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Phytochemistry 63 (2003) 415–420
PHYTOCHEMISTRY
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4α-Methyl-24β-ethyl-5α-cholesta-14,25-dien-3β-ol and 24β-ethylcholesta-5, 9(11), 22E-trien-3β-ol, sterols from *Clerodendrum inerme*

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Received 6 September 2002; received in revised form 12 February 2003

Abstract

From the aerial parts of *Clerodendrum inerme*, two new sterols (4α-methyl-24β-ethyl-5α-cholesta-14, 25-dien-3β-ol and 24β-ethylcholesta-5, 9(11), 22E-trien-3β-ol) and a new aliphatic ketone (11-pentacosanone) were isolated together with another known aliphatic ketone (6-nonacosanone) and a diterpene (clerodermic acid). The structure elucidations were based on analyses of physical and spectroscopic data.

Keywords: *Clerodendrum inerme*; Verbenaceae; Sterols

1. Introduction

Clerodendrum inerme (L.) Gaertn. (Verbenaceae) is a common shrub that grows in India, both in the wild and as a garden hedge. Its leaves are used as alterative, febrifuge and as a substitute for *Swertia chirayita*. Its leaves have been shown to possess antimicrobial activity (Prasad et al., 1995) and are reported to be cardiovascular system active. They also stimulate uterine motility in rats and inhibit intestinal motility (Husain et al., 1992). The plant contains mainly iridoids, flavonoids, diterpenes, sterols, triterpenes and neolignans (Akihisa et al., 1990a; Rehman et al., 1997; Kanchanapoom et al., 2001).

The genus *Clerodendrum* is known to contain 24β-ethylsterols possessing a Δ^{25} bond as the dominant sterols (Akihisa et al., 1990a). 4 α-Methylsterols viz. 4α, 24, 24-trimethyl-5α-cholesta-7, 25-dien-3 β-ol and 4α-methyl-24β-ethyl-5α-cholesta-7, 25-dien-3 β-ol together with two other common sterols have also been isolated from this plant (Akihisa et al., 1990a). This paper describes further investigation on 4α-methylsterols, which are important intermediates in 4-demethyl sterol biosynthesis (Nes and Mckean, 1977; Benveniste, 1986) in *Clerodendrum inerme*. It led to the isolation and characterization of a new 4α-methylsterol, 4α-methyl-24β-ethyl-5α-cholesta-14, 25-dien-3β-ol (**1a**) and another new sterol, 24β-ethylcholesta-5, 9(11), 22E-trien-3β-ol (**2a**) along with a new aliphatic ketone 11-pentacosanone (**3**), 6-nonacosanone (**4**) and a diterpene clerodermic acid (**5**).

2. Results and discussion

The compound **1a** isolated from the fractions eluted with hexane–ethyl acetate (95:5) mp 170–172°, $[\alpha]_D + 78°$ possessed molecular formula $C_{30}H_{50}O$ (MS) and υ_{max} 3400 and 1040 cm^{-1} for an equatorial OH group; 1640 and 880 cm^{-1} for double bond and 2940, 2860, 1460, 1380 cm^{-1} for CH, CH_2 and CH_3 functions (Gupta et al., 1981). The ^1H NMR spectrum displayed two signals at δ 0.89 and 0.83 for C-18 and C-19 angular methyl groups, respectively, typical of a Δ^{14}-sterol (Kokke et al., 1981; Dow et al., 1983). Signals corresponding to C-21 and C-30 methyl groups appeared at δ 0.92 (*d*) and 0.94 (*d*), respectively. C-15 olefinic proton appeared at δ 5.17 (Kokke et al., 1981). Another double bond signals at δ 4.57 and 4.69 together with C-29 and C-26 methyls

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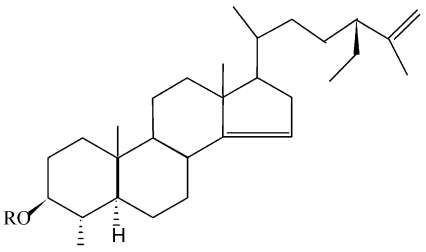
doi:10.1016/S0031-9422(03)00146-8



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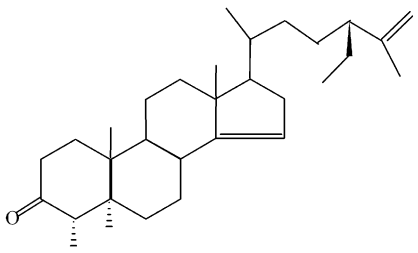

