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Alkyl esters, γ-lactone and triglucosidic triacetates from the oleo-gum resin of *Commiphora myrrha* (Nees) Engl.

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ABSTRACT

*Commiphora myrrha (Nees) Engl. (Burseraceae) is a small tree producing an oleo-gum-resin known as myrrh. The resin is used in perfumery and is an ingredient of toothpastes, mouthwashes and dentifrices. Phytochemical investigation of the methanolic extract of the oleo-gum resin (myrrh) resulted in the isolation of two new dihydroxy alkyl esters, a γ-lactone and two alkyl triglucosides characterized 2β, 3β-dihydroxy-tridecanyl n-octadec-9,12,15-trien-1-oate (**1**), 1,8β,11β-trihydroxy-tetracosan-23,24-olide (**2**), glyceryl-n-1-tetracontanoate (**3**), n-docosanyl-β-D-glucopyranosyl-(2'→1'')-β-D-glucopyranosyl-(2''→1''')-β-D-(2‴,3‴,4‴-triacetyl)-glucopyranoside (**4**) and n-octacos-21-onyl-β-D-glucopyranosyl-(2'→1'')--β-D-glucopyranosyl-(2''→1''')-)-β-D-(2‴,3‴,4‴-triacetyl)-gluco -pyranoside (**5**). The structures of all the isolated phytoconstituents have been estabilished on the basis of spectral data analysis and chemical reactions.*

Keywords: *Commiphora myrrha*, oleo-gum resin, aliphatic esters, γ-lactone, alkyl triglucosides.

INTRODUCTION

Commiphora myrrha (Nees) Engl. (Burseraceae) is a small tree which grows in small sandy and rocky regions of Somalia, Sudan, Ethiopia, Kenya and Saudi Arabia. The schizogenous cavities of the stem and branches of this tree produce a scented oleo-resin which is known as myrrh. It is imported into India since long time and used in perfumery as food additive, fragrance, incense, antiseptic, astringent, stimulant, stomachic, tonic and for embalming. It is an ingredient of toothpastes, mouthwashes and dentifrices. Myrrh tincture is useful in menstrual disorders and chlorosis. In China, it is prescribed to treat wounds, inflammation and menstrual pain due to blood stagnation [1]. Cadinenes, calamenes, triacont-1-ene [2], commiphoric acids, furanosesquiterpenoids [3-10], eudesmol and triterpenoids [11] and volatile oil [12] have been reported from the oleo-resin of *C. myrrha*. This paper describes the isolation and structure elucidation of two dihydroxy aliphatic esters, a tetracosanyl γ-lactone and two alkyl triglucosidic triacetates from the oleo-resin obtained from the Khari Baoli market of Delhi.

EXPERIMENTAL SECTION

Instrumentation and techniques

Melting points were determined on a Perfit melting apparatus (Haryana, India) and are uncurrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Kong) spectrophotometer using KBr pellets; γ_{max} values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were screened on Avance DRX 400, Bruker spectrospin 400 and 100 MHz instruments (Karlesruthe, Germany) using TMS as an internal standard. Mass spectra were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with

direct inlet probe system. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualised by exposing to iodine vapours, UV radiation and spraying with ceric sulphate.

Plant material

The crude oleo-gum-resin of *C. myrrha* was procured from the local market of the Khari Baoli, Delhi. The sample was authenticated by Dr. H.B. Singh, Taxonomist, NISCAIR, CSIR, New Delhi. A voucher specimen of the sample (No. N/R/C/-06-07/803/120) was deposited in the NISCAIR, RHM Division, Dr. K.S. Krishnan Marg, New Delhi.

Extraction and isolation

The air dried oleo-gum-resin (2.5 kg) was coarsely powdered and extracted with methanol at room temperature for one week. The extract was filtered and concentrated under reduced pressure to get 185 g (7.4% yield) of dark brown mass. The concentrated extract of the oleo-resin was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) to form a slurry. The slurry was air-dried and loaded on silica gel column (1.6 m × 16 mm × 2 mm) load in petroleum ether and then eluted successively with different solvents in increasing order of polarity in various combinations, such as petroleum ether, petroleum ether- chloroform (9:1, 3:1, 1:1, 1:3), chloroform, chloroform-methanol (19.9:0.1, 99:1, 97:3, 19:1, 93:7, 9:1, 17:3, 3:1, 3:2, 2:3) and methanol. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of *C. myrrha* oleo- resin:

