839. *Barringtogenol and Barringtogenic Acid, Two New Triterpenoid Sapogenins.*

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Two new triterpenoid sapogenins have been isolated from the fruits of *Barringtonia racemosa* Roxb. The evidence suggested that one, barringtogenol, was 2 : 3 : 23 : 28-tetrahydroxyolean-12-ene (I; R = CH_2·OH) and the other, barringtogenic acid, 2 : 3-dihydroxyolean-12-ene-23 : 28-dioic acid (I; R = CO_2H). Confirmation was obtained by identifying the product of the reduction of arjunolic acid [1] by lithium aluminium hydride with barringtogenol and with the product of similar reduction of barringtogenic acid.

THE ripe fruits of the tree *Barringtonia racemosa* Roxb. contain a large quantity of saponins, from which, after hydrolysis, two new triterpenoid sapogenins, barringtogenic acid and barringtogenol have been isolated.

Barringtogenic acid, $C_{30}H_{46}O_6$, m. p. 334°, readily yielded a dimethyl ester, diacetyl and dibenzoyl derivatives, and a dimethyl ester diacetate; this accounts for all the oxygen atoms. The presence of the carboxy group or an equivalent carbonyl function was also apparent from the band at 5·8—6·0 μ in the infrared absorption spectrum. The acid resisted hydrogenation over platinum oxide, but was unsaturated towards tetranitromethane. In reaction with perbenzoic acid, the equivalent of one double bond was consumed. Moreover, the rate of uptake of the per-acid was characteristic of members of the β-amyrin group possessing a hindered double bond; barringtogenic acid also failed to react with osmium tetroxide. These facts and the small molecular-rotation difference between the acid and its dimethyl ester (−27 units) [2] point to barringtogenic acid's being a member of the β-amyrin group.

Barton and Jones [2] have shown that, with those members of the α- and the β-amyrin series which possess a $C_{(23)}$- or $C_{(24)}$-carboxyl group, large decreases in molecular-rotation differences are observed between the acid and the 3-acetyl, 3-benzoyl, and 3-oxo-derivatives. Barringtogenic acid and its derivatives show this behaviour (cf. Table) and it can therefore

	$10^{-2}M[\alpha]$						
	Alcohol	Acetate 1	Benzoate 2	Ketone 3	$10^{-2}\Delta_1$	$10^{-2}\Delta_2$	$10^{-2}\Delta_3$
Barringtogenic acid	+361°	+264°	+142°	—	− 97°	−219°	—
Dimethyl barringtogenate	+334°	+199°	—	+202°	−135°	—	−132°

be reasonably concluded that one of the carboxyl groups is at $C_{(23)}$ or $C_{(24)}$. The position of the double bond with respect to the second carboxyl group was deduced from the ready formation of a bromo-lactone. Easy lactonisation is characteristic of carboxylic acids of the β-amyrin series having a double bond γδ to the carboxyl group,[3] and it was accordingly inferred that barringtogenic acid is unsaturated at the 12 : 13-position as in oleanolic acid and also that the second carboxyl group is at $C_{(28)}$. Confirmatory evidence was secured as barringtogenic acid readily yielded a lactone triacetate under conditions devised by Winterstein and Wiegand.[4]

The position of the other hydroxyl group was indicated by the quantitative oxidation of the acid with lead tetra-acetate, the disappearance of 1 mol. of the reagent establishing the existence of an αβ-diol. Further evidence that the two hydroxyl groups are secondary came from chromic acid oxidation of barringtogenic acid; although the product was non-crystalline, it could be converted into a crystalline dimethyl dioxo-ester which was characterised as the dioxime.

[1] King, King, and Ross, *J.*, 1954, 3995.
[2] Barton and Jones, *J.*, 1944, 659.
[3] See *e.g.*, Jeger, *Fortschr. Chem. Org. Naturstoffe*, 1950, **7**.
[4] Winterstein and Wiegand, *Z. physiol. Chem.*, 1931, 199, 46.

Since further development of the structure depends on the reactions of its companion triterpene barringtogenol these will be considered now.

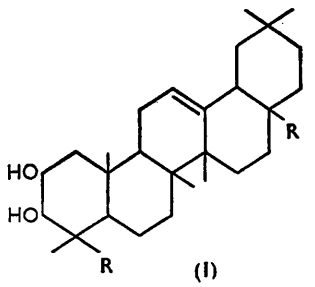
Barringtogenol, $C_{30}H_{50}O_4$, readily yielded tetra-acetyl and tetrabenzoyl derivatives, which could not be hydrolysed with alcoholic potash under ordinary conditions. The triterpene gave a yellow colour with tetranitromethane but resisted hydrogenation with Adams's catalyst. That it contained one double bond was evident from its oxidation with perbenzoic acid.