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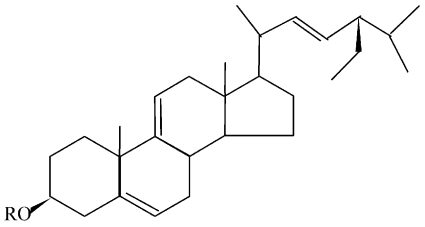

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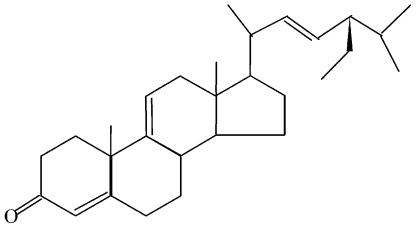

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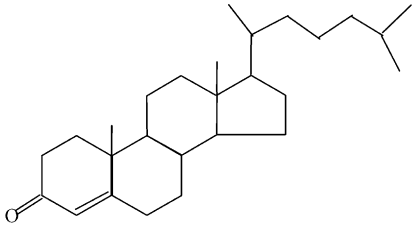

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at δ 0.82 (*t*) and 1.68 (*s*) are in good agreement with the double bond at Δ^{25} (Akihisa et al., 1988a). A multiplet assignable to C-3 axial methine appeared at δ 3.22 (Gupta et al., 1981). In its ^{1}H NMR spectrum the observed value (δ 0.83) for C-19 methyl group has close resemblance with the value calculated by Zürchers' rule for 5α-androstan (calculated value δ 0.82) than for a 5β-androstan (calculated value δ 0.95), suggesting a 5α-H in **1a** (Zürcher, 1963). The side chain proton signals were closely correlated with those of 24 β-ethyl-cholesta-5, 25-dien-3 β-ol (Akihisa et al., 1988a), which made it possible to determine the configuration at C-24 of **1a** to be β. The mass spectrum of **1a** showed molecular ion peak at *m*/*z* 426 ($C_{30}H_{50}O$), loosing a water molecule at *m*/*z* 408 and a methyl group at *m*/*z* 411. Loss of side chain ($C_{10}H_{19}$) from the molecular ion resulted in an ion at *m*/*z* 287. Ion at *m*/*z* 218 was due to C ring cleavage. Base peak at *m*/*z* 121 was due to the loss of C_7H_{13} of the side chain from the ion at *m*/*z* 218.

The ^{13}C NMR spectrum also showed the presence of 30 carbon signals in the molecule. The DEPT spectrum exhibited six methyls, eleven methylene and nine methine, while the remaining four signals in the broad band spectrum were due to the quaternary carbon atoms. It showed six methyl group resonances at δ 15.4, 15.5, 16.8, 18.0, 18.4 and 20.9. Two sets of olefinic carbon resonances occurred at δ (121.7, 145.2) and (109.3, 151.0) corresponding to trisubstituted and disubstituted double bonds at C_{14} and C_{25}, respectively. The signal at δ 79.0 was due to the carbon bearing oxygen. These data suggested (Kokke et al., 1981; Akihisa et al., 1988a, 1990a) the presence of a 4α-methyl and two double bonds at Δ^{14} and Δ^{25}.

Acetylation of **1a** afforded a monoacetate **1b**, υ_{max} 1733 and 1253 cm^{-1}. The singlet nature of the latter 'acetate band' established the equatorial orientation of the C-3 hydroxyl group (Gupta et al., 1981; Jones et al., 1951). The C-3 methine signal was shifted downfield by about 1.3.

Jones' oxidation of **1a** furnished a ketone **1c**, in which the C-18 and C-19 methyl groups appeared as singlet at δ 0.87 and 1.10, respectively. Its IR and UV spectra

lacked absorption for an α, β-unsaturated ketone indicating the absence of a double bond at C-5.

Thus, on the basis of above spectral evidences the structure of sterol **1a** was formulated as 4α-methyl-24β-ethyl-5α-cholesta-14, 25-dien-3β-ol.

