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THE STRUCTURE AND STEREOCHEMISTRY OF MOLLUGOGENOL A

A NEW SAPOGENIN FROM *MOLLUGO HIRTA*

P. CHAKRABARTI

Bose Institute, Calcutta 9, India

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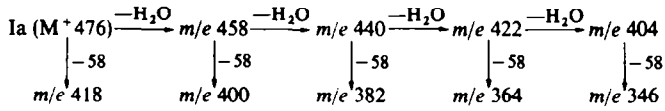
Abstract—The constitution of mollugogenol A, a new triterpenoid sapogenin isolated from *Mollugo hirta*, has been established as 3β, 6α, 16β, 22-tetrahydroxyisohopane.

IN A preliminary communication[1] the structure (Ia) of mollugogenol A, a new triterpenoid sapogenin from *Mollugo hirta* (syn. *M. lotoides*), has been reported. The present paper details the experiments leading to this structure.

Mollugogenol A (Ia), $C_{30}H_{52}O_4$, M^+ 476, m.p. 250–252°, $[\alpha]_D^{26}$ +58·5° (pyridine), gives a pink → brown colour in the Liebermann–Burchard test. Its UV spectrum does not show any band in the region 200–360 mμ. Its IR spectrum shows a broad band at 3293–3400 cm^{-1} for OH groups.

On heating with acetic anhydride and pyridine mollugogenol A furnishes two acetates (Ib and IIb). Only IIb gives a yellow colour with tetranitromethane. The presence of an isopropenyl group ($CH_3—\underset{|}{C}=CH_2$) in IIb is shown by its IR and NMR spectra. Its IR spectrum (Nujol mull) shows bands at 1735 cm^{-1} and 1240–1250 cm^{-1} (acetoxy), 1640 and 900 cm^{-1} (=CH_2). Its NMR spectrum (60 Mc, $CHCl_3$) shows signals at 0·82 (3H), 0·9 (3H), 0·98 (3H), 1·04 (3H), 1·06 (3H) and 1·16 (3H) δ for six tertiary Me groups; three singlets at 1·86 (3H), 2·02 (3H) and 2·03 (3H) δ for three acetoxy groups ($C\underline{H}_3 \cdot CO \cdot O$); a sharp singlet at 1·69 δ (3H, $C\underline{H}_3—\underset{|}{C}=CH_2$) and a broad singlet at 4·62 δ (2H, $CH_3—\underset{|}{C}=C\underline{H}_2$). The presence of a tertiary OH group in the second acetate (Ib) is shown by the band at 3500 cm^{-1} in its IR spectrum and by its resistance to oxidation. Its NMR spectrum (60 Mc, $CHCl_3$) shows sharp singlets at 2·02 (6H) and 1·88 δ (3H) for three acetoxy groups. The signals at 1·32 and 1·41 δ may be attributed to two Me groups attached to carbon bearing the tertiary OH group.

The mass spectrum of mollugogenol A shows a very weak molecular ion at *m/e* 476 and four other peaks at *m/e* 458, 440, 422 and 404 which corresponded to the successive loss of four molecules of water from the molecule. One interesting feature in the mass spectrum is shown below:



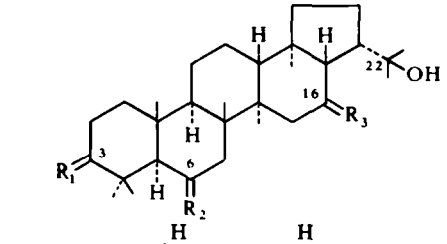
An intense peak at *m/e* 59 is due to the ion $(CH_3)_2C{=}\overset{+}{O}H$ formed by the cleavage of the hydroxy-isopropyl group. The origin of the peaks at *m/e* 418, 400, 382, 364 and 346, could be due to the loss of a terminal hydroxy-isopropyl group from respective ions with transfer of one H atom. Thus the presence of a hydroxyisopropyl group in mollugogenol A has been established from NMR and mass spectral data. Mollugogenol A may, therefore, be a triterpene of either the hopane or lupane series. Mollugogenol A was finally degraded to zeorininone (III),[2,3] which was obtained from zeorine (IVa), a triterpene of the hopane series.

