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The Structure and Stereochemistry of Spergulagenol—A New Triterpenoid Sapogenol from *Mollugo spergula*

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A new triterpenoid sapogenol, spergulagenol, has been isolated from the ethanolic extract of *Mollugo spergula*. It has been shown to be 3β,12β,16β,22-tetrahydroxy-(21α-H)-hopane.

MOLLUGO spergula (family Ficoidaceae) is used as a green vegetable in India and is extremely bitter in taste. The bitter principles have been characterized as triterpenoid saponins and the isolation of several new triterpenoid sapogenins, spergulagenic acid[1-3], spergulagenin A[4-11], spergulagenin C (x-hydroxy spergulagenin A)[12] and spergulatriol[13] have been reported. In a short communication[14] the isolation of another new sapogenin called spergulagenol (**Ia**) from the same plant was reported from this laboratory and the present paper details the experiments leading to the structure (**Ia**) of spergulagenol.

The ether insoluble part of the ethanolic extract of *Mollugo spergula* (whole plant) was hydrolysed with ethanolic hydrochloric acid. The neutral fraction of the crude sapogenin thus obtained on column chromatography yielded a new sapogenol called spergulagenol (**Ia**), $C_{30}H_{52}O_4$, m.p. 295-298°, $[\alpha]_D$+46° (Py). It gave a violet colour with Liebermann-Burchard reagent but no colour with tetranitromethane indicating the absence of unsaturation. The presence of hydroxyl groups was shown by bands at 3320 and 3440 cm^{-1} in its ir spectrum (Nujol mull). Its mass spectrum showed the molecular ion peak at m/e 476 and a peak at m/e 59 for the ion species $(Me)_2C{=}\overset{+}{O}H$ formed by the cleavage of the hydroxy isopropyl side chain. Such loss of 59 mass unit has been observed in case of mollugogenol A[15,16] which also contains a hydroxy isopropyl side chain at C-21 and a β-hydroxyl group at C-16. There were also peaks at m/e 458 and 400 for the ion species *a* and *b*, respectively.

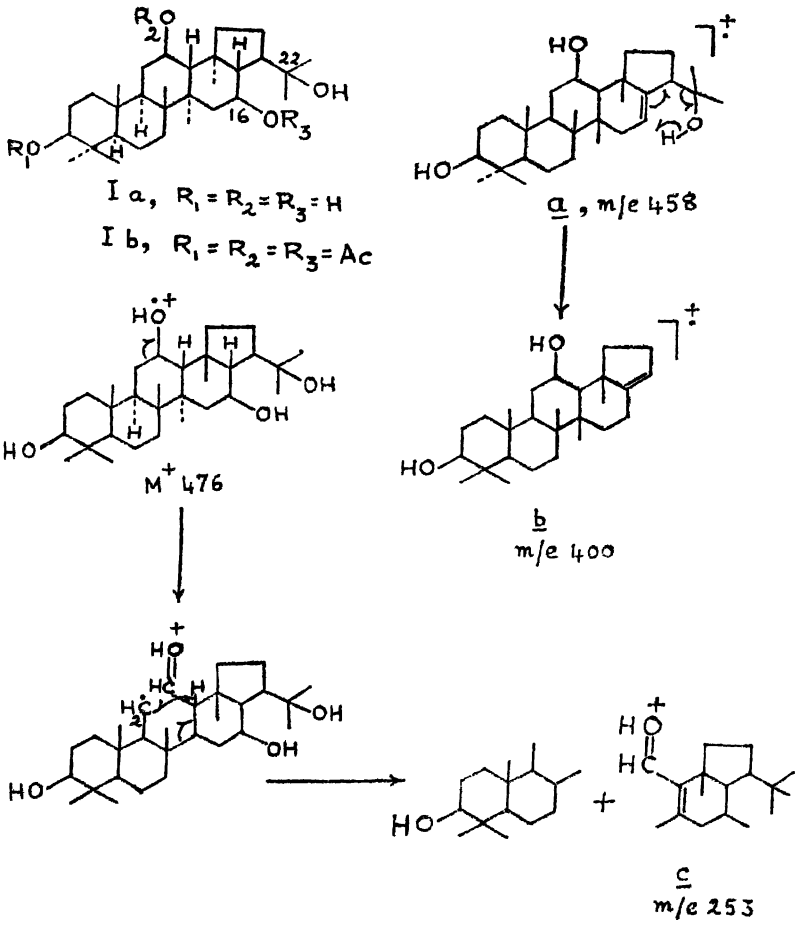
On treatment with acetic anhydride and pyridine at 0°, spergulagenol furnished a triacetate (**Ib**), $C_{36}H_{58}O_7$, m.p. 230-232°. Its mass spectrum did not show the molecular ion peak at m/e 602 but showed a peak at m/e 584 for the $(M\text{-}H_2O)^+$ ion. Its ^1H nmr spectrum ($CDCl_3$, 90 MHz) showed singlets at δ 0.87 (9H), 0.90 (3H), 1.03 (3H), 1.15 (3H) and 1.19 (6H) for the eight quarternary methyl groups ($-\overset{|}{\underset{|}{C}}-CH_3$). The singlets at δ 2.02 (3H), 2.03 (3H) and 2.07 (3H) were attributed to the three acetoxy groups (-O-CO-CH_3). The broadsignals centred at δ 4.49 (1H, m), 5.25 (1H, m) and 4.88 (1H, m) were assigned to 3-αH, 12-αH and 16-αH, respectively. The signal for the 22-OH group appeared at δ 3.5 and disappeared on D_2O exchange. That the fourth hydroxyl group in spergulagenol is tertiary was evident by its resistance to oxidation. Spergulagenol triacetate was recovered unchanged after treatment with CrO_3 in glacial acetic acid.

