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Studies on the Constitution of Spergulagenic Acid. A New Sapogenin from *Mollugo Spergula*

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Spergulagenic acid, a new triterpenoid acid sapogenin, has been isolated for *Mollugo spergula*. It is a monohydroxy dicarboxylic acid having the molecular formula $C_{30}H_{46}O_3$. It has been shown to be a pentacyclic triterpene of the oleanane group, having a typical 12,13-double bond.

In connection with our work on *Mollugo spergula*, the isolation of two new triterpenoid sapogenins, namely, spergulagenin[1] and spergulagenic acid[2], was reported. The present communication deals with our study on spergulagenic acid.

The bitter glycosidic mixture, obtained from the ethanolic extract of the defatted plant material, furnished both acidic and neutral aglycones on acid hydrolysis. The crude acidic fraction showed positive colour reaction with ferric chloride, showing presence of phenolic constituents. Crystallisation from methanol, however, afforded a colorless crystalline compound, m.p. 280-300°, which did not respond to ferric chloride test. Thin-layer chromatography over silica gel G showed the above product to be a mixture. Hence it was esterified with ethereal diazomethane and subsequent chromatographic resolution over acid-washed alumina furnished a fraction, m.p. 235-37°, which was crystallised from chloroform-benzene mixture; the compound $C_{32}H_{50}O_5$ melts at 235-37°, $[\alpha]_D^{32}+101.3°$ ($CHCl_3$). The homogeneity of this fraction was checked by thin-layer chromatography over silica gel G. Vigorous saponification with caustic potash and ethylene glycol[3] furnished a *dicarboxylic acid*, $C_{30}H_{46}O_3$, m.p. 347-50° (decomp.), $[\alpha]_D^{26.5}+121.2°$ (py). This acid appeared to be a new triterpenoid sapogenin and was named as spergulagenic acid. Re-esterification of spergulagenic acid with diazomethane regenerated dimethyl spergulagenate.

Both spergulagenic acid and its methyl ester developed a pink → violet colour in the Burchard-Lieberman test. The IR spectrum of dimethyl spergulagenate showed bands at 3500 cm^{-1} (hydroxyl group) and 1715 cm^{-1} (methoxycarbonyl group). On heating with acetic anhydride and pyridine, dimethyl spergulagenate afforded a monoacetate, $C_{34}H_{52}O_6$, m.p. 285-87°. Thus all the oxygen functions in spergulagenic acid were accounted for as forming part of one hydroxyl and two carboxyl groups. This was further supported by the formation of a crystalline triol, $C_{30}H_{50}O_3$, m.p. 278-80°, on lithium aluminium hydride reduction of dimethyl spergulagenate.

Both dimethyl spergulagenate and its acetate developed a pale yellow colour with tetranitromethane and consumed slowly one mole of perbenzoic acid, thus indicating the presence of one double bond. The UV spectrum of dimethyl spergulagenate showed a

1. Chakrabarti and Barua, this *Journal*, 1965, **42**, 137.
2. Chakrabarti *et al.*, *Ind. J. Chem.*, 1965, **3**, 283.
3. Djerassi *et al.*, *J. Amer. Chem. Soc.*, 1955, **77**, 3579.

terminal absorption at 208 mμ (ε, 4,996) for a tri-substituted double bond. The double bond is hindered as it could not be hydrogenated over Adams' catalyst under normal conditions.

Spergulagenic acid, having the molecular formula $C_{30}H_{46}O_5$ with one hydroxyl and two carboxyl groups and a double bond, must be a pentacyclic triterpene. The molecular rotation difference between acetyl dimethyl spergulagenate and dimethyl spergulagenate (Δ_1, *i.e.* $[M]_{OAc}-[M]_{OH}=-2$) is consistent with the values (Δ_1) for similar changes in 3-β-hydroxylated triterpenes of the α- or β-amyrin group[4]. The consumption of one mole of perbenzoic acid by dimethyl spergulagenate and its acetate suggests spergulagenic acid to be a member of the β-amyrin group, having a typical 12:13 double bond, as those of α-amyrin group are inert to this reagent[5]. This conclusion was further corroborated by the fact that acetyl dimethyl spergulagenate on oxidation with selenium dioxide in acetic acid under reflux furnished a heteroannular diene, $C_{34}H_{50}O_6$, m.p. 236-39°, which showed 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 β-amyrin group[6-8].

Further work on the constitution of spergulagenic acid is in progress.

EXPERIMENTAL*

Isolation of the Sapogenins.—Air-dried, powdered material (whole plant, 1kg.) was defatted with light petroleum and then extracted with ethanol (95%). The extract was concentrated to a small volume and the crude saponin was precipitated with ether as a dark brown gum. The precipitate was dissolved in ethanol (75%, 500 ml) and refluxed on a steam bath for 5 hr. with HCl (conc., 100 ml). Ethanol was removed, keeping the volume constant with water and the crude aglycone (20 g.), thus precipitated, was filtered, dried, and extracted with ether at the room temperature. The ether extract was washed with aqueous 0.5% NaOH solution (500 ml). The alkaline layer on acidification provided an appreciable amount of a pale yellow precipitate which was filtered and washed thoroughly with water. The crude acidic fraction (4 g.) developed a deep violet colour with EtOH-$FeCl_3$. This, however, on crystallisation from methanol furnished a colorless crystalline compound, m.p. 280-300° (2.6 g.) which did not respond to the ferric reaction. The above colorless product was dissolved in methanol (50 ml) and esterified with ethereal diazomethane. The crude ester was crystallised from chloroform-benzene mixture, m.p. 228-36°. The above methyl ester was dissolved in chloroform (20 ml) and adsorbed on a column of acid-washed alumina (70 g.). Elution with light petroleum afforded a colorless, crystalline fraction, m.p. 220-32°. Further elution with light petroleum-benzene mixture (2:1, 6 l; 1:1, 7 l) furnished a highly crysytalline material, m.p. 235-37°. All the fractions

