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TRITERPENOIDS—X

THE CONSTITUTION OF ALBIGENIC ACID—A NEW TRITERPENOID SAPOGENIN FROM *ALBIZZIA LEBBECK* BENTH.

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Abstract—The constitution of albigenic acid—a new triterpenoid sapogenin from the beans of *Albizzia lebbeck* Benth., has been established as 3β:16α-dihydroxyolean-13(18)-en-28-oic acid and its partial synthesis from echinocystic acid is described.

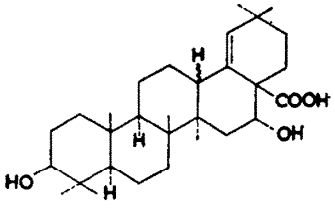
THE isolation of "albigenic acid" a new triterpene acid and echinocystic acid from the beans of *A. lebbeck* Benth., has been reported in an earlier communication.[1] Albigenic acid (Ia), $C_{30}H_{48}O_4$, gave a monomethyl ester (Ib), which formed a diacetate (Ic). It showed a violet → pink colouration in the Liebermann–Burchardt reaction. As albigenic acid was found to occur in association with echinocystic acid, which also has the same molecular formula, there seemed to be a close relationship between the two acids. Owing to its laevorotation, albigenic acid was thought to be a 3α-epimer of echinocystic acid, since it has generally been observed that the 3α-OH has a negative molecular rotatory contribution.[2] This possibility was overruled by the fact that the diketone obtained by the oxidation of methyl albigenate was different from methyl diketo echinocystate. Methyl diketoalbigenate gave a positive Zimmermann colour reaction proving the presence of 3-keto group.[3] Under comparable conditions the rate of hydrolysis of methyl albigenate was found to be almost the same as that of methyl echinocystate. The rate of hydrolysis of the triterpene esters of the oleanane series having the carboxyl group at the C_{17} position is usually very low whereas the presence of hydroxyl groups β to the carbomethoxyl group facilitates the hydrolysis.[3] That one of the hydroxyl groups is β to the carboxyl group is shown by the fact that methyl diketoalbigenate (VI) undergoes decarboxylation on saponification.

The fact that albigenic acid and its derivatives gave a pale yellow colouration with tetranitromethane and that methyl albigenate consumed only one mole of perbenzoic acid showed the presence of one double bond. The relative rapidity with which the uptake of perbenzoic acid was complete (24 hr) indicated that the ethylenic linkage in albigenic acid was not at the usual 12:13-position, in which case the consumption of the oxidant is very slow.[4] The double bond in albigenic acid, however, resisted hydrogenation under standard conditions in presence of platinum oxide catalyst and was not attacked by osmic acid. The rapid thermal decarboxylation of albigenic acid at its melting point indicated the possibility of the double bond being in the $\beta\gamma$-position to the carboxyl group, as $\beta\gamma$-unsaturated acids are known to be readily decarboxylated

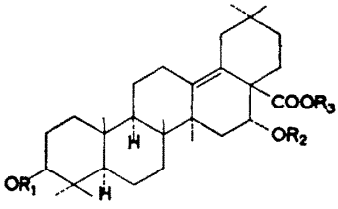
[1] A. K. Barua and S. P. Raman, *Sci. and Cult.* **23**, 435 (1958).
[2] W. Klyne and W. M. Stokes, *J. Chem. Soc.* 1979 (1954).
[3] D. H. R. Barton and P. De Mayo, *J. Chem. Soc.* 887 (1954).
[4] L. Ruzicka, H. Silbermann and M. Furter, *Helv. Chim. Acta* **15**, 482 (1932).

on pyrolysis.[5] Albigenic acid, unlike oleanolic acid, neither lactonized nor formed a bromolactone, under conditions used for the formation of the derivatives of oleanolic acid.[6]

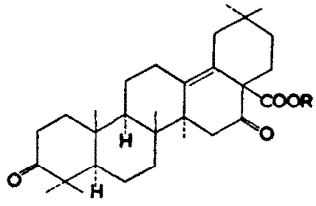
The first evidence bearing on the positions of the ethylenic double bond and the carboxyl group in albigenic acid came from the formation of a lactone diacetate identical with the 18-iso-echinocystic acid lactone diacetate (VII).



