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THE CONSTITUTION OF SPERGULAGENIC ACID

A NEW SAPOGENIN FROM *MOLLUGO SPERGULA*

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Abstract—The constitution of spergulagenic acid—a new triterpenoid sapogenin has been established as 3β-hydroxyolean-12-ene-28, 29-dioic acid).

ISOLATION of two new triterpenoid sapogenins, spergulagenin A and spergulagenic acid, has been reported earlier.[1,2] In the present paper,* the complete structure and stereochemistry of spergulagenic acid have been described.

Spergulagenic acid (Ia) is a monohydroxy dicarboxylic acid. It forms a dimethyl ester (Ib) and a monoacetyl dimethyl ester (Ic). On LAH reduction, dimethyl spergulagenate (Ib) furnishes a triol (II).

The presence of a trisubstituted double bond in spergulagenic acid is evident from the terminal absorption at 208 mμ (log ε, 3·7) and by the consumption of one mole of perbenzoic acid by dimethyl spergulagenate and its acetate. The hindered double bond in spergulagenic acid resists catalytic hydrogenation under normal conditions. Selenium dioxide oxidation of Ic leads to a hetero-annular diene (III) which shows triple UV absorption maxima at 242, 250 and 260 mμ (log ε, 4·27, 4·32 and 4·13), typical of the $\Delta^{11:12,13:18}$-dienes of the oleanane series. On the basis of the above experiments, spergulagenic acid is a triterpene of the oleanane series with a 12:13 bouble bond.[2]

Oxidation of acetyl dimethyl spergulagenate (Ic) with CrO_3 and glacial acetic acid under reflux furnishes an α,β-unsaturated ketone (IV, λ_{max} 249 mμ, log ε, 4·1). Dimethyl spergulagenate (Ib), on oxidation with CrO_3–pyridine complex, furnishes a colourless ketone (V) which responds to Zimmermann's colour test for a 3-keto group.[3] The almost quantitative reduction of V to dimethyl spergulagenate (Ib) by sodium borohydride and also the molecular rotational differences[4] ($[M]_{(Ic)} - [M]_{(Ib)} = -2°$; $[M]_{(V)} - [M]_{(Ib)} = +59·5°$) establish the equatorial (β) orientation of the C-3 hydroxyl group.

Djerassi *et al.*[5] have made a comparative study of the rate of saponification of some triterpene methyl esters having the normal Δ^{12}-oleanane skeleton and observed that the angular carbomethoxyl groups are more hindered than those attached to either C-4 or C-20. Dimethyl spergulagenate (Ib) on saponification under comparable conditions[5] (10% alcoholic caustic potash) furnishes a dicarboxylic monomethyl ester

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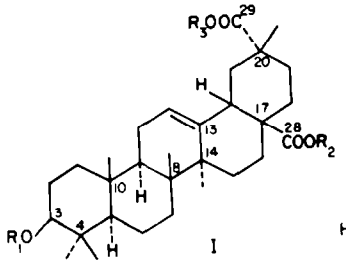
[1] P. Chakrabarti and A. K. Barua, *Indian J. Chem.* 2, 339 (1964)
[2] P. Chakrabarti, D. K. Mukherjee, R. Chatterjee and A. K. Barua, *Indian J. Chem.* 3, 283 (1965).
[3] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.* 887 (1954).
[4] D. H. R. Barton and E. R. H. Jones, *J. Chem. Soc.* 659 (1944).
[5] C. Djerassi and H. G. Monsimer, *J. Amer. Chem. Soc.* 79, 2901 (1957).

(Id, yield 99%). The above product (Id) can also be obtained in 42% yield by saponification with 1% alcoholic caustic potash for 3 hr. The compound (Id) on further saponification with caustic potash in ethylene glycol under reflux gives the dicarboxylic acid (Ia). The differential behaviour of the two carbomethoxyl groups in dimethyl spergulagenate towards saponification would locate the less hindered ester group either at C-4 or C-20 and the more hindered one at any one of the angular positions, 8, 10, 14 or 17. Spergulagenic acid, like oleanolic acid,[6] forms a monobromo-γ-lactone (VI) with bromine in acetic acid and this would normally locate one of the carboxyl groups at C-17. Such a bromolactone formation is, however, not impossible, both mechanistically and stereochemically, if the carboxyl group is attached to C-8 but this is considered to be less probable biogenetically as no triterpene having a carboxyl or any oxygen function attached to C-8 has yet been reported.

