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Studies on the Constitution of Spergulagenin A—A Novel Triterpene from *Mollugo spergula*

(Miss) Parul Chakrabarti

Spergulagenin A, a new triterpenoid sapogenin isolated from *Mollugo spergula*, was previously[1] shown to be a completely saturated pentacyclic triterpene containing a keto and three hydroxyl groups. It was assigned the molecular formula $C_{30}H_{50}O_4$ which has now been confirmed by molecular weight determination (M^+=474) by mass spectrometry†. Further studies on the constitution of spergulagenin A are described in this paper.

Spergulagenin A on potassium borohydride reduction furnishes a tetraol, $C_{30}H_{54}O_4$, m.p. 335-340°(dec.). Spergulagenin A triacetate forms an oxime, $C_{36}H_{57}O_7N$, m.p. 213-215° (dec.) which on treatment with acetic anhydride and fused sodium acetate furnishes an oxime tetra-acetate, $C_{38}H_{59}O_8N$, m.p. 207-208° (dec.). Its IR spectrum shows band at 1760 and 1200 cm^{-1} (=N—OAc); 1720 and 1255 cm^{-1} (·OAc) and 1625 cm^{-1} (–C=N–). The formation of the above oxime tetra-acetate confirms that the carbonyl group in spergulagenin A is a ketone and not an aldehyde as in the latter case a nitrile would have been formed under the above conditions. Moreover, spergulagenin A tribenzoate[1] is recovered unchanged after oxidation with CrO_3 in acetic acid. The NMR spectrum of spergulagenin A triacetate, which will be discussed later in detail, does not show any signal for aldehydic proton. All these facts conclusively prove the presence of a keto group in spergulagenin A.

All the three hydroxyl groups in spergulagenin A are most probably secondary since no acidic product is obtained when spergulagenin A is oxidized with CrO_3 in acetic acid. Moreover, the NMR spectrum of spergulagenin A triacetate shows the presence of three protons on acetoxy bearing carbon H–C–OAc; broad unresolved bands in the region 4.3-5.4δ). Spergulagenin A forms the triacetate on treatment with acetic anhydride and pyridine at 0°. The ease of acetylation suggests that all the three secondary hydroxyl groups are equatorially orientated.

On oxidation with Kiliani's reagent in acetone, spergulagenin A furnishes a colourless compound $C_{30}H_{44}O_4$[1], m.p. 279-282° (dec.), $[\alpha]_D^{34°}$+13.4°. It does not give any colour with alcoholic ferric chloride. Its IR spectrum shows a strong broad unresolved band in the region of 1690-1710 cm^{-1} for carbonyl groups. It shows end absorption at 205 mμ (ε 3,078), indicative of an isolated double bond particularly in view of the absence of any such UV absorption in case of spergulagenin A. The oxidation product, however, does not

1. Chakrabarti and Barua, *J. Ind. Chem. Soc.*, 1965, **42**, 137.

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develop any colour with tetranitromethane for unsaturation. The above negative colour reaction cannot, however, completely rule out the possibility of the presence of unsaturation as many unsaturated triterpenoid compounds are known (*cf.* isoaescigenin oxide[2], dumortierigenin[3] etc.) which do not respond to this colour test. The NMR spectrum of the above oxidation product does not show any signal for vinyl proton which suggests that the double bond, if present, must be tetrasubstituted. Further work is being done to clarify this point. The oxidation product on refluxing with alcoholic caustic potash (5%) furnishes a colourless product, $C_{30}H_{44}O_3$, m.p. 296-300°, $[\alpha]_D^{32°}$-121°, which shows UV absorption maxima at 239 mμ (ε14,790) but no end absorption at 205 mμ. Its IR spectrum shows bands at 1690 and 1705 cm^{-1} (non-conjugated ketone) and 1615 cm^{-1} (conjugated ketone). Thus the IR and the UV spectra show the presence of α,β-unsaturated keto group in the alkali treated product. It does not develop any colour either with tetranitromethane or alcoholic ferric chloride. Neither spergulagenin A nor its oxidation product consumes periodic acid.