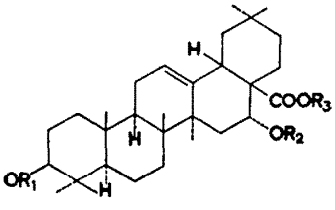
II



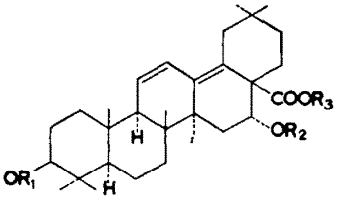
Ia, $R_1 = R_2 = R_3 = H$
Ib, $R_1 = R_2 = H$; $R_3 = CH_3$
Ic, $R_1 = R_2 = CH_3CO$; $R_3 = CH_3$
Id, $R_1 = CH_3CO$; $R_2 = H$; $R_3 = CH_3$



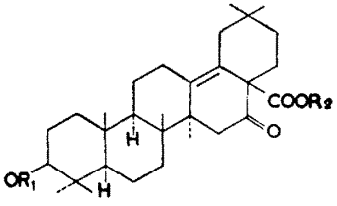
VI $R = CH_3$



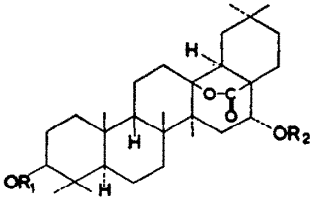
III $R_1 = R_2 = CH_3CO$
$R_3 = CH_3$



IVa, $R_1 = CH_3CO$; $R_2 = H$; $R_3 = CH_3$
IVb, $R_1 = R_2 = CH_3CO$; $R_3 = CH_3$



V $R_1 = CH_3CO$
$R_2 = CH_3$



VII $R_1 = R_2 = CH_3CO$

The above observations clearly show albigenic acid to be a dihydroxy pentacyclic triterpene acid of the oleanane series with the carboxyl group at the usual C_{17} position and closely related to echinocystic acid having the structure Ia or II. In order to decide between these structures, the compound (Ic) was prepared from echinocystic acid on the following lines. Oxidation of diacetyl methyl echinocystate (III) with

[5] D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.* 257 (1951).
[6] A. Winterstein and G. Stein, *Z. physiol. Chem.* **199**, 56, 64 (1931).

selenium dioxide in glacial acetic acid furnished IVa which showed triple ultra-violet absorption maxima at 244, 252 and 260·5, (log ε 4·4, 4·44, 4·26) typical of the $\Delta^{11:12,\ 13:18}$ dienes of the β-amyrin series.[5,7] Hydrogenation of this diene gave Id, which on acetylation furnished albigenic ester diacetate (Ic).

It is evident that in the formation of IVa from III the 16-acetyl group is eliminated. This is proved by the fact that Id on oxidation gave a keto compound V, which gives no colour in the Zimmermann reaction indicating the absence of a 3-keto group.[3] This clearly establishes the validity of the structures IVa, V and Id assigned to the respective compounds. The glassy mass obtained as one of the selinium dioxide oxidation products, on hydrogenation yielded diacetyl methyl albigenate (Ic) and hence should have the structure IVb. On the basis of this unequivocal partial synthesis the structure and stereochemistry of albigenic acid must be represented by Ia. The molecular rotation difference in the case of albigenic acid and echinocystic acid is −249° which is, however, far below the observed value for the shift of the double bond from 12:13- to 13:18-position in the oleanane series. (cf. δ-oleanolic acid,[8] δ-amyrin,[9] and δ-erythrodiol.)[5]

We believe that the recently reported[10] discrepancy in the properties of echinocystic acid isolated from the seeds of *A. lebbeck* Benth., is due to the non-homogeneity of the compound since we have observed that it is very difficult to separate echinocystic acid from this source, from the closely allied albigenic acid, the isolation of which has not been reported by the previous workers. We also encountered oleanolic acid in the sapogenin mixture.