Mollugogenol A (Ia) on oxidation with CrO_3–acetic acid furnishes the monohydroxy triketone (Ic) which shows a band at 3400 cm^{-1} in the IR spectrum for a free OH group. It does not develop any colour with alcoholic ferric chloride but gives a positive Zimmermann's test for a 3-keto group[4] indicating the presence of a secondary OH group at C-3 position. The monohydroxy triketone on Wolff–Kishner reduction furnished mixtures of two hydroxy monoketones (V and VI) which are epimeric at the C-17 position. This mixture, on treatment with ethanolic hydrogen chloride furnished the compound III identified as zeorininone,[2] a degradation product of zerorine (IVa), by mixed m.p., TLC and IR spectra. This correlation not only shows the nature of the carbon skeleton of mollugogenol A but also fixes one of the secondary OH groups at the C-6 position.

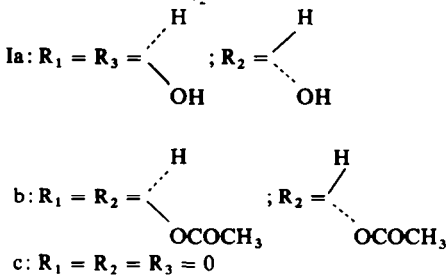
The triacetate (IIb) on mild saponification furnishes the triol (IIa). The mass spectrum of IIa does not show loss of a hydroxy-isopropyl group either from the molecular ion (M^+ 458) or from the ions $(M-H_2O)^+$, $(M-2H_2O)^+$ and $(M\,3H_2O)^+$. The triol (IIa) on oxidation with CrO_3–pyridine complex yields the triketone (IIc) which was readily isomerized to the α,β-unsaturated ketone (VII), [λ_{max}^{EtOH} 255 mμ, ν_{max}^{Nujol} 1668 cm^{-1}] by refluxing with ethanolic hydrogen chloride. Compound VII on LAH reduction followed by acid treatment furnishes the diene (VIII) [λ_{max}^{EtOH} 243, 251 and 261 mμ][5] thus clearly locating one of the OH groups at the C-16 position.

Of the four OH groups in mollugogenol A, three are placed at C-6, C-16 and C-22. The fourth OH group has been placed at C-3 on the following considerations. Both the triketones (Ic and IIc) respond to Zimmermann's colour test for a 3-keto group. The mass spectrum of mollugogenol A shows a peak at *m/e* 223 which may arise either from the part containing A and B ring (ion *a*, R = H) or that containing D and E (ion *b*, R = H) by the splitting of ring C, since both the fragments contain two OH groups. But in case of the acetate (Ib), the triol (IIa) and the hydrogenolysis product (IX, *vide supra*) the difference in the masses of the two fragments permits assignment of the peak at *m/e* 307 (in case of Ib) and at *m/e* 223 (in case of IIa and IX) to the ion *a*. The other part appears at *m/e* 265 (ion *b*) and *m/e* 207 (ion *c*) in Ib and IX respectively. This type of fragmentation is compatible with the cracking pattern associated with 3-hydroxy-4,4-dimethyltriterpenes having an additional OH group in ring A or B.[6] In the NMR spectrum of IIb the C-3α proton (axial) appears at 4·5 δ (broad triplet), usually observed in 3β-acetoxytriterpenes.[7] The above observations indicate that mollugogenol A contains a 3β-OH group.

Since mollugogenol A has been degraded to zeorininone (III) only the configuration of the hydroxy-isopropyl group needs to be determined. Recently the configuration of this group in hopane derivatives has been shown to be β from X-ray analysis[8] and other degradative experiments[9] and on this basis it is considered to be α in isohopane derivatives. Hydrogenolysis of the aforesaid group in mollugogenol A is very slow and furnishes mainly unreacted product together with a small amount of the saturated compound (IX M^+ 460). In this respect mollugogenol A behaves like isohopane rather than as a hopane derivative.[10] Moreover both compounds V and VI obtained from Ic by Wolff–Kishner reduction differ from zeorinone (IVb). The three secondary OH groups in mollugogenol A appear to be equatorial as these can be acetylated with acetic anhydride and pyridine at 0°. On the basis of the observations made so far mollugogenol A may be represented as 3β, 6α, 16β, 22-tetrahydroxyisohopane (Ia).