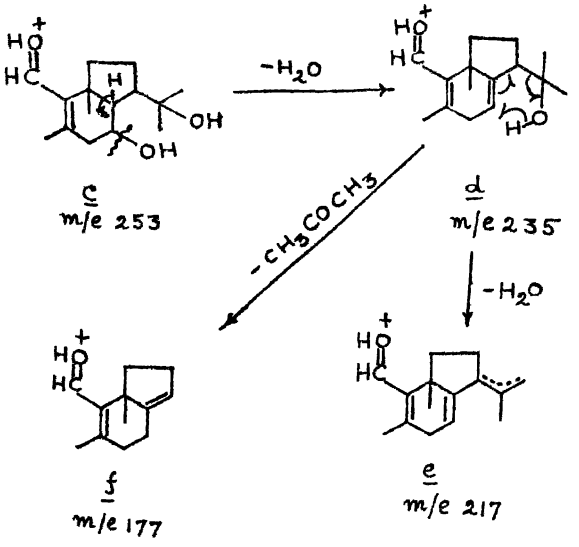
The mass spectrum of spergulagenol showed interesting fragmentation pattern giving rise to peaks at m/e 253, 235, 217 and 177 for the ion species *c*, *d*, *e* and *f*, respectively indicating the presence of a hydroxyl group at C-12.

Spergulagenol on oxidation with CrO_3 in acetic acid at room temp. gave a monohydroxy triketone (**II**), $C_{30}H_{46}O_4$, m.p. 254-256°. Its ir spectrum (Nujol mull) showed bands at 3350 (OH) and 1695-1700 cm^{-1} for six membered ring ketone. It gave positive Zimmerman colour reaction characteristic of 3-keto group and did not respond to the ferric chloride colour test. Its mass spectrum did not show the molecular ion peak at m/e 470 but showed a strong peak at 452 $(M\text{-}H_2O)^+$. The mass spectral fragmentation pattern was very characteristic of 12-keto compounds (cf. spergulagenin A[4-11]). There was a peak at m/e 247 for the ion species *g* and at m/e 219 arising out of loss of 28 mass unit (CO) from the ion species *g*. There was also a peak at m/e 205 for the ion *h* arising by the usual cleavage through ring C. This indicated the presence of a 12-keto group in the triketone and hence a hydroxyl group at C-12 in spergulagenol.

