

24 β -Ethyl Cholest-5, 22-Dien-3 β -OL from *Salvinia natans* L.

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ABSTRACT

A sterol characterised as 24 β -ethyl, 5,22diene-3 β -ol, has been isolated from *Salvinia natans*. This is the first report regarding the occurrence of the compound from this plant.

Keywords: *Salvinia natans*, 24 β -ethyl Cholest-5,22-dien-3 β -ol, Dal Lake

INTRODUCTION

Salvinia natans is an annual free-floating fern belonging to family Salvinaceae. There is no authentic record of the time of invasion of *Salvinia* in Kashmir waters. The first report regarding the presence of the fern in Dal lake, Srinagar and its fruiting of the species during the autumn is well documented as reported by Biswas and Calder (1936) and Zutshi and Vass (1971). The plant is known to contain Dihydro Cholesterol and some sterols as well and reported by Miralles *et al.*, (1989).

MATERIAL AND METHODS

The plant *Salvinia natans*, collected from Ashai Bagh Bridge Hazratbal in Dal Lake, was air dried in the shade. The plant material was extracted with 95% alcohol (Ethanol) and defatted with petrol. The defatted plant material was extracted with EtoH in a Soxhlet and the resulting extract, after drying in vacum, was chromatographed over silica-gel. The column was eluted using graded solvent systems. The fractions were pooled on the basis of their TLC pattern. The development of the column with chloroform and methanol (95:5) gave the compound, SNE-1. For further purification the isolated compound was subjected to chromatography over silica-gel and purity was checked on TLC using silic-gelG. Mps:Uncorr. IR spectra were recorded in KBr discs. ¹HNMR were run at 200MHz.

RESULTS AND DISCUSSION

An alcoholic extract of the plant was chromatographed on a silica gel column using graded elution of solvents. Elution with chloroform-Methanol (95:5) afforded

the compound SNE-1 analysed for $C_{29}H_{48}O$, m.p (119-20°C). The compound responded positively to tests for sterol and it was found a single entity on TLC.

Its IR spectrum displayed characteristic bands at 3408(OH), 2884, 1638, 1596, 1558 cm^{-1} for C=C, and 1380, 1262, 1174, 1098 cm^{-1} for gemdimethyl and isopropyl group.

The mass spectrum of compound SNE-1 giving a molecular ion peak at m/z 412 is analyzed for $C_{29}H_{48}O$. The index of unsaturation is six, out of which four are accommodated due to the presence of steroid nucleus, while the remaining two exist as double bonds one in the steroid nucleus and the other in the side chain as evident from the expected chemical shift in 1H and ^{13}C NMR. On the basis of MS the mass spectrum of SNE-1 indicated the presence of diagnostically important peaks at m/z 397 (M-Me), 302(M- $C_{18}H_{20}$), 273 (M-side chain). The spectrum shows a prominent peak at m/z 397 due to loss of one methyl from the side chain. After losing the side chain it loses a molecule of water; this confirms the alcoholic group in the molecule. A peak corresponding to m/z 254 [M-side chain - H_2O] $^+$ is present in the spectrum which confirms the presence of hydroxyl function in the molecule. The base peak fragment at m/z 271 is observed due to loss of side chain and two hydrogen from the nucleus characteristic of Δ^{22} bond. This confirms the position of one unsaturated center at Δ^{22} . A significant peak at m/z 302 is observed which shows the loss of $C_{18}H_{20}$ from the ion probably due to vinylic fission, which confirms that the double bond is present in the side chain. The base peak fragment at m/z 55 is characteristic of cholestane having cis fused A/B ring system. This reflects the stereochemistry at C-5 as β . A significant peak at m/z 254 is observed due to loss of methyl group arising from fragment ion at m/z 273, obtained after the loss of the side chain. β configuration of ethyl at C-24 was decided by the comparison of the spectra with other Δ^{22} compounds. The loss of 43 amu from the molecule ion is found to be common with 24 β -ethyl containing compounds. Thus compound SNE-1 is given the structure as 24 β -Ethyl Cholest-5, 22-Dien-3 β -0L.

