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Note

Chemical examination of the resinous exudate from *Azadirachta indica. A. Juss*

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A marine sterol namely (24ξ)-Isopropenyl cholesterol **2** and its biosynthetic precursor Fucosterol **1** have been isolated for the first time from the species *Azadirachta indica. A. Juss.*

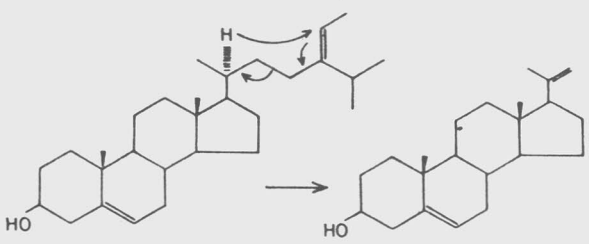
Azadirachta indica. A. Juss. of the family Meliaceae has been extensively investigated because of its pesticidal[1] and therapeutic[2] properties. We have recently reported,[3] for the first time, the presence of a new flavanoid viz.,8-prenyl-5, 7-dihydroxy-3'-(3-hydroxy-3, 3-dimethylbutyl)-4'-methoxyflavanone, in the resinous exudate of the glands in tender leaves of *A. indica.* Two steroidal components **1** and **2** are now isolated from the same part.

Results and Discussion

The acetone extract of the resinous exudate was concentrated and chromatographed over a column packed with neutral alumina. Petroleum ether eluant fractions gave the steroids **1** and **2**.

The steroid **1** had a molecular formula $C_{29}H_{48}O$. Its IR spectrum resembled that of fucosterol with peaks at 840 and 800 cm^{-1} for the Δ^5-bond[4]. The peak at 1380 cm^{-1} showed the presence of propyl group. ^{1}H-NMR spectrum showed a doublet at δ 5.36 and mutiplet at δ 3.52 which is the characteristic pattern of the Δ^5-3β-hydroxy-sterols[5]. A quartet at δ 5.10 for the proton at C-28, established a double bond between C-24 and C-28. The other signals in the region δ 0.67 to 2.27 suggested the presence of CH_2 and CH_3 entity on the steroid nucleus and on the side chain.

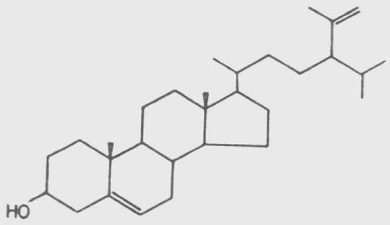
The mass spectrum showed the peaks at m/z 271, 255, 231 and 213 which demonstrated[6] the presence of Δ^5-3β-hydroxy-sterol nucleus with one degree of unsaturation in the side chain. The other



1

m/e 3/4

McLafferty Rearrangement in Fucosterol



2

fragment ions are at m/z 397 [M^+-CH_3], 394 [M^+-H_2O], 379 [M^+-(CH_3+H_2O)], 314 [M^+- part of side chain (C_7H_{14})]; 273 [M^+-(side chain)] and 271 [M^+-(side chain+2H)].The loss of part of side chain (C_7H_{14}) is characteristic of sterol with a $\Delta^{24(28)}$ bond. This may be explained by postulating 1:6 hydride shift associated with a double bond between C-24 and C-28.

On the basis of all the spectral evidences and on comparison with its literature melting point (123-24°C)[4], the structure of **1** was confirmed as fucosterol.

The other steroid **2** had a molecular formula $C_{30}H_{50}O$. Its ^{1}H-NMR and IR data revealed that **2** was a sterol containing the same basic skeleton as that of fucosterol. In its ^{1}H-NMR spectrum, a doublet at δ 5.24, a multiplet at δ 3.2 confirmed the presence of Δ^5-double bond and C_3-β-hydroxy group[5]. Further the ^{1}H-NMR showed the terminal methylene proton resonances as doublets at δ 4.66 and 4.71; this was further confirmed by its IR spectrum showing absorption bands at 1620 and 910 cm^{-1} . The mass spectrum showed a molecular ion peak at m/z 426 and major fragments at m/z

411 (22), 393 (11), 299, 271, 229, 215, 213, 119 (100), 55 and 41.

The steroid **2** was confirmed as (24ξ)-isopropenyl cholesterol on the basis of above spectroscopic data and on the evidence of the report[8] of biological transformation of fucosterol to (24ξ)-isopropenyl cholesterol by means of S-adenosylmethionine (SAM).

Experimental Section

Melting points were determined on Boetius Microheating table and Mettler FP-51 melting point apparatus and are uncorrected. Aluminium oxide (neutral) was used for column chromatography and TLC was performed using glass plates coated with silica gel-G [incorporating $CaSO_4$ (13%) as binder]. Benzene, chloroform and ethyl acetate were used as the solvents. Spots were detected with iodine. IR spectra in KBr were recorded on Perkin-Elmer-597 spectrophotometer. ^1H NMR spectra on Varian EM 390 (200 MHz) spectrometer using TMS as an internal reference (chemical shifts in δ, ppm) and mass spectra on Jeol-D300 instrument.

Isolation of steroids 1 and 2

The young reddish leaves of neem (1 kg) were collected in August 1995 in the Bharathiar University campus which is located at the foot of Maruthamalai Hills Western Ghats, South India. The leaves were soaked in cold acetone (3 L) for 3 days. While soaking, care was taken to avoid the dissolution of the sap and also the leaves carrying artificially induced gums. The acetone extract of the resinous exudate was concentrated and chromatographed by a column packed with neutral alumina. The first fraction of pet. ether (60-80°C) eluant gave the steroid **1** and second fraction gave the steroid **2**.

Fucosterol **1** was obtained as colourless powder (pet. ether); m.p. 123-24°C ; IR (KBr): 3450 (br), 2950, 1640, 1460, 1380, 1040, 840 and 800 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 3.52 (1H, m, H-3), 5.10 (1H, q, H-28), 5.36 (1H, d, H-6); m/z $[M]^+$ 412 (39), 397 (8), 394 (13), 379 (15), 314 (18), 273 (26), 271 (12), 159, 145, 107, 91, 81, 69, 55 (100).

(24ξ)- isopropenyl-cholesterol **2** was obtained as colourless powder (pet. ether): m. p. 122-23°C; IR(KBr): 3450 (br), 2950, 1640,1440, 1380, 1000, 840 and 800 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.80 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 3.2 (1H, m, H-3), 4.66 (1H, br, s, H-30), 4.71 (1H,br s, H-30), 5.24 (1H, d, H-6); m/z $[M]^+$ 426 (10), 411 (21), 393 (11), 271(5), 255, 147, 119 (100), 109, 91, 81, 69, 55.

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