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TRITERPENOIDS—XIX

THE CONSTITUTION OF BARRINGTOGENOL C—A NEW TRITERPENOID SAPOGENIN FROM *BARRINGTONIA ACUTANGULA* GAERTN

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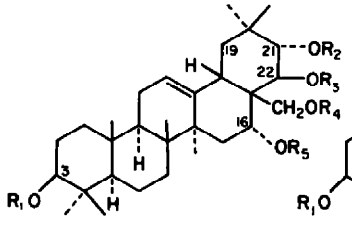
Abstract—The constitution of barringtogenol C—a new triterpenoid sapogenin isolated from the the fruits of *Barringtonia acutangula* Gaertn has been established as 3β, 16α, 21α, 22β, 28-pentahydroxyolean-12-ene.

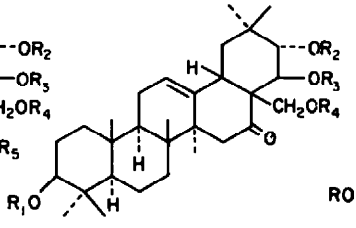
ISOLATION of a number of new sapogenins from the fruits of *Barringtonia actangula* Gaertn and the constitution of barringtogenol D, have previously been reported.[1,2] In the short communications[3,4] the constitution of another new sapogenol, barringtogenol C(Ia) was described and the present paper details the experiments leading to the structure of the latter compound.

Barringtogenol C (Ia) forms a tetra-acetate[5] (Ib) and a tetrabenzoate[5] (Ic). These compounds give the corresponding ketones (IIa and IIb) on oxidation with either CrO_3-pyridine complex or CrO_3 in acetic acid. All the oxygen functions in barringtogenol C are, therefore, hydroxyl groups, one of which is more hindered than the rest.



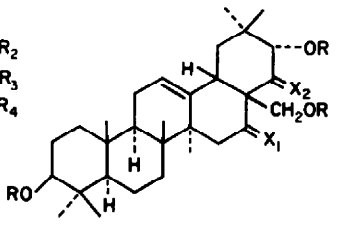
I

a, $R_1 = R_2 = R_3 = R_4 = R_5 = H$
b, $R_1 = R_2 = R_3 = R_4 = COCH_3$, $R_5 = H$
c, $R_1 = R_2 = R_3 = R_4 = COC_6H_5$, $R_5 = H$
d, $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = C(C_6H_5)_3$
e, $R_1 = R_2 = R_4 = COC_6H_5$, $R_3 = R_5 = H$



II

a, $R_1 = R_2 = R_3 = R_4 = COCH_3$
b, $R_1 = R_2 = R_3 = R_4 = COC_6H_5$



III

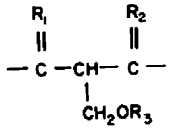
a, $R = COC_6H_5$, $X_1 = X_2 = O$
b, $R = COC_6H_5$, $X_1 = O$, $X_2 =$ 

[1] A. K. Barua, P. C. Maiti and S. K. Chakraborti, *J. Pharm. Sci.* **50**, 937 (1961).
[2] S. K. Chakraborti and A. K. Barua, *Tetrahedron* **19**, 1727 (1963).
[3] A. K. Barua, S. K. Chakraborti, P. Chakrabarti and P. C. Maiti, *J. Ind. Chem. Soc.* **40**, 483 (1963).
[4] A. K. Barua and P. Chakrabarti, *Sci. and Cult.* **30**, 332 (1964).
[5] Referred to as the penta-acetate and pentabenzoate in our earlier communications.[1,3,4] We are grateful to Dr. R. Tschesche, Bonn University, West Germany, for kindly pointing out to us that the penta-acetate is actually a tetra-acetate from a study of its IR spectrum.