The molecular-rotation difference between barringtogenol and its tetra-acetate (+12 units) was characteristic for the β-amyrin group of triterpenes, but that between it and the tetrabenzoate (+4 units) was anomalous. Reaction of barringtogenol tetra-acetate with selenium dioxide also suggested that the triterpene belonged to the β-amyrin group, the crystalline diene tetra-acetate having characteristic ultraviolet absorption ($\lambda_{max.}$ 250 and 162 mμ).[5,6]

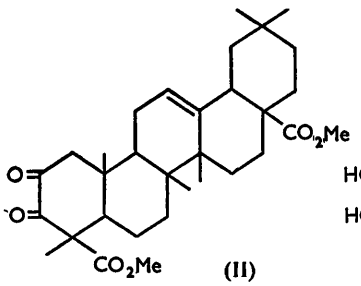
Like barringtogenic acid, barringtogenol is considered to contain an αβ-diol group, from its reaction with periodic acid and with lead tetra-acetate and by formation of an *iso*propylidene derivative. That this group is a 3 : 23-diol was shown by heating barringtogenol with a copper catalyst at 270—290°, small but consistent amounts of formaldehyde being obtained. This behaviour is generally considered typical of the 3 : 23-diol system in triterpenes.[1,7]

The nature of the hydroxyl groups was shown by chromic acid oxidation of barringtogenol. Although the acidic product obtained did not crystallise, it gave a crystalline dimethyl ester which formed a dioxime. Thus, of the four hydroxyl groups two are primary and two secondary.

The available evidence therefore indicated a close structural relation between barringtogenol and barringtogenic acid and so an attempt was made to interconvert the two compounds. Dimethyl barringtogenate (I; R = CO_2Me) was reduced with lithium aluminium hydride giving barringtogenol (analysis, etc., and mixed m. p.s of the product and its tetra-acetate). Further, the dimethyl esters (II) obtained by methylation of the acidic products of the oxidation of the two genins with chromic acid were identical.



(I)



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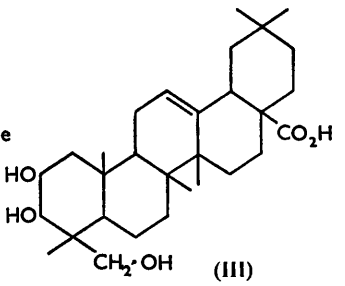

(III)

It is concluded, therefore, that barringtogenol and barringtogenic acid have the structures 2 : 3 : 23 : 28-tetrahydroxyolean-12-ene (I; R = CH_2·OH) and 2 : 3-dihydroxyolean-12-ene-23 : 28-dioic acid (I; R = CO_2H). If these structures are correct, then it is apparent that arjunolic acid,[1] 2 : 3 : 23-trihydroxyolean-12-en-28-oic acid (III), should give a lithium aluminium hydride reduction product identical with barringtogenol and with the lithium aluminium hydride reduction product of dimethyl barringtogenate. Professor F. E. King, F.R.S., kindly provided a sample of the reduction product of arjunolic acid, and this gave no depression in m. p. on admixture with either barringtogenol or with the lithium aluminium hydride reduction product of dimethyl barringtogenate.

Experimental

Rotations were measured in a 2-dm. tube at room temperature, and ultraviolet absorption measurements with a Uvispek spectrophotometer. For analysis, samples were dried at 110°

[5] Ruzicka and Jeger, *Helv. Chim. Acta*, 1942, **25**, 775.
[6] Barton and Holness, *J.*, 1952, 78.
[7] Tsuda and Kitagawa, *Ber.*, 1938, **71**, 1604.

for 2 hr. in a vacuum unless otherwise stated. For chromatography, Merck's alumina, standardised according to Brockmann, was used. Light petroleum signifies material of b. p. 60—80°.

