uv absorption for a 1,2 or a 1,3-diketone, and hence the second carbonyl group could not be placed in ring A. The signal for H-18 in compound **1** appeared as a singlet at δ 0.94 in the ^{1}H-nmr spectrum. In 11-keto-9β,19-cycloandrostane, this proton resonates much upfield at δ 0.77 (5), which ruled out the possibility of an 11-keto group in the compound. Because the H-18 signal is similar to cycloartenol, the keto group could not be at position 12. The second hydroxyl group must therefore be in ring B. Because the methine proton of the second C*H*OH group was shown to be equatorial the hydroxyl must be either 6β or 7α. The 6β position was clearly shown by the ^{13}C-nmr spectrum as well as by the 2D-nmr studies. The downfield shift of one of the cyclopropane protons to δ 0.90 as compared with cycloartenol in which they appear at δ 0.33 and 0.56 (6) could only be explained if the hydroxyl is β oriented. This is also justified by the γ-shielding of C-8 and C-10 by 5.9 and 2.2 ppm and the β-deshielding of C-5 and C-7 by 3.9 and 4.4 ppm as compared with pollinastanol ace-

tate (3β-acetoxy-14α-methyl-9β,19-cyclo-5α-cholestane) (4). The orientation of the hydroxyl group at C-6 caused C-19 to move downfield to δ 30.2 as compared to δ 25.7 for pollinastanol acetate. This is similar to going from androstane to 6β-androstanol (7) where C-19 methyl moves downfield by 3.5 ppm.

Roxburghiadiol B [**2**], mp 168–170°, $[\alpha]D$ +56° ($c=2$, $CHCl_3$) analyzed for $C_{29}H_{48}O_2$. It showed the same functional groups as in compound **1** and it also formed a diacetate **5**. The previous structure assigned for roxburghiadiol B [**2**] was confirmed by the ^{13}C-nmr and 2D-nmr studies. Compound **2** showed the H-19 signals at δ 0.14 and 0.30. This is in agreement with the values reported for 24-dehydropollinastanol and 24-methylenepollinastanol (8). H-3 and H-6 appeared as broad multiplets of $W½=25$ Hz at δ 3.70 and 3.10, respectively, thereby showing that both are axial and two diaxial interactions are involved in each case. As expected, the 6α-OH in **2** caused more β-deshielding of C-5 and C-7 when compared with the 6β-OH in roxburghiadiol A (Table 1). Compared with pollinastanol acetate, the introduction of a 6α-OH as in **2** made C-19 move downfield by 2.00 ppm. Furthermore in both compounds **1** and **2** the shift of C-9 is virtually unaffected, showing that both must be epimeric at C-6. Compelling evidence for the assigned structures **1** and **2** was obtained from the isolation of the same diketone **4** on Sarett oxidation of both compounds.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. Optical rotations were recorded in $CHCl_3$ and ir spectra in $CHCl_3$ or KBr disc. ^1H-nmr spectra were recorded at 300 MHz and ^{13}C-nmr at 75.5 MHz in $CDCl_3$ with TMS as the internal standard. Eims were obtained at 70 eV using a direct inlet system.

PLANT MATERIAL.—The plant *A. roxburghiana* (fruits and leaves) was collected at Tirupathi Hills in Andhra Pradesh at an altitude of about 900 m during September. It was identified by Mrs. P. Brindha, the botanist of our Institute. A voucher specimen has been deposited in the herbarium of Captain Srinivasa Murti Drug Research Institute for Ayurveda, Madras 106.

EXTRACTION AND FRACTIONATION.—The shade-dried and coarsely powdered plant material (2 kg) was extracted in the cold with *n*-hexane and $CHCl_3$ (48 h). The hexane extract was chromatographed over Si gel. Elution with *n*-hexane–C_6H_6 (1:1) gave cycloartenol (420 mg), mp 99°, $[\alpha]D$ +54° ($c=2$, $CHCl_3$), $[M]^+$ 426. Further elution with C_6H_6 gave β-sitosterol, mp 134°.

