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TRITERPENOIDS—XVI

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

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Abstract—The constitution of barringtogenol D—a new triterpenoid sapogenin isolated from the fruits of *Barringtonia acutangula* Gaertn has been shown to be 3β:22β:28-trihydroxy-16α:21 α-oxido-olean-12-ene.

IN TWO preliminary communications[1,2] the isolation of a number of new sapogenins from the fruits of *Barringtonia acutangula* Gaertn and the constitution of one of them, barringtogenol D, have been reported from this laboratory. The present paper deals with the details of our investigations leading to the structure of the latter. Barringtogenol D (Ia) $C_{30}H_{48}O_4$, isolated in a poor yield (0·05%), showed a violet→pink colouration in the Liebermann–Burchard reaction. It formed a triacetate (Ib) showing the presence of three hydroxyl groups in the molecule. The probability of the fourth oxygen atom being present as a keto or a hydroxyl function was ruled out by the absence of any characteristic band for either in the I.R. spectra of barringtogenol D and its triacetate respectively. A band at 1150 cm^{-1} in the latter, however, indicated the presence of an ether function. That the ether function is not present as methoxyl or ethoxyl group was shown by their absence in Zeisel method of determination. On the other hand, the triacetate formed a crystalline tetraacetate, $C_{38}H_{58}O_8$ on treatment in acetic anhydride either with *p*-toluene sulphonic acid under reflux or hydrogen chloride and the band at 1150 cm^{-1} disappeared. This tetraacetate on hydrolysis yielded a tetrol, $C_{30}H_{50}O_4$. These observations clearly showed that the fourth oxygen atom in barringtogenol D must be present as an internal oxide possibly as a five or six-membered ring. This type of oxide when treated with *p*-toluene sulphonic acid in presence of acetic anhydride is expected to give a pentaacetate or a tetraacetate containing one additional double bond while with hydrogen chloride one of the above compounds or a tetraacetate containing a chlorine atom would be the normal product. Our tetraacetate, however, did not contain any additional double bond or any halogen. It appeared, therefore, that the oxide linkage in barringtogenol D opened-up in rather a complex manner (reminiscent of aescigenin[3]) under the above acid-catalysed processes which calls for further investigation.

The ethylenic linkage in barringtogenol D, as indicated by tetranitromethane

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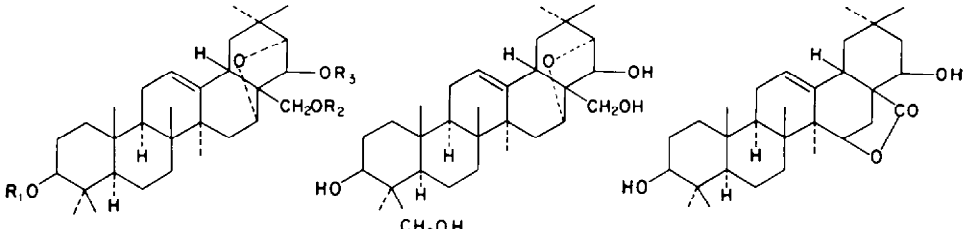
colouration, is hindered. It resisted hydrogenation in presence of Adams' catalyst under normal conditions. It consumed nearly 1 mole of perbenzoic acid at an extremely slow rate. The corresponding tetrol, however, consumed one mole at a faster rate (*vide* Experimental) which is comparable to that of other members of the β-amyrin group having a 12:13-double bond. The extreme hindrance can possibly be attributed to the shielding effect of the oxide linkage on the double bond in barringtogenol D and finds remarkable parallel in the case of aescigenin (II) (*loc.cit*). The triacetate (Ib) could be recovered unchanged after refluxing with selenium dioxide in glacial acetic acid under conditions in which $\Delta^{11:12,13:18}$-dienes[4] are obtained from typical β-amyrin type compounds. The similar observations in cases of dumortierigenin[5] (III) and



aescigenin make it apparent that substitution in ring D and E affects the reactivity of the 12:13-double bond. Evidence for the presence of typical 12:13-double bond in barringtogenol D was obtained by oxidation of the triacetate (Ib) with CrO_3 in acetic acid leading to an $\alpha:\beta$-unsaturated ketone (IV). It showed a maxima at 241 mμ (log ϵ 4·1) like the corresponding compound obtained from aescigenin which is about 6 mμ lower than the usual value[6] for the 11-keto-Δ^{12}-triterpenes of β-amyrin group.