Hopanes and (21α-H)-hopane derivatives having hydroxyl groups at C-22 and C-16 are known to give

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hop-15,17(21)-dienes on treatment with ethanolic hydrogen chloride[15,16]. Under similar conditions spergulagenol yielded a diene, not isolated in pure state, which showed uv absorption maxima at 243, 251 and 261 nm, very characteristic of hop-15,17 (21)-dienes. This clearly showed the presence of a secondary hydroxyl group at C-16 and a tertiary hydroxyl group at C-22. Hydroxy-hopane derivatives[17] are known to undergo hydrogenolysis in the presence of Adam's platinum catalyst. Under similar conditions spergulagenol did not undergo hydrogenolysis and this indicated the probability of spergulagenol being a 22-hydroxy-(21α-H)-hopane derivative.

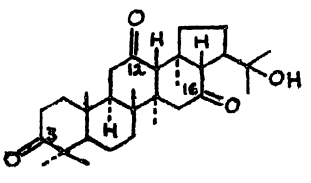
Huang Minlon reduction of the monohydroxy triketone (II) gave a compound, m.p. 225-227°, $C_{30}H_{52}O$, which was characterized as 22-hydroxy-(21α-H)-hopane by direct comparison of m.p., tlc and ir spectrum with an authentic sample.

Spergulagenol formed the triacetate (Ib) on treatment with acetic anhydride and pyridine at 0°. The ease of acetylation showed the equatorial orientation of the secondary hydroxyl groups in spergulagenol. On the basis of the data presented above the structure and stereochemistry of spergulagenol is represented as (Ia).

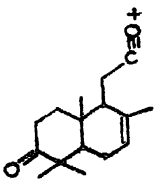
Experimental

Melting points reported are uncorrected. Petroleum ether used was in the boiling range of 60-80°.

Isolation of spergulagenol: Air dried powdered plants (whole plant, 5 kg) were defatted with petro-

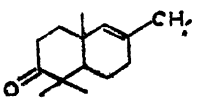


II



g

m/e 247



h

m/e 205

leum ether and then extracted with ethanol (95%) in a Soxhlet apparatus. The ethanolic extract was concentrated by distillation and the crude saponin was precipitated by addition of excess ether to the concentrated ethanolic extract. Solvent was decanted off and the gummy precipitate was washed with ether. It was dissolved in 50% aqueous ethanol (400 ml) and conc. hydrochloric acid (80 ml) was added to it. It was then refluxed for 0.5 hr on steam bath. The crude sapogenin thus obtained was taken up in ether and separated into neutral and acid sapogenins in the usual way. Spergulagenol was isolated by repeated column chromatography over silica gel and crystallized from aq. methanol, m.p. 295-98°. (Found : C, 75.42 ; H, 11.11% ; Mol. wt. (M^+ 476). $C_{30}H_{52}O_4$ requires C, 75.58 ; H, 10.99% ; Mol. wt. 476).

Acetylation of spergulagenol : Spergulagenol (50 mg) was dissolved in pyridine (2 ml) and acetic anhydride (2 ml) and left overnight at 0°. The product was worked up in the usual way, charcoalised and then crystallised from aqueous methanol, m.p. 228°. (Found : C, 71.62 ; H, 9.84. $C_{36}H_{56}O_7$ requires C, 71.73 ; H, 9.70%).

Oxidation of spergulagenol : Spergulagenol (20 mg) was dissolved in glacial acetic acid (2 ml) and a solution of CrO_3 in glacial acetic acid [prepared from CrO_3 (180 mg), glacial acetic acid (7.5 ml) and water (0.5 ml)] was added to it slowly at room temp. with constant stirring for 2 hr till the brown colour persisted. The product was worked up in the usual way and crystallised from ethanol, m.p. 254-56° (decom). (Found : C, 76.23 ; H, 9.93% ; $(M-H_2O)^+$ 452. $C_{30}H_{46}O_4$ requires C, 76.65 ; H, 9.98% ; Mol. wt. 470).

