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TRITERPENOIDS—XXV

THE CONSTITUTION OF ENTAGENIC ACID—A NEW TRITERPENOID SAPOGENIN FROM *ENTADA PHASEOLOIDES* MERRILL

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Abstract—The constitution of entagenic acid—a new triterpenoid sapogenin isolated from the seed kernels of *Entada phaseoloides* Merrill—has been established as 3β,21α,22α-trihydroxyolean-12-ene-28-oic acid.

ENTAGENIC ACID, a new sapogenin isolated from the seed kernels of *Entada phaseoloides* Merrill, was shown to be either 3,15,16-trihydroxyolean-12-ene-28-oic acid or 3,21,22-trihydroxyolean-12-ene-28-oic acid.[1] The present paper gives the final structure.

Entagenic acid (Ia) forms a monomethyl ester (Ib) and a triacetate (Ic). It gives a pink → violet coloration in the Liebermann–Burchard test. The presence of one double bond in entagenic acid is shown by positive tetranitromethane colour test and by the consumption of nearly 1 mole of perbenzoic acid by methyl entagenate. The double bond resists hydrogenation in presence of Adam's catalyst under normal conditions. Entagenic acid, like oleanolic acid,[2] forms a monobromo-γ-lactone (II) with bromine in acetic acid. IR spectrum of the above compound shows a band at 1770 cm^{-1} for a γ-lactone. On the basis of these experiments, entagenic acid is considered to be a triterpene of the oleanane series having a 12:13 double bond and a carboxyl group at C-17.

The rate of saponification of methyl esters of triterpene acids of the oleanane series having a carboxyl group at C-17 position is usually very low whereas the presence of a hydroxyl group β to the carbomethoxyl group facilitates the hydrolysis.[3] In case of methyl echinocystate and methyl oleanolate the rate of saponification with ethanolic caustic potash (20%) for 3 hr has been found to be 60 and 5% respectively. Under comparable conditions the rate of saponification of methyl entagenate is 37%, and this suggests the presence of a hydroxyl group at β-position with respect to the carboxyl group in entagenic acid. Methyl entagenate consumes 1 mole of periodic acid thus showing the presence of an α-glycol system. The dialdehyde III obtained by the above process does not cyclize to give an α,β-unsaturated aldehyde[4] (IV) and this eliminates ring A as the possible site for the α-glycol system. Oxidation of methyl entagenate with CrO_3 in glacial acetic acid gives methyl entagentrione (V), which does not give any positive ferric chloride colour test but furnishes the diosphenol (VI,

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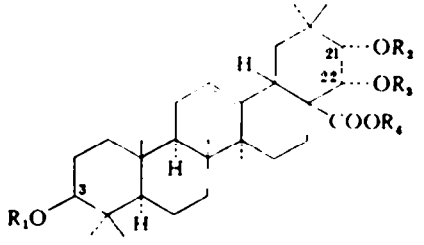
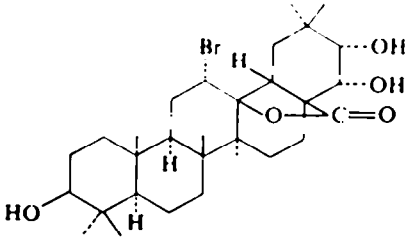
[3] D. H. R. Barton and P. De Mayo, *J. chem. Soc.* 887 (1954).
[4] R. B. Woodward, F. Sondheimer, T. David, K. Heusler and W. M. Mclamore, *J. Amer. Chem. Soc.* **74**, 4223 (1952).

UV(EtOH) 283 mμ, ε 12,060; UV(EtOH–KOH) 318 mμ, ε 9,263) on heating with alkali. The formation of ketone V which contains a non-enolizable α-diketo system requires the two hydroxyl groups of the α-glycol moiety in entagenic acid to be either at C-15 and C-16 or C-21 and C-22. Methyl entagentrione (V) responds to Zimmermann's colour reaction for 3-keto group[5] thus locating a secondary hydroxyl group at C-3 position in entagenic acid. Methyl entagentrione on Clemmensen reduction furnishes a hydrocarbon identical with "oleanene-III[5,6] (VII) obtained from either nor-echinocystenone or nor-echinocystendione.[7,8] On the basis of these observations entagenic acid may be represented either as 3,15,16-trihydroxyolean--12-ene-28-oic acid or 3,21,22-trihydroxyolean-12-ene-28-oic acid.

