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THE STRUCTURE OF MOLLUGOGENOL-F, A NEW TRITERPENOID SAPOGENIN FROM *MOLLUGO HIRTA*

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Key Word Index—*Mollugo hirta*; Ficoidaceae; new pentacyclic triterpenoid sapogenol; isohopane series; mollugogenol-F; 3β,16β,22-trihydroxyisohopane.

Abstract—The constitution of mollugogenol-F, a new triterpenoid sapogenin isolated from *Mollugo hirta* (family: Ficoidaceae), has been established as 3β,16β,22-trihydroxyisohopane.

INTRODUCTION

The plant *Mollugo hirta* is scattered throughout India. It is used as a medicine against diarrhoea, bilious attacks and as a purgative, for curing boils, wounds and pains in the limbs. The juice of the plant is taken internally to strengthen weak children [1]. In a preliminary communication [2], the structure of mollugogenol-F (**1**) from *M. hirta* has been reported. The present paper details the experiments leading to the structure. In this publication the β-orientation of the side chain at C-21 (C-21α-H) has been represented as in the isohopane series [3].

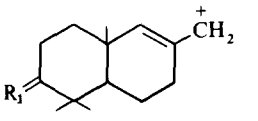
RESULTS AND DISCUSSION

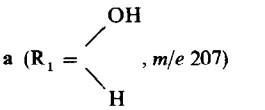
Mollugogenol-F (**1**), $C_{30}H_{52}O_3$, [(M − H_2O)$^+$442], mp 211–212°, $[\alpha]_D^{24}$ + 41.26° ($CHCl_3$), gave a pink, turning to violet, colour in the Liebermann–Burchard test. It neither showed any band in the region 200–360 nm in the UV nor did it respond to the TNM test. Its IR spectrum (nujol) showed a broad band at 3240 cm^{-1} for OH groups.

On acetylation (Ac_2O–Py) at 0°, mollugogenol-F furnished a monohydroxy diacetate (**2**), $C_{34}H_{56}O_5$ (M$^+$ 544), mp 225–226°. Its IR spectrum (nujol) showed bands at 3600 (tertiary OH), 1735, 1755 and 1240–1250 cm^{-1} (acetoxy). The ^{1}H NMR spectrum (60 MHz, $CDCl_3$, TMS) of **2** showed signals at δ 0.85 (3H, *s*, 18α-Me), 1.05 (3H, *s*, 4β-Me), 1.1 (3H, *s*, 4α-Me), 1.16 (3H, *s*, 10β-Me) and 1.19 (6H, *s*, 8β and 14α-Me) for six tertiary methyl groups [4, 5]. Two methyl groups in a hydroxyisopropyl side chain [$\underline{CH_3}$—C—(OH)—$\underline{CH_3}$] appeared at δ 1.50 (3H, *s*, C-29) and 1.52 (3H, *s*, C-30). The singlet at δ 2.92 (1H), which disappeared with D_2O, was due to the tertiary OH group. The signals at δ 2.06 (3H, *s*) and 2.08 (3H, *s*) were attributed to the two acetoxy methyl groups (2$\underline{CH_3}$—CO—O—). The 3-axial (α) proton appeared as a doublet at δ 4.2 (1H, J_{aa} = 7.5 Hz). The large coupling constant showed the *trans* (diaxial) relationship of this proton with the 2β-proton. A weak broad multiplet was centred at δ 5.55 (1H) for the 16α (axial) proton, as is usually observed in 3β and 16β-acetoxy triterpenes [6].

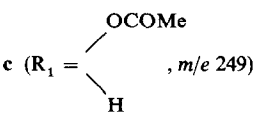
The MS of mollugogenol-F (**1**) did not show the molecular ion peak at *m/e* 460 but three important peaks were observed at *m/e* 442, 424 and 406 which corresponded to the successive loss of three molecules of H_2O from the molecule. An intense peak at *m/e* 59 was due to the ion $(CH_3)_2\overset{+}{C}{=}OH$ formed by the cleavage of the hydroxyisopropyl side chain. It showed other peaks at *m/e* 207 (ion **a**) and *m/e* 223 (ion **b**). The MS of the diacetate (**2**) showed a molecular ion peak at *m/e* 544 together with peaks at *m/e* 526 (M$^+$ − H_2O), 466 (M$^+$ − H_2O − HOAc), 426 (M$^+$ − HOAc-58), 424 (M$^+$ − 2HOAc), 249 (ion **c**), 265 (ion **d**) and an intense peak at *m/e* 59. The above MS fragmentation pattern was compatible with mollugogenol-A [7, 8] belonging to the isohopane series isolated from the same source.