The normal Δ^{12}-oleanane skeleton is attributed to barringtogenol C as the selenium dioxide oxidation of Ib leads to a hetero-annular diene showing triple UV absorption maxima (243, 252 and 261 mμ) characteristic of $\Delta^{11:12, 13:18}$-dienes of the β-amyrin series.[6,7] Barringtogenol C forms a monotrityl ether (Id) indicating the presence of one primary hydroxyl group. Consumption of one mole of periodic acid shows the presence of an α-glycol system. Elimination of formaldehyde by pyrolysis of barringtogenol C with copper-bronze at 350° indicates the presence of a formaldehydogenic group (—CHOH—$\ddot{C}$—CH_2OH).[3] Oxidation of barringtogenol C with CrO_3 in acetic acid furnishes acidic and neutral products neither of which could be obtained in the pure state. The former gives a positive ferric chloride colour test whereas the latter responds to the above test only after alkali treatment. The neutral product gives a positive Zimmermann colour reaction for a 3-keto group[8] thus showing the presence of one secondary hydroxyl function at the C-3 position in barringtogenol C. Partial benzoylation of barringtogenol C furnishes a tribenzoate (Ie) which does not consume any periodate, hence at least one of the hydroxyl groups of the α-glycol moiety is blocked by a benzoyl group (*loc. cit.*). The preparation of the tribenzoate is the crucial factor in the structure elucidation of barringtogenol C. The tribenzoate on oxidation with CrO_3 in acetic acid at room temperature furnishes a colourless neutral diketone (IIIa) which does not give an alcoholic ferric chloride test and its spectral properties require the absence of an enolizable α or β-diketo system. On mild treatment with alkali, the diketone yields formaldehyde and a yellow amorphous product. Elimination of formaldehyde is obviously due to a retro-aldol type reaction[8] necessitating the presence of a system —CO—$\ddot{C}$—CH_2OR ($R = COC_6H_5$) in the diketotribenzoate (IIIa). The amorphous product obtained above could not be crystallized. It gives an intense violet colour with alcoholic ferric chloride and shows absorption maxima at 274 and 244 mμ. The latter band remains unaffected in the presence of alkali while the former (274 mμ) shifts to 310 mμ in accordance with an enolizable β-diketone. It forms a highly crystalline enolacetate, showing characteristic absorption at 243 mμ ($\log \varepsilon$, 3·91). The product obtained by alkali treatment of the diketotribenzoate is most probably a mixture of two compounds, one showing absorption at 274 mμ and the other at 244 mμ. On heating with methanolic caustic potash (5%) the former is most probably converted into the latter as the final product shows a single absorption at 244 mμ and still gives a colour with alcoholic ferric chloride. The nature of the latter compound is not clear and calls for further investigation. The formation of an enolizable β-diketone necessitates the presence of a system (IVa) in the diketotribenzoate and hence the tribenzoate and barringtogenol C should have the moiety IVb and IVc respectively.

A system such as IVc can be incorporated in an intact oleanane skeleton only in ring D and E and hence two possible structures (V) 3, 19, 21, 29 (30), x-pentahydroxyolean-12-ene and (VI) 3, 16, 22, 28, x-pentahydroxyolean-12-ene may be proposed for barringtogenol C. The former structure (V) is rejected on the following grounds. The C-19 hydroxyl group (α or β)[9,10] is known to be one of the most hindered hydroxyl

[6] L. Ruzicka, G. Muller and H. Schellenberg, *Helv. Chim. Acta* **22**, 767 (1939).
[7] D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.* 257 (1951).
[8] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.* 887 (1954).
[9] J. Simonsen and W. C. J. Ross, *The Terpenes* Vol. 5; p. 286. Cambridge University Press (1957).
[10] L. R. Row and G. S. R. S. Rao, *Tetrahedron Letters* No. 27, 12 (1960).

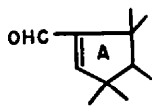


IV

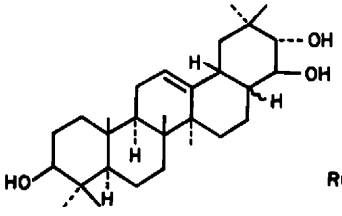
a, $R_1 = R_2 = O$; $R_3 = COC_6H_5$

b, $R_1 = R_2 =$  ; $R_3 = COC_6H_5$

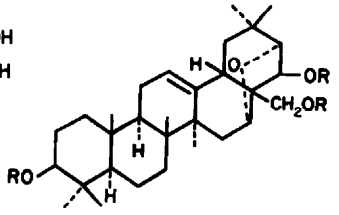
c, $R_1 = R_2 =$  ; $R_3 = H$



X



XI



XII

a, R = H

b, R = $COCH_3$

groups in the oleanene series and cannot be esterified with acetic anhydride and pyridine. If the hindered hydroxyl group in barringtogenol C is located at C-19 position, then the compounds (IIa and IIb), described before would be β,γ-unsaturated ketones which should readily isomerize with alkali to α,β-unsaturated ketones[11,12] but their UV-spectra in alkaline solution show no selective absorption for such a chromophore.

