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Note

Lupeol ester from *Clerodendrum phlomidis* L.

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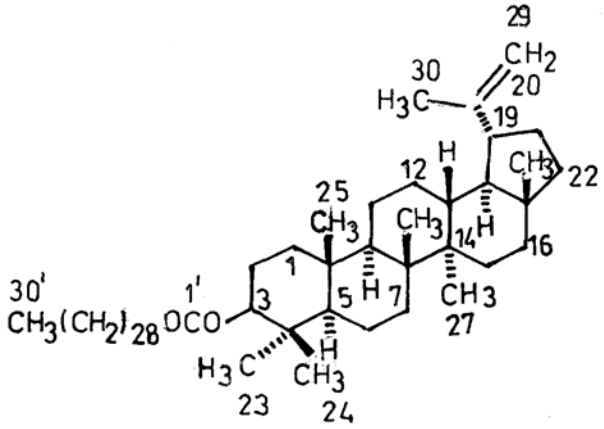
A new triterpene ester, together with tetratriacontanol and 24β-ethylcholesta-5,22*E*,25-triene-3β-ol, has been isolated from the aerial parts of *Clerodendrum phlomidis* L. Occurrence of a triterpene fatty acid ester lup-20(29)-en-3-triacontanoate in this plant is of taxonomic importance. Structures of all the isolated compounds have been established by spectroscopic and chemical studies.

Keywords: Lupeol ester, *Clerodendrum phlomidis* L**,** triterpene ester, tetratriacontanol and 24β-ethylcholesta-5,22*E*,25-triene-3β-ol.

Clerodendrum phlomidis Linn. (Family: *Verbenaceae*), also commonly known as Arani, is a common shrub growing in plains all over India especially in the drier parts of Uttar Pradesh, Bihar and Orissa. Various medicinal properties are attributed to it in the Indian System of Medicine[1]. It is widely used in the indigenous system of medicine as Kshudra Agnimantha[2], which is one of the components of the well known Ayurvedic medicine Dasamula[3]. The plant is reported to possess antifungal and antipyretic[4] also inhibit several plant and human pathogenic fungi[5]. In addition antimicrobial[6], hypoglycemic[7] and anti-diarrhoeal[8] activities were also reported. The major groups of compounds isolated from the plant are sterols and flavonoids[9-11]. Continuing our work on plants used in traditional medicine[12-15] we isolated a novel triterpene ester from aerial parts of *C. phlomidis*, the structural determination of which is described herein. Two other known compounds, a fatty alcohol and sterol, were also encountered during the course of the present investigation.

Results and Discussion

Chromatographic resolution of the hexane fraction of ethanolic extract of aerial parts of the plant yielded a crystalline solid compound **1**. The molecular formula was deduced as $C_{60}H_{108}O_2$ on the analysis of its ^{13}CNMR and mass spectral data. The IR spectrum indicated it to contain two centres of unsaturation (ν_{max}1660, 883 for exocyclic disubstituted double bond[16] and 1731 for ester group) as well as an absorption band at 719 cm^{-1} indicating that the ring in the compound carries a long aliphatic side chain. The ^{13}C NMR spectra indicated that the compound was a triterpenoid with a lupane skeleton[17]. The identification of the triterpenoid group was established as lupeol-type triterpene by the characteristic signals displayed at δ 4.68 (1H, *d*, *J* = 3.0 Hz, H - 29) and 4.57 (1H, *d*, *J* = 3.0 Hz, H - 29) in the ^{1}H NMR together with the values of unsaturated carbons at δ 109.2 and 150.8 as well as signals for the seven tertiary methyl groups. On comparison of the NMR data of the compound **1** and lupeol the major difference was observable in the H-3 and C-3 values which appeared more downfield in compound **1**. The C - 3 signal was observed at a lower field (δ 80.5) than that for lupeol (δ 78.8, ref.18). When the spectral values of the compound were compared with acetyl derivative of lupeol[19] it was observed that the carbonyl group was deshielded. Moreover, a new upfield signal due to methyl group of a fatty acid ester moiety at δ 13.9 was visible while no signal for the methyl group of an acetoxyl group was observed. In addition to these characteristic signals of a long chain fatty acid unit were noticeable in both the NMR spectra. These data indicated that the compound is a fatty acid ester derivative of lupeol. This inference was supported by the ^{1}H NMR which contained resonance signals characteristic of lup - 20(29) - ene[20] with a 3β - ester moiety (δ 4.47, 1H, *dd*, *J* = 9.6, 6.6



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Hz, H–3, ref. 21). This linkage was confirmed by the observed HMBC correlation between the oxygenated methine proton at δ 4.47 to the ester carbonyl at δ 173.5 (C - 1'). Signals as two one-proton doublets at δ 2.21 (J = 7.5 Hz) and 1.99 (J = 7.5 Hz) were due to methylene protons α to the carbonyl group. On alkaline hydrolysis the compound yielded the corresponding triterpene, lupeol and fatty acid, triacontanoic acid. Lupeol and triacontanoic acid were confirmed by comparison of their spectra and co-TLC with an authentic sample.