The oxidation of mollugogenol-F (**1**) with CrO_3–Py at 0° [9] furnished a monohydroxy diketone (**3**), $C_{30}H_{48}O_3$, mp 231–234° and a dihydroxy monoketone (**4**), $C_{30}H_{50}O_3$, mp 228–229°. The latter compound (**4**)

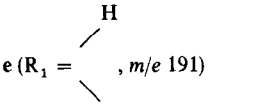




a (R_1 = , *m/e* 207)



c (R_1 = , *m/e* 249)



e (R_1 = , *m/e* 191)

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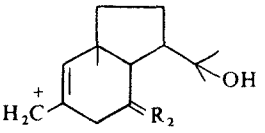
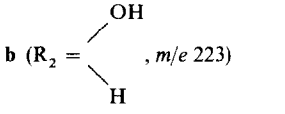
could be oxidized to the former (3). Both mono and diketones responded to Zimmerman's colour test for a 3-keto group [10] thereby locating a secondary OH group at C-3. The IR spectrum (nujol) of the monohydroxy diketone (3) showed bands at 1715 $\diagdown$ (C=O) $\diagup$ and 3540 cm^{-1} (OH) indicating the presence of a six-membered ring ketone and one tertiary OH group, respectively and consequently ruled out the possibility of the OH function being at C-19 or C-20 in ring E.

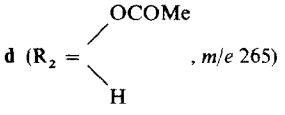
Mollugogenol-F (1), on treatment with dry EtOH–HCl (5%) for 3 hr (reflux), produced a conjugated 15,17(21)-diene (6) (λ_{max}^{EtOH} 243,251 and 261 nm) [8, 11, 12]. The diketone (3) readily isomerized to the α,β-unsaturated ketone (7), $C_{30}H_{46}O_2$, mp 233–236° [λ_{max}^{EtOH} 257 nm (log ε 3.91), ν_{max}^{nujol} 1682 cm^{-1} for an α,β-unsaturated six-membered ring ketone and 1715 cm^{-1} for a six-membered ring ketone] by refluxing with dry EtOH–HCl (5%) for 3 hr. The UV and IR spectra of (7) further supported the absence of any five-membered ring ketone and obviously the absence of an OH group in ring E (C-19 or C-20) in mollugogenol-F (1). The easy formation of the dienic compound (6) and facile isomerization to the α,β-unsaturated ketone (7) clearly indicated the presence of two other OH groups at C-16 (secondary) and C-22 (tertiary), respectively. During oxidation of mollugogenol-F (1), the C-16 OH group showed resistance towards oxidation which was obviously due to the intramolecular hydrogen bonding between the C-16 and C-22 OH groups [12]. The 15,17(21)-conjugated diene (6) was also produced when the monoketone (4) was refluxed with *p*-toluene sulphonic acid in C_6H_6 solution containing a little diethylene glycol.

Wolff–Kishner reduction [13] of diketone (3) gave a product (8), $C_{30}H_{52}O$, mp 176–178°, in very poor yield which might be epimeric at C-17 [8]. This showed the same R_f value on TLC as an authentic sample of 22-hydroxyisohopane (5), whereas 22-hydroxyhopane (21β-H) showed a very close but less polar spot than (5). The product (8) and 22-hydroxyisohopane (5) showed broad bands at 3440 and 3300 cm^{-1}, respectively, in their IR spectra (KBr). The MS of (8) showed a molecular ion peak at m/e 428 as expected together with peaks