In the partial structure (VI) four of the five hydroxyl groups have been accommodated. The position of the fifth hydroxyl group, which being a part of the α-glycol moiety, can only be placed at C-2, C-15 or C-21 positions and hence three different structures (VII) 2,3,16,22,28-pentahydroxyolean-12-ene, (VIII) 3,15,16,22,28-pentahydroxyolean-12-ene and (IX) 3,16,21,22,28-pentahydroxyolean-12-ene, must be considered for barringtogenol C. As the neutral product obtained by oxidation of barringtogenol C gives a positive Zimmermann colour reaction for a 3-keto group, there must be a free methylene group at C-2 and this is contrary to the structure VII. Further, the position C-2 is excluded as the lead tetraacetate oxidation product of barringtogenol C can not be cyclized to give an α,β-unsaturated aldehyde[13] (X). It has already been mentioned that one of the hydroxyl groups of the α-glycol moiety has been blocked in the preparation of the tribenzoate (Ie). If the structure VIII is correct then in the tribenzoate formation the 16-hydroxyl group must be blocked as the 15-hydroxyl group (α or β)[14] is known to be highly hindered. But the formation of

[11] P. Bilham, G. A. R. Kon and W. C. J. Ross, *J. Chem. Soc.* 540 (1942).

[12] L. Ruzicka, A. Grob, R. Egli and O. Jeger, *Helv. Chim. Acta* **26**, 1218 (1943).

[13] R. B. Woodward, F. Sondheimer, T. David, K. Heusler and W. M. Mclamore, *J. Amer. Chem. Soc.* **74**, 4223 (1952).

[14] C. Djerassi, C. H. Robinson and D. B. Thomas, *J. Amer. Chem. Soc.* **78**, 5687 (1956).

the non-enolizable β-diketone (IIIa) requires both the hydroxyl groups at C-16 and C-22 to be free and hence the structure VIII for barringtogenol C is untenable. Thus IX is the only structure which explains all the reactions of barringtogenol C. The formation of the tribenzoate (Ie) requires 16 axial (α) and 22 axial (β) orientation of the two free hydroxyl groups as the 16β and 22α-hydroxyl functions are easily esterified (cf. chichipegenin[15]). The tribenzoate (Ie) on oxidation with CrO_3–pyridine complex furnishes a monoketone. Molecular rotational data indicates this to be a 16-keto compound (IIIb) which on Wolff-Kishner reduction furnishes a nor-compound. The nor-compound (XI) consumes one mole of periodate thus showing the α-glycol group to be still intact. Obviously, XI is formed by the reduction of the 16-keto group preceded by retro-aldolization.[16,17] Wolff-Kishner reduction of the monoketotetrabenzoate (IIb) also furnishes the above nor-compound (XI) thus establishing the validity of the structures (Ib, Ic, IIa and IIb) assigned to these compounds.

The α-glycol group in barringtogenol C was shown to be *trans* by the slow rate of lead tetra-acetate oxidation. Barringtogenol C forms a mono-acetonide which is obtained in the pure state only as its acetate. The acetonide formation obviously involves the 22β, 28-diol system, the α-glycol group being *trans* and the acetonide formation between 16α, 28-diol being impossible. The 22α, 28-diol does not form an acetonide (cf. chichipegenin[15]). *trans* Orientation of the α-glycol group in barringtogenol C requires the C-21 hydroxyl group to be α as the C-22 hydroxyl group is β. Assuming normal equatorial (β) orientation of the C-3 hydroxyl group, the stereochemistry of barringtogenol C may be represented as 3β, 16α, 21α, 22β, 28-pentahydroxyolean-12-ene (Ia).