Compound **2a**, mp 160–162°, $[\alpha]_D$ −47°, was isolated from the fractions eluted with hexane-ethyl acetate (95:5). The EI mass spectrum of **2a** exhibited a peak at *m*/*z* 410 compatible with the molecular formula $C_{29}H_{46}O$. Other significant peaks were observed at *m*/*z* 392 $[M–H_2O]^+$, 395 $[M–CH_3]^+$, 271 $[M–C_{10}H_{19}$ (side chain)$]^+$, 253 $[M–H_2O–C_{10}H_{19}]^+$, 229 $[M–C_{10}H_{19}$–D ring cleavage $(C_3H_6)]^+$, 190 [C ring cleavage]$^+$, 176 [C ring cleavage]$^+$and 138 [B ring cleavage]$^+$ indicating that it is a C_{29} sterol with three double bonds, one of which was in the C_{10} side chain and the other two were in the skeleton. Absence of double bond in the D ring was indicated by the fragment ions at *m*/*z* 190 and 176, resulted by C ring cleavage. Ion at *m*/*z* 138 was due to B ring cleavage. The IR spectrum displayed the presence of hydroxyl (3432 and 1059 cm^{-1}) and olefinic (1640 cm^{-1}) groups. Absorption maxima at 2939, 2865, 1458, 1376 cm^{-1} were due to the stretching and bending vibrations of CH, CH_2 and CH_3 groups.

In the ^{1}H NMR spectrum signals corresponding to methyl groups at C-21, C-26, C-27 and C-29 appeared at δ 1.02 (*d*), 0.79 (*d*), 0.84 (*d*) and 0.80 (*t*), respectively (Akihisa et al., 1988a, b). The olefinic protons signals at δ 5.01 (1H, H-23) and 5.19 (1H, H-22) were due to the presence of a double bond at C-22 (Akihisa et al., 1988b) while the signal at δ 5.21 (1H, *t*, H-11) indicated the presence of a double bond at C-9 (Akihisa et al., 1990b). The angular methyl groups at C-18 and C-19 resonated at δ 0.70 (*s*) and 1.01 (*s*), respectively, typical of a Δ $^{9(11)}$ sterol (Gupta et al., 1981). The signals at δ 5.35 (1 H, br *d*, H-6) and the multiplet at δ 3.52 (1H, *m*, H-3) were characteristic of a Δ^5-3β-hydroxyl sterols (Goswami et al., 1996). The stereochemistry at C-22 was established to be E (*trans*) since the 21-H_3 doublet appeared at δ 1.02; whereas a Z (*cis*) double bond resonates at δ 0.94–0.95 (Akihisa et al., 1988a). The configuration at C-24 was established as 24β by comparing side chain protons of a 24β sterol, 4α-methyl-24β (S)-ethyl-5α-cholest-22E-en-3β-ol (Tam Ha et al., 1982).

The ^{13}C NMR spectrum showed 29 signals corresponding to 29 carbon atoms in the molecule. The DEPT spectrum showed the presence of six methyl, eight methylene and eleven methine groups while the remaining four carbon atoms existed in the quaternary form. Resonances due to the presence of six methyl groups were seen at δ 12.0, 12.2, 19.4, 20.2, 20.8 and 21.2. Three sets of olefinic carbon resonances occurred at δ 121.7, 140.7; 129.3, 138.3 and 130.0, 137.2 corresponding to two trisubstituted and one disubstituted double bonds at $\Delta^{5,\ 9(11),\ 22}$ (Akihisa et al., 1988b; Shirane et al., 1990; Lin et al., 1988). The signal at δ 71.8 was due to the carbon bearing oxygen.

Acetylation of **2a** yielded a monoacetate **2b**, υ_{max} 1735 and 1260 cm^{-1} and the singlet nature of the latter 'acetate band' established the equatorial orientation of the C-3 hydroxyl group (Gupta et al., 1981; Jones et al., 1951). The C-3 methine signal was shifted to δ 4.60.

Authentic cholesterol (Δ^5, 3β-hydroxysterol), purchased from Sigma, India, was oxidised with Jones' reagent to yield Δ^4-cholesten-3-one (**6**), an α,β-unsaturated ketone. Comparison of ^{1}H NMR and UV spectral data of α,β-unsaturated ketone **2c**, obtained after Jones' oxidation of **2a,** and **6** suggested a double bond at C-5 in **2a** similar to cholesterol (^{1}H NMR: δ 6.17 (*s*) in **6** for C-4 of α,β-unsaturated ketone; δ 6.17(*s*) in **2c** for C-4. UV: 247 nm in **6**; 248 nm in **2c**). Proton signals for C-6 H at δ 5.34 (**2a)** and δ 5.35 (cholesterol) disappeared in the respective oxidation products **2c** and **6**.

