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STRUCTURE OF MARSILEAGENIN A; A NEW HEXAHYDROXY TRITERPENE FROM *MARSILEA MINUTA* LINN[a]

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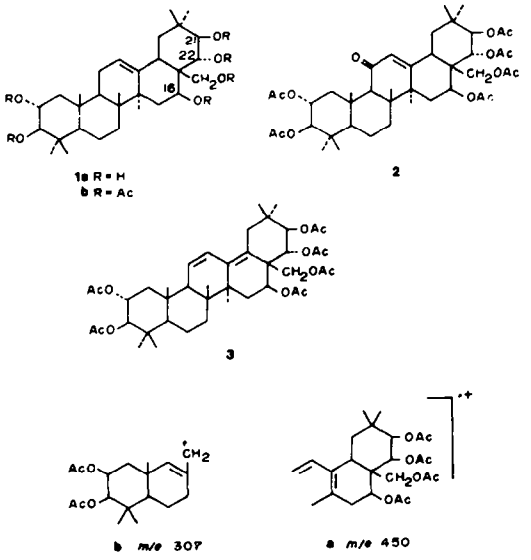
Abstract—The crude saponin obtainable from *Marsilea minuta* Linn on acid hydrolysis yielded a mixture of sapogenols. The major sapogenol named Marsileagenin A was found to be a hexahydroxy triterpene of oleanene series. From a study of various spectrometric data together with chemical reactions the structure of this sapogenol has been assigned as olean-12-ene-2α, 3β, 16β, 21β, 22α, 28-hexol (**1a**).

In a recent communication[1] isolation and characterisation of a number of known compounds together with a new hydroxy ketone from *Marsilea minuta* Linn was reported. The present paper deals with the isolation and structure elucidation of a new hexahydroxy triterpenoid sapogenol the major sapogenol constituent designated marsileagenin A. It analysed for $C_{30}H_{50}O_6$, m.p. 332–33° $[\alpha]_D$ + 48° and gave a pink coloration in Liebermann Burchard test. It forms a hexaacetate (**1b**), $C_{42}H_{62}O_{12}$, m.p. 285° $[\alpha]_D$ + 37·5°. Thus all the six O atoms in marsileagenin A are present as OH functions. That one of the hydroxyls is primary was revealed by the formation of a monotrityl derivative, m.p. 205–7° with trityl chloride and pyridine. Moreover, it consumed two moles of periodic acid and the hexaacetate (**1b**) consumed one mole of perbenzoic acid. From these the presence of two α-glycol systems and a double bond in the molecule may be inferred. Marsileagenin A hexaacetate on refluxing with chromium trioxide furnished an unsaturated ketone, m.p. 287° presumably the 11-keto compound (**2**) indicating the presence of a 12:13 double bond. The 11-keto compound displayed UV absorption maximum at 239 nm against usual position at about 249 nm.[2] This hypsochromic shift is reasonably attributed to the presence of a number of acetoxyl groups in the molecule.

The MS of marsileagenin A hexaacetate (**1b**) did not show the molecular ion peak but it gave the M^+–AcOH peak at *m/e* 698 due to the facile loss of a molecule of acetic acid in the ionising chamber. The retro Diels–Alder fragments involving 12:13 double bond[3] were well discernible at *m/e* 307 (fragment *b*) and at 390 (*a*–AcOH). The other significant peaks were at 375 (*a*–AcOH–CH_3), 315 (*a*–2AcOH–CH_3), 302 (*a*–AcOH–CH_2OAc–CH_3), 270 (*a*–3AcOH), 197 (*a*–3AcOH–CH_2OAc), 247 (*b*–AcOH) and 187 (*b*–2AcOH) a fragmentation pattern characteristic of either Δ^{12}-oleanene or Δ^{12}-ursene derivatives.

The detection of seven quaternary C-Me groups as sharp singlets in the NMR spectrum of the hexaacetate (**1b**) suggested that marsileagenin A is a oleanene-type triterpene alcohol. Furthermore, the hexaacetate (**1b**) when refluxed with SeO_2 in glacial AcOH yielded the $\Delta^{11,13(18)}$-*trans* heteroannular diene (**3**) showing triple UV absorption maxima at 241, 250 and 259 nm (log ϵ 4·43, 4·46 and 4·38 respectively) characteristic of Δ^{12}-oleanenes.[4] The locations of the 6 OH groups in **1a** were revealed from the following considerations:



