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MASS SPECTRAL STUDIES OF PHYTOSTEROLINS AND A KETONE FROM *TRIANTHEMA PENTANDRA*

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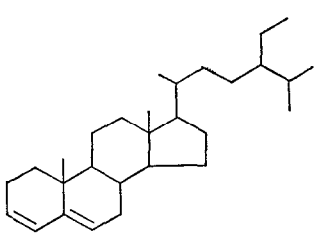
Key Word Index—*Trianthema pentandra*; Aizoaceae; sitosterol and stigmasterol glucosides; nonacos-1-en-4-one.

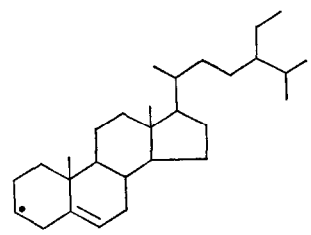
Abstract—Mass spectral data of a phytosterolin and its tetraacetate from *Trianthema pentandra* show that sitosterol-D(+)-glucoside reported earlier[1] was a mixture of sitosterol-D(+)-glucoside and stigmasterol-D(+)-glucoside. The structure of a ketone has been suggested as nonacos-1-en-4-one, on the basis of physicochemical data. The positions of the double bond and the carbonyl group have been established by MS and NMR data. This is the first report in plants of a C_{29} ketone with a terminal double bond and carbonyl group at position 4.

INTRODUCTION

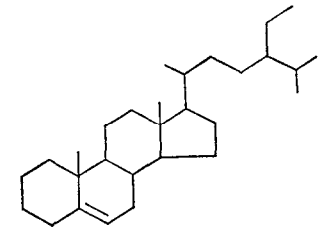
WE HAVE earlier reported the isolation of hentriacontane (I), a ketone (II), hentriacontol (III) from light petrol. extract and sitosterol-D(+)-glucoside (IV) from alcoholic extract of the roots and stems of *Trianthema pentandra*.[1] This paper deals mainly the MS studies of the ketone and phytosterolin.



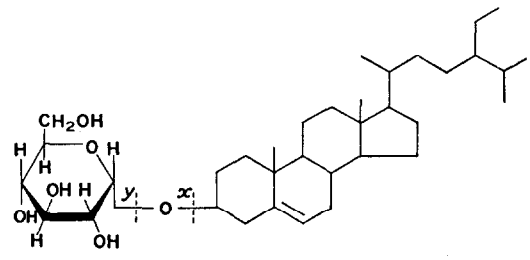
(i) *m/e* 396



(ii) *m/e* 397



(iii) *m/e* 398



(IV)

[1] A. N. MISRA and H. P. TIWARI, *Phytochem.* **11**, 1176 (1972).

RESULTS AND DISCUSSION

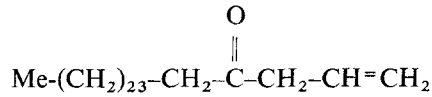
Ketone (II)

Elemental analysis indicates the molecular formula $C_{29}H_{56}O$. This molecular formula is supported by molecular ion peak at *m/e* 420. The compound gives a yellow colour with tetranitromethane. The IR spectrum of II showed a strong peak at 1730 cm^{-1} showing ketonic function. The peak at 1665 cm^{-1} indicates C=C and that at 722 and 715 cm^{-1} shows there is an *n*-alkane chain. II formed an orange yellow crystalline 2,4-dinitrophenyl hydrazone, m.p. 89–90°. $LiAlH_4$ reduction gave a compound m.p. 85–87°, the IR spectrum of which shows peaks at 3625, 1055, 1066 and 1015 cm^{-1} characteristic of secondary alcohol.[2] The peak at 1665 cm^{-1} characteristic of C=C is also present in the spectrum of the reduction product. The alcohol on acetylation gave an acetyl derivative m.p. 65°. The IR spectrum of acetate shows peaks at 1735 cm^{-1} (acetate CO) and peak at 1252 cm^{-1} (O–COMe). The UV spectrum of II shows a very weak absorption band at 227 nm, which rules out the possibility of the double bond being in conjugation with keto group.

MS study of ketone (II)

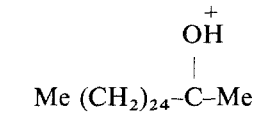
In the MS of the ketone (II), no significant $(M-15)^+$ peak was observed and the ratio $(M-15)^+/M^+$ was close to zero. This indicates that ketone has a straight chain.[3] MS showed $(M+1)^+$ peak characteristic of asymmetrical ketones.[4] The ratio of M^+ to $(M+1)^+$ is two.

The most intense peak obtained in the spectrum is at *m/e* 394. The next intense peak is at *m/e* 379. Other significant peaks are at *m/e* 85 and 69. These mass fragments can be explained on the basis of the fragmentation pattern given by Wollrab for nonacosan-7-one derived from secondary alcohol,[2] by assigning the following structure to the ketone (II)



(II)

Peak at *m/e* 395 may arise from following fragment[5]



The NMR spectrum is also in agreement with the proposed structure II. This spectrum shows signal at 9·12 τ assigned to three methyl protons and a strong signal at 8·72 τ due to methylene protons. A multiplet centered at 7·60 τ is assigned to methylenic protons next to the carbonyl function. Signal at 5·80 τ is assigned to the allylic protons which are also next to carbonyl group. The signal for the olefinic protons appear as a multiplet centered at τ 4·44.