*All m.p.s are uncorrected and recorded in a bisulphate bath. Brockmann's alumina (S. Merck) was used for chromatography and acid-washed refers to Brockmann's alumina deactivated with 5% of 10% acetic acid. Petroleum used throughout had b.p. 60-80°.

4. Klyne and Stokes, *J. Chem Soc.*, 1954, 1979.
5. Barton, "Progress in Organic Chemistry", Ed. J. W. Cook, Vol. II, 1953, p. 67, Butterworth, London.
6. Ruzicka *et al.*, *Helv. Chim. Acta*, 1939, 22, 767.
7. Barton and Brooks, *J. Chem. Soc.*, 1951, 257.
8. Barua and Raman, *Tetrahedron*, 1959, 7, 21.

having m.p. 235-37° were mixed together and crystallised from chloroform-benzene, m.p. 235-37°, $[\alpha]_D^{32}+101.3°$ ($CHCl_3$). (Found: C, 74.71; H, 10.05, OMe, 12.00; M.W., 500. $C_{32}H_{50}O_5$ requires C, 74.71; H, 9.73; OMe, 12.06%; M.W., 514).

Spergulagenic Acid.—A solution of dimethyl spergulagenate (640 mg.) in ethylene glycol (50 ml) was refluxed with KOH (12 g.) for 27 hr. The mixture was then poured on crushed ice, acidified, and worked up in the usual way. The crude material was repeatedly crystallised from absolute ethanol (charcoal). The crystalline product had m.p. 347-50° (decomp.). (Found: C, 74.13; H, 9.64. $C_{30}H_{46}O_5$ requires C, 74.07; H, 9.46%). The above acid on esterification with diazomethane regenerated dimethyl spergulagenate.

Acetyl Dimethyl Spergulagenate.—A solution of dimethyl spergulagenate (500 mg.) in dry pyridine (10 ml) and acetic anhydride (5 ml) were heated on a steam bath for 3 hr. The reaction mixture was poured on crushed ice and the precipitate was filtered, washed with water, and dried. The crude product was dissolved in benzene (7 ml) and adsorbed on a column of acid-washed alumina (10 g.). Elution with light petroleum-benzene mixture (2:1, 1:2) afforded a colorless crystalline material, m.p. 284-87°. This on crystallisations from chloroform-methanol mixture furnished needles, m.p. 285-87°; $[\alpha]_D^{31.5}+93.1°$ ($CHCl_3$). (Found: C, 73.41; H, 9.77. $C_{34}H_{52}O_6$ requires C, 73.38; H, 9.35%).

$LiAlH_4$-*reduction of Dimethyl Spergulagenate.*—To a slurry of $LiAlH_4$ (4 g.) in dry ether (150 ml) a solution of dimethyl spergulagenate (550 mg.) in dry ether (400 ml) was added. The mixture was refluxed for 7 hr. The reaction product was worked up in the usual way and then extracted with ether. The ethereal layer was washed with dilute alkali and water. Neutral ether layer on evaporation provided a highly crystalline solid which was digested successively with hot petroleum and benzene; the insoluble residue was crystallised from methanol (charcoal), m.p. 278-80°. (Found: C, 78.56; H, 10.45. $C_{30}H_{50}O_3$ requires C, 78.62; H, 10.92%).

Perbenzoic Acid Oxidation of Dimethyl Spergulagenate and its Acetate.—Both dimethyl spergulagenate (50 mg.) and its acetate (50 mg.) were dissolved separately in chloroform (5 ml) and a solution of perbenzoic acid (0.7*N*, 5 ml) was added to each and kept at 0°. Iodometric titration showed the consumption of 0.9 mole of per-acid in each case after 7 days and there was no further uptake up to 15 days.

Attempt for the Hydrogenation of Dimethyl Spergulagenate.—Hydrogenation of dimethyl spergulagenate in glacial acetic acid solution in presence of Adams' catalyst at the room temperature and pressure was attempted. On working up the product, only unchanged material could be recovered.

Selenium Dioxide Oxidation of Monoacetyl Dimethyl Spergulagenate.—To a solution of acetyl dimethyl spergulagenate (200 mg.) in glacial acetic acid (15 ml) freshly prepared selenium dioxide (200 mg.) was added and the mixture was refluxed for 4 hr. The mixture was filtered hot to remove the deposited metallic selenium and the filtrate was poured on crushed ice and worked up in the usual way. The crude product was dissolved in benzene (5 ml) and adsorbed on a column of deactivated alumina (10 g.). Elution with benzene afforded a product which was crystallised from benzene-petroleum mixture, m.p. 236-39°. (Found: C, 73.71; H, 9.10. $C_{34}H_{50}O_6$ requires C, 73.65; H, 9.02%).

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