From the mass fragmentation pattern of **1b** one can conclude that one primary and three secondary OH groups are located on rings D and E and two secondary OH groups are present on rings A and B. Abundant peaks at *m/e* 302 and 197 (obtainable by loss of CH_2OAc from fragment *a*) indicate the primary acetoxyl group being present at C-28. This is supported by the appearance of a broad singlet at δ 3·96 assignable to methylene protons of C-28 acetoxymethyl group in the NMR spectrum of **1b**. Marsileagenin A contains two 1,2-glycol systems and one of the four OH groups present on rings D and E is primary, it follows, therefore, that only one α-glycol moiety is present in this part. The presence of a 1,2-glycol at C-15 and C-16 is ruled out, because it has been observed[5] that an OH group at

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C-15 whether axial or equatorial is resistant to acetylation under mild conditions whereas marsileagenin A furnished a hexaacetate under such conditions. On the other hand, the AB quartet at 5·0 (1H, d, J = 11 Hz) and at 5·39 (1H, d, J = 11 Hz) in the NMR of **1b** is better ascribed to the trans diaxial protons alpha to the acetylated 1,2-glycol systems at C-21 and C-22 rather than at C-15 and C-16.[6] The C-19 OH group (axial or equatorial) is known to be one of the most hindered OH groups in the oleanene series and cannot be esterified with Ac_2O and pyridine.[7] Consequently, the presence of the third secondary OH group on rings D and E at C-16 with equatorial configuration was indicated. This was supported by the presence of a triplet like multiplet at 5·75 assignable to C-16 axial proton[8] (deshielding effect due to 1,3-interaction with the C-27 Me group) in the NMR of **1b**. The presence of an OH group at C-3 is assumed on biogenetic ground and as there are two OH groups on rings A and B constituting a 1,2-glycol system, the other OH group is reasonably placed at C-2. Diequatorial relationship of the glycol system is revealed from the NMR of **1b** which shows signals at 4·68 (1H, m) and 4·84 (1H, d, J = 10 Hz) ascribable to diaxial protons at C-2 and C-3 respectively.

These combined evidences have led to a conclusion that marsileagenin A may be formulated as olean-12-ene-2α, 3β, 16β, 21β, 22α, 28-hexol (**1a**).

EXPERIMENTAL

M.ps were determined in open capillary tubes in a sulphuric acid bath and are uncorrected, UV data are for EtOH solns. IR spectra for Nujol mulls and rotations in $CHCl_3$ solns unless stated otherwise. NMR spectra were recorded at 60 MHz in $CDCl_3$ with TMS as internal reference and mass spectra were determined at an ionizing potential of 70 eV. Petroleum used had b.p. 60–80° and TLC was performed on silica gel G (E. merck).

Isolation of marsileagenin A (**1a**). Dried stem and leaves of *Marsilea minuta* Linn were powdered (640 g) and successively extracted with light petroleum, chloroform and EtOH. The ethanolic extract on removal of solvent yielded a dark residue (80 g) which was extracted repeatedly with BuOH. BuOH was distilled off under reduced pressure wheen the crude saponin was obtained as a dark brown gummy mass (60 g). It is interesting to note that the tongue loses taste for sugar when a little of the crude saponin is taken on it.

The crude saponin (55 g) was hydrolysed with 6% ethanolic HCl (380 ml) under reflux for 16 hr. Alcohol was evaporated off on water bath keeping the volume the same by frequent addition of water. It was then filtered and the residue was washed with water until free from acid and dried (30 g). On TLC examination the residue was found to contain three sapogenols designated as marsileagenin A, B and C according to the increasing order of their R_f values. The latter two however, were present in very small quantities. Alumina (neutral Brockmann, 600 g) column chromatography of the crude sapogenol mixture (27 g) developing successively with benzene, benzene-chloroform mixture (1 : 1) and chloroform–methanol mixture (90 : 10) gave three fractions: fraction 1 (eluted with benzene, 4·1 g), fraction 2 (eluted with benzene–chloroform mixture 1 : 1, 1·15 g), fraction 3 (eluted with chloroform–methanol mixture 90 : 10, 0·9 g).

Fractions 1 and 2 were gummy materials and no crystalline matter could be obtained from them. Fraction 3 was rechromatographed on alumina (100 g) elution being carried out with chloroform–methanol mixture (90 : 5) and two fractions were separated—an early fraction (A) (110 mg) and a later fraction (B) (440 mg). TLC examination of fraction (A) revealed that it was a mixture of marsileagenin B and marsileagenin C. Separation and characterisation of these two constituents is in progress.

Fraction (B) on crystallisation twice from EtOH afforded marsileagenin A as micro needles (260 mg) m.p. 332–33° $[\alpha]_D$ + 48° (EtOH). (Found: C, 71·23; H, 9·91. $C_{30}H_{50}O_6$ requires: C, 71·11; H, 9·95%).