The 1H -NMR spectrum of compound exhibit one proton doublet at δ 5.38(d, 5.7Hz) assigned to H-5 proton. A multiplet around δ 5.14, can be assigned to the 22-H and 23-H olefinic protons. Four doublets integrating three protons each at δ 0.80, δ 0.70, δ 0.79, δ 0.79, were the exhibits for C-21, C-26, C-29 and C-27 secondary methyls respectively. Remaining two tertiary methyl's resonated as singlets at

δ 1.03(3H, 19- Me) and 0.68(3H, 18-Me). A broad multiplet at δ 1.41 with coupling constant 24.3Hz was assigned to the 17- α proton. The appearance of all methyl's in the region δ 1.08 - 0.68 suggested that these functionalities were attached to a saturated carbon. The remaining methylene and methine groups appeared in the region δ 2.27 -1.07.

Further evidence for the structure was provided by its ^{13}C -NMR spectral data which showed the existence of 29 carbon atoms in the molecule. Signal at δ c140.12, 121.07 and 73.06 were assigned to unsaturated carbon C-5,C-6 and C-3 Carbinol respectively. Signal at δ c128.04,96.75 were also assigned to unsaturated carbon C-22, C-23. The degree of proto-nation of each carbon atom was determined by Dept. experiment and the assignments of the carbon chemicals shifts were made by comparison with the δ c values of the corresponding carbon atoms in the structurally similar sitosterols.

Table 1. ^1H - NMR of SNE-1

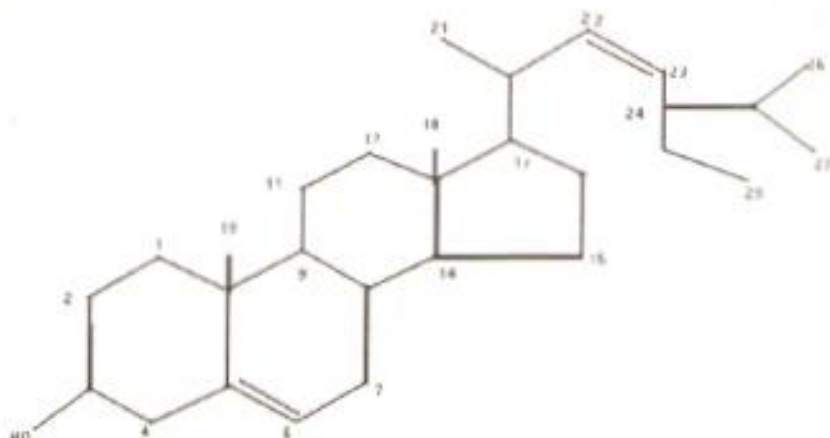
Assignment	δ (Chemical shift)	Assignment	δ (Chemical shift)
H-1	1.31	H-16	-1.83
2	1.86	17	1.44
3	1.81	18	0.68
4	1.83	19	1.03
5	5.36	20	1.51
6	1.56	21	0.80
7	1.52	22	5.14
8	1.15	23	1.52
9	1.43	24	1.18
10	1.49	25	1.22
11	2.27	26	0.70
12	1.03	27	0.79
13	1.48	28	1.16
14	1.15	29	0.79
15	1.08	---	-----

Table 2. ^{13}C -NMR of SNE-1

Carbon	δc	Carbon	δc
1	36.6	16	11.34
2	28.52	17	56.23
3	73.06	18	18.3
4	39.14	19	18.7
5	140.12	20	41.68
6	121.07	21	22.4
7	31.03	22	128.04
8	31.03	23	96.75
9	49.50	24	45.20
10	35.50	25	33.31
11	20.44	26	18.14
12	35.86	27	19.17
13	41.66	28	25.14
14	29.60	29	11.34
15	23.66	---	-----

Conclusive evidence for the structure I was derived from the results of chemical reactions. Acetylation of structure I with AC_2O -Pyridine at room temperature afforded a mono-acetyl derivative.

On the basis of the spectral and chemical evidences the structure I was established as 24, β -ethyl-Cholest- Δ 5,22diene-3 β -ol.



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