Only after elucidation of the structure (Ia) did the striking similarity in the constitution of barringtogenol C and barringtogenol D become evident. The structure of the latter was previously shown to be XIIa[2]. Formation of barringtogenol D from barringtogenol C by dehydration appears quite probable. In fact, on refluxing pure barringtogenol C with ethanolic hydrochloric acid (8%), barringtogenol D is obtained in 42% yield. This suggests that barringtogenol D is an artifact derived from barringtogenol C by dehydration during acid hydrolysis of the saponin. It is formed only in poor yield (0·05%) during the isolation process most probably because aqueous ethanol (60%) is used for the hydrolysis of the saponin. The above interconversion finally establishes the structure and stereochemistry of barringtogenol C as Ia.

EXPERIMENTAL

The m.ps are uncorrected and recorded in a bisulphate bath. Petroleum ether used throughout had b.p. 60–80°. Brockmann's alumina (E. Merck) was used for chromatography and acid-washed alumina refers to Brockmann's alumina deactivated with 5% of 10% acetic acid. Optical rotations are in $CHCl_3$ solution unless otherwise specified.

Oxidation of the tetra-acetate (Ib) *to* (IIa)

(a) A cold solution of the tetra-acetate (Ib, 200 mg) in pyridine (5 ml) was added slowly to a slurry of CrO_3 (from 250 mg of CrO_3 and 7 ml of pyridine) at −5° and the mixture was kept at room temp overnight and then poured onto crushed ice and worked up. The product was crystallized

[15] A. Sandoval, A. Manjarrez, P. R. Leeming, G. H. Thomas and C. Djerassi, *J. Amer. Chem. Soc.* **79**, 4468 (1957).

[16] J. Polonsky, *C.R. Acad. Sci., Paris* **233**, 93 671 (1951).

[17] C. Djerassi and H. G. Monsimer, *J. Amer. Chem. Soc.* **79**, 2901 (1957).

from methanol (charcoal) as needles (150 mg), m.p. 279–281°, $[\alpha]_D^{33.5°}$ −35·4°. (Found: C, 68·89; H, 8·34. $C_{38}H_{56}O_9$ requires: C, 69·51; H, 8·54%).

(b) To a solution of the acetate (Ib, 200 mg) in glacial acetic acid (5 ml), a solution of CrO_3 (200 mg) in acetic acid (90%, 6 ml) was added until a brown colour persisted and left overnight at room temp. A neutral colourless product, m.p. 279–281°, was obtained which did not depress the m.p. of IIa when admixed.

Oxidation of the tetrabenzoate (Ic) *to* (IIb)

A solution of Ic (200 mg) in glacial acetic acid (40 ml) was treated with a solution of CrO_3 (200 mg) in acetic acid (90%, 6 ml) as described for IIa. On working up followed by crystallization from chloroform–methanol mixture, colourless crystalline compound (IIb, 160 mg), m.p. 298–300°, $[\alpha]_D^{33.5°}$ −30·5°, was obtained. (Found: C, 76·3; H, 7·21. $C_{58}H_{44}O_9$ requires: C, 76·99; H, 7·08%).

Oxidation of Ic with CrO_3-pyridine complex as described for IIa also furnished IIb.

Oxidation of barringtogenol C (Ia)

A solution of Ia (1 g) was treated with CrO_3 (1·1 g) in acetic acid (90%, 25 ml). The product was taken up in ether and washed with NaOH aq (1%, 150 ml). The alkaline extract on acidification gave appreciable amount of a precipitate which was taken up in ether. This acidic fraction (150 mg) could not be crystallized but it gave a reddish-violet colour with alcoholic $FeCl_3$. The neutral ether layer gave a yellow glassy residue which was dissolved in benzene (5 ml) and chromatographed over deactivated alumina. Elution with benzene–pet. ether mixture (2:1, 300 ml) gave a yellow glassy residue which from benzene–pet. ether furnished a pale yellow amorphous product, m.p. 205–215 (21 mg). It gave a positive Zimmermann colour reaction and a positive $FeCl_3$ test only after treatment with alkali.