ROXBURGHIADIOL A [**1**].—Elution of the column with $CHCl_3$ gave a gum which on repeated crystallization from Et_2O/hexane afforded compound **1** (160 mg), 173° mp $[\alpha]D$ +51° ($c=2$, $CHCl_3$), $C_{29}H_{48}O_2$ $[M]^+$ 428. *Anal.* found C 81.29, H 11.25; calcd C 81.32, H 11.21. Ir ν max (KBr) 3325, 3030, 2950, 2925, 2853, 1635, 1470, 1390, 1300, 1100, 1050, 983, 970, 887 cm^{-1}; ^1H nmr δ 0.20, 0.90 (1H each, d, $J=3.5$ Hz, H-19), 0.90 (3H, d, $J=6.6$ Hz, H-21), 0.94 (3H, s, H-18), 1.04 (3H, s, H-32), 1.02, 1.03 (3H each, d, $J=6.8$ Hz, H-26 and H-27), 3.75 (1H, m, $W½=25$ Hz, H-3), 3.90 (1H, m, $W½=9$ Hz, H-6), 4.66, 4.72 (1H each, d, $J=1.5$ Hz, $>=CH_2$); ^{13}C-nmr see Table 1; eims *m/z* (rel. int. %) $[M]^+$ 428 (2.8), $[M-Me]^+$ 413 (2.1), $[M-H_2O]^+$ 410 (7.6), 409 (20.7), $[M-Me-H_2O]^+$ 395 (6.2), 394 (13.8), $[M-Me-2H_2O]^+$ 377 (6.2), 327 (6.2), $[M-\text{side chain}-H_2O]^+$ 285 (6.9), $[M-\text{side chain}-2H_2O]^+$ 267 (6.9), 225 (6.8), 211 (6.9), 197 (10.3), 191 (17.2), 173 (22.1), 149 (20.0), 147 (20.7), 143 (20.7), 133 (24.1), 123 (26.2), 121 (26.2), 119 (27.6), 109 (34.5), 107 (31.0), 105 (35.9), 97 (38.0), 95 (76.0), 94 (55.2), 91 (47.0), 85 (27.6), 83 (48.3), 81 (62.1), 79 (41.4), 77 (27.6), 71 (48.3), 67 (50.3), 57 (100), 55 (96.5).

ACETYLATION OF **1**.—Compound **1** (50 mg) was dissolved in pyridine (1 ml) and Ac_2O (1 ml). The mixture was kept overnight, and usual workup gave the diacetate **3** as an amorphous compound: ^1H-nmr δ 0.26, 0.91 (1H each, d, $J=4$ Hz, H-19), 0.86 (3H, d, $J=7.0$ Hz, H-21), 0.93 (3H, s, H-32), 1.01–1.04 (9H, H-18, H-26, and H-27), 4.70, 4.75 (1H each, d, $J=2$ Hz, $>=CH_2$), 4.85 (1H, m, $W½=25$ Hz, H-3), 5.05 (1H, m, $W½=9$ Hz, H-6); ^{13}C nmr δ 29.64 (C-1), 29.05 (C-2), 71.83 (C-3 or C-6), 35.54 (C-4), 39.73 (C-5), 73.46 (C-6 or C-3), 29.34 (C-7), 41.11 (C-8), 22.77 (C-9), 27.77 (C-10), 26.39 (C-11), 34.20 (C-12), 45.59 (C-13), 48.3 (C-14), 32.67 (C-15), 28.05 (C-16), 52.23 (C-17), 19.31 (C-18), 31.20 (C-19), 36.04 (C-20), 18.29 (C-21), 34.90 (C-22), 31.20 (C-23), 156.82 (C-24), 33.76 (C-25),

21.90 (C-26), 21.84 (C-27), 105.98 (C-28), 18.09 (C-32), 170.53 (2×OCOMe), 21.16, 21.38 (2×$OCOCH_3$).

OXIDATION OF **1**.—Compound **1** (30 mg) was dissolved in 1.0 ml of pyridine and added to 100 mg of CrO_3 in 3 ml of pyridine at ice-cold temperature. It was left overnight, and usual workup gave the diketone **4**: mp 110° $[M]^+$ 424 ($C_{29}H_{44}O_2$); ir ν max (KBr) 2925, 2850, 1725, 1705, 1455, 1220, 1120, 1050, 900, 883 cm^{-1}; ^1H nmr δ 0.26, 0.62 (1H each, d, J = 4 Hz, H-19), 0.87 (3H, s, H-32), 0.92 (3H, d, J = 6.5 Hz, H-21), 0.97 (3H, s, H-18), 1.02, 1.03 (3H each, d, J = 6.5 Hz, H-26 and H-27), 2.80 (2H, dd, J = 9.5, 5.5 Hz, H-5), 4.70, 4.75 (1H each, d, J = 2.0 Hz, >=CH_2); ^{13}C nmr δ 30.3 (C-1), 40.1 (C-2), 210.9 (C-3 or C-6), 38.1 (C-4), 49.6 (C-5), 210.7 (C-6 or C-3), 41.3 (C-7), 40.1 (C-8), 25.4 (C-9), 30.0 (C-10), 27.2 (C-11), 33.4 (C-12), 45.7 (C-13), 49.6 (C-14), 32.7 (C-15), 27.5 (C-16), 51.2 (C-17), 17.8 (C-18), 29.7 (C-19), 36.1 (C-20), 18.5 (C-21), 34.9 (C-22), 31.2 (C-23), 156.6 (C-24), 33.7 (C-25), 22.0 (C-26), 21.8 (C-27), 106.1 (C-28), 14.5 (C-32).

