Tetrahedron. Vol. 24, pp. 1113 to 1117. Pergamon Press 1968. Printed in Great Britain

TRITERPENOIDS—XXIX*

THE STRUCTURE OF BARRINGTOGENOL B—A NEW TRITERPENOID SAPOGENIN FROM *BARRINGTONIA ACUTANGULA* GAERTN†

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(*Received in UK 9 June* 1967; *accepted for publication 22 June* 1967)

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

THE new sapogenins, barringtogenol B, C and D from the fruits of *B. acutangula* Gaertn were isolated earlier[1,2] and the structure and stereochemistry of the latter two compounds elucidated as 3β, 16α, 21α, 22β, 28-pentahydroxy-olean-12-ene [3,4] and 3β, 22β, 28-trihydroxy-16α:21α-oxido-olean-12-ene.[5,6] Barringtogenol D which is actually a dehydration product of barringtogenol C was directly correlated with a degradation product Ia of aescigenin,[7] which is again a dehydration product of protoaescigenin.[8] In 1964, Kuhn and Löw reported the isolation of a triterpene, aescinidin,[9] from the saponin of horse chestnut (*Aesculus hippocastanum* L.). Aescinidin was later shown by Tschesche and Wulff[10] to be identical with barringtogenol C. Very recently, the configuration of the C-21, C-22 α-glycol system in both protoaescigenin and barringtogenol C has been shown[11–13] to be *trans* diequatorial instead of *trans* diaxial as suggested by the previous workers in both cases and therefore barringtogenol C and D are represented by the structures IIa and Ib, respectively. The present paper deals with the structure of barringtogenol B (IIb).

The method of isolation of barringtogenol B has now been slightly modified (Experimental). The previously assigned molecular formula, $C_{30}H_{50}O_6$, for this compound has now been revised to $C_{35}H_{56}O_6$ on the basis of mass spectral studies on barringtogenol B (IIb) and its dihydro derivative IIc. Barringtogenol B shows a UV absorption max at 213 mμ (log ε 4·72) indicating the presence of an α,β-unsaturated ester function. This was further corroborated by the IR spectrum of barringtogenol B (KBr) which shows bands at 1705 cm^{-1}, 1645 cm^{-1} (C=O and C=C of an α,β-unsaturated ester function) and 3440 cm^{-1} (OH groups).

* Part XXVIII of this series, *Tetrahedron* **24**, 1107 (1968).

† This paper was presented at the joint Convention of Indian Chemical Society and Chemical Research Committee, C.S.I.R., India, held at Aligarh, India, during December, 1965.

On catalytic hydrogenation, barringtogenol B gave a dihydro derivative IIc which does not show any characteristic UV absorption max for an α,β-unsaturated ester group. On acetylation in presence of perchloric acid, barringtogenol B furnished a tetraacetate IId indicating the presence of four acylable OH groups. On saponification under reflux with alcoholic caustic potash, barringtogenol B furnished an acid and a neutral alcohol which formed a tetrabenzoate. The neutral alcohol and its tetrabenzoate were characterized as barringtogenol C (IIa) and its tetrabenzoate (IIe) by m.p., mixed m.p. and IR spectra. The acid fraction was identified as tiglic acid by comparative TLC,[14] m.p. and mixed m.p. The isolation of tiglic acid from barringtogenol B under the above condition leaves open the question whether the latter is actually an ester of tiglic acid or angelic acid since influence of alkali at an elevated temperature is known to isomerize angelic acid to the more stable *trans* form (i.e. tiglic acid). To settle this issue, barringtogenol B was saponified at room temperature.[15] The acid fraction thus obtained was actually proved to be angelic acid by TLC,[14] m.p. and mixed m.p. Evidently barringtogenol B is a monoangeloyl derivative of barringtogenol C.