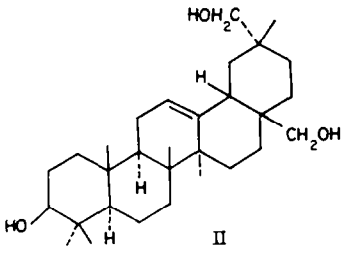
On the basis of the above analysis, Ia is probably the structure of spergulagenic acid. But the above conclusion which is based on a process of elimination needs confirmation by correlating spergulagenic acid to a known triterpene or degradation product. This has been achieved by taking advantage of the differential behaviour of the two carbomethoxyl groups in dimethyl spergulagenate towards saponification thus enabling selective conversion of C-20 carboxyl group to methyl leading to oleanolic acid along the following lines.

The acetyl derivative (Ie) of dicarboxylic monomethyl ester (Id) converted to its acid chloride was subjected to Rosenmund reduction. The aldehyde (VII), thus obtained, was directly reduced by the Wolff-Kishner method to oleanolic acid (VIIIa) identified through its methyl ester (VIIIb) and the corresponding acetate (VIIIc).

Thus the structure and stereochemistry of spergulagenic acid is established except



I



II

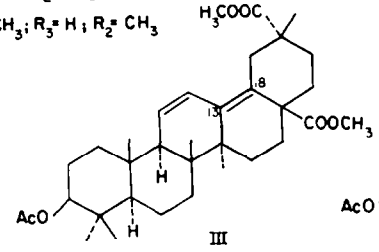
a, $R_1 = R_2 = R_3 = H$

b, $R_1 = H$; $R_2 = R_3 = CH_3$

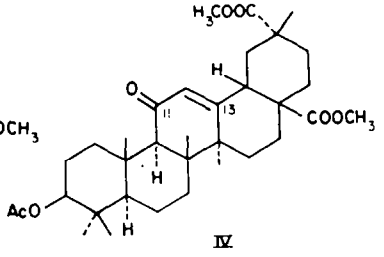
c, $R_1 = COCH_3$; $R_2 = R_3 = CH_3$

d, $R_1 = R_3 = H$; $R_2 = CH_3$

e, $R_1 = COCH_3$; $R_3 = H$; $R_2 = CH_3$



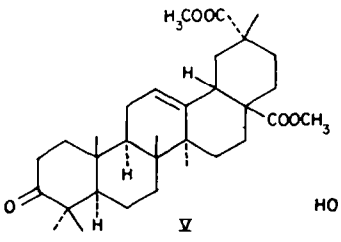
III



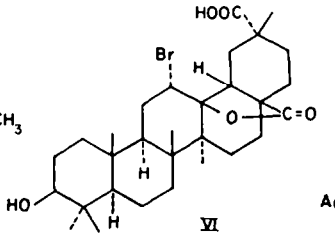
IV

[6] A. Winterstein and G. Stein, *Z. Physiol. Chem.* **199**, 56, 64 (1931).

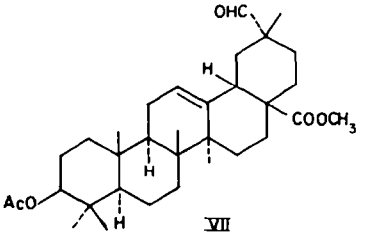
for the configuration of the carboxyl group at C-20, which should be equatorial (α, C-29) due to the ease of saponification of the carbomethoxyl group attached to C-20 in dimethyl spergulagenate and this finds a close parallel in methyl katonate[7] (IX). The axial (β, C-30) carbomethoxyl group is definitely more resistant to saponification (cf. methyl glycerrhetate[5]). The above conclusion was further corroborated by the fact that 3-keto dimethyl spergulagenate (V) and dicarboxylic acid monomethyl ester (Id) differ from the Xb and Xc respectively, prepared from queretaroic acid[8] (Xa) where oxygenation at C-30 has been established definitely. Consequently the structure and stereochemistry of spergulagenic acid is established as 3β-hydroxyolean-12-ene-28,29-dioic acid (Ia).