Barringtogenol D did not react with periodic acid but readily formed a monoacetonide (Va) pointing to the presence of a 1:3-glycol system. Oxidation of Va with CrO_3-pyridine complex furnished Vb which showed a band at 1700 cm^{-1} typical of a six membered ring ketone. It also responded to Zimmermann's colour test for 3-keto group[7]. Moreover, the size and shape of the optical rotatory dispersion curve* of this ketone is so similar to that of β-amyrone[8,9] that one is tempted to represent the (partial) structure and stereochemical environment in ring A of the ketone as VI. The molecular rotational data[10] ($M_{(Vb)} - M_{(Va)} = +22{\cdot}22$) showed the β-orientation of the 3-hydroxyl group in barringtogenol D. The indifference to alcoholic ferric chloride of the colourless ketone Vb showed the absence of any α- or β-diketone system. Again, the possibility of the presence of any primary hydroxyl group at C_{23} or C_{24} was ruled out as in that case, contrary to the fact, the compound Vb should have been a

* By the courtesy of Prof. Carl Djerassi, Stanford University, U.S.A.

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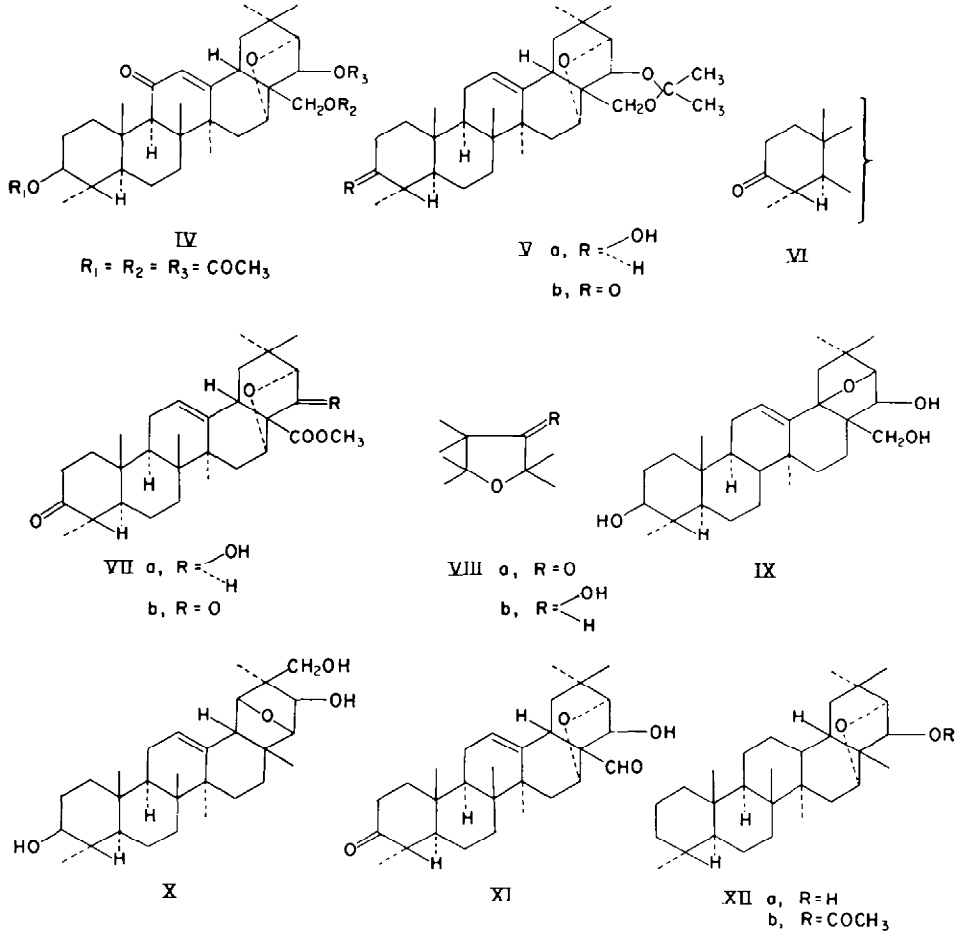
[11] D. H. R. Barton and P. de. Mayo, *loc. cit.*

β-ketoaldehyde which would have lost formaldehyde on treatment with acid or alkali (cf. hederagenin and icterogenin[11] etc.). All the above observations permit the conclusion that ring A contains one and only one hydroxyl group at C_3.

