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THE STRUCTURE AND STEREOCHEMISTRY OF BARRIGENIC ACID, A NEW TRITERPENE ACID SAPOGENIN FROM *BARRINGTONIA ACUTANGULA*

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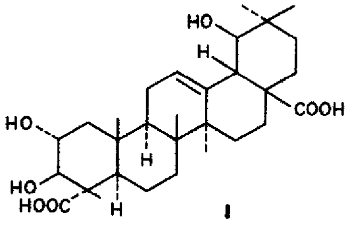
Key Word Index—*Barringtonia acutangula*; Barringtoniaceae; new triterpene acid; barrigenic acid; 2α,3β,19β-trihydroxyolean-12-en-23,28-dioic acid.

Abstract—A new triterpene acid, barrigenic acid, was isolated from the fruits of *Barringtonia acutangula*. Its structure was established as 2α,3β,19β-trihydroxyolean-12-en-23,28-dioic acid.

From the branch wood of *Barringtonia acutangula* the isolation of a new triterpene acid sapogenin, called barrinic acid, was reported previously from this laboratory [1]. This paper reports the isolation of its 19β-isomer, barrigenic acid (1), from the fruits of the plant.



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Barrigenic acid, $C_{30}H_{46}O_7$, mp 286–90° (dec), $[\alpha]_D$ +80.2° (Py) furnished a dimethyl ester, $C_{32}H_{50}O_7$, mp, 252–54°, $[\alpha]_D^{33°}$ +75° ($CHCl_3$), with ethereal diazomethane, which gave a pale yellow colouration with tetranitromethane (UV 205 nm (log ε 3.43), trisubstituted double bond; IR, ν_{max} cm^{-1}: 3420 (–OH), 1730 and 1700 (–COOMe). Dimethyl barrigenate treated with Ac_2O–pyridine at 100° gave an amorphous triacetate, $C_{38}H_{56}O_{10}$, mp, 145–50°, and a crystalline diacetate, $C_{36}H_{54}O_9$, mp 212–14°, $[\alpha]_D^{27°}$ +12.5° ($CHCl_3$), the latter being the major product. Dimethyl barrigenate consumed nearly 1 mole of periodic acid indicating an α-glycol system. Oxidation of the diacetate with CrO_3 in acetic acid furnished a ketone, $C_{36}H_{52}O_9$ (M^+ 628), mp 247–49°, $[\alpha]_D^{27°}$ +55.3° ($CHCl_3$) (UV showed this not to be an α:β-unsaturated ketone), which on treatment with ethanolic caustic potash gave a conjugated ketone, $C_{32}H_{48}O_7$, mp 192–94°, $[\alpha]_D^{27°}$ −169.3° ($CHCl_3$); UV 249 nm. This showed that the hindered OH group was at C-19 and hence the ketone diacetate was identical with that formed from diacetyl dimethyl barrinate. Thus the new compound was probably the 19β-isomer. This was confirmed by the following experiments. The diacetate, on treatment with $POCl_3$ in pyridine, gave an unresolvable mixture, which showed no absorption maxima (UV) for either a $\Delta^{11,13(18)}$-diene or a $\Delta^{12,18}$-diene system. The mixture was saponified with alcoholic KOH and the NMR spectrum of the product showed signals at δ 6.63 and 4.77 for exomethylene protons ($\rangle C{=}C\underline{H}_2$) and also another signal at δ 1.68 for a double bond attached methyl group ($\rangle C{=}\overset{|}{C}{-}C\underline{H}_3$) suggesting that dehydration led to the expected products formed by a Wagner–Meerwein type rearrangament [2–4].

The above result suggested that the 19–OH group in barrigenic acid is β. The MS of both dimethyl barrigenate and its diacetate showed no molecular ion peak at *m/e* 546 or 630 but a peak at *m/e* 528 or 612 due to the facile loss of water. Other peaks in the MS of both compounds showed loss of COOMe from C-4 and *m/e* 278 equivalent to the C/D ring system.

The NMR spectrum ($CDCl_3$, 60 MHz) of diacetyl dimethyl barrigenate showed six quarternary methyl groups ($\equiv C{-}C\underline{H}_3$) as sharp singlets at δ 0.75, 0.87, 0.91, 1.08, 1.17 and 1.27. The high field shift of one of the methyl signals to δ 0.75 showed the presence of a carbomethoxyl group at C-17. The two vicinal acetoxy groups ($-O{-}COC\underline{H}_3$) at C-2 and C-3 gave singlets at δ 1.98 and 2.07; the singlets at δ 3.63 (3H) and at 3.7 (3H), being due to the C-28 and C-23 $COOC\underline{H}_3$ groups respectively. The presence of a C-23 carbomethoxyl group caused a downfield shift of the signal for the 24-methyl group [5] to δ 1.27. The 3-axial (α) proton appeared as a doublet at δ 4.84 (*J* 10.5 Hz). The large coupling constant showed the *trans* (diaxial) relationship of this proton with the 2β-proton which appeared as a broad multiplet centred at δ 5.7.