Thus, on the basis of above spectral evidences structure of compound **2a** was established as 24β-ethylcholesta-5, 9(11), 22E-trien-3β-ol.

Compound **3**, mp 66–68°, possessed IR absorption bands at 2910, 2855, 1705, 720 cm^{-1} for a long chain aliphatic ketone. Molecular ion at *m*/*z* 366 suggested its molecular formula as $C_{25}H_{50}O$. Presence of strong ions at *m*/*z* 225, 197, 169, 141, and at 240, 184 were due to α and β fissions, respectively indicating a carbonyl group at C-11 (Gupta and Verma, 1991). A double rearrangement ion seen at *m*/*z* 58 was characteristic of a ketone having γ H in both alkyl fragments. In its ^{13}C NMR, the terminal methyls appeared at δ 14.1, –CH_2–CO–CH_2–at δ 33.9 and the carbonyl group at δ 178.0. Thus, the structure of compound **3** was assigned as 11-pentacosanone. This compound is being reported for the first time in nature.

Compound **4**, mp 74–76°, showed IR bands at 2920, 2860, 1702, 720 cm^{-1}. Molecular ion peak at *m*/*z* 422 suggested the molecular formula as $C_{29}H_{58}O$. Presence of strong ions at *m*/*z* 351, 323, 99, 71, and 366, 337 due to α and β fissions, respectively indicated the carbonyl group at C-6 (Gupta and Verma, 1991). Similar to **3** a double rearrangement ion at *m*/*z* 58 was due to the presence of γ H in both alkyl fragments. This is the first report of the occurrence of this compound in this plant although it has been reported earlier from another natural source, *Cassia auriculata* (Lohar et al., 1981).

3. Experimental

3.1. General

Mps: uncorr. The 300 MHz NMR spectra were recorded in $CDCl_3$ with tetramethyl silane (TMS) as internal standard. The ^{13}C NMR (broad band and DEPT) spectra were recorded at 75 MHz. The DEPT

experiments were used to determine the multiplicities of carbon atoms. TLC was performed on 60 F_{254} pre-coated silica gel plates (Merck) and the spots were visualized by exposure to I_2 vapours or by spraying with vanillin: sulphuric acid: ethanol (1 g: 5 ml: 95 ml) reagent followed by heating the plate at 110 °C for 15 min.

3.2. Plant material

Aerial parts of *Clerodendrum inerme* were collected from Lucknow in October 2000 and a voucher specimen (No. CIMAP-8199) has been deposited in the herbarium of this institute.

3.3. Extraction and isolation

Air dried and finely powdered aerial parts of the plant (8.2 kg) were exhaustively extracted at room temperature (25–26 °C) with ethanol (10 l×3 times) and the ethanolic extract was concentrated in vacuo to give a residue. Water (500 ml) was added and it was then partitioned with *n*-hexane, chloroform and *n*-butanol. The *n*-hexane extract (80.46 g) was subjected to column chromatography on silica gel, 1.5 kg (60–120 mesh). Elution was carried out in varying percentage of EtOAc in hexane. Fractions eluted with hexane–ethyl acetate (95:5) afforded compounds **1a** and **2a**. Compound **1a** (0.06 g) was obtained in crystalline form after crystallization from $CHCl_3$–CH_3COCH_3. Compound **2a** (0.35g) was obtained as colourless crystals from $CHCl_3$–CH_3COCH_3. Compounds **3** (0.04 g) and **4** (0.22 g) were obtained from earlier fractions eluted with hexane–EtOAc (95:5) and were crystallised from $CHCl_3$-MeOH. The fractions eluted with hexane–EtOAc (70:30) were crystallised from hexane–EtOAc to provide **5** (0.09 g). Clerodermic acid and 6-nonacosanone were identified by comparison of their spectroscopic data with published data (Achari et al., 1990; Lohar et al., 1981).