Marsileagenin A hexaacetate (**1b**). A soln of **1a** (300 mg) in pyridine (3 ml) and Ac_2O (6 ml) was left standing at 40° for 24 hr, poured into ice-water and extracted with ether. Alumina column chromatography of the ether extract followed by crystallisation from MeOH yielded **1b** (250 mg, single spot on TLC), m.p. 285°. $[\alpha]_D$ + 35·5°, ν_{max} 1740 (br), 1242 (br.) cm^{-1} (acetate carbonyl), MS *m/e* 698 (M^+–AcOH), 683 (M^+–AcOH–CH_3), 638 (M^+–2AcOH), 578 (M^+–3AcOH, base peak), 536 (M^+–3AcOH–C_2H_2O), 518 (M^+–4AcOH), 476 (M^+–4AcOH–C_2H_2O), 458 (M^+–5AcOH), 445 (M^+–4AcOH–CH_2OAc), 385 (M^+–5AcOH–CH_2OAc), 375 (*a*–AcOH–CH_3), 315 (*a*–2AcOH–CH_3), 302 (*a*–AcOH–CH_2OAc–CH_3), 270 (*a*–3AcOH), 197 (*a*–3AcOH–CH_2OAc), 247 (*b*–AcOH), 187 (*b*–2AcOH) (Found: C, 66·39; H, 8·26. $C_{42}H_{62}O_{12}$ requires: C, 66·46; H, 8·24%).

Preparation of the α,β-unsaturated ketone (**2**). A soln of **1b** (50 mg) in glacial AcOH (10 ml) was heated under reflux and then CrO_3 (50 mg) in AcOH (85%, 5 ml) was added gradually over a period of 1 hr. Refluxing was continued for another hr, cooled and diluted with water. The ppt was filtered off, purified by chromatography and crystallised from MeOH as colourless needles (20 mg), m.p. 287°, λ_{max} 239 nm (log ϵ 4·12), ν_{max} 1670 cm^{-1} (α,β-unsaturated ketone) (Found: C, 65·28; H, 7·79. $C_{42}H_{60}O_{13}$ requires: C, 65·26; H, 7·83%).

SeO_2 *Oxidation of* **1b**, *formation of* **3**. The hexaacetate (**1b**) (50 mg) in glacial AcOH (3 ml) was refluxed with freshly sublimed SeO_2 (50 mg) for 4 hr. The soln was filtered hot, diluted with water and worked up in the usual way. The crude product was purified by chromatography over alumina and crystallised from MeOH as flakes (20 mg), m.p. 285–86°, λ_{max} 241, 250 and 259 nm (log ϵ 4·43, 4·46 and 4·38 respectively) (Found: C, 66·57; H, 7·89. $C_{42}H_{60}O_{12}$ requires: C, 66·64; H, 7·99%).

Periodic acid oxidation of **1a**. A soln of **1a** (60 mg) in MeOH (20 ml) was treated with 5 ml of 0·5M periodic acid aq and left for 24 hr. A blank experiment was performed side by side. Excess of periodic acid was titrated with standard sodium arsenite soln. Consumption of periodic acid per mole of the compound was found to be two moles.

Perbenzoic acid oxidation of **1b**. The hexaacetate (35 mg) in $CHCl_3$ was treated with a soln of perbenzoic acid (0·35 N, 5 ml) at 0°. The consumption of the per acid was measured by titrating with standard thiosulphate soln. It was found that consumption of perbenzoic acid per mole of the compound was one mole.

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REFERENCES

[1]D. Chakravarti and N. B. Deb Nath, *J. Indian Chem. Soc.* **51**, 260 (1974).
[2]G. G. Allan and C. S. Chopra, *Phytochemistry* **10**, 1363 (1971).
[3]H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Am. Chem. Soc.* **85**, 3688 (1963).
[4]L. Ruzicka, G. Muller and H. Schellenberg, *Helv. Chim. Acta* **22**, 767 (1939).
[5]C. Djerassi, C. H. Robinson and D. B. Thomas, *J. Am. Chem. Soc.* **78**, 5685 (1956).
[6]G. Subba Rao and J. E. Sinsheimer, *Chem. Comm.* 1681 (1968).
[7]J. Simonsen and W. C. J. Ross, *The Terpenes*, Vol. 5, p. 286. Cambridge University Press (1957).
[8]M. Shamma, R. E. Glick and R. O. Mumma, *J. Org. Chem.* **27**, 4512 (1962).