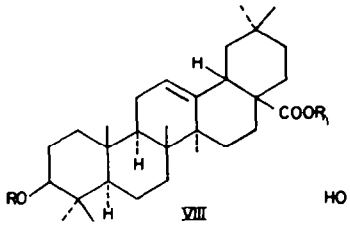
V



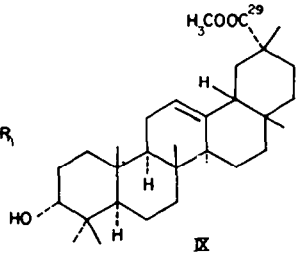
VI



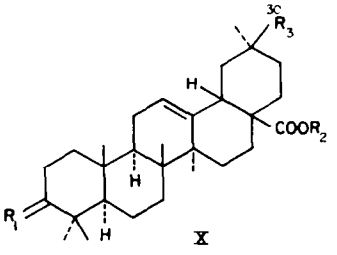
VII



VIII



IX



X

a, R = R$_1$ = H

b, R = H; R$_1$ = CH_3

c, R = $OOCH_3$ R$_1$ = CH_3

a, R =  ; R$_2$ = H, R$_3$ = CH_2OH

b, R$_1$ = O, R$_2$ = CH_3; R$_3$ = $COOCH_3$

c, R$_1$ =  , R$_2$ = CH_3, R$_3$ = COOH

EXPERIMENTAL

The m.ps are uncorrected and recorded in a bisulphate bath. Pet. ether used throughout had b.p. 60–80°. Brockmann's alumina (S. Merck) was used for chromatography and acid-washed alumina refers to Brockmann's alumina deactivated with 5% of 10% acetic acid unless noted otherwise. Optical rotations are in $CHCl_3$ and UV absorption spectra in EtOH solution.

11-*Keto acetyl dimethyl spergulagenate* (IV). A boiling solution of 1c (200 mg) in glacial acetic acid (25 ml) was treated dropwise with a solution of CrO_3 (200 mg) in acetic acid (85%, 10 ml). The mixture was refluxed for 1½ hr and then poured onto crushed ice. The product was dissolved in benzene (5 ml) and chromatographed over deactivated alumina (5 g). Elution with pet. ether–benzene (1:1, 250 ml) gave a product which crystallized from $CHCl_3$–MeOH as needles, m.p. 293–295°, $[\alpha]_D^{25.5°}$ +118°. (Found: C, 71·74; H, 8·60. $C_{34}H_{50}O_7$ requires: C, 71·58; H, 8·77%.)

3-*Keto dimethyl spergulagenate* (V). A cold solution of Ib (400 mg) in dry pyridine (10 ml) was added to a slurry of CrO_3–pyridine complex (500 mg CrO_3, 15 ml dry pyridine) kept at 0°. The mixture was kept overnight at room temp and then poured onto crushed ice. The crude product was

[7] F. E. King and J. W. W. Morgan, *J. Chem. Soc.* 4738 (1960).

[8] C. Djerassi, J. A. Henry, A. J. Lemin, T. Rios and G. H. Thomas, *J. Amer. Chem. Soc.* **78**, 3783 (1956).

dissolved in benzene (10 ml) and adsorbed on a column of deactivated alumina (10 g). Elution with pet. ether–benzene (3:1, 400 ml) gave a colourless glassy material which was crystallized from $CHCl_3$–MeOH as needles (300 mg), m.p. 204–206°, $[\alpha]_D^{33°}$ +113°. (Found: C, 74·72; H, 9·44. $C_{32}H_{48}O_5$ requires: C, 75·0; H, 9·37%.)

A solution of V (100 mg) in dry dioxan–MeOH (1:1, 30 ml) was treated with excess KBH_4 (500 mg), and left overnight at room temp. The product was crystallized from $CHCl_3$–benzene, m.p. 235–237° (94 mg). It did not depress the m.p. of dimethyl spergulagenate when admixed.

A portion (100 mg) of V was refluxed with methanolic KOH (20%, 30 ml) for 5 hr. Then the mixture was diluted with cold water, acidified and the precipitate, filtered off. The crude product was esterified with diazomethane. The ester was purified by chromatography over acid-washed alumina and only 3-keto dimethyl spergulagenate, m.p. 204–206° (89 mg) obtained.

Bromolactone (VI) *of spergulagenic acid* (Ia). To a solution of spergulagenic acid (200 mg) and sodium acetate (800 mg) in glacial acetic acid (20 ml) was added dropwise a solution of Br_2 in acetic acid (3%, 6 ml). It was kept at room temp for 2 hr and the mixture then poured into water containing sodium thiosulphate to discharge excess Br_2. The precipitate was filtered off, washed thoroughly with water and dried. Crystallization from MeOH (charcoal) gave shining flakes, m.p. 298–300°, $[\alpha]_D^{26°}$ +118°. (Found: C, 64·20; H, 7·86. $C_{30}H_{45}O_5$ Br requires: C, 64·3; H, 7·96%.)