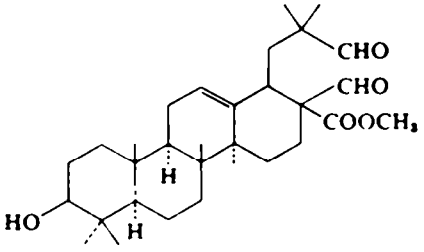


I

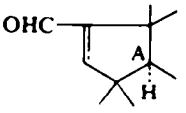


II

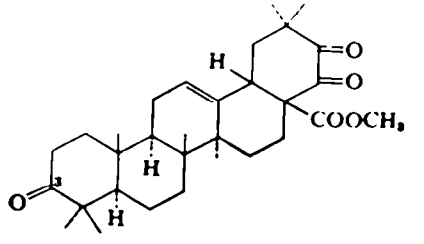
a, $R_1 = R_2 = R_3 = R_4 = H$
b, $R_1 = R_2 = R_3 = H$; $R_4 = CH_3$
c, $R_1 = R_2 = R_3 = COCH_3$; $R_4 = H$
d, $R_1 = COCH_3$; $R_2 = R_3 = H$; $R_4 = CH_3$



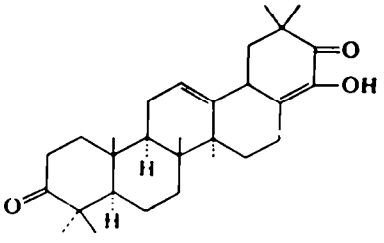
III



IV



V



VI

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[6] J. Simonsen and W. C. J. Ross, *The Terpenes* Vol. V, p. 278. Cambridge Univ. Press (1957).
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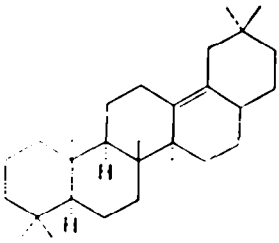
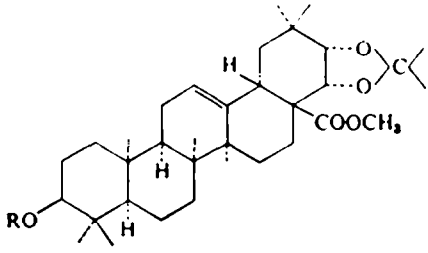


VII



VIII

a, R = H
b, R = $COCH_3$

Entagenic acid forms triacetate Ic on treatment with pyridine and acetic anhydride at 0°. The two hydroxyl groups of the α-glycol system are therefore at C-21 and C-22; the hydroxyl group at C-15, whether α or β, cannot be acetylated under the above condition.[9] Methyl entagenate forms an acetonide (VIIIa) which furnishes the acetate (VIIIb). Compound VIIIb on treatment with acid gives 3-acetyl methyl entagenate (Id). The molecular rotational difference[10] ($[M]_{Id} - [M]_{Ib} = -17°$) suggests the β-orientation of the C-3 hydroxyl group in entagenic acid. The fact that entagenic acid forms triacetate Ic under very mild conditions suggests the C-22 hydroxyl group to be α as the 22β-hydroxyl group is more resistant to esterification under the above conditions (cf. barringtogenol C[11]). Formation of acetonide VIIIa requires α-orientation of the C-21 hydroxyl group. Thus the structure and stereochemistry of entagenic acid may be represented as 3β,21α,22α-trihydroxyloean-12-ene-28-oic acid (Ia).

EXPERIMENTAL

The m.ps are uncorrected and were determined in a bisulphate bath. UV spectrum was measured in EtOH soln and optical rotations are in chf unless otherwise specified. Pet. ether, b.p. 60–80°, was used for chromatography unless mentioned otherwise.

Isolation of sapongenins. Air-dried powdered seed kernels of *Entada phaseoloides* Merrill (600 g) were defatted with pet. ether and then exhaustively extracted with hot EtOH (90%). The extract was concentrated under red. press. and ether was added to the concentrated extract. A dark brown gum precipitated which gave characteristic tests for saponins. The gum was dissolved in 90% EtOH (700 ml) and refluxed for 5 hr after addition of conc. HCl (140 ml). The EtOH was removed over a steam bath, the volume being kept constant by addition of water. The chocolate-coloured crude sapogenin thus obtained was collected and extracted in a Soxhlet apparatus with ether for 12 hr. The extract was washed with 5% NaOHaq (3 × 200 ml). The alkali washings on neutralization gave a white ppt which was filtered and dried (3·36 g). The neutral ether layer gave negligible amount of residue.

