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TRITERPENOIDS—XII

THE CONSTITUTION OF ALBIGENIN—A NEW TRITERPENE FROM *ALBIZZIA LEBBECK* BENTH

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Abstract—The constitution of albigenin—a new triterpenoid sapogenin isolated from the beans of *Albizzia lebbeck* Benth has been shown to be 3β-hydroxy-16-oxo-13(18)-en-28-noroleanane.

THE constitution of albigenic acid (Va) isolated from the acid sapogenin fraction of the beans of *A. lebbeck* Benth., along with oleanolic and echinocystic acids, has been dealt with in a previous communication.[1] It has now been possible to isolate another new triterpene in low yield (0·012 per cent) from the neutral sapogenin fraction and has been named "albigenin" (Ia). It gives a violet → pink colouration in the Liebermann–Burchardt reaction. Molecular weight determination and combustion data indicate either $C_{29}H_{46}O_2$ or $C_{30}H_{48}O_2$ as the molecular formula, and although the latter is common for natural triterpenes, the C_{29} formulation has been found to be correct.

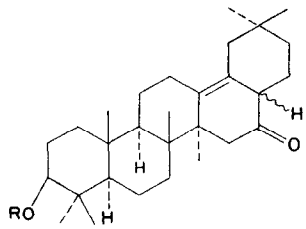
The infra-red spectrum of albigenin shows the presence of *gem* dimethyl groups (bands at 1365 and 1385 cm^{-1}), hydroxyl (3655 cm^{-1}) and carbonyl groups (1695 cm^{-1}).

Albigenin gives a monoacetate (Ib) and a mono-2,4-dinitrophenylhydrazone. The ease of formation of the hydrazone suggests the relatively unhindered nature of the carbonyl function. This carbonyl must be a keto group as albigenin does not respond to the usual tests for aldehydes. Albigenone, the oxidation product of albigenin, responds positively to the Zimmermann colour reaction whereas albigenin does not, thus proving that the hydroxyl group is at the C_3-position.[2] Albigenone, a colourless compound, gives neither a colour with alcoholic ferric chloride nor shows an absorption maxima in the ultra-violet region characteristic of an α- or a β-diketo system proving thereby that the two oxygen functions in albigenin are located in different rings.

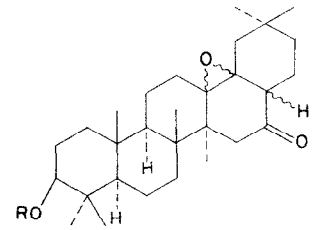
Albigenin and its acetate give a pale yellow colouration with tetranitromethane and consume only one mole of perbenzoic acid within 24 hours. The monoepoxide of albigenin acetate (IIa) is saturated to tetranitromethane. These observations prove the presence of only one double bond, but the ethylenic linkage resists hydrogenation under standard conditions and is inert to osmic acid treatment. In all these respects the double bond in albigenin closely resembles the one in albigenic acid.[1]

Conversion of albigenin to a known hydrocarbon by removal of the oxygen functions by usual methods were not made owing to the paucity of the starting

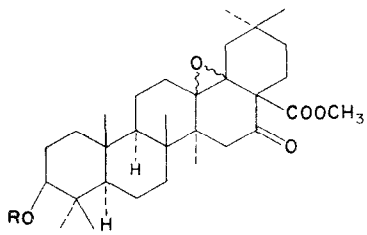
[1] A. K. Barua and S. P. Raman, *Tetrahedron* **7**, 19 (1959).
[2] D. H. R. Barton and P. De Mayo, *J. Chem. Soc.* 887 (1954).