The NMR spectrum* of spergulagenin A triacetate is highly informative. It shows four sharp signals at 1.9 (3H), 2.0 (3H), 2.02 (3H) and 2.17 (3H)δ. The signals at 1.9,2.0, and 2.02δ are attributed to the nine protons of three acetoxy methyl groups (–O–CO–CH_3). The signal at 2.17δ is assigned to the methyl protons of an acetyl group (CH_3.CO) on the basis of the following facts. Firstly, the NMR spectrum of spergulagenin A triacetate, as mentioned earlier, shows signals only for three protons on acetoxy bearing carbon (–$\overset{H}{\underset{|}{C}}$–O–$COCH_3$). Secondly, spergulagenin A triacetate on Wolff-Kishner reduction furnishes a triol, $C_{30}H_{52}O_3$, m.p. 246-248°, $[\alpha]_D^{32°}$+37.3° (EtOH), which forms a triacetate, $C_{36}H_{58}O_6$, m.p. 245-247°, $[\alpha]_D^{32°}$+45.4°. The NMR spectrum of this triacetate shows signals at 2.08δ (6H, sharp singlet, unsplit) and 2.18δ (3H, singlet) for the nine methyl protons of the three acetoxy group (–O–$\overset{O}{\overset{\|}{C}}$–$CH_3$) but the signal at 2.17δ attributed to an acetyl group (CH_3.CO), found in the spectrum of spergulagenin A triacetate, is not observed in this case. These facts clearly show that the carbonyl function in spergulagenin A is present as an acetyl group (CH_3.CO) i.e. methyl ketone and not as a six-membered ring ketone as thought previously[1]. Finally, the mass spectral studies, as discussed below, also show the presence of an acetyl group in spergulagenin A.

The mass spectrum of spergulagenin A, shows the molecular ion peak at m/e 474 together with the ion species corresponding to the loss of a molecule of water, i.e., m/e 456 $(M\text{-}H_2O)^+$. Besides these there is a weak peak at m/e 431 and an intense peak at m/e 413 which correspond to the loss of mass unit 43 (CH_3.CO) from the molecular ion and the ion species $(M\text{-}H_2O)^+$ respectively. This is further corroborated by the fact that the mass spectrum of the triol, obtained by Wolff-Kishner reduction of spergulagenin A triacetate, does not show any loss of 43 mass unit either from the molecular ion (M^+ 460) or the $(M\text{-}H_2O)^+$ peak; instead there are peaks at m/e 431 and m/e 413 corresponding to the loss of an ethyl group (CH_3CH_2) from the molecular ion and the $(M\text{-}H_2O)^+$ peak respectively. Ethyl group is obviously formed by the reduction of the acetyl (CH_3.CO) group in spergula-

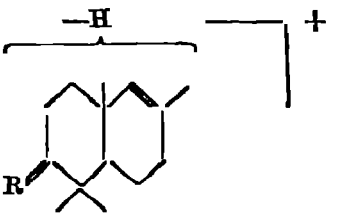
2. Thomas, *Tetrahedron*, 1966, **22**, 351.
3. Djerassi *et al.*, *J. Amer. Chem. Soc.*, 1956, **78**, 5685.

*The NMR and mass spectra of spergulagenin A triacetate were obtained by courtesy of Prof. R. Tschesche, Bonn, West Germany, to whom the author's thanks are due.

genin A during Wolff-Kishner reduction. Thus the NMR data and the mass spectral analyses definitely establish the presence of an acetyl group ($CH_3.CO$) in spergulagenin A.

In the mass spectrum** of spergulagenin A triacetate (M+ 600), the splitting of three acetoxy groups is evident from the peaks at m/e 540 $(M-ACOH)^+$, 480 $(M-2\times ACOH)^+$ and 420 $(M-3\times ACOH)^+$ respectively. Each ion species again splits off very easily an acetyl group giving rise to peaks at m/e 497, 437 and 377 respectively.

The mass spectra of spergulagenin A, its triacetate and its oxidation product show peaks at m/e 207, 249 and 205 which correspond to the ion species Ia (R=⟨H, OH⟩), Ib (R=⟨H, OAC⟩) and Ic (R=O) respectively. The ion fragments (Ia) and (Ib) further lose one molecule of water and acetic acid respectively giving rise to the peak at m/e 189. This type of fragmentation is very suggestive of 3-hydroxy-4, 4-dimethyl triterpenes[4]. Mass spectral studies also show the absence of any other oxygen function in ring A and B of spergulagenin A.



(I)

The optical rotatory dispersion curve† of spergulagenin A triacetate demonstrates a positive Cotton effect [$(\Phi)304 + 2630°$ and (Φ) 268—28°].