3.4. 4α-Methyl-24β-ethyl-5α-cholesta-14, 25-dien-3β-ol (*1a*)

Crystalline solid; mp 170–172°; $[\alpha]_D$ +77° ($CHCl_3$); IR υ_{max} cm^{-1}: 3400, 2940, 2860, 1640, 1460, 1380, 1040, 880; ^1H NMR (300 MHz, $CDCl_3$): δ 0.82 (3H, *t*, *J* = 9.0 Hz, H-29), 0.83 (3H, *s*, H-19), 0.89 (3H, *s*, H-18), 0.92 (3H, *d*, *J* = 8.0 Hz, H-21), 0.94 (3H, *d*, *J* = 8.0 Hz, H-30), 1.68 (3H, *s*, H-26), 3.22 (1H, *m*, H-3), 4.57 (1H, *br s*, H-27), 4.69 (1H, *d*, *J* = 2.1 Hz, H-27), 5.18 (1H, *m*, H-15); ^{13}C NMR ($CDCl_3$): δ 15.4 (C-29), 15.5 (C-18), 16.8 (C-30), 18.0 (C-26), 18.4 (C-19), 20.9 (C-21), 23.5 (C-11), 26.2 (C-28), 29.7 (C-6), 29.8 (C-23), 31.1 (C-2), 32.5 (C-16), 32.6 (C-7), 33.3 (C-22), 34.3 (C-8), 34.7 (C-10), 35.6 (C-20), 37.1 (C-1), 38.8 (C-12), 40.0 (C-4), 46.8 (C-13), 47.2 (C-5), 50.4 (C-24), 55.2 (C-9), 55.3 (C-17), 79.0 (C-3), 109.3 (C-27), 121.7 (C-15), 145.2 (C-14), 151.0 (C-25); EIMS *m*/*z* (rel. int.): 426$[M]^+$ ($C_{30}H_{50}0$, 3.4), 411 $[M–Me]^+$ (1.5), 408 $[M–H_2O]^+$ (2.5), 287 $[M–C_{10}H_{19}$ (SC)$]^+$ (0.5), 218 [C ring cleavage]$^+$(14.8), 121 [218-C_7H_{13} of SC]$^+$(100).

3.5. Acetylation of *1a*

Compound **1a** (0.012 g) was dissolved in pyridine (2 ml), and Ac_2O (2 ml) was added. It was left overnight at room temp. and then diluted with cold H_2O (50 ml) and extracted with Et_2O (4×50 ml). The Et_2O extract was washed successively with dil. HCl (2×50ml), $NaHCO_3$ soln (2×50ml) and H_2O (2×50 ml), and dried over Na_2SO_4. Crystallisation from acetone provided **1b** (0.007 g), mp 212–214 °C. IR υ_{max} cm^{-1}: 2943, 2855, 1733, 1635, 1462, 1367, 1253 and 1027. ^1H NMR (300 M Hz, $CDCl_3$): δ 0.80 (3H, *t*, *J* = 9.0Hz, H-29), 0.83 (3H, *s*, H-19), 0.87 (3H, *s*, H-18), 0.91 (3H, *d*, *J* = 8.0Hz, H-21), 0.94 (3H, *d*, *J* = 8.0 Hz, H-30), 1.66 (3H, *s*, H-26), 2.05 (3H, *s*, $COOCH_3$), 4.50 (1H, *m*, H-3), 4.56 (1H, *brs*, H-27), 4.68 (1H,br*s*, H-27), 5.17 (1H, *m*, H-15); EIMS *m*/*z*: 468 $[M]^+$ ($C_{32}H_{52}0_2$).

3.6. Jones' oxidation of *1a*

Compound **1a** (0.015 g) was dissolved in CH_3COCH_3 (250 ml) and 8 N chromic acid was added dropwise with constant shaking. Completion of the reaction was indicated by the persistence of the yellow colour in the supernatant liquid even after 10 min. The residue was conc. to 50 ml in vacuo diluted with H_2O (100 ml), extracted with Et_2O (4×50 ml). The Et_2O extract was washed with H_2O (100 ml) and dried (Na_2SO_4). It was purified by preparative TLC (benzene–acetone; 95:5) to give a viscous mass, 7 mg. Due to the small amount it resisted crystallization. IR υ_{max} cm^{-1}: 2938, 2855, 1705, 1635, 1450, 1370, 1022, 900; ^1H NMR (300 MHz, $CDCl_3$): δ 0.81 (3H, *t*, *J* = 9.0 Hz, H-29), 0.87 (3H, *s*, H-18), 0.92 (3H, *d*, *J* = 8.0 Hz, H-21), 1.04 (3H, *d*, *J* = 8.0 Hz, H-30), 1.10 (3H, *s*, H-19), 1.68 (3H, *s*, H-26), 4.56 (1H, *br s*, H-27), 4.69 (1H, *d*, *J* = 2.1 Hz, H-27), 5.18 (1H, *m*, H-15); EIMS *m*/*z*: 424$[M]^+$ ($C_{30}H_{48}0$).