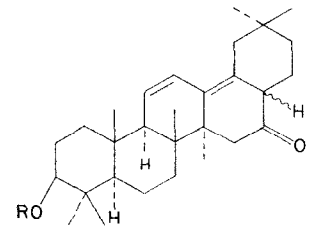
Ia R = H
Ib R = CH_3CO



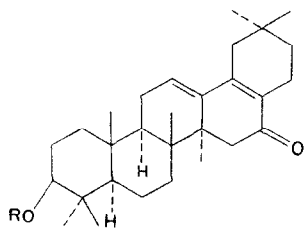
IIa R = $COCH_3$



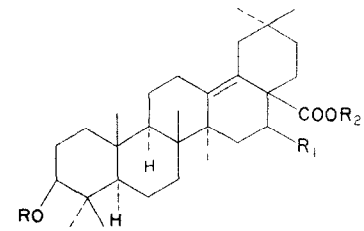
IIb R = $COCH_3$



III R = $COCH_3$



IV R = $COCH_3$



Va R = H; R_1 = OH; R_2 = H
Vb R = $COCH_3$; R_1 = O; R_2 = CH_3

material and due to the uncertainty of the resulting hydrocarbon since albigenin is a C_{29} triterpenoid. The nature of the carbon skeleton, together with the possible location of the double bond and the carbonyl function, was shown by a compartively simple reaction. Epoxides obtained from triterpenes of the oleanane series with unsaturation at 11:12, 13:18 or 18:19 are known to give the $\Delta^{11:12,\ 13:18}$ dienes on treatment with mineral acids.[3] Mineral acid fission of the epoxide of albigenin acetate (IIa) gives III and IV. The compound III shows triple ultra-violet absorption maxima at λ241·5, 252, and 257·5 mμ (log ε 4·1, 4·3 and 4·0) typical of the $\Delta^{11:12,\ 13:18}$ dienes of the β-amyrin series.[4,5] The presence of an $\alpha\beta$-unsaturated keto group with an extended conjugation in compound IV is shown by the sharp ultra-violet absorption maxima at λ299·5 mμ (log ε 4·1). The spectral properties of compounds III and IV clearly establish the validity of the structures assigned to these compounds.

The formation of the conjugated ketone (IV) is difficult to explain unless the usual

[3] L. Ruzicka, O. Jegger and J. Norymberski, *Helv. Chim. Acta* **25**, 457 (1942).
[4] D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.* 257 (1957).
[5] L. Ruzicka, G. Muller and H. Schellenburg, *Helv. Chim. Acta* **22**, 767 (1939).

$-CH_3$ group at the C_{17} position is replaced by hydrogen in the case of albigenin. As stated earlier, albigenin appears to be a C_{29}-compound instead of the usual C_{30} which can now be accounted for by the loss of the methyl group at C_{17} position. Such a loss is easily visualized as being the result of a decarboxylation of the corresponding β-keto acid and seems highly probable in view of the close association of albigenin with albigenic acid and echinocystic acid both of which are β-hydroxy acids. The experiments on the fission of the epoxide of albigenin acetate clearly indicate that albigenin is probably a nor-compound of the oleanane series having the structure Ia. The position C_{22} as the seat of the keto group is not considered on biogenetic grounds. An examination of the structures of albigenic acid (Va) and albigenin (Ia) suggests the possibility of the latter being formed in nature by the oxidation of the C_{16}-OH group in albigenic acid followed by decarboxylation. Finally, albigenin is correlated with albigenic acid along the following lines. On treatment with perbenzoic acid at 0°, 3-acetyl-16 keto methyl albigenate (Vb) gives the epoxide (IIb) which on mild hydrolysis followed by acetylation gives albigenin acetate oxide (IIa). This partial synthesis of albigenin acetate oxide from albigenic acid establishes the structure and stereochemistry of albigenin as Ia. No evidence has yet been obtained as regards the configuration of the H at C_{17} but it seems more likely to have the β-configuration.

This structure for albigenin contains a $\beta\gamma$-unsaturated keto group which is expected to rearrange to an $\alpha\beta$-unsaturated ketone under mild alkaline conditions but no such rearrangement takes place during the saponification of albigenin acetate, (vide experimental). It is interesting to consider whether albigenin, a nor-compound, occurs as such in the plant or is an artifact derived from the corresponding keto-ester during isolation. Model experiments in which 3-acetyl-16-keto methyl albigenate (Vb) was treated with acid under conditions of hydrolysis of the saponins shows that the keto-ester is unaffected.

Albigenin is of biogenetic interest as the first naturally occuring triterpenoid with a carbonyl function at the C_{16} position and as a nor-compound with a double bond at the more thermodynamically stable 13[18]:8 position.

EXPERIMENTAL

The m.p. are uncorrected and optical rotations are in chloroform solutions unless otherwise specified. Ultra-violet spectra were measured in ethanol solutions with a Beckmann DU instrument. The infra-red spectra were taken in a Baird IR spectrophotometer in $CHCl_3$ solutions. 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°.

Isolation of albigenin (1a). The air-dried pods of *A. lebbeck* Benth; containing the seeds (5 kg) after defatting with pet ether for 50 hr were Soxhleted for 72 hr with alcohol (90%) and the saponin obtained by ether precipitation was hydrolysed to give the neutral sapogenin fraction as described. The yield (0·012%) of the neutral sapogenin was obtained as a thick brown liquid. A benzene solution (30 cc) was adsorbed over acid-washed alumina (250 g) and elution with pet ether–benzene (1:1, 450 cc) gave solids m.p. 186–204°. Re-chromatography of this fraction, after one crystallization from methanol, over acid-washed alumina (100 g) and elution with benzene (250 cc) gave albigenin (Ia) crystallizing as sharp needles from methanol, m.p. 226–228°, $[\alpha]_D^{34°}$ − 114°. With tetranitromethane it gave a pale yellow colouration and a violet→pink colouration in the Liebermann–Buchardt reaction. It consumed exactly one mole of perbenzoic acid in 20 hr at 0° and there was no further up take after 15 days. The compound could be recovered unchanged from its acetic acid solution after shaking in an atmosphere of hydrogen in presence of platinum oxide catalyst at room temp and at atm. press. Albigenin was also quantitatively recovered after treatment with osmic acid in dry ether or pyridine solution for 7 days at room temp. (Found: C, 81·42, 81·51, 81·38; H, 10·89, 10·72, 10·78; M.W.

