Lipid constituents from *Centratherum anthelminticum* (seeds)

Manju Verma, Shrilakshmi Deshiraju, Mustazi Lafri & B K Mehta

School of Studies in Chemistry, Vikram University, Ujjain 456010 India

Molecular Biology Unit, Chemistry Department, Indian Institute of Science, Bangalore 560 012

Received 31 July 2001; accepted (revised) 15 January 2003

Ethanolic extract of *Centratherum anthelminticum* seeds yields six new compounds - hexatetracontane-16-ol 1, 6, 9-icosadiene 2, butyl 11-hydroxystearanoate 3, hexyl 3-hydroxynonanoate 4, hexyl 9-hydroxyheptatriacontanoate 5, heptadecyl nonadecanoate 6 and one known compound (24aS) - stigmasta-5,22-diene-3β-ol 7 characterised by spectral data and chemical evidences.

**IPC:** Int.CI.' A 61 K 35/78

*Centratherum anthelminticum* (Willd.) Kuntze (Hindi-Kalijiri) belongs to family compositae, and it has been reported to possess febrifugal, alterative, anthelmintic, antiphlegmatic, cardiac, diuretic, digestive, antifertility, antimicrobial, antifilarial and anthelmintic activities, etc. Fifteen 4-demethyl sterol, five dimethyl sterols and eight 4,4-dimethyl sterols (triterpene alcohols) were identified from the seeds of *Vernonia anthelmintica* var. *Centratherum anthelminticum*. A new flavone glycoside characterised as aca­ceti n-7-O-α-D-glucopyranosyl (1→4)-α-D-xylopyranoside was reported from the seeds. Herein we describe the isolation and structure elucidation of six new and one known compound, from the ethanolic extract of the seeds.

**Results and Discussion**

**Compound 1:** Hexatetracontane-16-ol, *M*+ 662, C₄₆H₇₀O. IR showed absorption bands at 730-720 cm⁻¹ diagnostic of (CH₂)ₙ (where n is more than four) and at 3500 cm⁻¹ assignable to hydroxyl group. The IR bands of the monoacetate 1a at 1742 and 1240 cm⁻¹ with the conspicuous absence of the band at 3500 cm⁻¹ exhibited by the parent compound indicated the presence of only one esterifiable hydroxyl group.

The signal at δ 0.83 (t, 6H) in the ¹H NMR is assignable to the presence of 2 terminal -CH₃ groups while the multiplet at δ 3.5 (1H) accounts for a proton of the carbon linked to the hydroxyl group. A broad singlet at δ 1.3 (82H) showed the presence of 41 methylene groups and a singlet at δ 1.5 was recorded for OH proton (D₂O exchangeable). The methylene protons α to carbinolic carbon appeared at δ 1.55 as broad peak. Thus, the parent compound may be a saturated C₄₆ aliphatic alcohol.

Mass spectrum showed [M]+ at *m/z* 662. Appearance of number of ion peaks at a regular difference of 14 mass units in the MS of 1 and 1a confirmed the straight chain nature of the compound. The presence of α-fission peaks at *m/z* 211, 451, 241 and 421 sug-
gested the location of one hydroxyl group at C-16. This was finally confirmed by the presence of α-cleavage peaks at m/z 255, 101 and 57 were due to α-cleavage. The 13C NMR (BB, DEPT I, II) spectra have shown peaks at 174.2, 63.4, 34.7, 32.3, 32.2, 31.7, 29.4, 29.2, 24.6, 22.8 and 16.2 ppm. Compound 3 on alkaline hydrolysis yielded a mixture of alcohol, identified as butanol and an acid which confirmed the structure of compound 3 as butyl 11-hydroxyoctadecanoate being reported first time. Acetylation of 3 yielded a monoacetate; IR: 1741 cm⁻¹.

Compound 2: 6,9-Icosadiene, M⁺ 278, C₁₀₀H₁₈₂. The IR spectrum showed the presence of unsaturation (1637, 1617 cm⁻¹) and long chain aliphatic nature of the molecule (1080, 735-725 cm⁻¹). 1H NMR spectrum showed a six proton triplet at δ 0.83 for the terminal methyl groups and a 22 proton broad singlet at δ 1.30 for 11 methylene units present in identical environment. The olefinic protons resonated at δ 4.8 (J=8.0 Hz) as triplet. The methylene group between the two double bonds was resonated as multiplet at δ 3.16, while methylene groups adjacent to olefinic protons resonated as broad peak at δ 1.62. In the mass spectrum, separation of most of the peaks by 14 mass units and appearance of C₆H₁₄₊, and C₆H₁₂₊ ion series confirmed its long chain aliphatic nature. Abundant fragments at m/z 207, 167, 137 and 97 indicated the position of unsaturation. The 13C NMR (BB, DEPT I, II) have shown peaks at 121.6, 38.7, 38.4, 29.5, 29.4, 18.6, 18.4 and 17.1. Thus, compound 2 was assigned as 6,9-icosadiene and it is a new compound.