That albigenic acid is not an acid-induced isomerization product[11] of echinocystic acid is borne out but the fact that echinocystic acid itself in model experiments was found to be unaffected under the conditions of the isolation process. Albigenic acid, incidentally, is the first example of a naturally occurring triterpene acid with the double bond at the more thermodyanamically stable 13:18-position and therefore is of biogenetic interest.

EXPERIMENTAL*

Isolation of sapogenins. Air-dried powdered beans of *A. lebbeck*† Benth. containing the seeds (1·5 kg) were Soxhleted for 50 hr with alcohol (90%) and the viscous dark brown residue, left on evaporation of the solvent *in vacuo*, was repeatedly washed with ether (5 × 500 cc). The brown ether insoluble gummy mass, which gave characteristic tests for saponins, was dissolved in alcohol (1 l., 70%) and refluxed for 4 hr after addition of conc HCl (200 cc). The alcohol was evaporated over a steam bath, the volume being kept constant by addition of water. The chocolate-coloured crude sapogenin thus obtained was extracted in a Soxhlet apparatus with ether for 40 hr and the ether extract washed with aqueous caustic soda (2%, 5 × 200 cc). The alkali washings on neutralization gave a voluminous white precipitate, (12·5 g).

* The m.p.s are uncorrected and were determined in a bisulphate bath. Optical rotations are in chloroform solutions unless otherwise specified. Ultra-violet spectra were measured in ethanol solutions with a Beckmann 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. Petroleum ether, b.p. 60–80°, was used for chromatography.

† We wish to thank Dr. S. K. Mukherjee, Keeper, Central National Herbarium, Sibpur, Calcutta, for botanical identification of the plant material.

[7] L. Ruzicka, G. Muller and H. Schellenberg, *Helv. Chim. Acta* **22**, 767 (1939).
[8] L. Ruzicka and O. Jegger, *Helv. Chim. Acta* **24**, 1236 (1941).
[9] O. Jegger, J. Norymberski and L. Ruzicka, *Helv. Chim. Acta* **27**, 1532 (1944).
[10] Ch. Sannie, H. Lapin and I. P. Varshney, *Bull. Soc. Chim. Fr.* 1440 (1957).
[11] G. Brounlie, M. B. E. Fayez, F. S. Spring, R. Stevenson and W. S. Strachan, *J. Chem. Soc.* 1377 (1956).

Methyl albigenate (Ib). The above acid sapogenin fraction was esterified in the usual way using excess diazomethane in ether solution and a benzene solution of the crude ester was adsorbed over a column of acid-washed alumina (400 g). Petroleum ether eluate (600 cc) gave a fraction which on several crystallizations from methanol gave a compound (1 g), $C_{31}H_{50}O_3$, m.p. 199–200°, $[\alpha]_D^{30}$ +73°, which showed no change in m.p. when admixed with an authentic sample of methyl oleanolate. Eluting the column with petroleum ether–benzene mixture (3 : 1, 20 l.) gave solids of m.p. 203–207°, (7 g), which on crystallization from methanol yielded needles, $C_{31}H_{50}O_4$, m.p. 212–214°, $[\alpha]_D^{30}$ +33·5° (ethanol 95%). This was shown to be identical with methyl echinocystate by direct comparison with an authentic sample.* Further elution of the column with the same solvent mixture (1 : 1, 5 l.) gave crystals, m.p. 219–224°, (0.9 g), which on repeated crystallizations from methanol furnished methyl albigenate (Ib), m.p. 225–226°, $[\alpha]_D^{27}$ −10°, (Found: C, 76·3, 76·73; H, 10·08, 10·45; OMe, 6·8; M.W. (Rast's) 480, 491; $C_{31}H_{50}O_4$ requires: C, 76·49; H, 10·35; OMe, 6·37% M.W. 486). Methyl albigenate in chloroform solution consumed exactly 1 mole of perbenzoic acid in 24 hr and there was no further uptake for 15 days. The ester could be recovered unchanged from its acetic acid solution after shaking for 12 hr, in an atmosphere of hydrogen in presence of platinum oxide catalyst at room temp and atmospheric pressure. The ester was also quantitatively recovered after treatment with osmic acid in dry ether or pyridine solution for 7 days at room temp.