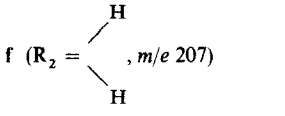




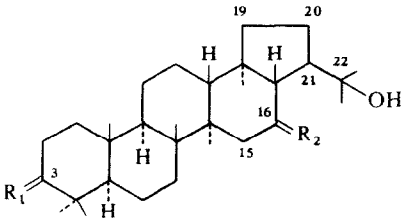
b (R_2 = , *m/e* 223)

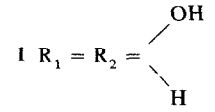


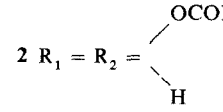
d (R_2 = , *m/e* 265)



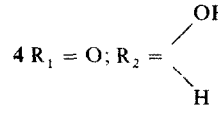
f (R_2 = , *m/e* 207)

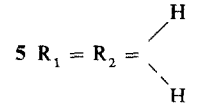


1 $R_1 = R_2$ = 

2 $R_1 = R_2$ = 

3 $R_1 = R_2 = O$

4 $R_1 = O$; R_2 = 

5 $R_1 = R_2$ = 

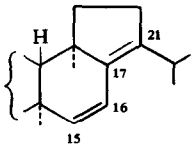
at *m/e* 410 ($M^+ - H_2O$), 207 (ion **f**), 191 (ion **e**) and an intense peak at *m/e* 59.

The two secondary OH groups in mollugogenol-F appeared to be equatorial (β) as these could be easily acetylated at 0°. The hydroxyisopropyl side chain at C-21 was considered to be β from the biogenetic point of view. On the basis of these observations, mollugogenol-F may be represented as 3β,16β,22-trihydroxyisohopane (1).

EXPERIMENTAL

Mps are uncorr. TLC plates were sprayed with Liebermann reagent (Ac_2O, 10 ml; H_2SO_4, 10 ml; EtOH, 80 ml) and the spots were developed by heating at 100° for *ca* 10–15 min.

Isolation of mollugogenol-F (1). The air-dried powdered plants of *M. hirta* (4 kg) were defatted with petrol (bp 60–80°) and then extracted with 95% EtOH in a Soxhlet apparatus for 48 hr. The EtOH extract was concd and the saponin was pptd by addition of Et_2O. The saponin was dissolved in EtOH–H_2O (1:1, 300 ml), conc HCl (30%, 50 ml) was added and the mixture was hydrolysed by refluxing the soln (with 5% aq. EtOH–HCl) for 30 min. The sapogenin was completely pptd by removal of EtOH on the steam bath with subsequent addition of H_2O. It was filtered, made acid-free and dried (25 g). The crude sapogenin was extracted with $CHCl_3$ and separated into neutral and acid parts by aq. KOH (2%, 400 ml). The neutral part (12 g) was chromatographed over Si gel (900 g). Elution with $CHCl_3$ furnished a fraction (3.2 g) which was re-chromatographed over Si gel (100 g) and elution with C_6H_6–Et_2O (9:1) yielded a product which on repeated crystallization from $CHCl_3$–MeOH gave mollugogenol-F (600 mg), mp 211–212°, $[\alpha]_D^{24}$ + 41.26°. ($CHCl_3$). (Found: C, 78.01; H, 11.16, $(M - H_2O)^+$ 442. $C_{30}H_{52}O_3$ requires: C, 78.21; H, 11.38%).



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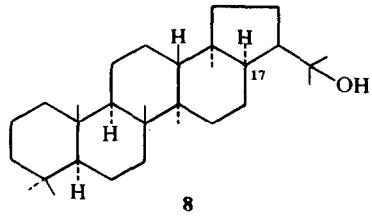

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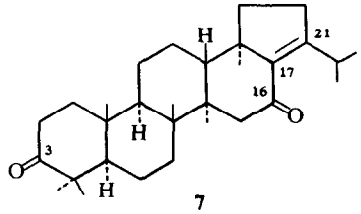
Acetylation of mollugogenol-F. Mollugogenol-F (200 mg) (**1**) was acetylated with dry Py (8 ml) and Ac_2O (20 ml) at 0° (overnight). The reaction product was poured into crushed ice and worked up in the usual way. The crude acetate (190 mg) was dissolved in C_6H_6 (15 ml) and adsorbed on a column of Si gel (20 g). Elution with C_6H_6–$CHCl_3$ (3:1) gave a glassy material (145 mg), crystallized from MeOH, diacetate (**2**), mp 225–226°. (Found: C, 74.82; H, 10.54. M^+ 544. $C_{34}H_{56}O_5$ requires: C, 74.96; H, 10.36%).