Barringtogenol D when treated with chromium trioxide-sulphuric acid in acetone[12] furnished both neutral and acidic products. The former could not be obtained in the pure state but on further oxidation with the same reagent furnished additional quantities of the acidic products. The acid fraction on treatment with diazomethane afforded two compounds characterized as methylhydroxy ketoester (VIIa) and methyl diketoester (VIIb) which requires the presence of one primary hydroxyl group in barringtogenol D. The β-keto acid corresponding to ester VIIb did not undergo decarboxylation under the condition of our experiment which it normally should have. Ester VIIa showed bands at 3400 cm^{-1} (hydroxyl), 1700 cm^{-1} (six membered ring ketone) and at 1725 cm^{-1} (carbomethoxyl group). Ester VIIb besides showing peaks for six-membered ring ketone and carbomethoxyl group showed strong band at 1765 cm^{-1} which was attributed to a five membered ring ketone VIIIa involving an oxide function (cf. aescigenin, *loc. cit.*). Formation of the system VIIIa requires



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one secondary hydroxyl group as shown in VIIIb and the monoacetonide Va formation fixes the primary hydroxyl group at 1:3 position with respect to the above secondary hydroxyl group in VIIIb.

On the basis of the above observations barringtogenol D can be represented by either of the three structures Ia, IX or X. To scale molecular model (Catalin type) experiment favoured structure Ia most for barringtogenol D and final confirmation was achieved as in the sequel. Barringtogenol D was oxidized with CrO_3 in acetic acid and the neutral oxidation product XI, which was not isolated in the pure state, on Huang Minlon variant of Wolf-Kishner reduction[13] furnished a compound XIIa that formed an acetate XIIb. The identity of XIIa and XIIb with 22 β-hydroxy-16α:21α-oxido-olean-12-ene and its acetate respectively, was shown by comparison of their mixed melting point with corresponding authentic samples.

Hence the structure and stereochemistry of barringtogenol D should be represented as Ia.

EXPERIMENTAL

The m.ps. are uncorrected and optical rotations are in chloroform solutions unless otherwise specified. U.V. spectra were measured in ethanol solutions with a Beckman DU instrument. 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. Pet. ether refers to b.p. 60–80°.

Barringtogenol D *triacetate* (Ib). Barrintogenol D (Ia, 200 mg) was heated over a steam bath with acetic anhydride (5 ml) and pyridine (5 ml) for 2 hr. Pouring into ice gave solids which was worked up in the usual way. It crystallized from ethanol (Ib, 140 mg), m.p. 233–234°, $[\alpha]_D^{28°}$ +74°. (Found: C, 72·16; H, 8·98. $C_{36}H_{54}O_7$ requires: C, 72·20; H, 9·09%).

Preparation of the tetraacetate. (a) To a boiling solution of the triacetate (Ib, 300 mg) in acetic anhydride (15 ml) was slowly added *p*-toluene sulphonic acid (150 mg) over a period of ½ hr. The mixture was then refluxed on a sand bath for 1 hr and left at room temperature overnight. The reaction mixture was diluted with water and boiled for 15 mins and the granular precipitate was filtered and washed with hot water. It was crystallized from methanol as needles (40 mg) m.p. 284–285°, $[\alpha]_D^{28°}$ −12·5° (Found: C, 70·95; H, 8·79; $C_{38}H_{58}O_8$, requires: C, 71·02; H, 9·40%).

(b) A solution of the triacetate (Ib, 500 mg) in acetic anhydride (25 ml) was saturated with hydrogen chloride and heated on steam bath for 2 hr and left at room temperature over night. It was poured into crushed ice and worked up in the usual way. The brown solid residue was crystallized from ethanol (charcoal) m.p. 278–82° (60 mg). This was repeatedly crystallized from methanol as fine needles m.p. 284–285°. It did not depress the melting point of the tetraacetate obtained by method (a).