Albigenic acid (Ia). Methyl albigenate (500 mg) was refluxed with an alcoholic solution of caustic potash (20%, 50 cc) for 3 hr and yielded an acidic fraction (68%) which on crystallization from chloroform methanol mixture gave albigenic acid (Ia), m.p. 246–248 (dec),† $[\alpha]_D^{31}$ −13° (ethanol). (Found: C, 75·99; H, 10·02; Eq. Wt. 468, 470. $C_{30}H_{48}O_4$ requires: C, 76·22; H, 10·24%; Eq. Wt. 472). The acid on esterification gave methyl albigenate (Ib). The neutral fraction obtained after saponification was found to be unreacted methyl albigenate. The rate of hydrolysis of methyl echinocystate under identical conditions was 62%. Albigenic acid in chloroform solution gave a pale yellow colouration with tetranitromethane and was recovered unchanged after treatment adequate for the formation of a lactone or bromolactone of oleanolic acid.[6]

Diacetyl methyl albigenate (Ic). Methyl albigenate (Ib, 500 mg) was heated with pyridine (5 cc) and acetic anhydride (5 cc) over a steam bath for 3 hr and a benzene solution of the product passed through a column of alumina (50 g). Benzene–petroleum ether (1 : 1, 250 cc) eluted fractions which crystallized from rectified spirit to give diacetyl methyl albigenate as plates, (Ic, 420 mg), m.p. 189–190°, $[\alpha]_D^{27}$ −54°. (Found C, 74·0; H, 9·68; AC, 13·98; $C_{35}H_{54}O_6$ requires: C, 73·64; H, 9·53; Ac, 15·04%). It gave a pale yellow colour with tetranitromethane in chloroform solution and on mild hydrolysis (5% alcoholic caustic potash) gave methyl albigenate.

Methyl diketoalbigenate (VI). A cold solution of methyl albigenate (Ib, 300 mg) in pyridine (10 cc) was added slowly to a slurry of chromium trioxide–pyridine complex[12] (from 400 mg of CrO_3 and 10 cc of pyridine) at −15°, and the mixture kept at room temp for 24 hr after which it was poured into crushed ice. The separated solids were filtered, washed repeatedly with hot water and dried. The product after adsorption over acid washed alumina (50 g) using benzene, was eluted by benzene–petroleum ether mixture (2 : 1, 200 cc) and on repeated crystallization from methanol gave the diketo-ester (VI) as sharp needles, m.p. 207–209°, $[\alpha]_D^{29}$ −156° (dioxane), λ_{max} 294 mμ (log ε 2·14). (Found: C, 77·16; H, 9·82. $C_{31}H_{46}O_4$ requires: C, 77·13; H, 9·61%). This compound gave a yellow colouration with tetranitromethane and a violet colour in the Zimmermann test. Mixed m.p. with an authentic sample of methyl diketoechinocystate (m.p. 167–168°) was 138–156°.

The diketoester (VI, 200 mg) did not furnish any acidic material on refluxing for 8 hr with alcoholic caustic potash (20%, 50 cc) but gave a neutral gummy product which could not, however, be obtained in a crystalline state.

Pyrolysis of albigenic acid(Ia). Albigenic acid (200 mg) was heated in a metal bath at 250° for 2 min when the evolution of CO_2 subsided. The cold melt in ether furnished an entirely neutral material as an oil which failed to crystallize even after repeated chromatography.

Lactone diacetate of albigenic acid (VII). A solution of albigenic acid (Ia, 300 mg) in glacial acetic acid (10 cc) was treated with hydrobromic acid (48%, 10 cc) and acetic anhydride (3 cc) and the

* We express our sincere thanks to Prof. Carl Djerassi of Wayne University, Michigan, U.S.A., for sending us an authentic sample of methyl echinocystate.

† The m.p. of albigenic acid recorded in the preliminary communication (*loc. cit*) was 233–236°, which however on prolonged drying over P_2O_5 in high vacuo rose to 246–248° (dec).