The mass spectrum* (Fig. 1) of barringtogenol B (IIb) is in complete agreement with the structure assigned to it. Barringtogenol B shows the molecular ion peak at *m/e* 572. The ions *m/e* 554 and *m/e* 536 correspond to the loss of one and two molecules

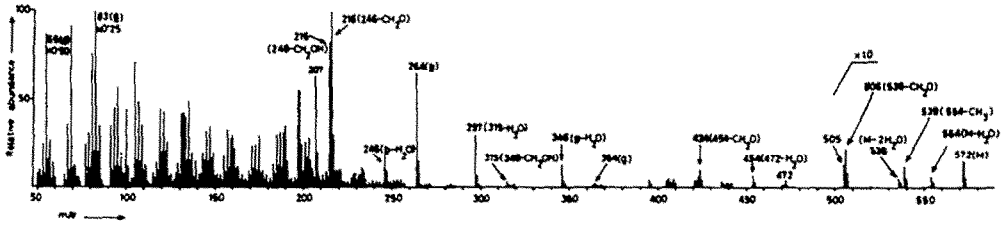


FIG. 1.

of water, respectively, from the molecular ion. Further loss of CH_2O and $—CH_2OH$ from the $M—2H_2O$ ion (*m/e* 536) results in the peaks at *m/e* 506 and 505, respectively. Elimination of angelic acid from the molecular ion leads to the fragment *m/e* 472 which again loses one molecule of water showing the peak at *m/e* 454. Subsequent loss of CH_2O and $—CH_2OH$ gives the peaks at *m/e* 424 and *m/e* 423, respectively.

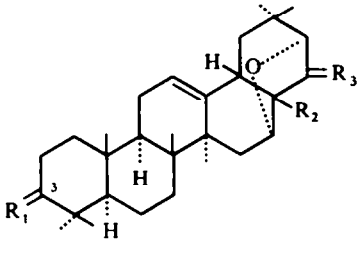
Retro-Diels Alder fragmentation[16] of the molecule leads to a small peak at *m/e* 364 (*a*). Elimination of water from *a* produces the fragment *m/e* 346 which by loss of $—CH_2OH$ and then a molecule of water gives rise to ions *m/e* 315 and *m/e* 297, respectively. Similar fragmentation is also observed after elimination of angelic acid from *a* showing peaks at *m/e* 264 (*b*), 246, 216 (246-CH_2O), 215 (246-CH_2OH), 198, 197. A fragment containing rings A and B[16] occurs at *m/e* 207 which undergoes further loss of water resulting in the peak at *m/e* 189. The two intense peaks at *m/e* 83 and *m/e* 55 originating from the angeloyl ester group correspond to the ions *c* and *d*, respectively.

The mass spectrum of dihydro barringtogenol B (IIc) shows the expected molecular ion peak at *m/e* 574 and in agreement with the interpretation given above, all the fragments retaining the ester group are shifted by two mass units, while no such shift was observed for those devoid of the ester chain.

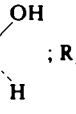
* Obtained with an A.E.I. MS9 mass spectrometer operating at 70 eV.

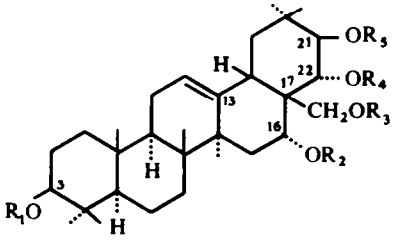
The mass spectra of both barringtogenol B and dihydrobarringtogenol B clearly locate the ester chain in either ring D or E of barringtogenol B. Loss of 31 (—CH_2OH) mass units from the *m/e* 346 and 246 ions (Fig. 1) indicated the C-17 hydroxymethylene group to be free. Barringtogenol B consumed one mole of periodic acid showing the C-21, C-22 α-glycol system to be unsubstituted. The amorphous product thus obtained exhibits the UV absorption max at 214 mμ indicating the α,β-unsaturated ester moiety to be still intact. This leaves C-16 as the only possible site for the ester group. Thus barringtogenol B is represented as 3β, 21β, 22α, 28-tetrahydroxy-16α-angeloyloxy-olean-12-ene (IIb). This is further supported by a study of the NMR spectrum (60 Mc/s in $CDCl_3$) of barringtogenol B tetraacetate (IId). In the low field region, the spectrum shows a signal at 3·85 δ (2H; S) assignable to the two methylene protons (—CH_2OAc) of the 17β-acetoxy methyl group and a multiplet centred at 4·50 δ (1H) for the axial proton at C-3.[17] Though not very well resolved, five protons are still discernible from the multiple signals around the region 5·27 δ–5·73 δ which may be attributed to: (i) the olefinic β-proton of the angeloyl moiety,[18] (ii) the C-12 vinyl proton[19] and (iii) three >CH—O— protons at C-16, C-21 and C-22.[17]

By chromatography of the crude sapogenin mixture obtained from the fruits of *B. acutangula*, it has been possible to isolate another fraction (called fraction A) which has been found by TLC to be a mixture of two closely related compounds. Individual components of this fraction have not, so far, been isolated in a pure state, but on saponification, it gave only barringtogenol C and angelic acid. Evidently both the components of this fraction are angelic acid esters of barringtogenol C but are different from barringtogenol B. Further attempts are being made for the identification and characterization of the components.