Isolation and Hydrolysis of the Saponins.—Dried ripe fruits were coarsely powdered (after removal of the husk and testa) (4·5 kg.) and extracted successively with 95% and 70% ethanol. The combined extract was evaporated and the residue dissolved in 90% ethanol and reprecipitated by the addition of an equal volume of ether. By repeating the dissolution and precipitation twice, a fairly pure product was obtained, which was completely soluble in water. The solution effected a 100% mortality of fish (*Lebistis reticulata*) in 12 min. (1 in 6000). The purified saponins (600 g.) and 50% ethanol containing 7% sulphuric acid were refluxed for 36 hr. The yield of sapogenin was 195 g.

Isolation of Barringtogenic Acid.—The sapogenin mixture (50 g. lots) was extracted (Soxhlet) successively with carbon tetrachloride and carbon tetrachloride–benzene (1 : 1) for 60 hr. each. The first extract gave a brown (14 g.) and the second a dark brown powder (15·5 g.). These were separately treated (3 times) with 10% aqueous sodium carbonate (500 c.c.) on a water-bath for ½ hr., and filtered. The combined filtrate from each fraction was acidified with dilute hydrochloric acid. From the first extract, only a trace of the acid genin was obtained, while the second gave 12 g. This was readily soluble in ethanol, methanol, and dioxan, but the solutions could not be decolorised without serious loss of material. However, when an alcoholic solution was diluted to turbidity with water and the alcohol partially removed, a precipitate was formed which was appreciably paler. By repeating these operations, a nearly colourless product was obtained, which, after recrystallisation from methanol, gave thick prisms of *barringtogenic acid* (2 g.), m. p. 332—334° (decomp.), $[\alpha]_D$ +72° (*c*, 1·12 in MeOH). The pure genin crystallised from pyridine as small needles. A further 1 g. of the pure material was obtained by chromatography of the residues from the mother-liquors in methanolic solution on purified activated animal charcoal. 12·5 g. of the barringtogenic acid were obtained from 195 g. of crude sapogenin mixture.

Isolation of Barringtogenol.—The residues from the solvent extractions of the mixture of sapogenins were combined after removal of the acid genin, well washed, and dried (yield, 65 g.). The yellowish-brown powder (16 g.) was refluxed with 80% ethanol (300 c.c.) for 4 hr. On cooling, the non-acid genin separated as a gel. This was filtered off and again refluxed with 80% ethanol. The white amorphous powder (10·6 g.) was chromatographed in benzene solution (250 c.c.) on alumina (300 g.); on elution with light petroleum and light petroleum–benzene, *barringtogenol* was obtained. After two crystallisations from acetone and drying in a vacuum this formed needles (5·3 g.), m. p. 290—291°, $[\alpha]_D$ +18° (*c*, 1·22 in pyridine). In all 23 g. of barringtogenol were obtained from the sapogen mixture.

Barringtogenic Acid.—The acid [Found, on sample dried at 180°: C, 71·4, 71·6; H, 9·4, 9·3%; *M* (2 carboxyl groups being assumed), 500, 506 (by titration with alkali); 502, 502 (by analysis of the silver salt). $C_{30}H_{46}O_6$ requires C, 71·7; H, 9·2%; *M*, 502] showed the usual solubility of a triterpene acid. Its solution in sulphuric acid was yellow, becoming orange-brown on storage; Salkowsky reaction, acid layer reddish green; Liebermann–Burchardt reaction, pink, immediately becoming red-brown. Addition of tetranitromethane to a solution of the acid caused yellow coloration. The number of double bonds was estimated by reaction of the acid with perbenzoic acid. The equivalent of 1 double bond was consumed in 120 hr., and the reaction then ceased.

The *diacetate*, prepared by using acetic anhydride and pyridine, formed needles (85%) (from acetone), m. p. 334—336° (decomp.), $[\alpha]_D$ +45° (*c*, 0·86 in MeOH) [Found, on sample dried at 180°: C, 69·8, 69·8; H, 8·4, 8·3%; *M*, 584 (by titration with alkali). $C_{34}H_{50}O_8$ requires C, 69·6; H, 8·6%; *M*, 586]. The *dibenzoate*, obtained from the acid with pyridine and benzoyl chloride, was crystallised successively from acetone–methanol and pyridine, giving clusters of needles (yield, 70%), m. p. 343—344°, $[\alpha]_D$ +20° (*c*, 1·53 in MeOH) [Found, on sample dried at 180°: C, 74·6, 74·5; H, 7·5, 7·5%; *M*, 713 (by titration). $C_{44}H_{54}O_8$ requires C, 74·3; H, 7·7%; *M*, 710]. *Dimethyl barringtogenate* was obtained as silky needles, m. p. 253—254°, $[\alpha]_D$ +63° (*c*, 1·12 in MeOH) (Found, on sample dried at 150°: C, 72·0, 72·2; H, 9·5, 9·5. $C_{32}H_{50}O_6$ requires C, 72·4; H, 9·5%), by treatment of the acid with ethereal diazomethane or dimethyl sulphate. It was not hydrolysed by boiling 10% ethanolic potassium hydroxide during 4 hr. The *ester diacetate*, prepared in the usual way was crystallised from ethanol, and then from acetone–methanol, and formed thick, stout needles (yield, 80%), m. p. 239—240°, $[\alpha]_D$ +32° (*c*, 0·69 in MeOH) (Found, for sample dried at 150°: C, 70·7, 70·5; H, 8·6, 8·6. $C_{36}H_{54}O_8$ requires C, 70·3; H, 8·85%).