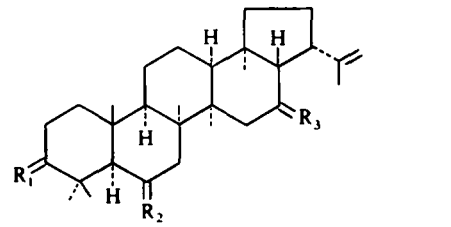


Ia: $R_1 = R_3 =$; $R_2 =$

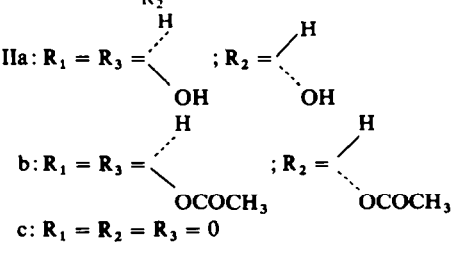


b: $R_1 = R_2 =$; $R_2 =$

c: $R_1 = R_2 = R_3 = 0$

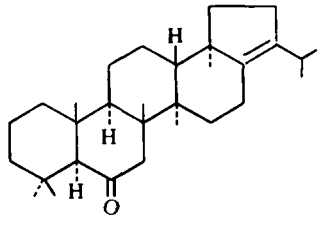


IIa: $R_1 = R_3 =$; $R_2 =$

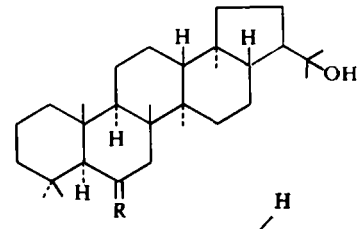


b: $R_1 = R_3 =$; $R_2 =$

c: $R_1 = R_2 = R_3 = 0$

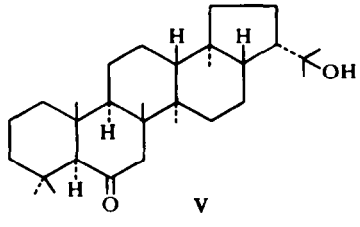


III

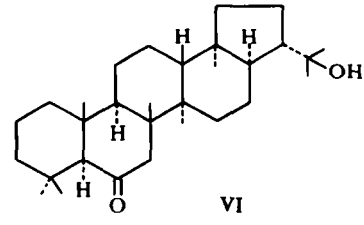


IVa: R =

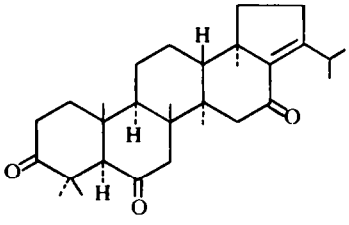
b: R = O



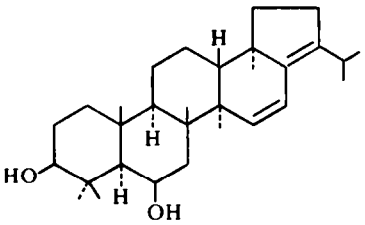
V



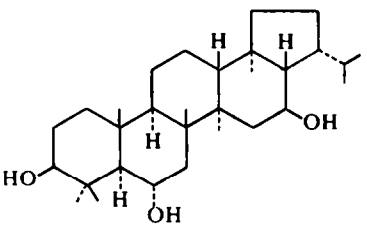
VI



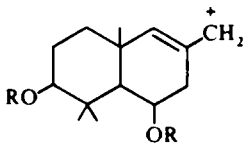
VII



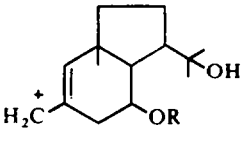
VIII



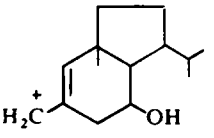
IX



a (R = H, *m/e* 223; R = $COCH_3$, *m/e* 307)



b (R = H, *m/e* 223; R = $COCH_3$, *m/e* 265)



c (*m/e* 207)

EXPERIMENTAL

M.ps are uncorrected and recorded in a H_2SO_4 bath. Pet. ether used has b.p. 60–80°. UV spectra (Beckmann's DK-2 spectrophotometer).