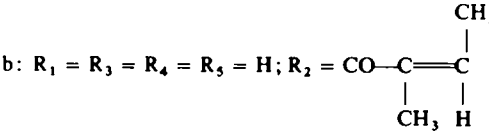


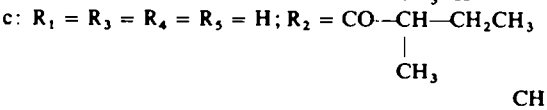
Ia: $R_1 = H_2$; $R_2 = CH_3$; R_3 = 

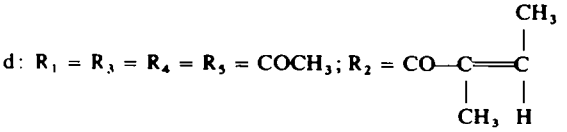
b: R_1 =  ; $R_2 = CH_2OH$; R_3 = 



IIa: $R_1 = R_2 = R_3 = R_4 = R_5 = H$

b: $R_1 = R_3 = R_4 = R_5 = H$; R_2 = 

c: $R_1 = R_3 = R_4 = R_5 = H$; R_2 = 

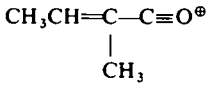
d: $R_1 = R_3 = R_4 = R_5 = COCH_3$; R_2 = 

e: $R_1 = R_3 = R_4 = R_5 = COC_6H_5$; $R_2 = H$

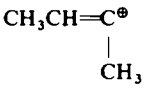
a: *m/e* 364
↓ $-H_2O$
m/e 346
↓ $-CH_2OH$
m/e 315
↓ $-H_2O$
m/e 297

m/e 216 ← $-CH_2O$ — b: *m/e* 264
↓ $-H_2O$
m/e 198

↓ $-H_2O$
m/e 246
↓ $-CH_2OH$
m/e 215
↓ $-H_2O$
m/e 197



c: *m/e* 83



d: *m/e* 55

EXPERIMENTAL

The m.ps are uncorrected and were recorded in a bisulphate bath. UV spectra and optical rotations were measured in EtOH soln unless otherwise specified. Silica gel (supplied by National Chemical Laboratory, Poona, India) was used for chromatography. Homogeneity of all the products was checked by TLC over silica gel G (E. Merck).

Isolation of barringtogenol B (IIb). The air-dried powdered fruits of *B. acutangula* (1·2 kg) were defatted with pet. ether (b.p. 60–80°) and then extracted with EtOH. The extract was concentrated and the saponin was precipitated by addition of ether. The saponin was hydrolysed by refluxing with 5% ethanolic HCl for 15 min. The crude sapogenin thus obtained was extracted with ether and the ether extract was separated into acid and neutral parts. The neutral part was chromatographed over silica gel. Two fractions were isolated. The first one (fraction A) was eluted with $CHCl_3$ and was crystallized from $CHCl_3$–EtOH m.p. 196–210°. It was found to be a mixture of two compounds by TLC. A second fraction (Barringtogenol B) was eluted with $CHCl_3$–MeOH (49:1). It was crystallized from 95% EtOH (yield, 110 mg) m.p. 245–247°, $[\alpha]_D^{32°}$ +3°. (Found: C, 73·42; H, 9·73; M^+ 572. Calc. for $C_{35}H_{56}O_6$: C, 73·43; H, 9·79%; Mol. wt. 572.) It was found to be identical with barringtogenol B isolated earlier (*loc. cit.*).

Dihydrobarringtogenol B (IIc). Barringtogenol B (500 mg) was dissolved in EtOH (85 ml) and hydrogenated in presence of Pd–C (200 mg) for 8 hr. After working up the product was crystallized from aq. EtOH, m.p. 239–241°, $[\alpha]_D^{31°}$ +2°. (Found: C, 73·45; H, 9·98; M^+ 574. Calc. for $C_{35}H_{58}O_6$: C, 73·17; H, 10·10%; Mol. wt. 574.)