Hydrolysis of the tetraacetate to tetrol. The tetraacetate (50 mg) was refluxed with 5% ethanolic caustic potash (20 ml) for 4 hr. On working up the product and crystallization from aqueous methanol shining needles m.p. 293–295°, $[\alpha]_D^{28°}$ +48°, was obtained. (Found: C, 75·80; H, 10·55. $C_{30}H_{50}O_4$ requires: C, 75·90; H, 10·62%).

Perbenzoic acid oxidation of the triacetate (Ib) *and the tetraacetate.* The triacetate (Ib) and the tetraacetate (40 mg each) in chloroform solution was treated separately with a solution of perbenzoic acid (0·35 N; 5 ml in each case) at 0°. The consumption of the per acid was measured by titrating with thiosulphate at intervals (*vide* Table 1).

Attempted oxidation of the triacetate (Ib) *with selenium dioxide.* The triacetate (Ib, 350 mg) in glacial acetic acid (15 ml) was refluxed on a sand bath with selenium dioxide (400 mg) for 17 hr. On working the reaction product and subsequent chromatography over acid-washed alumina gave back the original compound (300 mg) m.p. and mixed m.p. 232–33°.

Preparation of the α:β-unsaturated ketone (IV). To a refluxing solution of the triacetate (Ib, 200 mg) in glacial acetic acid (20 ml), chromium trioxide (200 mg) in acetic acid (85%, 10 ml) was added drop-wise over a period of 1 hr. Refluxing was continued further 1 hr. and then diluted with water. The precipitate was filtered and crystallization from chloroform-methanol gave colourless

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Table 1

Compound	Moles of perbenzoic acid consumed			
	24 hr	3 days	7 days	15 days
Triacetate (Ib)	nil	0·45	0·7	0·9
Tetraacetate	0·39	0·75	1·0	1·1

needles m.p. 248–55°. This was chromatographed over acid-washed alumina and elution with benzene gave a fraction which crystallized from chloroform-methanol as colourless needles m.p. 256–258°. It did not give any colouration with tetranitromethane. (Found: C, 70·02; H, 8·52. $C_{36}H_{58}O_8$ requires C, 70·58; H, 8·49%).

Preparation of the monoacetonide (Va). To a solution of barringtogenol D (Ia, 100 mg) in 15 ml of dioxane-acetone mixture (1:2), 4 drops of conc. hydrochloric acid was added and left overnight. It was poured into cold water and the product worked up in the usual manner and crystallized from ethanol (charcoal) m.p. 230–35°. It was dissolved in benzene (5 ml) and adsorbed on a column of Brokmann's alumina. Elution with benzene gave a fraction which crystallized from ethanol m.p. 233–236°, $[\alpha]_D^{25°}$ +33°. (Found: C, 77·25; H, 10·21. $C_{33}H_{52}O_4$ requires: C, 77·29; H, 10·22%).

Oxidation of the monoacetonide (Va) *to the ketone* (Vb). A solution of the monoacetonide (Va, 100 mg) in pyridine (5 ml) was cooled and added slowly to a slurry of chromium trioxide-pyridine complex (from 100 mg of CrO_3 and 5 ml pyridine) kept below 10°. The mixture was kept at room temperature overnight and then poured into crushed ice. The separated solid was filtered and washed with water. It crystallized from methanol in long needles m.p. 212–213°, $[\alpha]_D^{25°}$ −57°. (Found: C, 77·59; H, 9·42. $C_{33}H_{50}O_4$ requires C, 77·6; H, 9·86%).