Lead tetra-acetate oxidation of barringtogenol C (Ia)

A solution of Ia (0·001 mole) in glacial acetic acid (5 ml) was mixed with 15–16 times the molar excess of lead tetra-acetate and the volume made up to 25 ml with glacial acetic acid. An aliquot sample (5 ml) was taken out at definite intervals and added to an aqueous solution (10 ml) of NaI (200 mg) and sodium acetate (2 g) and the liberated I_2 titrated with 0·02N $Na_2S_2O_3$. All oxidations were carried out at room temp and blank experiments were performed side by side. Consumption of lead tetra-acetate was complete within 4½ hr.

Barringtogenol C (500 mg) in glacial acetic acid (20 ml) was treated with a solution of freshly prepared lead tetra-acetate (1 g) in glacial acetic acid (20 ml). The mixture was left overnight and then poured into large volume of ice water. The white precipitate was filtered, washed with water and dried. The amorphous product, m.p. 115–155°, could not be crystallized.

A solution of the above oxidation product (100 mg) in dry benzene (20 ml) and piperidine (5 drops) was heated on an oil bath (temp, 60°) for 1 hr in N_2 atm. The amorphous product which was obtained did not show any characteristic absorption for $\alpha\,\beta$-unsaturated aldehyde in the UV spectrum.

Diketotribenzoate (IIIa)

The tribenzoate (Ie, 1·5 g) in glacial acetic acid (110 ml) was treated with CrO_3 (1·6 g) in acetic acid (90%, 35 ml) over a period of 5 hr at room temp. The reaction mixture was left overnight, the product dissolved in benzene (10 ml) and filtered through a column of deactivated alumina (5 g). Elution with benzene furnished the diketone (IIIa) which crystallized from chloroform–methanol as colourless needles (400 mg), m.p. 284–286° (dec), $[\alpha]_D^{30.5°}$ −11·9°. (Found: C, 76·76; H, 7·32. $C_{51}H_{58}O_8$ requires: C, 76·69; H, 7·26%). It did not respond to alcoholic ferric chloride or Zimmermann tests.

A solution of IIIa (390 mg) in methanolic KoH (5%, 70 ml) was kept overnight at room temp. Water (40 ml) was added and after acidifying with dil. HCl a yellow precipitate was obtained which was filtered and washed with $NaHCO_3$ aq and then with water. The above product could not be crystallized but it gave an intense violet colour with alcoholic $FeCl_3$. λ_{max}^{EtOH} 274 and 244 mμ; λ_{max}^{KOH} 310 and 244 mμ. From the filtrate formaldehyde was separated as its dimedone derivative, m.p. 188–189°.

The above amorphous product (100 mg) was refluxed with methanolic KOH (5%, 10 ml) for 1 hr and worked up as in the previous case. The yellowish white material still gave a violet colour with alcoholic $FeCl_3$ and showed a single absorption at 244 mμ.

The crude nor-diketone (100 mg) obtained by alkali treatment of IIIa at room temp formed an enolacetate which was crystallized from benzene–pet. ether (b.p. 40–60°) as rosettes, m.p. 256–261° (dec), λ_{max}^{EtOH} 243 mμ (log ε, 3·91). (Found: C, 72·0; H, 8·61. $C_{35}H_{50}O_7$ requires: C, 72·16; H, 8·59%).

Oxidation of (Ie) *to* (IIIb)

A cold solution of the tribenzoate (Ie, 1 g) in dry pyridine (20 ml) was added to a slurry of CrO_3 in pyridine (CrO_3 1·2 g and pyridine 40 ml) at 0° with stirring. The crude product was dissolved in benzene (10 ml) and adsorbed on a column of deactivated alumina (20 g). Elution with benzene gave colourless crystalline material (200 mg) which was crystallized from chloroform–methanol as needles (IIIb), m.p. 279–281° (dec), $[\alpha]_D^{25°}$ +21·7°. (Found: C, 76·35; H, 7·71. $C_{51}H_{60}O_8$ requires: C, 76·5; H, 7·5%). Molecular rotational difference (i.e. $[M]_{(IIIb)} - [M]_{(Ie)}$) was −197·6°.