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

mixture kept at room temp for 5 days. The dark red solution was diluted with water, extracted with ether, washed with a saturated solution of sodium carbonate, dried and evaporated. A solution of the residue in benzene (10 cc) was chromatographed over alumina (50 g). Elution of the column with benzene gave a product, (220 mg) which gave no coloration with tetranitromethane, m.p. 281–282°, $[\alpha]_D^{28} -24°$. The m.p. of this compound when admixed with an authentic sample of 18-iso-echinocystic acid lactone diacetate was undepressed.[13] (Found: C, 73·1; H, 9·4. $C_{34}H_{52}O_6$ requires: C, 73·4; H, 9.4%).

Selenium dioxide oxidation of diacetyl methyl echinocystate (III). To a solution of diacetyl methyl echinocystate (m.p. 199–200°; $[\alpha]_D^{30} -16°$; 2 g) in glacial acetic acid (50 cc), freshly prepared and resublimed selenium dioxide (200 mg, 0·5 mole) was added and the solution refluxed for 17 hr on a sand-bath. The solution, after filtration of the separated red selenium and on pouring into water, gave solids, which were taken up in ether, washed with alkali, dried and evaporated. The residue in benzene (15 cc) was filtered through a column of alumina (200 g). Elution with petroleum ether–benzene mixture (2 : 1, 200 cc) gave (200 mg) m.p. 199–200°, of unreacted diacetate of methyl echinocystate. Petroleum ether–benzene mixture (1 : 1, 1 l.) eluted a fraction, as a glassy mass which showed no tendency to crystallize even after repeated chromatography. Benzene (500 cc) eluate gave a third fraction (IVa), crystallizing as colourless plates from dilute ethanol, m.p. 236–237°, $[\alpha]_D^{33} -134°$ (500 mg). It gave a yellow colour with tetranitromethane. λ_{max} 244, 252, 260 mμ (log ε 4·40, 4·44, 4·26 respectively). (Found: C, 75·0; H, 9·49. $C_{33}H_{50}O_5$ requires: C, 75·24; H, 9·56%).

Hydrogenation of the diene (IVa) *to* (Id). A solution of the diene (IVa, 400 mg) in glacial acetic acid (50 cc) was shaken with platinum oxide catalyst (300 mg) in an atmosphere of hydrogen at room temp and at atmospheric pressure, till one mole of the gas was consumed. Filtration of the catalyst and evaporation of the filtrate *in vacuo* left a residue, which after purification by chromatography over alumina, crystallized as shining leaflets, (Id, 380 mg) m.p. 205–206°, $[\alpha]_D^{32} -6°$, and showed no absorption maxima above λ 220 mμ. It gave a pale yellow colouration with tetranitromethane. (Found: C, 74·98; H, 9·82. $C_{33}H_{52}O_5$ requires: C, 74·96; H, 9·91%).

Oxidation of the above compound (Id) to (V). A solution of the compound (Id, 150 mg) in pyridine (5 cc) was added to a suspension of chromium trioxide–pyridine complex prepared from 200 mg of CrO_3 and 5 cc of pyridine at 15°, and the mixture kept for 24 hr. The product after chromatography over alumina, furnished colourless needles (V) from methanol, m.p. 251–253°, $[\alpha]_D^{28} -151°$ which showed no coloration in the Zimmermann test. (Found: C, 75·5, H, 9·48; $C_{33}H_{50}O_5$ requires: C, 75·24; H, 9·56%).

Acetylation of (Id) *to form diacetyl methyl albigenate* (Ic). 200 mg of the compound (Id) was heated over a steam-bath with pyridine (2 cc) and acetic anhydride (3 cc) for 3 hr. The product m.p. 181–188° (180 mg) was adsorbed on a column of alumina (25 g). Elution with benzene–petroleum ether mixture (1 : 1, 150 cc) gave needles crystallizing from dilute ethanol m.p. 189–190°, $[\alpha]_D^{28} -53°$, which showed no change in m.p. or specific rotation when admixed with a pure sample of diacetyl methyl albigenate (Ic).

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[13] P. Bilham and G. A. R. Kon, *J. Chem. Soc.* 552 (1941).