Barringtogenol B tetraacetate (IId). Barringtogenol B (250 mg) was dissolved in Ac_2O (5 ml) and kept at room temp for 48 hr after addition of one drop of perchloric acid. After working up, the product was chromatographed over silica gel. The fraction eluted with $CHCl_3$–benzene mixture (2:1) was crystallized from aq. EtOH, m.p. 248–250°. (Found: C, 69·60; H, 8·72. Calc. for $C_{43}H_{64}O_{10}$: C, 69·73; H, 8·65%.)

Hydrolysis of barringtogenol B

(a) Barringtogenol B (500 mg) was dissolved in 10% ethanolic KOH (10 ml) and refluxed on a water bath for 4 hr. The reaction mixture was separated into neutral and acid fractions. The neutral fraction was purified by crystallizing from aq. EtOH, m.p. 315–320°, $[\alpha]_D^{30°}$ +34° (dioxan); mixed m.p. with barringtogenol C (IIa), 315–320°. (Found: C, 73·35; H, 10·14. Calc. for $C_{30}H_{50}O_5$: C, 73·47; H, 10·20%.) This neutral product (100 mg) was dissolved in dry pyridine (2 ml) and heated on a steam bath for 5 hr after addition of benzoyl chloride (2 ml). After working up, the product was crystallized from $CHCl_3$–MeOH to yield

barringtogenol C tetrabenzoate, m.p. 315–317°, $[\alpha]_D^{30°}$ +32° (dioxan); mixed m.p. with authentic IIe, 315–317°. (Found: C, 76·56; H, 7·07. Calc. for $C_{58}H_{66}O_9$: C, 76·82; H, 7·28%.)

The acid fraction from the hydrolysate was purified by distillation at 92–100°/12 mm of Hg, m.p. 64–65°; mixed m.p. with authentic tiglic acid, 64–65°.

(b) Barringtogenol B (500 mg) was dissolved in methanolic KOH (1N; 10 ml) and kept overnight at 24–25°. The reaction mixture was concentrated under vacuum at room temp, diluted with water and separated into acid and neutral fractions. The neutral fraction was purified, m.p. 315–320°, $[\alpha]_D^{30°}$ +34·5° (dioxan). The acid part was purified by distillation at 83–90°/12 mm of Hg. A viscous liquid was obtained which solidified on cooling, m.p. 44–45°; mixed m.p. with authentic angelic acid 44–45°. It was identified as angelic acid by comparative TLC over silica gel G impregnated with 5% ammoniacal $AgNO_3$ soln.

Saponification of fraction A. Fraction A (100 mg) was saponified with methanolic KOH (1N; 5 ml) at 24–25°. The hydrolysate was separated into neutral and acid parts and they were identified as barringtogenol C and angelic acid.

Periodic acid oxidation of barringtogenol B (IIb). Barringtogenol B (100 mg) was dissolved in MeOH (10 ml) and kept for 24 hr at room temp after addition of periodic acid soln (3 ml, 0·5M aq. methanolic soln). The excess periodic acid was determined by titration with standard sodium arsenite soln in the usual way. On dilution of the reaction mixture with water an amorphous product, m.p. 152–172° (dec) was obtained which could not be crystallized. It was found to be different from barringtogenol B by TLC over silica gel G. It formed an amorphous DNP derivative, m.p. 240–250°, with methanolic solution of 2,4-dinitrophenylhydrazine sulphate. It could not be further purified.

Acknowledgements—The authors are grateful to Dr. D. M. Bose, Director, Dr. A. Sen, Head of the Department of Chemistry, Bose Institute and Prof. E. Lederer, Director, Institut de chimie des Substances Naturelles for their interest in this work. Thanks are due to Dr. N. Dutta Gupta, Roswell Park Memorial Institute, Buffalo, U.S.A. and to Dr. S. C. Pakrashi, Scientist, Indian Institute for Experimental Medicine, Calcutta, for the NMR and IR spectra respectively of the compounds reported. We are also indebted to Prof. S. C. Bhattacharya, I.I.T., Bombay and Dr. Sukh Dev, Asst. Director, National Chemical Laboratory, Poona, for authentic samples of angelic acid and tiglic acid respectively. The microanaylses were carried out by Drs. G. Weiler and F. B. Strauss, Oxford, U.K.

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