ROXBURGHIADIOL B [**2**].—The $CHCl_3$ extract was chromatographed over Si gel. The solid from EtOAc eluates on crystallization from aqueous EtOH gave compound **2** (150 mg), mp 168–170°, $[\alpha]_D$ +56° (c = 2, $CHCl_3$), $[M]^+$ 428 ($C_{29}H_{48}O_2$). *Anal.* found C 81.35, H 11.18; calcd C 81.32, H 11.21. Ir max (KBr) 3250, 3030, 2950, 2900, 2850, 1630, 1470, 1375, 1280, 1115, 1050, 975, 887 cm^{-1}; ^1H nmr δ 0.14, 0.30 (1H each, d, J = 4 Hz, H-19), 0.90 (3H, d, J = 6.6 Hz, H-21), 0.93 (3H, s, H-18), 0.96 (3H, s, H-32), 1.02, 1.03 (3H each, d, J = 6.8 Hz, H-26 and H-27), 3.10 (1H, m, W½ = 25 Hz, H-6), 3.70 (1H, m, W½ = 25 Hz, H-3), 4.65, 4.71 (1H each, d, J = 2 Hz, >=CH_2); ^{13}C nmr see Table 1; eims *m/z* (rel. int. %) $[M]^+$ 428 (1.2), $[M-Me]^+$ 413 (1.7), $[M-H_2O]^+$ 410 (13.1), $[M-Me-H_2O]^+$ 395 (7.6), $[M-Me-2H_2O]^+$ 377 (2.7), $[M-$side chain $-H_2O]^+$ 285 (4.0), $[M-$side chain $-2H_2O]^+$ 267 (3.2), 123 (19.4), 121 (26.0), 119 (21.9), 109 (27.8), 107 (34.5), 105 (32.2), 95 (100), 94 (23.2), 93 (55.2), 91 (37.0), 83 (23.5), 81 (52.3), 79 (47.1), 77 (17.2), 69 (60.2), 67 (42.7), 57 (14.1), 55 (89).

ACETYLATION OF **2**.—Compound **2** (50 mg) was acetylated by Ac_2O/pyridine similarly to compound **1**. The diacetate **5** was obtained as a gum: ^1H-nmr δ 0.22, 0.45 (1H each, d, J = 4.2 Hz, H-19), 0.87 (3H, d, J = 7.5 Hz, H-21), 0.91 (3H, s, H-32), 0.95 (3H, s, H-18), 1.01–1.03 (6H, H-26 and H-27), 4.35 (1H, m, W½ = 25 Hz, H-6), 4.70, 4.75 (1H each, d, J = 2.0 Hz, >=CH_2) 4.82 (1H, m, W½ = 25 Hz, H-3); ^{13}C nmr δ 30.44 (C-1), 29.64 (C-2), 73.94 (C-3 or C-6), 31.48 (C-4), 41.74 (C-5), 72.86 (C-6 or C-3), 30.81 (C-7), 46.14 (C-8), 23.16 (C-9), 31.60 (C-10), 26.46 (C-11), 33.54 (C-12), 45.30 (C-13), 48.76 (C-14), 32.46 (C-15), 27.89 (C-16), 52.09 (C-17), 18.96 (C-18), 26.94 (C-19), 36.05 (C-20), 18.31 (C-21), 34.92 (C-22), 31.24 (C-23), 156.75 (C-24), 33.75 (C-25), 21.96 (C-26), 21.83 (C-27), 105.96 (C-28), 17.45 (C-32), 170.56, 170.98 (2×OCOMe), 21.29, 21.38 (2×$OCOCH_3$).

OXIDATION OF **2**.—Compound **2** (50 mg) was oxidized by the Sarett method similarly to compound **1**. The oxidation product was found to be identical to compound **3** (mmp, co-tlc, and superimposable ir).

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