3.7. 24β-Ethylcholesta-5, 9 (11), 22E-trien-3β-ol (*2a*)

Crystalline solid; $[\alpha]_D$−47° ($CHCl_3$); mp 158–160°; IR υ_{max} cm^{-1} : 3432, 2939, 2865, 1640, 1458, 1376, 1244, 1059, 966; ^1H NMR (300 MHz, $CDCl_3$): δ 0.70 (3H, *s*, H-18), 0.79 (3H, *d*, *J* = 6.3 Hz, H-26), 0.84 (3H, *d*, *J* = 6.9 Hz, H-27), 0.80 (3H, *t*, *J* = 7.0 Hz, H-29), 1.01 (3H, *s*, H-19), 1.02 (3H, *d*, *J* = 6.9 Hz, H-21), 3.52 (1H, *m*, H-3), 5.01 (1H, *dd*, *J* = 8.4, 15.3 Hz, H-23), 5.19 (1H, *dd*, *J* = 8.4, 15.3 Hz, H-22), 5.21 (1H, *t*, *J* = 8.1 Hz, H-11), 5.35 (1H, *br d*, *J* = 4.8 Hz, H-6);^{13}C NMR ($CDCl_3$):

δ 12.0 (C-18), 12.2 (C-29), 19.4 (C-19), 20.2 (C-26), 20.8 (C-27), 21.2 (C-21), 24.3 (C-15), 25.7 (C-28), 28.7 (C-16), 28.9 (C-2), 31.6 (C-8), 31.9 (C-7), 36.5 (C-25), 37.2 (C-1), 39.7 (C-12), 40.2 (C-10), 40.3 (C-20), 40.5 (C-13), 42.3 (C-4), 50.1 (C-24), 55.9 (C-17), 56.8 (C-14), 71.8 (C-3), 121.7 (C-6), 129.3 (C-11), 130.0 (C-23), 137.2 (C-22), 138.3 (C-9), 140.7 (C-5); EIMS *m/z* (rel. int.): 410 $[M]^+$ ($C_{29}H_{46}O$, 71.2), 395 $[M–CH_3]^+$(9.3), 392 $[M–H_2O]^+$(34.0), 367 $[M–C_3H_7]^+$(18.9), 271 $[M–C_{10}H_{19}$ (SC)$]^+$(59.2), 253 $[M–H_2O–SC]^+$(100), 229 [M–SC–C_3H_6 (D ring cleavage)]$^+$(16.4), 211 [M–H_2O–SC–C_3H_6]$^+$(34.7), 190 [M–SC–C_6H_9 (C ring cleavage)]$^+$(2.4), 176 [M–SC–C_7H_{11}]$^+$(9.8), 172 [M–H_2O–SC–C_6H_9]$^+$(21.1), 158 [M–H_2O–SC–C_7H_{11}]$^+$(63.6), 138 [M–SC–$C_{10}H_{13}$ (B ring cleavage)]$^+$(14.2), 120 [M–H_2O–$C_{20}H_{32}$]$^+$(25.4).