It may be mentioned here that in any known C-30 pentacyclic triterpene skeleton an acetyl group ($CH_3.CO$) cannot be accommodated as such. Triterpene embodying this system and having a C-30 formulation is yet to be reported. In this respect spergulagenin A appears to be a novel type of pentacyclic triterpene.

Further work is in progress

Procedure††

Potassium Borohydride Reduction of Spergulagenin A.—To a solution of spergulagenin A (200 mg) in ethanol (40 ml), potassium borohydride (200 mg) was added and the mixture was kept overnight at room temperature and then poured onto crushed ice. It was worked up in the usual way. The product was crystallized from ethanol, and had m.p. 335-340° (dec.). (Found: C, 75.35; H, 11.08. $C_{30}H_{52}O_4$ requires: C, 75.63; H, 10.92%).

Preparation of the Oxime of Spergulagenin A Triacetate.—A mixture of spergulagenin A triacetate (200 mg) and hydroxylamine hydrochloride (300 mg) in pyridine (3 ml) was heated on a steam bath for 16 hr and kept at room temperature for 24 hr and then worked

4. Budzikiewicz *et al.*, *J. Amer. Chem. Soc.*, 1963, **85**, 3688.

††M. P.'s are uncorrected and recorded in bisulphate bath. Optical rotations are in chloroform solutions unless mentioned otherwise.

up in the usual way. It was crystallized from methanol, and had m.p. 213-215° (dec.) (Found: C, 69.61; H, 9.17. $C_{36}H_{57}O_7N$ requires: C, 70.24; H, 9.26%).

Oxime Tetra-acetate.—The oxime (150 mg) was heated on steam bath with fused sodium acetate (300 mg) and acetic anhydride (5 ml) for 3 hr and then worked up in the usual way. Crystallization from methanol yielded a colourless compound, m.p. 207-208° (dec.). (Found: C, 69.11; H, 8.63. $C_{38}H_{59}O_8N$ requires C, 69.41; H, 8.98%).

Oxidation of Spergulagenin A Tribenzoate with CrO_3 in Acetic Acid.—A solution of spergulagenin A tribenzoate (200 mg) in glacial acetic acid was treated at room temperature with a solution of CrO_3 (400 mg) in acetic acid (90%, 10 ml) till brown colour persisted (3 hr.). On working up in the usual way, only unchanged tribenzoate (m.p. 317-319°) could be obtained.

Spergulagenin A Triacetate.—A solution of spergulagenin A (500 mg) in dry pyridine (3 ml) and acetic anhydride (3 ml) was kept at 0° for 16 hr. On working up only spergulagenin A triacetate, m.p. 236-238°, $[\alpha]_D^{33°}+58°$ was obtained.

Treatment of the Chromic Acid Oxidation Product of Spergulagenin A.—The oxidation product, $C_{30}H_{44}O_4$[1], (500 mg) was refluxed with alcoholic caustic potash (5%, 50 ml) for 1 hr. On working up, a highly crystalline material, m.p. 296-300° (dec.) was obtained. (Found: C, 79.35; H, 9.20%; M^+ 450. $C_{30}H_{42}O_3$ requires: C, 80.0; H, 9.33%; mol. wt. 450).

A methanolic solution (20 ml) of the oxidation product (15 mg) was treated with an aqueous solution (2ml) of periodic acid (20 mg) overnight at room temperature. On working up only unchanged product was obtained.

Under similar condition spergulagenin A also was recovered unchanged.

Wolff-Kishner Reduction of Spergulagenin A Triacetate—A solution of spergulagenin A triacetate (1 g.) in absolute alcohol (30 ml) and diethylene glycol (30 ml) was refluxed with hydrazine hydrate (85%, 30 ml) for ½hr. Then solid caustic potash (8 g.) was added and solvent distilled off until the mixture refluxed freely at 195-200°, where it was kept for 4hr. On working up in usual way and crystallization from aqueous methanol a compound m.p. 246-248°, was obtained. (Found: C, 78.24; H, 11.28%; M^+ 460. $C_{30}H_{52}O_3$ requires C, 78.26; H, 11.30%; mol. wt. 460).

The above triol (500 mg) forms a triacetate, m.p. 245-247°, on heating with pyridine (5 ml) and acetic anhydride (5 ml). (Found: C, 74.11; H, 10.14. $C_{36}H_{58}O_6$ requires C, 73.72; H, 9.89%).

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Department of Chemistry,
Bose Institute,
Calcutta—9.

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