Wolff-Kishner reduction of (IIIb) *to* (XI)

The monoketone (IIIb, 200 mg) in dioxane–ethanol (10 ml dioxane, 5 ml ethanol) was refluxed with hydrazine hydrate (85%, 10 ml) and diethylene glycol (13 ml) for 1 hr. Solid KOH (1·5 g) was added and the mixture concentrated by distillation until the temp was 198–200° where it was maintained for 4½ hr. The reaction product on crystallization from ethanol (charcoal) furnished a highly crystalline material (XI), m.p. 273–276° (dec). (Found: C, 78·23; H, 11·0. $C_{38}H_{48}O_8$ requires C, 78·38; H, 10·81%).

A solution of XI (30 mg) in methanol (20 ml) was treated with sodium metaperiodate (0·2 M, 2 ml) at room temp for 4 days. Excess periodate was titrated with standard sodium arsenite solution. The consumption of periodate was equivalent to one mole.

Wolff-Kishner reduction of (IIb) *to* (XI)

The Wolff-Kishner reduction of IIb was carried out exactly as described in the case of IIIb, yielding a product m.p. 273–276° (dec) which did not depress the m.p. of XI when admixed.

Acetonyl derivative of barringtogenol C (Ia)

To a suspension of barringtogenol C (1 g) in dry acetone (200 ml), acetone (25 ml) saturated with dry HCl was added slowly with continuous stirring until the solid dissolved. The mixture was kept overnight and the solvent removed *in vacuo*. The residue was washed successively with petroleum ether, $NaHCO_3$ aq and water. It crystallized from acetone (charcoal) as colourless needles, m.p. 275–290° (dec). The m.p. could not be raised by repeated crystallization.

The above impure product was heated with pyridine (3 ml) and acetic anhydride (3 ml) on steam bath for 4 hr and the mixture was poured onto crushed ice. The product was dissolved in benzene (5 ml) and chromatographed over deactivated alumina (30 g). Elution with benzene–pet. ether (2:1, 400 ml) gave the tetra-acetate (Ib, 320 mg) and further elution with benzene–chloroform (2:1, 700 ml) furnished a colourless crystalline mass which was crystallized from aqueous ethanol as shining scales, m.p. 262–264°, $[\alpha]_D^{31°}$ −17·1°. (Found: C, 71·46; H, 9·65. $C_{37}H_{58}O_7$ requires: C, 72·31; H, 9.42%).

Conversion of barringtogenol C (Ia) *to barringtogenol* D (XIIa)

Pure barringtogenol C (Ia, Ig) in absolute ethanol (500 ml) was refluxed with conc. HCl (160 ml) for 8 hr. Then the solvent was evaporated off keeping the volume constant by addition of water. The solid was filtered, washed with water and crystallized from dioxane–methanol, m.p. 280–305° (dec).

The above crude product was benzoylated with benzoyl chloride (5 ml) and pyridine (5 ml) over a steam bath for 3 hr. The product, (1·5 g) after chromatography over deactivated alumina (80 g) furnished two distinct fractions. Elution with pet. ether (1·5 l.) gave an waxy pet. ether soluble fraction, m.p. 124–160° (780 mg). Further elution with benzene–pet. ether (2:1, 2l.) furnished a colourless crystalline material, m.p. 300–310°, which was crystallized from chloroform–methanol as needles, m.p. 314–317°, and it did not depress the m.p. of the tetrabenzoate (Ic).

The fraction (m.p. 124–160°) was hydrolysed with alcoholic KOH (10%, 50 ml). The crude product was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) over steam bath for 2 hr. The product after chromatography over deactivated alumina (30 g) furnished a colourless glassy material when eluted with pet. ether. This was crystallized from aqueous ethanol as needles, m.p. 233–234°, $[\alpha]^{32°}$ +75°. It did not depress the m.p. of barringtogenol D triacetate (XIIb) when admixed.

The above acetate (whole amount) was saponified with methanolic KOH (5%) and the product followed by crystallization from ethanol gave a compound, m.p. 305–310°, $[\alpha]_D^{32°}$ +58° (dioxane) yield 420 mg (42% on the basis of the starting material i.e. barringtogenol C). It did not depress the m.p. of an authentic sample of barringtogenol D (XIIa).

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