(Rast's) 414, 430, 421; $C_{29}H_{46}O_2$ requires: C, 81·63; H, 10·88%. M.W. 426·65; $C_{30}H_{48}O_2$ requires: C, 81·76; H, 10·98%. M.W. 440·68).

Albigenin acetate (Ib). Albigenin (Ia, 150 mg) was heated over a steam bath with acetic anhydride (5 cc) and pyridine (2 cc) for 3 hr. Pouring into crushed ice gave solids and a benzene solution of this (10 cc) was passed through a column of acid-washed alumina (50 g). Pet ether–benzene (1:3, 120 cc) eluted fractions which crystallized as colourless rectangular plates from methanol, (Ib, 120 mg), m.p. 211–212°, $[\alpha]_D^{30°}$ −101°. (Found: C, 78·94, 79·12; H, 10·13, 10·08; M.W. (Rast's) 460, 471; $C_{31}H_{48}O_3$ requires: C, 79·43; H, 10·32%. M.W. 468·69.) It gave a pale yellow colour with tetranitromethane and on hydrolysis with 2% alcoholic caustic potash at room temp for 8 hr albigenin was recovered quantitatively.

Albigenin benzoate. Albigenin (Ia, 100 mg) was dissolved in pyridine (3 cc) and heated over a steam bath for 1 hr after addition of freshly distilled benzoyl chloride (3 cc). A solution of the product in benzene (5 cc), was chromatographed over alumina (25 g). Pet ether–benzene (4:1, 80 cc) eluted the benzoate crystallizing as sharp needles from chloroform–methanol, m.p. 312–315°, $[\alpha]_D^{33°}$ −72°. (Found: C, 81·28, 81·41; H, 9·42, 9·47. $C_{36}H_{50}O_3$ requires: C, 81·46; H, 9·49%). The above benzoate (100 mg) was refluxed with alc caustic potash (2%; 25 cc) for 1 hr on a water bath and after purification by chromatography over acid-washed alumina furnished albigenin, m.p. 225–228° (63 mg).

Preparation of 2,4-dinitrophenylhydrazone of albigenin. To a solution of albigenin (Ia, 50 mg) in acetone free methanol (20 cc) was added a methanolic solution of 2,4-dinitrophenylhydrazine sulphate (8 mg of 2,4-dinitrophenylhydrazine dissolved in 2 cc of a 1:1 mixture of conc H_2SO_4 and H_2O) and kept aside for 1 hr. The pale yellow precipitate was washed repeatedly with water and crystallized from chloroform–methanol as yellow silky needles, m.p. 266–268° (dec). (Found: C, 69·4; H, 8·2; N, 9·4. $C_{35}H_{50}O_5N_4$ requires: C, 69·28, H, 8·30; N, 9·23%).

2,4-dinitrophenylhydrazone of albigenin acetate. Albigenin acetate (Ib, 100 mg) was dissolved in acetone free methanol (40 cc) and to this was added a methanolic solution of 2,4-dinitrophenylhydrazine sulphate (3 cc) and after ½ hr the fine orange red precipitate was purified by chromatography over acid washed alumina (5 g). The product could be eluted with pet ether–benzene (1:1, 25 cc) crystallizing as fine orange-red needles from chloroform–methanol m.p. 259–260°. (Found: C, 68·3; H, 8·1; N, 8·8. $C_{37}H_{52}O_6N_4$ requires: C, 68·49; H, 8·08; N, 8·63%).

Oxidation of albigenin to albigenone. A solution of albigenin (Ia, 200 mg) in dry pyridine (5 cc) was cooled and added slowly to a slurry of chromium trioxide–pyridine complex[6] (from 200 mg of CrO_3 and 10 cc of pyridine) kept below 10°. The mixture was kept at room temp overnight and then poured into crushed ice. The separated solids in benzene (8 cc) was passed through a column of acid-washed alumina (25 g). Benzene (70 cc) eluted, albigenone, the product crystallizing as colourless from ethanol (165 mg) m.p. 200–204°, $[\alpha]_D^{30°}$ −98°, λ_{max} 286 mμ (log ε 1·98). It gave a deep violet colour in the Zimmermann test while albigenin the parent compound developed no colour under identical conditions. Albigenone gave a pale yellow colouration with tetranitromethane in $CHCl_3$ solution and developed no colour with alcoholic ferric chloride. (Found: C, 82·0, 81·91, 81·86; H, 10·11, 9·98, 10·0. $C_{29}H_{44}O_2$ requires: C, 82·02, H, 10·44%).