Compound 3: Butyl 11-hydroxyoctadecanoate, M⁺ 356, C₁₃₂H₂₈₄O₉. It was characterized as hexyl 3-hydroxynonanoate being reported first time reported. Acetylation of compound 3 yielded a monoacetate; IR: 1751 cm⁻¹.

Compound 4: Hexyl 9-hydroxyheptatriacontanoate, M⁺ 650, C₅₃H₁₀₆O₃. IR spectrum has shown it to be an aliphatic ester alcohol (1737, 1019 and 730-720 cm⁻¹). 1H NMR spectrum showed a triplet at δ 0.94 (J=6.0 Hz) for two terminal methyl groups. A multiplet at δ 3.60 accounts for the methine proton. Alcoholic protons and methylene protons β to hydroxyl and ester groups were also resonated at δ 1.60 as singlet (9H). Methylene protons of -CH₂-O-CO- and -CH₂-CO-O- moieties were resonated as triplets at δ 4.05 and 2.30 respectively. Rest of the methylenes were resonated at δ 1.25 as an intense singlet. The base peak at m/z 119 was due to the β-cleavage with respect to both ester and hydroxyl group, thus confirming the position of ester and hydroxyl group in the molecule. Other major fragments obtained at m/z 146, 127 and 101 were also inconsistent with the proposed structure. Thus compound 4 was characterized as hexyl 3-hydroxyoctadecanoate being first time reported. Acetylation of compound 4 yielded a monoacetate; IR: 1751 cm⁻¹.

Compound 5: Hexyl 9-hydroxyheptatriacontanoate, M⁺ 650, C₅₃H₁₀₆O₃. IR spectrum has shown it to be an aliphatic ester alcohol (1737, 1019 and 730-720 cm⁻¹). 1H NMR spectrum showed a triplet at δ 0.94 (J=6.0 Hz) for two terminal methyl groups. A multiplet at δ 3.60 (1H) was due to carbinolic proton. A broad singlet (9H) was due to methylene protons β to hydroxyl and ester group and -OH proton. The triplets at δ 4.09 and 2.35 (J=8.0 Hz) indicated the presence of methylene protons of -CH₂-O-CO- and -CH₂-CO-O- moieties respectively. Rest of the methylenes were resonated at δ 1.30 as sharp singlet. Mass spectrum showed parent ion M⁺ peak at m/z 650. The base peak at m/z 257 was due to the α-cleavage indicated the position of hydroxyl group where as peak at m/z 285 was obtained due to β-cleavage. The peak at m/z 521 was due to the α-cleavage indicated the position of the ester group.
$^{13}$C NMR spectrum showed a peak at 174.0 ppm corresponding to the carbon of the ester group. The peak at 34.5 and 32.0 ppm corresponds to the methylene carbons $\alpha$ and $\beta$ to the ester group. The carboxilic carbon attached to the hydroxyl group was observed at 64.4 ppm. The methylene carbons adjacent to the carboxilic carbon were resonated at 32.0 ppm. The peak at 14.2 ppm corresponds to the end methyl carbons while peaks at 29.7-22.7 ppm corresponds to the remaining methylene carbons.

Thus on the basis of above evidences the compound 5 was identified and characterized as hexyl 9-hydroxyheptatriacontanoate and being first time reported. Acetylation of compound 5 yielded a monoacacetate; IR: 1740 cm$^{-1}$.

Compound 5 on alkaline hydrolysis$^{13}$ yielded a mixture of alcohol, identified as 1-hexanol$^{12}$ and an acid respectively.

**Compound 6:** Heptadecyl nonadecanoate, M$^{+}$ 536, C$_{46}$H$_{94}$O$_{2}$. The IR spectrum showed bands at 2919, 2850 and 720 cm$^{-1}$ for aliphatic nature and at 1737 cm$^{-1}$ for ester group. $^1$H NMR spectrum showed a triplet at $\delta$ 0.90 ($J=6.0$ Hz) for terminal methyl groups. Methylene protons of -CH$_2$-CO- and -CH$_2$-CO-O- moieties were resonated as triplets at $\delta$ 4.05 and 2.30 ($J=8.0$ Hz) respectively. Methylene protons $\beta$ to ester group were resonated at $\delta$1.60 as singlet. Rest of the methylenes were resonated at $\delta$ 1.25 as an intense singlet.