The above acid sapogenin fraction was esterified in the usual way using diazomethane in ether soln. The crude ester was dissolved in benzene (30 ml) and adsorbed over a column of Brockmann's alumina (150 g). Pet. ether eluate (700 ml) gave a fraction which on crystallizations from MeOH gave a comp (310 mg), m.p. 199–200°, $[\alpha]_D^{30°}$ +73°, which showed no depression in m.p. when admixed with an authentic sample of methyl oleanolate. (Found: C, 79·2; H, 10·5. $C_{31}H_{50}O_3$ requires: C, 79·1; H, 10·7%.) It formed an acetate, m.p. 220°, $[\alpha]_D^{30°}$ +69°, which did not depress the m.p. of an authentic sample of acetyl methyl oleanolate (Found: C, 77·1; H, 10·4. $C_{33}H_{52}O_4$ requires

[9] C. Djerassi, C. H. Robinson and D. B. Thomas, *J. Amer. Chem. Soc.* **78**, 5687 (1956).
[10] W. Klyne and W. M. Stokes, *J. Chem. Soc.* 1979 (1954).
[11] A. K. Barua and P. Chakrabarti, *Tetrahedron* **21**, 381 (1965).

C, 77·3; H, 10·2%.) Further elution of the column with benzene–ether (5:1, 1·5 l.) gave a fraction which was crystallized from benzene–pet. ether (b.p. 40–60°) and then from MeOH, methyl entagenate Ib, m.p. 251–252°, $[\alpha]_D^{30°}$ +38°. (Found: C, 74·2; H, 10·0; M.W. (Rast's) 498. $C_{31}H_{46}O_6$ requires: C, 74·1; H, 10·0%; M.W. 502.)

Compound Ib in chf soln consumed 0·91 mole of perbenzoic acid within 10 days and there was no further uptake for 15 days. The ester could be recovered unchanged from its AcOH soln after shaking for 10 hr, in an atm of H_2 in presence of PtO_2 at room temp and atm. press.

Compound Ib (1 g) was saponified with 20% alcoholic KOH (50 ml) for 3 hr whereby an acidic fraction (370 mg) was obtained which on crystallization from EtOH (95%) gave entagenic acid (Ia), m.p. 310–315° (dec); $[\alpha]_D^{28°}$ +35° (EtOH). (Found: C, 73·8; H, 9·9. $C_{30}H_{44}O_6$ requires: C, 73·8; H, 9·8%.) Re-esterification of Ia with diazomethane furnished Ib. Both Ia and Ib were found to be homogeneous in TLC experiments.

Entagenic acid triacetate (Ic). Pure Ia (1 g) was dissolved in pyridine (8 ml) and Ac_2O (12 ml) and heated on a steam bath for 1½ hr. It was then poured onto crushed ice and the ppt was washed with water and dried. The product could not be crystallized from any solvent. It was purified by repeated precipitation from EtOH with water, m.p. 192°, $[\alpha]_D$ +6° (EtOH).

Compound Ic could also be obtained when acetylation was carried out at 0°. (Found: C, 70·4; H, 9·0. $C_{36}H_{50}O_9$ requires: C, 70·4; H, 8·8%.)

Bromo-lactone (II) *of* Ia. A soln of Ia (500 mg) and AcONa (2 g) in AcOH (4·5 ml) and water (5 ml) was treated dropwise with 3% Br_2 in AcOH (15 ml). The mixture was shaken for a few min and left for 2 hr at room temp after which it was poured onto water containing $Na_2S_2O_3$ when white crystalline mass separated. It was collected and washed with 10% K_2CO_3 (100 ml) and then with water. The product was repeatedly crystallized from glacial AcOH, m.p. 263–264°. (Found: C, 63·1; H, 8·5. $C_{30}H_{47}O_6Br$ requires: C, 63·5; H, 8·3%.)

Periodic acid oxidation of Ib. Compound Ib (76 mg) was dissolved in MeOH (10 ml), 0·5 M periodic acid aq (5 ml) was added and left for 24 hr. A blank experiment was performed side by side. Excess periodic acid was titrated with sodium arsenite soln. Consumption of periodic acid per mole of ester was found to be 1 mole.

Compound Ib (550 mg) was dissolved in MeOH (50 ml) and to this was added a soln of periodic acid (1 g) in water (8 ml) and left at room temp for 16 hr. The mixture was diluted with water when fine colourless product separated. It was filtered, washed with Na_2CO_3 soln and then with water. The product was crystallized from MeOH, III, m.p. 195°, $[\alpha]_D^{24°}$ −11·7°. (Found: C, 74·5; H, 9·6. $C_{31}H_{46}O_6$ requires: C, 74·4; H, 9·6%.) The above dialdehyde III gave positive Angeli Rimini test.