3.8. Acetylation of **2a**

Compound **2a** (0.050g) was acetylated in the same manner as compound **1a**. When crystallizated from acetone it gave crystals (0.025 g); mp 136–138°. IR υ_{max} cm^{-1} : 2943, 2865, 1735, 1640, 1460, 1366, 1260, 1036, 969; ^1H NMR (300 MHz, $CDCl_3$): δ 0.70 (3H, *s*, H-18), 0.79 (3H, *d*, *J* = 6.3 Hz, H-26), 0.84 (3H, *d*, *J* = 6.9 Hz, H-27), 0.80 (3H, *t*, *J* = 7.0 Hz, H-29), 1.02 (3H, *s*, H-19), 1.03 (3H, *d*, *J* = 6.9 Hz, H-21), 2.03 (3H, *s*, $COOCH_3$), 4.60 (1H, *m*, H-3), 5.03 (1H, *dd*, *J* = 8.4, 15.3 Hz, H-23), 5.18 (1H, *dd*, *J* = 8.4, 15.3 Hz, H-22), 5.20 (1H, *t*, *J* = 8.1 Hz, H-11), 5.38 (1H, *br d*, *J* = 4.8 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 12.0 (C-18), 12.2 (C-29), 19.3 (C-19), 20.2 (C-26), 20.8 (C-27), 21.2 (C-21), 24.3 (C-15), 25.7 (C-28), 28.7 (C-16), 28.9 (C-2), 31.6 (C-8), 31.9 (C-7), 36.6 (C-25), 37.0 (C-1), 39.6 (C-12), 40.2 (C-10), 40.3 (C-20), 40.5 (C-13), 42.2 (C-4), 50.0 (C-24), 55.9 (C-17), 56.8 (C-14), 74.0 (C-3), 122.6 (C-6), 129.3 (C-11), 130.0 (C-23), 137.2 (C-22), 138.3 (C-9), 139.6 (C-5); EIMS *m/z* (rel. int.): 452 $[M]^+$ ($C_{31}H_{48}O_2$, absent), 392 $[M–CH_3COOH]^+$(85.9), 377 $[M–AcOH–Me]^+$(5.7), 253 [M–AcOH–$C_{10}H_{19}$ (SC)]$^+$(37.5), 211 [M–AcOH–SC–C_3H_6 (D ring cleavage)]$^+$ (4.8).

3.9. Jones' oxidation of **2a**

Compound **2a** (0.050 g) was oxidized using Jones reagent in a similar manner as compound **1a**. Crystallization from methanol yielded a solid (0.020 g); mp 146–148°. UV λ_{max} 248 nm (α, β unsaturated ketone); IR υ_{max} cm^{-1} : 2959, 2870, 1683, 1638, 1457, 1379, 1224, 968; ^1H NMR (300 MHz, $CDCl_3$): δ 0.74 (3H, *s*, H-18), 0.79 (3H, *d*, *J* = 6.3 Hz, H-26), 0.84 (3H, *d*, *J* = 6.9 Hz, H-27), 0.80 (3H, *t*, *J* = 7.0 Hz, H-29), 1.04 (3H, *d*, *J* = 6.9 Hz, H-21), 1.17 (3H, *s*, H-19), 5.04 (1H, *dd*, *J* = 8.4, 15.3 Hz, H-23), 5.14 (1H, *dd*, *J* = 8.4, 15.3 Hz, H-22), 5.20 (1H, *t*, *J* = 8.1 Hz, H-11), 6.17 (1H, *s*, H-4); EIMS *m/z* : 408 $[M]^+$ ($C_{29}H_{44}0$).

Cholesterol (0.05 g) was oxidized as above to yield an α, β-unsaturated ketone; UV λ_{max} 247 nm ; ^1H NMR (300 MHz, $CDCl_3$): δ 6.17 (1H, *s*, H-4).

3.10. 1-Pentacosanone (**3**)

Solid; mp 66–68 °C; IR υ_{max} cm^{-1}: 2910, 2855, 1705, 1460, 1380, 720; ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (6H, *t*, *J* = 7.5 Hz, CH_3), 1.25 (*m*,-$(CH_2)_n$-), 1.63 (*m*), 2.35 (4H, *t*, *J* = 7.5 Hz,–CH_2CO–); ^{13}C NMR ($CDCl_3$): δ 14.1 (C-1, C-25), 22.7 (C-2 or C-24), 24.7 (C-2 or C-24), 29.1–31.9 (C-3 to C-9, C-13 to C-23), 33.9 (C-10, C-12), 178.0 (C-11); EIMS *m/z* (rel. int.): 366 $[M]^+$ ($C_{25}H_{50}O$, 4.0), 240 $[M–C_9H_{18}]^+$ (9.0), 225 $[M–C_{10}H_{21}]^+$ (5.7), 197 $[M–C_{11}H_{21}O]^+$ (2.5), 184 $[M\text{-}C_{13}H_{26}]^+$ (22.1), 169 $[M–C_{14}H_{29}]^+$ (5.1), 141 $[M–C_{15}H_{29}O]^+$ (7.4), 58 $[C_4H_{10}]^+$ (8.5), 57 $[C_4H_9]^+$ (60.5), 43 $[C_3H_7]^+$ (100).

Acknowledgements

We are thankful to Director, CIMAP, for keen interest during the course of work and RSIC unit, Central Drug Research Institute, Lucknow for providing the spectral analysis.

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