Acetylation of mollugogenol A. Mollugogenol A (Ia, Ig) was heated with Ac_2O (20 ml) and pyridine (8 ml) for 30 hr and then worked up in the usual way. The product was dissolved in benzene and adsorbed on a column of silica gel (100 g). Elution with benzene gave a colourless glassy material (350 mg) which was crystallized from MeOH (charcoal), m.p. 235–237° (IIb). (Found: C, 73·45; H, 9·47. $C_{36}H_{56}O_6$ requires: C, 73·93; H, 9·65%). Further elution with benzene–chloroform (2:1) gave another glassy colourless product (450 mg) which was crystallized from aqueous MeOH, m.p. 230–232° (Ib). (M^+ 602; mol. wt. of $C_{36}H_{58}O_7$ is 602) Ib was previously thought to be a tetracetate.

Saponification of the triacetate (IIb) *to the triol* (IIa). The triacetate (300 mg) was treated with 2% alcoholic KOH (40 ml) overnight at room temp. The resulting triol (IIa) was crystallized from EtOH, m.p. 243–246°, (Found: C, 78·75; H, 10·53; M^+ 458. $C_{30}H_{50}O_3$ requires: C, 78·55; H, 10·99%).

Oxidation of IIa *to* IIc. A cold solution of IIa (150 mg) in pyridine (5 ml) was added to a slurry of CrO_3–pyridine complex (from 200 mg of CrO_3 and 4 ml of pyridine) at 0° and left overnight at room temp. The product was purified by chromatography over silica gel. It was crystallized from MeOH, m.p. 255–257° (IIc).

Conversion of IIc *to* VII. Compound IIc, (80 mg) was heated under reflux with alcoholic HCl (3%, 10 ml) for 3 hr. The product was poured onto crushed ice and the ppt was filtered off and crystallized from aqueous MeOH, m.p. 244–248° (dec).

LAH reduction of VII *to* VIII. A slurry of LAH (50 mg) in dry ether (50 ml) was heated under reflux with a solution of VII (10 mg) in dry ether (5 ml) for 6 hr. After decomposing the excess reagent with ice–water the product was treated with 2N H_2SO_4 and then extracted with ether. The product thus obtained showed absorption at λ_{max} 243, 251 and 261 mμ in the UV spectrum. Compound VIII, though homogeneous in TLC, could not be crystallized because of the small quantity of material used for reduction.

Wolff-Kishner reduction of Ic. A soln of Ic, (500 mg) in a mixture of abs alcohol (20 ml) and diethylene glycol (20 ml) was refluxed with hydrazine hydrate (85%, 20 ml) for 2 hr on a steambath. Solid KOH (4 g) was added and the solvent was distilled off till the mixture refluxed freely at 200°. The mixture was kept at this temp for 4 hr and then worked up in the usual way. After purification, a product, m.p. 225–232°, was found to be a mixture of V and VI by TLC and both were differed from zeorinone (IVb).

The above mixture (100 mg) was refluxed with alcoholic HCl (5%, 10 ml) for 3 hr and the product was diluted with water. The separated solid was filtered off and repeatedly crystallized from pet. ether–acetone, m.p. 178–180°, (III). (Found: C, 88·21; H, 11·62. $C_{30}H_{48}O$ requires: C, 88·16; H, 11·84%).

Hydrogenolysis of mollugogenol A (Ia) *to* (IX). A soln of mollugogenol A (150 mg) in glacial AcOH (30 ml) and ether (Na-dry, 10 ml) was added to glacial AcOH (10 ml) containing Adams' catalyst (50 mg)

and the mixture was stirred for 8 hr in presence of H_2 at room temp. The product was dissolved in $CHCl_3$ and adsorbed on a column of silica gel (15 g). Elution with $CHCl_3$–MeOH (49:1) gave a minor product (10 mg) which was crystallized from aqueous MeOH (charcoal), m.p. 248–252° (IX). (Found: C, 78·11; H, 11·51. $C_{30}H_{52}O_3$ requires: C, 78·21; H, 11·38%). Further elution with $CHCl_3$–MeOH (46:4) gave a major product (100 mg), which crystallized from EtOH, m.p. 250–252° alone or when admixed with mollugogenol A.

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