Dimethyl didehydrobarringtogenate. To a cold solution of barringtogenic acid (2·3 g.) in glacial acetic acid (40 c.c.) was added dropwise a solution of chromic anhydride (1·5 g.) in acetic acid–water (15 c.c.); the mixture was then set aside at room temperature for 20 hr. The excess of chromic acid was destroyed by the addition of a little methanol and the product extracted with aqueous sodium carbonate. The extract was filtered and then acidified. Since the product could not be crystallised it was esterified; the *ester* (1 g.) formed fine needles (from ethyl acetate), m. p. 218—220°, $[\alpha]_D$ +38° (*c*, 0·81 in MeOH) (Found: C, 73·1; H, 8·9. $C_{32}H_{46}O_6$ requires C, 72·95; H, 8·8%). Its *dioxime* was prepared by refluxing the ester (300 mg.) and hydroxylamine hydrochloride (500 mg.) in pyridine–methanol for 6 hr. The slender needles (from acetone) (200 mg.) had m. p. 288—290°, $[\alpha]_D$ +46° (*c*, 1·02 in MeOH) (Found: C, 69·3; H, 8·7; N, 5·0. $C_{32}H_{48}O_6N_2$ requires C, 69·0; H, 8·8; N, 5·0%).

Lactones.—(*a*) Barringtogenic acid (500 mg.) in hydrobromic acid–acetic acid (50%; 7 c.c.) was set aside for 48 hr. The *diacetyl-lactone* was obtained as needles (from methanol–acetone), (300 mg.), m. p. 226° (decomp.), $[\alpha]_D$ +132° (*c*, 0·83 in MeOH) (Found: C, 69·85; H, 8·7. $C_{34}H_{50}O_8$ requires C, 69·6; H, 8·6%). The compound did not give a colour with tetranitromethane.

(*b*) A 5% solution of bromine in acetic acid (25 c.c.) was added dropwise to a solution of barringtogenic acid (1·5 g.) and sodium acetate (4 g.) in acetic acid (90%; 30 c.c.). After 1 hr. the mixture was poured into water containing sodium thiosulphate (3 g.), and the product crystallised from methanol and acetone–methanol. A *bromolactone* was obtained as needles (1·1 g.), m. p. 239—240° (decomp.), $[\alpha]_D$ +147° (*c*, 0·34 in MeOH) (Found: Br, 13·8. $C_{30}H_{45}O_6Br$ requires Br, 13·75%).

Attempted Oxidation of Dimethyl Di-O-acetylbarrintogenate with Osmium Tetroxide.—Dimethyl barringtogenate diacetate was recovered after 10 days' treatment with osmium tetroxide in pyridine.

Reduction of Dimethyl Barringtogenate with Lithium Aluminium Hydride: Barringtogenol.—A solution of lithium aluminium hydride (1 g.) in dry ether (100 c.c.) was added to one of dimethyl barringtogenate (500 mg.), and the mixture stirred for 2 hr. The product was worked up in the usual way and crystallised from pyridine, giving needles (320 mg.), m. p. 286—287°, $[\alpha]_D$ +17° (*c*, 1·3 in pyridine) (Found, on sample dried at 150°: C, 75·7; H, 10·5. $C_{30}H_{50}O_4$ requires C, 75·9; H, 10·6%). A mixture with natural barringtogenol of m. p. 290—291° had 288—289°; a mixture with the product, m. p. 286°, of the lithium aluminium hydride reduction of arjunolic acid had m. p. 288°. It readily formed a *tetra-acetate*, m. p. 269—270°, $[\alpha]_D$ +15° (*c*, 0·71 in pyridine) (Found, on sample dried at 150°: C, 70·8; H, 9·0. $C_{38}H_{58}O_8$ requires C, 71·0; H, 9·0%), which did not depress the m. p. of the tetra-acetate of natural barringtogenol.