A soln of III (180 mg) in dry benzene (20 ml) and glacial AcOH (3 drops) and piperidine (2 drops) was heated on an oil bath (60°) for 1 hr in N_2 atm. The product which was obtained did not show any characteristic absorption for α,β-unsaturated aldehyde in the UV spectrum.

Preparation of acetonide VIIIa. Compound Ib (500 mg) was dissolved in acetone (5 ml) and 3 drops of conc. HCl were added. Within 15 min fine needle-shaped crystals separated, which were recrystallized from acetone, m.p. 207–208°. (Found: C, 75·1; H, 10·0. $C_{34}H_{54}O_6$ requires: C, 75·3; H, 10%.)

3-*Acetyl methyl entagenate* (Id). Compound VIIIa (500 mg) was dissolved in pyridine (5 ml) and Ac_2O (5 ml) and left at room temp overnight. The product (VIIIb) was worked up in the usual way and crystallized from MeOH, m.p. 212–214°. (Found: C, 74·1; H, 9·5. $C_{34}H_{54}O_6$ requires: C, 73·9; H, 9·7%.) Compd VIIIb (250 mg) was dissolved in MeOH (5 ml) and refluxed on steam bath for 45 min after addition of HCl (1 ml). The mxiture was poured onto crushed ice and the precipitate was washed with water and then crystallized from MeOH, Id, m.p. 262–265°, $[\alpha]_D^{31°}$ +32°. (Found: C, 72·5; H, 9·5; Ac, 8·1. $C_{33}H_{48}O_6$ requires: C, 72·7; H, 9·6; A, 7·9%.)

Methyl entagentrione (V). To a soln of Ib (3 g) in glacial AcOH (150 ml) and water (15 ml) was added dropwise at room temp a soln of CrO_3 (1·8 g) in water (15 ml) and glacial AcOH (65 ml) during 25 min with constant stirring which was continued for 1½ hr. Excess CrO_3 was destroyed with MeOH and the solvent distilled under red. press. The residue was treated with dil. H_2SO_4 (100 ml) and then extracted with ether. The ethereal layer was washed with 5% NaOHaq and then with water and dried (Na_2SO_4). On removal of solvent fine yellow crystals were obtained (1·9 g), which were dissolved in benzene and the soln was filtered through a column of alumina. The residue obtained by removal of the solvent was crystallized MeOH, V, m.p. 212°, $[\alpha]_D^{27°}$ −9·5°. (Found: C, 74·9; H, 8·8. $C_{31}H_{44}O_5$ requires: C, 75·0; H, 8·9%.)

Compound V formed an orange coloured mono-2:4-dinitrophenyl hydrazone which was crystallized from AcOEt–EtOH m.p. 225–226°. (Found: C, 65·6; H, 7·0; N, 8·2. $C_{37}H_{48}O_6N_4$ requires: C, 65·7; H, 7·1; N, 8·3%.)

Saponification of V *to diosphenol* (VI). Compound V (500 mg) was refluxed with 5% ethanolic KOH (20 ml) for 5 hr on a steam bath. The mixture was then diluted with water and acidified with dil. HClaq. The ppt was taken up in ether and the ethereal layer washed with water and dried (Na_2SO_4) Removal of the solvent gave a yellowish solid residue which was taken up in benzene (50 ml) and filtered through a column of Brockmann's alumina. The solvent was removed and the residue crystallized from benzene–pet. ether (b.p. 40–60°) and then from EtOH VI, m.p. 209–210°. (Found: C, 79·4; H, 9·5. $C_{30}H_{48}O_3$ requires: C, 79·5; H, 9·6%.)

Clemmensen reduction of V *to oleanene* III (VII). To a soln of V (2·7 g) in 95% EtOH (180 ml), Zn amalgam (36 g) was added and the mixture refluxed on a steam bath. A slow stream of HCl was passed through the soln continuously. The refluxing was continued for 26 hr and then the mixture was poured onto water (300 ml). The ppt was filtered and the residue extracted with ether The ethereal layer was washed with water and dried (Na_2SO_4). The residue obtained after removal of solvent was repeatedly crystallized from MeOH and then from acetone, m.p. 223–224°, $[\alpha]_D$ +30·8° (toluene). (Found: C, 87·8; H, 12·1. $C_{30}H_{48}$ requires: C, 87·9; H, 12·1%.) The above hydrocarbon did not depress the m.p. of "oleanene-III" kindly supplied by Prof. C. R. Noller of Stanford Univ. Cal. U.S.A.

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