Sarett oxidation of mollugogenol-F. A cold soln of mollugogenol-F (290 mg) in dry Py (5 ml) was added to a slurry of CrO_3–Py complex (600 mg CrO_3 and 10 ml Py) at 0° with stirring and then left overnight at room temp. The reaction product was poured into crushed ice and worked up in the usual way. The oxidation product (165 mg) was dissolved in C_6H_6 (20 ml) and adsorbed on a column of Si gel (20 g). Elution with C_6H_6–$CHCl_3$ (3:1 and 2:1) gave a material (60 mg), crystallized from petrol–$CHCl_3$ as needles (**3**), mp 231–234°. (Found: C, 78.71; H, 10.68. $C_{30}H_{48}O_3$ requires: C, 78.90; H, 10.59%). Further elution with C_6H_6–$CHCl_3$ (1:1) and $CHCl_3$ furnished a glassy material (71 mg), crystallized from MeOH as fine needles (**4**), mp 228–229°. (Found: C, 78.70; H, 11.12. $C_{30}H_{50}O_3$ requires: C, 78.55; H, 10.99%).

Preparation of α:β-unsaturated ketone (**7**) *from* (**3**) *with dry* EtOH–HCl. The monohydroxy diketone (**3**) (50 mg) was dissolved in dry EtOH (4 ml) and dry EtOH–HCl (10%, 4 ml) was added. The soln was refluxed for 3 hr. The reaction product was poured into crushed ice and worked up in the usual way. (**7**) was crystallized from MeOH as needles (34 mg), mp 233–236°.

Treatment of mollugogenol-F (**1**) *with dry* EtOH–HCl. To the soln of mollugogenol-F (10 mg) in dry EtOH (2 ml) was added dry EtOH–HCl (10%, 2 ml) and the mixture refluxed for 3 hr. The reaction mixture was poured into crushed ice, extracted with $CHCl_3$ and worked up in the usual way. The product could not be crystallized due to the small quantity of material. However, it showed UV absorption maxima (EtOH) at 243, 251 and 261 nm.

Treatment of dihydroxy monoketone (**4**) *with* p-*toluene sulphonic acid.* To the C_6H_6 soln (30 ml) of dihydroxy monoketone (60 mg), diethylene glycol (0.2 ml) and *p*-toluene sulphonic acid (160 mg) were added and the mixture was refluxed at 110° (oil bath) for 6 hr in a Dean and Stark apparatus. A material that pptd in the flask was filtered, washed with C_6H_6 and this was characterized as *p*-toluene sulphonic acid. The filtrate was neutralized with NaOEt–EtOH, washed with H_2O, freed from alkali, dried over dry Na_2SO_4 and the solvent removed. Crystallization from MeOH did not furnish a very pure material. UV λ_{max}^{EtOH} nm: 243, 251, 261.



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Wolff–Kishner reduction of monohydroxy diketone. Monohydroxy diketone (200 mg) was dissolved in a mixture of EtOH (15 ml) and freshly distilled diethylene glycol (8 ml) by heating and to this soln was added hydrazine hydrate (85%, 10 ml) when a slight turbidity formed. The ppt. was dissolved by adding dioxan (6 ml) and the mixture was refluxed for 15 min. Solid KOH (2 g) was added and it was again refluxed for 15 min. Some solvent was distilled off at red. pres. and the mixture was heated at 196–200° (oil bath) for 3 hr. It was then left overnight at room temp. The reaction product was poured into crushed ice and worked up in the usual way. The crude product (145 mg) was dissolved in C_6H_6 (10 ml) and adsorbed on a column of Si gel (20 g). Elution with petrol–C_6H_6 (3:1) furnished a product (**8**) in very poor yield (35 mg), crystallized from MeOH, mp 176–178°, M^+ 428. The product showed the same R_f value as an authentic sample of 22-hydroxyisohopane (**5**) on TLC.

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