Mass spectrum showed the characteristic fragmentation pattern of long chain aliphatic compound. The abundant peak at m/z 282 was due to the $\alpha$-cleavage and loss of one hydrogen atom. The fragment at m/z 312 was due to McLafferty rearrangement showed the presence of ester group in the centre of the molecule.

Thus on the basis of above evidences the compound 6 was characterized as heptadecyl nonadecanoate and being first time reported by us.

**Compound 7:** (24oxS)-Stigmastera-5,22-diene-3-$\beta$-ol (stigmasterol), M$^{+}$ 412, C$_{29}$H$_{48}$O. Comparison of m.p., $^1$H NMR and $^{13}$C NMR of compound 7 with those reported in the literature, identified it as stigmasterol$^{6}$.

**Experimental Section**

M.ps are uncorrected. The IR spectra were recorded in KBr on Perkin Elmer-377, $^1$H NMR spectra were recorded on 300 MHz Varian XL spectrometer and 400 MHz Bruker WM spectrometer with TMS as internal standard, $^{13}$C NMR spectra on Varian XL 75 MHz spectrometer in CDCl$_3$ and EIMS on Jeol - JMS D 300 Mass spectrometer at 70 eV. The column chromatography was carried out on alumina Gr.III and TLC on silica gel G. Spots were visualized by exposure to iodine vapour or by spraying with H$_2$SO$_4$-vanillin solution followed by heating at 105°C for 5 minutes. Seeds of C. anthelminticum were collected from the nearby area of Ujjain city and identified by School of Studies in Botany, Vikram University, Ujjain.

**Extraction and isolation:** The seeds of C. anthelminticum (Willd.) Kuntze (5 Kg) were, dried, cleaned and powdered coarsely. It was extracted by ethanol and excess of solvent was removed by rotary film evaporator to afford dark brown solid extract (86 gm). The extract showed positive test for the presence of steroids and terpenoids. This extract was fractionated on alumina grade III column. The column was eluted with various solvent and their mixtures starting with n-hexane, benzene, ether, chloroform, ethanol and methanol. The fractions were collected in bulk and monitored by TLC. Repeated chromatography afforded 7 compounds in pure form.

**Hexatetracontan-16-ol 1:** M$^{+}$ 662, C$_{46}$H$_{94}$O (methanol, 28mg), m.p. 93-95°C, isolated from hexane : benzene (9:1) fraction of column. TLC (benzene : MeOH : AcOEt, 9:0.5:1.0, v/v, Rf 0.82). IR (KBr): 3500 (OH), 2910, 2865, 1460, 1380, 1130, 1080,1010 and 730-720 cm$^{-1}$. $^1$H NMR (400 MHz, CDC$_3$), $\delta$: 0.83 (6H, t, 2x-CH$_3$), 1.3 (82H, s, 41x-CH$_2$), 1.5 (1H, s, OH), 1.55 (4H, bs, 2xCH$_2$) and 3.5 (1H, m, J=8.8 Hz, -CH(OH)-) ; EIMS m/z (rel.int. ) : 662[M$^{+}$] (2.2), 619 (3.2), 605 (3.5), 577 (3.4), 549 (3.8), 521 (2.8), 465 (3.9), 451 (5.5), 421 (2.2), 407 (3.0), 379 (3.4), 351 (4.8), 323 (1.2), 295 (2.5), 283 (3.8), 255 (5.5), 241 (3.9), 225 (2.9), 211 (4.7), 197 (4.5), 169 (3.2), 141 (1.9), 113 (30.2), 85 (58.4), 71 (66.0), 57 (100), 43 (80.5); $^{13}$C NMR (75 MHz, CDCl$_3$, BB, DEPT I, II) : 63.5, 38.5, 34.9, 32.2, 29.7, 27.2, 18.6 and 16.5. Anal.Found: C, 83.42; H,14.21; O, 2.37.

Caled for C$_{46}$H$_{92}$O: C, 83.38; H,14.19; O, 2.43 %.