Thus, compound **1** was characterized as lup-20(29)-en-3-triacontanoate. This is the first report of occurrence of this compound in nature as well as the presence of fatty acid ester of a triterpene in this plant. Lupeol esters of this type have shown significant antimalarial activity[22].

Tetratriacontanol **2** and 24β-ethylcholesta-5, 22*E*, 25-triene-3β-ol **3**, were isolated from later fractions eluted with hexane-ethyl acetate. The structures of these known compounds were established by a comparison of their m.p. and spectral data with reported data[23,24]. Compound **2** is being reported for the first time from this plant. Compound **3** has been suggested by some workers to be a chemotaxonomic marker of the genus.

Experimental Section

Plant material. Aerial parts of *Clerodendrum phlomidis* were collected locally from Lucknow and identified by the Botany and Pharmacognosy department of our Institute. A voucher specimen (CIMAP No. 9054) has been deposited in the herbarium of the Institute.

Extraction and Isolation. The shade air dried aerial parts were ground to a fine powder (3.2 kg). Extraction with ethanol (10L × 3 times) yielded a viscous ethanolic extract (249 g) which was subsequently fractionated with solvents of differing polarity, in the order *n*-hexane, chloroform and *n*-butanol to provide the respective fractions. The hexane fraction (70 g) thus obtained was subjected to column chromatography on silica gel (Rankem, India, 60-120 mesh) using mobile solvent system of a mixture of hexane-ethyl acetate in varying proportion. Fractions obtained on elution with hexane-ethyl acetate (98:02) afforded compounds **1** and **2**. Compound **1**, 437 mg, was obtained in crystalline form on crystallization from chloroform-acetone while compound **2** (790 mg) was crystallized from acetone. Later fractions eluted with hexane-ethyl acetate (95:05) on crystallization from acetone yielded 327 mg of compound **3**.

Lup-20(29)-en-3- triacontanoate 1: Solid; m.p. 72-74°C; $[\alpha]_D$ + 11.2° ($CHCl_3$); IR: 2918, 2851, 1731, 1660, 1471, 1465, 1381, 883, 719 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.79 (3H, *s*, H_3-28), 0.87-0.84 (12H, H_3-23, H-24, H_3-25, H_3-30'), 0.94 (3H, *s*, H_3-26),1.03 (3H, *s*, H_3-27), 1.25 (52 H, *br s*, H-4' to H-29'), 1.63 (2H, *m*, H - 3'), 1.68 (3H, *s*, H_3-30), 2.29 (2H, *br t, J* = 7.5 Hz, H - 2'), 2.37 (1H, *t*, J = 8.0 Hz, H- 19), 4.47 (1H, *dd, J* = 9.6, 6.6 Hz, H-3), 4.57 (1H, *d, J* = 3.0 Hz, H-29), 4.68 (1H, *d, J* = 3.0 Hz, H - 29); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.3 (C-1), 23.6 (C-2), 80.5 (C-3), 37.7 (C-4), 55.3 (C-5), 18.1 (C-6), 34.2 (C-7), 40.8 (C-8), 50.3 (C-9), 37.0 (C - 10), 20.9 (C - 11), 25.1 (C-12), 38.0 (C-13), 42.7 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.8 (C-20), 29.8 (C-21), 39.9 (C-22), 27.8 (C-23), 16.4 (C - 24), 16.0 (C-25), 15.9 (C-26), 14.4 (C-27), 17.9 (C-28), 109.2 (C-29), 19.2 (C-30), 173.5 (C-1'), 34.8 (C-2'), 29.5 $(CH_2)_{27}$, 13.9 (C-30'); FAB MS: *m/z* 883 $[M + Na]^+$, 833 $[M + Na - 60]^+$, 408, 393, 218, 189.

Hydrolysis of 1: Compound **1** (50 mg) was refluxed with 5% alcoholic KOH (20 mL) for 4 hours. Volume of the alcohol was reduced by distillation and after dilution with water (25 mL) it was extracted with diethyl ether (4 × 25 mL). The diethyl ether extract was washed with water (2 × 25 mL) and dried over anhydrous Na_2SO_4. Removal of the solvent gave a residue which was crystallized from acetone to yield the alcohol portion (20 mg, m.p. 204-206°C) of the ester. The water soluble part was acidified (*p*H 3) with dil. HCl and subsequently extracted with ether

(4×25 mL), washed with water (2×25mL) and dried over anhydrous Na_2SO_4. Removal of solvent furnished a solid which was crystallized from acetone to yield the acid part of the ester, 7 mg, m.p. 76 - 78°C.

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