Albigenin acetate oxide (IIa). A solution of albigenin acetate (Ib, 250 mg) in chloroform (15 cc) was treated with a chloroform solution of perbenzoic acid (1·5 N; 20 cc) and kept for 48 hr at 0°. Solid sodium carbonate (1 g) and water (50 cc) were added and the organic layer washed with water and dried. Distillation of the solvent left a white solid, a benzene solution of which (5 cc) after adsorption over a column of acid-washed alumina (25 g) could be eluted by pet ether–benzene (1:1, 150 cc) to give the epoxide of albigenin acetate (IIa), crystallizing as colourless sharp needles (190 mg) from chloroform–methanol m.p. 242–244°, $\alpha_D^{35°}$ −27°. It was saturated to tetranitromethane. (Found: C, 76·64; H, 9·72. $C_{31}H_{48}O_4$ requires: C, 76·81; H, 9·72%).

Fission of the epoxide of albigenin acetate. To albigenin acetate oxide (IIa, 200 mg) dissolved in glacial acetic acid (20 cc) conc hydrochloric acid (0·4 cc) was added and the solution refluxed on a sand bath for 3 hr. Distillation in vacuum left a residue which was taken up in ether (50 cc) and the ether extract washed with alkali (0·25%; 2 × 10 cc). Neutralization of the alkali layer gave no solids. The residue from the ether extract was dried by azeotropic distillation with benzene and acetylated using pyridine (1 cc) and acetic anhydride (2 cc) for 1 hr over a steam bath. The crude acetate after crystallization from methanol gave a compound with a m.p. range 192–226°. It gave a deep brown

[6] G. L. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *J. Amer. Soc.* **75**, 422 (1953).

colour with tetranitromethane. The impure acetate in benzene (5 cc) was adsorbed over a column of acid washed alumina (25 g). Pet ether (80 cc) eluted a fraction which after crystallization from aqueous methanol gave III (42 mg) m.p. 210–214°. It gave a yellow colour with tetranitromethane. (Found: C, 79·82; H, 10·12. $C_{31}H_{46}O_3$ requires: C, 79·75; H, 9·94%). Elution with pet ether–benzene mixture (3:1, 50 cc) gave solids crystallizing as fine needles from methanol, IV, (67 mg) m.p. 224–230°. (Found: C, 79·64; H, 9·68. $C_{31}H_{46}O_3$ requires: C, 79·75; H, 9·94%).

3-*acetyl*-16-*keto- methyl albigenate oxide* (IIb). To a solution of 3-acetyl-16-keto methyl albigenate (Vb, 180 mg) in chloroform (10 cc) freshly prepared perbenzoic acid in chloroform (1·56 N; 10 cc) was added and kept at 0° for 48 hr. The product obtained as in preparation of IIa could be purified by chromatography over alumina (10 g) and elution with pet ether–benzene (2:7, 110 cc). The epoxide (IIb; 104 mg) was obtained as colourless needles from methanol m.p. 272–276°: It gave no colouration with tetranitromethane. (Found: C, 72·68; H, 9·0. $C_{33}H_{50}O_6$ requires: C, 73·00; H, 9·28%).

Saponification of IIb: *Formation of albigenin acetate oxide* (IIa) *from* IIb. The epoxide (IIb, 100 mg) was heated with alcoholic caustic potash (3%; 25 cc) over a water bath for 2 hr. The alcohol was evaporated over a water bath while keeping the volume constant by addition of water. It was then cooled to 0° and carefully neutralized with hydrochloric acid (1%) avoiding excess acid. The precipitated solids were extracted with ether (50 cc) and the layer washed with cold alkali (1%; 2 × 15 cc). Neutralization of the alkali washings gave a small amount of solids, which were not further investigated. The ether layer was washed free from alkali, dried and distilled. The semi-solid residue was dried in vacuum over P_2O_5 and acstylated using pyridine (1 cc) and acetic anhydride (5 cc) at room temp for 5 hr. The crude compound which was purified by passing a benzene solution (5 cc) over acid washed alumina column (15 g). Elution with pet ether–benzene (1:1, 150 cc) gave solids crystallizing as fine needles from methanol. m.p. 240–244°: $[\alpha]_D^{35°}$ −27° which was saturated to tetranitromethane. This showed no change in m.p. or optical rotation when admixed with a sample of albigenin acetate oxide (IIa).

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