Barringtogenol.—This triterpene (Found, on sample dried at 150°: C, 75·8, 75·8; H, 10·4, 10·4%) gave a cherry-red solution in sulphuric acid; Salkowsky reaction, acid layer deep orange; Liebermann–Burchardt reaction, pink, rapidly changing to violet, with strong fluorescence. It was unaffected by boiling 10% ethanolic potassium hydroxide. Reaction with perbenzoic acid (about 140 hr. were required for complete reaction) indicated the presence of one double bond. The number of 1 : 2-diol units was estimated with periodic acid in the usual way; the oxidation corresponded with 0·67, 0·97, and 0·99 unit after 3, 24, and 30 hr., respectively. A similar result was obtained by use of lead tetra-acetate.

The tetra-acetate (yield 70%), after chromatography and crystallisation from methanol–ether, formed small needles, m. p. 269—270°, $[\alpha]_D$ +15° (*c*, 2·06 in pyridine) (Found, on sample dried at 150°: C, 70·9, 70·9; H, 9·3, 9·1%). The *tetrabenzoate* after being chromatographed from benzene solution (alumina) and crystallised from methanol–ethyl acetate formed needles, m. p. 236—238°, $[\alpha]_D$ +10° (*c*, 0·86 in pyridine) (Found: C, 78·3; H, 7·4. $C_{58}H_{66}O_8$ requires C, 78·2; H, 7·5%). Treatment of barringtogenol (340 mg.) with a mixture of dry ether (200 c.c.), dry acetone (50 c.c.), and concentrated sulphuric acid (1 c.c.) gave the OO′-iso*propylidene derivative* (280 mg.), m. p. 150—154° (decomp.), $[\alpha]_D$ +20° (*c*, 1·2 in pyridine) (Found: C, 76·8; H, 10·5. $C_{33}H_{54}O_4$ requires C, 77·0; H, 10·6%).

Chromic Acid Oxidation of Barringtogenol: Dimethyl Didehydrobarringtogenate.—Barringtogenol (2·1 g.) was oxidised with chromic acid as described for barringtogenic acid. The acidic product was isolated as before, and purified by repeated precipitation from dilute methanol. The amorphous product (0·95 g.) (*M*, by titration with alkali, 492; *M*, by analysis of its silver salt, 496) was then esterified with diazomethane and the ester crystallised several times from ethyl acetate (charcoal). The dioxo-compound was obtained as fine needles (520 mg.), m. p. 219—220°, $[\alpha]_D$ +38° (*c*, 0·64 in pyridine) (Found: C, 72·9; H, 8·7%). It was identical with

that obtained from barringtogenic acid. Its dioxime, prepared as described before, had m. p. and mixed m. p. 287—289°, $[\alpha]_D$ +47° (*c*, 0·95 in pyridine) (Found : C, 69·3; H, 8·65%).

Pyrolysis of Barringtogenol.—Barringtogenol (1 g.) and finely divided copper (5 g.) were heated at 270° for 1 hr. The evolved gases were passed into a 2% solution of β-naphthol in 50% ethanol containing a few drops of concentrated hydrochloric acid. The solution gradually became turbid, and on gentle boiling, yielded a crystalline precipitate (63 mg.). This was recrystallised twice from dilute methanol and then melted at 187—188° (decomp.), undepressed by authentic di-2-naphthyloxymethane.

11 : 13(18)-*Dehydrobarringtogenol Tetra-acetate.*—Barringtogenol tetra-acetate (1·3 g.), selenium dioxide (2 g.), and acetic acid (20 c.c.) were heated under reflux for 6 hr. The product was worked up in the usual way and chromatographed in benzene on alumina. Elution with light petroleum and light petroleum–benzene gave 11 : 13(18)-*dehydrobarringtogenol tetra-acetate* as needles (from light petroleum–methanol) (250 mg.), m. p. 193—194°, $[\alpha]_D$ −60° (*c*, 1·8 in pyridine) (Found : C, 71·3; H, 8·7. $C_{38}H_{56}O_8$ requires C, 71·2; H, 8·8%). Light absorption in EtOH : max. 250, and 262 mμ; log ε 4·1 and 3·9.

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