Partial saponification of dimethyl spergulagenate (Ib) *to the dicarboxylic acid monomethyl ester* (Id)

(a) Dimethyl spergulagenate (Ib, 1 g) was refluxed with alcoholic KOH (10%, 400 ml) for 8 hr. Alcohol was removed on a steam bath keeping the volume constant by addition of water. The aqueous alkaline layer was washed with ether and acidified yielding a precipitate which was extracted with ether. Residue (Id, 990 mg), obtained on evaporation of solvent, crystallized from MeOH (charcoal), m.p. 292–294°, $[\alpha]_D^{32°}$ +97°. (Found: C, 74·29; H, 9·72; OCH_3, 6·5. $C_{31}H_{48}O_5$ requires: C, 74·4; H, 9·6; OCH_3, 6·2%.) The acid (Id) on esterification with diazomethane gave dimethyl spergulagenate.

(b) Dimethyl spergulagenate (Ib, 200 mg) was refluxed with alcoholic KOH (1%, 50 ml) for 3 hr. The reaction product was worked up as described in (a). The neutral fraction (110 mg) on crystallization from $CHCl_3$–benzene yielded unreacted dimethyl spergulagenate. The acidic fraction (84 mg) on crystallization from MeOH (charcoal) yielded a product, m.p. 292–294° which did not depress the m.p. of (Id) when admixed.

Acetyl derivative (Ie) *of dicarboxylic acid monomethyl ester* (Id). The Id (650 mg) was heated with dry pyridine (8 ml) and acetic anhydride (5 ml) on a steam-bath for 3 hr. The product after repeated crystallization from $CHCl_3$–MeOH yielded shining crystals (Ie, 550 mg), m.p. 260–263°, $[\alpha]_D^{32°}$ +94°. (Found: C, 73·21; H, 9·20. $C_{33}H_{50}O_6$ requires: C, 73·06; H, 9·23%.)

Rosenmund reduction of the acid chloride of (Ie) *to* (VII). Compound Ie (500 mg) was heated with freshly distilled thionyl chloride (3 ml) over a steam bath for 1 hr. The mixture was cooled to room temp, dry xylene (40 ml) added and the solvent removed under red. pressure.

The crude acid chloride was dissolved in dry xylene (120 ml) and catalyst (Pd–$BaSO_4$, 0·6 g) and catalyst poison (quinoline-sulphur, 0·075 ml) were added to the above solution. Dry H_2 gas was passed through this mixture for 3½ hr which was vigorously stirred keeping the temp at 120–130°. The mixture was left overnight and then filtered. Filtrate was distilled under red. pressure. The residue thus obtained was extracted with ether. Removal of solvent gave the crude VII which was not purified but directly reduced by the Wolff-Kishner method.

Wolff-Kishner reduction of (VII) *to oleanolic acid* (VIII). The crude VII was dissolved in diethylene glycol (30 ml) and absolute alcohol (55 ml) and the mixture refluxed with hydrazine hydrate (85%, 15 ml) for 1½ hr. Then solid KOH (6 g) was added and the mixture concentrated by distillation until the temp was 198–200° where it was maintained for 5 hr. The reaction product after acidification was extracted with ether and then esterified with diazomethane. Repeated chromatography of the crude product gave two fractions (i) m.p. 198–200° and (ii) m.p. 235–237°. The fraction (ii), m.p. 235–237°, was identified as dimethyl spergulagenate (Ib). The fraction (i) was crystallized from MeOH as needles, (VIIIb, 150 mg) m.p. 198–200°, $[\alpha]_D$ +72°. (Found: C, 79·23; H, 10·55. $C_{31}H_{50}O_3$ requires: C, 79·09; H, 10·71%.)

With pyridine (1 ml) and acetic anhydride (1 ml) the fraction (i) formed a monoacetate (VIIIc) m.p. 218–220°, $[\alpha]_D$ +69°. (Found: C, 77·01; H, 10·34. $C_{33}H_{52}O_4$ requires: C, 77·29; H, 10·23%.)

The above VIIIb and VIIIc did not depress the m.ps of authentic samples of methyl oleanolate and its acetate respectively.

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