Oxidation of barringtogenol D (Ia) *to esters* (VIIa) *and* (VIIb). A fine suspension of barringtogenol D (Ia, 0·8 g) in acetone (40 ml) was treated gradually with Kiliani oxidation mixture (2 ml; the mixture prepared by dissolving 2·66 g of CrO_3 in 7·7 ml water and 2·3 ml conc. H_2SO_4) until orange-brown colour persisted. The reaction mixture was kept in an ice bath and continually stirred. After 30 min the mixture was diluted with water when white solid separated. It was taken up in ether and the ethereal layer washed with caustic soda solution (1%, 150 ml) then with water and dried over anhydrous sodium sulphate. Removal of the solvent gave colourless solid (380 mg, neutral fraction). The alkali fraction was acidified and the colourless precipitate taken up in ether and subsequent esterification with ethereal diazomethane gave crystalline material (150 mg) which was chromatographed over Brockmann's alumina (25 g). Elution with benzene (250 ml) gave a fraction which crystallized from chloroform–methanol m.p. 200–250° and further elution with benzene ether (2:1; 230 ml) furnished crystalline material (45 mg) which was further crystallized from chloroform–methanol m.p. 274–277°, $[\alpha]_D^{21°}$ +77·5° (VIIa). (Found: C, 74·06; H, 9·33. $C_{31}H_{46}O_5$ requires: C, 74·65; H, 9·29%). It gave Zimmermann's test for 3-keto group and the ferric chloride test was negative. It formed a 2:4-dinitrophenyl hydrazone m.p. 257–258° (Found: C, 65·34; H, 7·46; N, 8·28; $C_{37}H_{50}O_8N_4$ requires: C, 65·49; H, 7·37; N, 8·26%). The fraction (m.p. 200–250) was rechromatographed over Brockmann's alumina (10 g) and elution with benzene:pet. ether mixture (1:1, 175 ml) gave a glassy material which crystallized from chloroform–methanol as colourless needles (15 mg) m.p. 217–218° (VIIb). (Found: C, 75·1; H, 9·13. $C_{31}H_{44}O_6$ requires: C, 74·96; H, 8·93%). The neutral fraction (380 mg) obtained above could not be purified but on further oxidation with the same reagent furnished acidic material which on esterification and subsequent chromatography over Brockmann's alumina furnished small quantities of esters (VIIa) and (VIIb).

Preparation of the compound (XIIa) *from barringtogenol* D (Ia). To a cold solution of barringtogenol D (Ia, 0·75 g) in glacial acetic acid (150 ml) and benzene (300 ml) a solution of CrO_3 (260 mg) in glacial acetic acid (25 ml) was added over a period of 1 hr with stirring which was continued for 16 hr. It was poured into cold water and the precipitate taken up in ether. The ethereal layer was washed with caustic soda solution (2%) and it gave small amount of acidic material on acidification. The ethereal layer was worked up in the usual way and furnished a colourless solid (640 mg) m.p. 255–270°. It responded to Zimmermann's colour test.

A mixture of the above oxidation product (640 mg), diethylene glycol (39 ml), absolute ethanol (17 ml) and hydrazine hydrate (85%, 15 ml) was heated to reflux in a paraffin bath for 2 hr. Solid caustic potash (3 g) was added to the mixture and distilled till the temp. of the mixture rose to 200°. After heating the mixture at 195–200° for 4 hr it was cooled and poured into crushed ice and the separated solid was filtered. Yield (550 mg). This was dissolved in benzene and chromatographed over acid-washed alumina (25 g). Elution with benzene gave a crystalline material m.p. 215–225° (8 mg) and further elution furnished colourless glassy mass which crystallized from methanol m.p. 198–200°, $[\alpha]_D^{25°}$ +61°, (130 mg). (Found: C, 81·67; H, 10·88. $C_{30}H_{48}O_2$ requires: C, 81·77; H, 10·92%). It did not depress the m.p. of an authentic sample of 22β-hydroxy-16α:21α-oxido-olean-12-ene (XIIa).

The above deoxy compound (XIIa, 50 mg) was acetylated in the usual way (pyridine-acetic anhydride) and crystallized from chloroform–methanol m.p. 201–203°, $[\alpha]_D^{25°}$ +76°, (Found: C, 80·06; H, 10·38. $C_{32}H_{50}O_3$ requires C, 79·61; H, 10·44%), undepressed when mixed with the authentic sample of 22β-acetoxy-16α:21α-oxido-olean-12-ene (XIIb). The authentic samples of (XIIa) & (XIIb) were obtained through the courtesy of Dr. D. Arigoni, Zurich, Switzerland.

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