Treatment of spergulagenol with ethanolic hydrogen chloride : Spergulagenol (7 mg) was refluxed with 3 ml of ethanolic hydrogen chloride (5%) for 3 hr. The product was worked up in the usual way. The uv spectrum was recorded with the reaction product without further purification.

Huang Minlon reduction of the triketone (II) : The triketone (II, 50 mg) was dissolved in absolute ethanol-diethylene glycol mixture (2 : 1 ; 21 ml) by heating. To it hydrazine hydrate (85% ; 6 ml) was added and the mixture refluxed for 1 hr. Solid KOH (1 g) was then added and refluxed for 0.5 hr. The solvent was partly distilled off and the mixture heated for 6 hr at 190-200°. It was then poured onto crushed ice and the precipitate was filtered off and washed with water. TLC showed three spots, one of which was major. The compound corresponding to the major spot was isolated by repeated column chromatography over silica gel column and crystallised from acetone, m.p. 225-27° [22-hydroxy-(21α-H)-hopane]. (Found : C, 84.39 ; H, 12.42. $C_{30}H_{52}O$ requires C, 84.04 ; H, 12.22%).

Acknowledgement

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References

1. P. CHAKRABARTI, D. K. MUKHERJEE and A. K. BARUA, *Tetrahedron*, 1966, 22, 1431.
2. P CHAKRABARTI, D. K. MUKHERJEE and A. K. BARUA and B. C. DAS, *Tetrahedron*, 1968, 24, 1107.
3. P. CHAKRABARTI and A. K. BARUA, *J. Indian Chem. Soc.*, 1969, 46, 626.
4. P. CHAKRABARTI and A. K. BARUA, *J. Indian Chem. Soc.*, 1965, 42, 137.
5. P. CHAKRABARTI, *J. Indian Chem. Soc.*, 1967, 44, 242.
6. P. CHAKRABARTI, A. BASAK and A. K. BARUA, "Ann. Report of Bose Institute", 1972-1974, p. 15.
7. A. BASAK, Part of Ph.D. Thesis, University of Calcutta, 1974.
8. I. KITAGAWA, M. SUZUKI and I. YOSIOKA, *Tetrahedron Lett.*, 1974, 1173.
9. I. KITAGAWA, H. SUZUKI, K. KITAZAWA, N. YAMO and I. YOSIOKA, *Chem. Pharm. Bull. Japan*, 1975, 23, 355.
10. P. CHAKRABARTI, A. BASAK and A. K. BARUA, *Trans. Bose Res. Inst.*, 1977, 40, 117.
11. A. K. BARUA, A. BASAK, S. K. BANERJEE, T. K. CHATTERJEE, K. BASU and P. CHAKRABARTI, *Trans. Bose Res. Inst.*, 1978, 41, 83.
12. A. K. BARUA, S. K. BANERJEE, C. DAS GUPTA, A. BASAK and P. CHAKRABARTI, 64th Session Indian Science Congress, January 1977, Abst., Org. p. 18.
13. I. KITAGAWA, H. YAMANAKA, T. NAKANISHI and I. YOSIOKA, *Tetrahedron Lett.*, 1976, 2327.
14. A. K. BARUA, S. K. BANERJEE, C. DAS GUPTA, K. BASU, L. BOSE and P. CHAKRABARTI, *Phytochem.*, 1980, 19, 1551.
15. P. CHAKRABARTI, *J. Indian Chem. Soc.*, 1969, 46, 98.
16. P. CHAKRABARTI, *Tetrahedron*, 1969, 25, 3301.
17. R. E. CORBETT and R. A. J. SMITH, *J. Chem. Soc.*, 1967, 1622.