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24-Ethyl-cholest-22-en-3α-ol and other constituents from the roots of *Holoptelea integrifolia*

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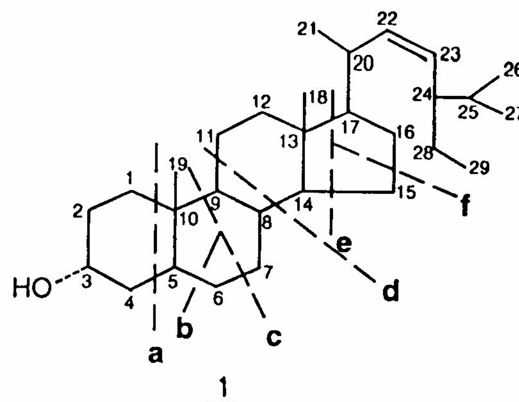
From the combined pet. ether-benzene extract of roots of *Holoptelea integrifolia*, a rare cholesterol derivative identified as 24-ethyl-cholest-22-en-3α-ol has been isolated alongwith *n*-tetracosane, α-amyrin, epifriedelinol, β-amyrin, β-sitosterol and friedelin. Characterization have been made on the basis of chemical and spectral evidences.

Holoptelea integrifolia (Roxb.) Planch. (Fam. Ulmaceae) is a large glabrous deciduous tree distributed throughout the greater parts of India up to an altitude of 800 m. A survey of literature revealed some work on the phytochemistry of leaves[1], stem bark[1-4], heart wood[5,6] and seeds[7] and antiviral activity of the crude leaf sap[8]. This communication describes the isolation and identification of phytochemicals from the roots.

The sterol **1**, white granules, m.p. 193-95°, showed M^+ at m/z 414 corresponding to the molecular formula $C_{29}H_{50}O$. It showed IR absorption bands for hydroxyl (3440 cm^{-1}), olefinic bond (1650 cm^{-1}) and *gem*-dimethyl group (1380 and 1370 cm^{-1}). Its ^1H NMR spectrum exhibited singlets at δ 0.67 and 0.78 due to angular methyl groups at C-18 and C-19, a doublet at δ 0.98 due to C-21 methyl group and multiplets at δ 5.12 (2H) corresponding to two olefinic protons, 4.04 for H-3 proton, 2.08 (2H) for the allylic protons at C-20 and C-24, 0.81 (9H) for the methyl group at C-29 and isopropyl group and in the region 1.24-1.88 (26H) for the remaining methine and methylene protons. A broad singlet at δ 2.55



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(1H) which disappeared on deuteration confirmed the presence of an -OH group. In the mass spectrum, in addition to the molecular ion peak at m/z 414, peaks due to characteristic steroid fragmentations **a**, **b**, **c**, **d**, **e** and **f** at m/z 342, 303, 290, 165, 233 and 248 respectively indicated the presence of a saturated steroid nucleus[9]. Peaks at m/z 275 [M^+-$C_{10}H_{19}$, side chain, 81%] and 273 [M^+ - side chain -2H, 43%] with the high intensity of the former which was formed by the allylic cleavage indicated the presence of a $\Delta^{22(23)}$ double bond[10]. The position of the olefinic bond was further confirmed by the peak at m/z 302 which was produced by cleavage of the C_{20}-C_{22} bond together with a one hydrogen transfer from the charge retaining fragment. The peaks at m/z 399 [M^+-CH_3], 396 [M^+-H_2O], 381 [M^+-(H_2O + CH_3)] and 371 [M^+-$CH(CH_3)_2$] were also observed. Based on these evidences, compound **1** appeared to be 24-ethyl-cholest-22-en-3-ol.

The orientation of hydroxyl group at C-3 was found to be α. In the ^1H NMR spectrum the H-3 signal appeared at δ 4.04 which fulfilled the requirement for 3β-H accounting for the fact that equatorial protons attached to hydroxyl substituted carbon atoms appear at lower fields than the axial protons in epimeric alcohols[11]. The same was also confirmed by the mass spectrum where an intense peak appeared at m/z 343, as only those structures with α-oriented hydroxyl group readily undergo cleavage of the A ring[12]. A survey of literature revealed that this steroid 24-ethyl-cholest-22-en-3α-ol has been isolated earlier from the sponge

Esperoipsis edwardii[11] but such compounds with 3α-hydroxy group are very rare in the higher plants.

Experimental Section

General. Melting points are uncorrected. IR spectra were recorded (as KBr pellets) on a Perkin-Elmer model 557 spectrophotometer, ^1H NMR and ^{13}C NMR spectra on model Jeol FX 90Q at 89.55 MHz and 22.49 MHz respectively, using $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal standard and EIMS spectra on a Hitachi Model RMU 6E Mass Spectrometer.

Column chromatography was carried out using silica gel (60-120 mesh). TLC was carried out on silica gel G chromatoplates and 2% ceric ammonium sulphate as spraying agent.

Plant material. The plant material was collected from the Campus, University of Rajasthan, Jaipur during July 1994 and identified at the Botany Department of our University.

Extraction. Air-dried, powdered *H. integrifolia* (1.5 kg, roots) was exhaustively extracted with ethanol (95%) on a steam bath for 8 × 3 hr. The concentrated ethanolic extract (60 g) was fractionated with pet. ether followed by benzene which on concentration afforded 2.5 g and 10.5 g fractions respectively. Both the fractions gave similar spots on TLC (benzene:EtOAc, 1:1), hence they were mixed together.

Isolation of compounds. The combined pet. ether-benzene fraction on CC over silica gel (260 g) gave following compounds.

***n*-Tetracosane.** M^+ 338, m.p. 52-54° (EtOAc) (Eluent:pet.ether, 0.44 g).

α-Amyrin. M^+ 426, m.p. 183-84° (MeOH) (Eluent: pet. ether: C_6H_6, 4:1, 0.76 g).

Epifriedelinol. M^+ 428, m.p. 277-78° ($CHCl_3$-MeOH), (Eluent: pet. ether: C_6H_6, 2:3, 0.56 g).

Lupeol. M^+ 426, m.p. 210-11° ($CHCl_3$-MeOH), (Eluent: pet. ether: C_6H_6, 2:3, 0.53 g).

β-Amyrin. M^+ 426, m.p. 196-97° ($CHCl_3$-MeOH), (Eluent:pet. ether: C_6H_6, 1:4, 0.52 g).

β-Sitosterol. M^+ 414, m.p. 135-36° ($CHCl_3$-MeOH), (Eluent: pet. ether: C_6H_6, 1:4, 0.42 g).

Friedelin. M^+ 426, m.p. 258-60° ($CHCl_3$-MeOH), (Eluent: C_6H_6, 0.98 g).

24-Ethyl-cholest-22-en-3α-ol 1. White granules, M^+ 414, m.p. 193-95° ($CHCl_3$-MeOH), acetate (Ac_2O/Py), m.p. 125-27° (MeOH), (Eluent: EtOAc, 0.72 g).

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