**Acetylation of 1 (Ia).** Compound 1 (10 mg), Ac$_2$O and C$_2$H$_3$N (2.0 mL each) was allowed to stand overnight at room temperature. On usual work-up, the mixture afforded white crystals of Ia (7 mg), m.p. 114°C. TLC (benzene : MeOH : AcOEt, 9:5:0.5:1.0, v/v, Rf 0.63). IR:1742, 1240 etc., 'H NMR: 81.99 (3H, s, OAc) and other proton signals; MS : m/z 704 [M$^{+}$] (2.0) for C$_{47}$H$_{95}$O$_{2}$, 661 (3.4), 667 (6.1), 619 (3.7), 591 (3.4), 563 (3.2), 507 (4.9), 493 (3.8), 421 (2.2), 407 (3.0), 379 (3.4), 351 (4.8), 325 (3.2), 323 (1.2), 297 (2.2), 295 (2.6), 283 (4.8), 225 (2.9), 211 (4.7), 197 (4.4), 169 (3.2), 141 (2.0), 113 (30.2), 85 (58.2), 71 (60.1), 57 (100), 43 (80.3).
6,9-icosadiene 2: C_{36}H_{78}, M^+ 278 (chloroform : methanol, 30 mg), m.p. 126-27 °C, isolated from benzenemethyl acetate (98:2, v/v) fraction of acetone eluate of the column. TLC (benzene : methanol : acetic acid, 9:1:0.5, Rf 0.59); IR (KBr): 2960, 2920, 2860, 1637, 1617, 1480,1080 and 735-725 cm^{-1}; \(^1\)H NMR (300 MHz, CDCl3): \(\delta\) 0.83 (6H, t, 2x-CH3), 1.30 (22H, br s, 11x-CH2), 4.8 (4H, t, 2x-CH=CH, J=8.0 Hz), 3.16 (2H, m, -CH2, \(\alpha\) to unsaturation), 1.64 (4H, bs, 2xCH2); EIMS m/z (rel. int., %): M+ 278 [M]+ (40.12), 250(29.4), 222(23.2), 207(12.3), 194(14.3), 187(21.2), 167(100), 166(71.0), 141(39.4), 137(37.5), 113(42.08), 111(46.2), 97(44.0), 83(56.4), 71(62.4), 57(75.5), 43(58.3).

Hexyl 9-hydroxyheptatriacontanoate 5: M^+ 356, C_{22}H_{42}O_3 (chloroform: methanol, 20 mg), m.p. 140-42° C, isolated from acetone fraction of acetone eluate of the column. TLC (benzene : methanol : acetic acid 7:3:1, Rf 0.43); IR (KBr): 3425 (OH), 2923, 2853, 1725 (C=O), 1480,1379, 1080-1010 and 730 cm^{-1}; \(^1\)H NMR (300 MHz, CDCl3): 6.09 (6H, t, 2x-CH3, J=0.60 Hz), 4.08 (2H, t, -CH=O-CO-., J=8.0 Hz), 3.62(1H, m, -CHOH), 2.25 (2H, t, -CH2-CO-O.-, J=8.0 Hz), 1.24 (24H, s, 12x-CH2), 1.60 (9H, bs, 4x-CH2, \(\beta\) to -OH, ester group and -OH); EIMS m/z (rel. int., %): 356 [M]+ (20.0), 313(80), 299(27.14), 285 (3.6), 271 (5.5), 257 (2.4), 255 (2.5), 241 (6.4), 239 (78.5), 227 (4.1), 213 (2.3), 199 (3.5), 185 (25.4), 171 (6.5), 157 (4.8), 143 (6.1), 129(29.30), 116(35.2), 115 (4.3), 101 (7.8), 99 (5.3), 98(66.1), 85 (40.8), 71(45), 67(95), 57(82.4), 43(100); \(^1\)C NMR (75 MHz, CDCl3, BB, DEPT 1, II): 174.2, 63.4, 34.7, 32.3, 32.2, 31.7, 29.4, 29.2, 24.6, 22.8 and 16.2ppm. Anal. Found: C, 74.13; H, 12.32; O, 13.55. Calcd for C_{22}H_{42}O_3: C, 74.15; H, 12.35; O, 13.5 %.

Acetylation of 3 (3a). Compound 3 (10 mg), Ac_2O (10 mL) on usual work-up, afforded white crystals (7 mg). TLC (benzene : methanol : acetic acid, 7:3:2, Rf 0.23), m.p. 125-26°C; IR 2921, 2855,1741, 1725, 1468,1357, 1080-1010 and 730-725 cm^{-1}.

Alkaline hydrolysis of 3 (3b). Compound 3 (5 mg) was refluxed with ethanolic KOH (2.5 mL, 5 %) for 1 hr. At the end of the reaction, the mixture was diluted with water (3.0 mL) and extracted with chloroform. The chloroform layer was dried over anhyd. magnesium sulphate and concentrated. To separate both the compounds it was put in deep freezer. After 4 days, some solid was separate out. After usual work-up it afforded an alcohol, identified as butanol (MS : M^+ 74 and other fragments), and an acid 3b respectively (IR : 1702 cm^{-1}).