Alcoholic extract of the roots and stems gave a crystalline compound (IV) m.p. 302–303° (decomp.), $[\alpha]_D$ −38° (pyridine). The compound gave a yellow colour with tetranitromethane. In the Liebermann–Burchard test a pink colour was observed which changed

[2] V. WOLLRAB, *Phytochem.* **8**, 623 (1969).
[3] B. STOIANOVA-IVANOVA and P. HADJIEVA, *Phytochem.* **8**, 1549 (1969).
[4] J. H. BEYON, G. R. LESTER, K. A. SAUNDERS and A. E. WILLIAMS, *Trans. Faraday Soc.* **57**, 1259 (1961).
[5] A. G. NETTING and M. J. K. MECEY, *Phytochem.* **10**, 1917 (1971).

to bluish green immediately. The compound gave positive Molish's test. Acetylation with acetic anhydride and pyridine gave acetyl derivative: m.p. 157°, $[\alpha]_D$ −30° ($CHCl_3$). The compound (IV) appears to be phytosterolin.

MS study of phytosterolin

Phytosterolin and its tetraacetate were unstable when run in a mass spectrophotometer even using difect inlet probe. The most intense peak is at *m/e* 396, which corresponds to the fragment (i) obtained after elimination of sugar from phytosterolin (IV). Peak at *m/e* 397 (ii) can arise by the cleavage of the carbon–oxygen bond at *x* (IV) and peak at *m/e* 398 (iii) being formed by cleavage of carbon–oxygen bond at *x* and a hydrogen rearrangement. Peak at *m/e* 414 which corresponds to mass of sitosterol may arise by cleavage of carbon–oxygen bond at *y* and a hydrogen rearrangement.

Peaks at *m/e* 394, 395, 396 and 412 in the MS may be explained if the compound under study is a mixture of sitosterol-D(+)-glucoside and stigmasterol-D(+)-glucoside. Mass peak at *m/e* 300 is characteristic of steroids having double bond between C_{22} and C_{23}[6,7] is present in the MS. The other significant peaks in the MS are at *m/e* 369 and 351. The occurrence of peaks at *m/e* 145, 127, 109, 73, 61 and 60 in the lower region of the MS indicates that the sugar of the phytosterolin is glucose.

MS of the acetate of phytosterolin besides other expected peaks shows peaks at *m/e* 331, 289, 271, 229, 211, 157, 115, 109 and 57. These fragments are characteristic of glucose tetraacetate.

The phytosterolin on hydrolysis gave a mixture of sitosterol and stigmasterol which were separated as their dibromo and tetrabromo derivatives respectively on neutral alumina column and the glucose was identified by PC.

EXPERIMENTAL

The dried powdered stems and roots (8 kg), collected locally, were extracted (So xhlet) with light petrol (60–80°) and then with EtOH.

Light petrol. extract. Concentration of this extract afforded a dark green solid. The solid was dissolved in EtOH–$CHCl_3$ (10:1), kept at 0°, filtered and chromatographed through neutral alumina grade 1. The following compounds were eluted: (I) hentriancontane (light petrol.), (II) nonacos-1-ene-4-one (later petrol. fractions), and (III) hentriancontol (benzene fraction).

Nonacos-1-ene-4-one (II). Crystallizations with $CHCl_3$–MeOH (1:1) gave crystalline solid m.p. 75°. (Found: C, 82·45; H, 13·34. $C_{29}H_{56}O$ required: C, 82·85; H, 13·57%). ν_{max}^{KBr} 2915, 2855, 1730, 1665, 1460, 1470, 1408, 1375, 1200, 1182, 1170, 722, 715 cm^{-1}, NMR bands at g 9·12, 8·72, 7·60, 5·80, 4·44.

$LiAlH_4$ *reduction of* (II) in ether was carried out by the standard procedure. The solid obtained was crystallized 2× with $CHCl_3$–MeOH (1:1) to give a compound (43 mg), m.p. 84–85°. $\nu_{max}^{CHCl_3}$ 3625, 2945, 2850, 1665, 1465, 1450, 1365, 1258, 1200, 1095, 1060, 1015 cm^{-1}. The reduction product on acetylation with Ac_2O in pyridine a monoacetate was formed, m.p. 65° from EtOH.

Ethanol extract. Further extracted with Et_2O. This extract on evaporation gave brown coloured solid. Repeated crystallizations with EtOH gave crystals m.p. 302–303°, $[\alpha]_D$ −38° (pyridine), ν_{max}^{KBr} 3400–3425, 2930, 2848, 1454, 1650, 1375, 1362, 1152–1115, 825–830 cm^{-1}. The compound on acetylation with Ac_2O and pyridine gave an acetyl derivative from EtOH m.p. 157°, $[\alpha]_D$ −30° (CHCl), NMR signals at τ 9·28 9·12, 9·19, 9·14, 8·94–7·66, 7·98, 5·81, 4·98–4·78 (deformed multiplet) and 4·50–4·75 (deformed envelop due to ethylenic protons).

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[6] M. Lenfant, R. Ellouz, B C. Das, E. Zissman and E. Lederer, *Europ. J. Biochem.* **7**, 159 (1969).
[7] M. C. Gerschengorn, A. R. H. Smith, G. Goulston, L. J. Goad, T. W. Goodwin and T. M. Haines, *Biochemistry* **7**, 1698 (1968).