The 19-axial (α) proton appeared as a doublet at δ 3.39 (*J* 11 Hz) by coupling with the 18β-H (2.58, *d*, *J* 11 Hz) which are in a *trans* diaxial relationship. The C-12 vinyl proton appeared as a triplet at δ 5.44 (*J* 3.5 Hz). Thus the NMR spectral data is in good agreement with

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the structure 1 for 2,3-diacetyl dimethyl barrigenate. The NMR spectrum of the triacetate was also in agreement.

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THE OCCURRENCE OF 24-METHYLENELANOST-8-EN-3β-OL IN THE SEEDS OF *BRASSICA NAPUS*

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Key Word Index—*Brassica napus*; Cruciferae; triterpene alcohol; 24-methylenelanost-8-en-3β-ol; seeds.

Plant and source. The seeds of *Brassica napus* were courteously supplied by Dr. A. Nishioka, Toyo Oil Manufacturing Co., Chiba, Japan. *Uses*. The seeds are widely used for the production of rape-seed oil. *Previous work on 4,4-dimethylsterols (triterpene alcohols)*. Cycloartanol [1], cycloartenol [1–4], 24-methylenecycloartanol [1–4], α-amyrin [2,3], β-amyrin [1–3], butyrospermol [3,4] and lupeol [3] in the commercially available rape-seed oil produced from the seeds of *Brassica* spp.

Present work. Extraction of the dried and ground seeds of *B. napus* (158 g) by CH_2Cl_2 using a Soxhlet apparatus gave a dark brown oil (64 g). The unsaponifiable fraction (1.22 g) extracted from the oil after saponification was fractionated by PLC on Si gel using hexane–Et_2O (4:1) as the developing solvent [1], to give the 4,4-dimethylsterol fraction (36 mg). On acetylation, the acetate fraction (33 mg) obtained was separated into two principal bands (R_f 0.39 and 0.24) by PLC on $AgNO_3$–Si gel (1:9) using CH_2Cl_2–CCl_4 (1:1) as the developing solvent. The acetate fraction (6 mg) recovered from the R_f 0.24 band gave two GLC peaks: The GC–MS for the faster eluted minor peak (20%) gave M^+ at *m/e* 482 ($C_{33}H_{54}O_2$) with fragment ions at *m/e* 467 (Table 1, ion a) and 407 (c) indicating that this compound was the acetate of a C_{31} triterpene alcohol. Ions at *m/e* 297 (d) and 301 (e) showed that the side chain had nine carbon atoms and one double bond. An ion at *m/e* 383 (g) indicated the presence of a 24-methylene group [5,6] in sterol devoid of the 9β,19-cyclopropane ring [6], and the ion e indicated the presence of a methyl group attached at C-14 [7]. The GLC and MS data of this triterpene acetate were identical with those measured for the acetate of 24-methylenelanost-8-en-3β-ol occurring in the acetylated 4,4-dimethylsterol fraction separated from an yeast, *Pichia* sp. [8]. The slower eluted major peak (80%) was identified as the acetate of 24-methylenecycloartanol by comparison of its GLC and MS data with those of the authentic specimen of 24-methylenecycloartanyl acetate [9]. The hydrogenated fraction (PtO_2 catalyst, Et_2O soln) afforded two constituents which had the GLC and MS data identical with those of the authentic specimens [9] of the acetates of 24-methyllanost-8-en-3β-ol and 24-methylcycloartanol, respectively.

Thus, 24-methylene-5α-lanost-8-en-3β-ol occurs as a

Table 1. GLC and MS data of triterpene acetates.

	GLC data RR_t (sitosterol = 1.00)			MS data (in GC–MS, 70 eV), *m/e* (>200) and relative intensities (%)							
	SE-30*	OV-17†	OV-225‡	M^+	a§	b	c	d	e	f	g
24-Methylenecycloartanyl acetate	1.77	1.67	1.45	482 (16)	467 (14)	422 (100)	407 (84)	297 (34)	301 (14)	300 (36)	—
24-Methylcycloartanyl acetate	1.82	1.62	1.37	484 (18)	469 (24)	424 (100)	409 (90)	297 (59)	—	302 (56)	—
24-Methylenelanost-8-en-3β-yl acetate	1.56	1.41	1.20	482 (39)	467 (100)	—	407 (89)	297 (6)	301 (28)	—	383 (11)
24-Methyllanost-8-en-3β-yl acetate	1.61	1.37	1.13	484 (20)	469 (100)	424 (7)	409 (98)	297 (7)	301 (7)	—	—

* 3% SE-30; 238°, N_2 50 ml/min. † 3% OV-17; 268°, N_2 50 ml/min. ‡ 10% OV-225; 260°, N_2 50 ml/min. § Major fragment ions: a, M^+–Me; b, M^+–AcOH; c, M^+–Me–AcOH; d, M^+–SC–AcOH; e, M^+–SC–56; f, M^+–ring A [6]; g, M^+–Me–84.

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