Butyl 11-hydroxyoctadecanoate 3: M^+ 356, C_{22}H_{42}O_3 (chloroform: methanol, 20 mg), m.p. 140-42° C, isolated from acetone fraction of acetone eluate of the column. TLC (benzene : methanol : acetic acid 7:3:1, Rf 0.43); IR (KBr): 3425 (OH), 2923, 2853, 1725 (C=O), 1480,1379, 1080-1010 and 730 cm^{-1}; \(^1\)H NMR (300 MHz, CDCl3): 6.09 (6H, t, 2x-CH3, J=0.60 Hz), 4.08 (2H, t, -CH=O-CO-., J=8.0 Hz), 3.62(1H, m, -CHOH), 2.25 (2H, t, -CH2-CO-O.-, J=8.0 Hz), 1.24 (24H, s, 12x-CH2), 1.60 (9H, bs, 4x-CH2, \(\beta\) to -OH, ester group and -OH); EIMS m/z (rel. int., %): 356 [M]+ (20.0), 313(80), 299(27.14), 285 (3.6), 271 (5.5), 257 (2.4), 255 (2.5), 241 (6.4), 239 (78.5), 227 (4.1), 213 (2.3), 199 (3.5), 185 (25.4), 171 (6.5), 157 (4.8), 143 (6.1), 129(29.30), 116(35.2), 115 (4.3), 101 (7.8), 99 (5.3), 98(66.1), 85 (40.8), 71(45), 67(95), 57(82.4), 43(100); \(^1\)C NMR (75 MHz, CDCl3, BB, DEPT 1, II): 174.2, 63.4, 34.7, 32.3, 32.2, 31.7, 29.4, 29.2, 24.6, 22.8 and 16.2ppm. Anal. Found: C, 74.13; H, 12.32; O, 13.55. Calcd for C_{22}H_{42}O_3: C, 74.15; H, 12.35; O, 13.5 %.
Alkaline hydrolysis of 5 (5b). Compound 5 (2.5 mg) was refluxed with ethanolic KOH (1.5 mL, 5%) for 1 hr. After usual work-up it afforded an alcohol, identified as 1-hexanol\(^{15}\) and an acid. TLC (hexane : ether : acetic acid 8:2:1); m.p. 112-13°C; IR: 3386, 2921, 2869, 1702, 1468, 1362, 1202, 1080 and 722 cm\(^{-1}\).

Heptadecyl nonadecanoate 6: M\(^{+}\) 536, C\(_{36}\)H\(_{72}\)O\(_2\) (chloroform: methanol, mg), m.p. 115-16°C, isolated from ethyl acetate fraction of ethyl acetate eluate of the column. TLC solvent system; hexane: ether: acetic acid (7:3:2, v/v); IR (KBr): 2919, 2850, 1737, 1462, 1383, 1018 and 730-720 cm\(^{-1}\); \(^{1}\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\): 0.90 (6H, t, \(2\times\)-CH\(_3\), \(J=6.0\) Hz), 4.05 (2H, t, -CH\(_2\)-O-CO-\(_2\), \(J=8.0\) Hz), 2.30 (2H, t, -CH\(_2\)-CO-O-, \(J=8.0\) Hz), 1.60 (4H, s, \(\beta\)-protons), 1.25 (58H, s, \(\alpha\)-CH\(_2\));

ElMS, m/z (rel. int., %): 536 [M\(^{+}\) (1.37), 530(2.1), 501(2.3), 485(2.6), 483(3.8), 454(5.2), 430(3.1), 425(5.7), 401(2.7), 396(2.9), 372(5.8), 364(3.4), 342(3.2), 312(11.2), 282(61.2), 186(9.2), 170(19.8), 142(11.1), 127(25.1), 112(50.7), 97(50.5), 69(82.5), 53(100). Anal. Found; C, 80.2; H, 13.5; O, 6.3. Calcd for C\(_{36}\)H\(_{72}\)O\(_2\): C, 80.5; H, 13.4; O, 6.1%.

(24aS)-stigmasta - 5, 22- diene - 3\(\beta\)-ol 7: M\(^{+}\) 412, C\(_{29}\)H\(_{48}\)O (chloroform: methanol, 24mg), m.p. 123-25°C.

Acknowledgement
Authors are thankful to CSIR, New Delhi for financial assistance and RSIC, CDRI, Lucknow, and RSIC, IIT- Bombay, Mumbai for NMR and mass spectra.

References