Dihydroxytridecanyl linolenate (1)

Further elution of the column with petroleum ether-chloroform (9:1) produced a pale yellow mass of **1**, recrystallized from methanol, 95 mg (0.0038% yield); R_f: 0.72 (chloroform-methanol; 97:3); m.p.: 70- 71°C; UV λ_{max} (MeOH): 208.5 nm (log ε 5.9); IR ν_{max} (KBr): 3313, 2923, 2845, 1746, 1645, 1450, 1380, 1215, 1030, 908, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.46 (1H, m, H-13), 5.43 (1H, m, H-10), 5.26 (1H, m, H-15), 5.24 (1H, m, H-9), 5.08 (1H, m, H-16), 4.87 (1H, m, H-12), 4.49 (2H, d, J=8.5 Hz, H_2-1′), 3.39 (1H, brm, $w_{1/2}$=18.7, Hz, H-3′α), 3.14 (1H, m, $w_{1/2}$=16.2 Hz, H-2′), 2.04 (2H, t, *J*=8.7 Hz, H_2–2), 1.94 (2H, m, H_2-11), 1.90 (2H m, H_2-14), 1.88 (2H, m, H_2-17), 1.70 (2H, m, H_2-8), 1.54 (2H, m, H_2-3), 1.35 (2H, m, CH_2), 1.25 (24H, brs, 12×CH_2), 0.82 (3H, t, *J* = 6.5 Hz, Me-18), 0.73 (3H, t, *J* = 6.1 Hz, Me-13′); ^{13}C NMR ($CDCl_3$): 171.61 (C-1), 136.32 (C-10), 125.04 (C-12), 122.06 (C-13), 121.45 (C-9, C-15), 120.49 (C-16), 68.16 (C-2′), 66.28 (C-3′), 62.13 (C-1′), 42.76 (C-2), 37.94 (C-11), 35.21 (C-14), 31.74 (C-17), 29.57 (13×CH_2), 22.54 (CH_2), 20.02 (CH_2), 15.29 (Me-18), 14.04 (Me-13′); +ve ion FAB MS *m/z (rel. int.)*: 492 $[M]^+$($C_{31}H_{56}O_4$) (11.2), 261 (26.3), 231 (35.8), 215 (31.5), 201 (32.1), 171 (48.6), 119 (80.3), 95 (100).

Myrrhatetracosanyl lactone (2)

Further elution of the column with petroleum ether-chloroform (3:1) yielded a pale yellow mass of **2**, recrystallized from acetone-chloroform (1:1), 200 mg (0.008% yield); R_f 0.83 (petroleum ether-chloroform; 9: 1); m.p.: 76- 77°C; UV λ_{max} (MeOH): 215 nm (log ε 5.1); IR ν_{max} (KBr): 3434, 3390, 2930, 2852, 1761, 1640, 1447, 1380, 1219, 1097, 1037, 725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.18, (2H, d, J=7.5 Hz, H_2-24′), 3.85 (1H, m, $w_{1/2}$=10.3 Hz. H-8α), 3.73 (1H, m, $w_{1/2}$= 10.6 Hz, H-11α), 3.11 (2H, t, J=9.5 Hz, H_2-1), 2.11 (2H, d, J=8.5 Hz, H_2-22), 1.81 (1H, brm, $w_{1/2}$=11.2 Hz, H-21α), 1.65 (2H, m, H_2-9), 1.60 (2H, m, H_2-10), 1.52 (2H, m, H_2-12), 1.49 (2H, m, H_2-7), 1.21 (26H, brs, 13×CH_2); ^{13}C NMR ($CDCl_3$): δ 173.16 (C-23), 71.28 (C-8), 69.05 (C-11), 63.87 (C-24), 61.22 (C-1), 54.17 (C-22), 46.23 (C-21), 31.39 (CH_2), 30.81 (CH_2), 29.42 (12 × CH_2), 27.34 (CH_2), 22.10 (CH_2); +ve ion FAB MS *m/z (rel. int.)*: 414 $[M]^+$ ($C_{24}H_{46}O_5$) (18.6), 329 (8.9), 299 (21.3), 241 (15.6), 211 (18.7), 203 (69.8), 173 (27.6), 145 (38.1), 115 (29.5), 95 (100), 85 (33.4).

Glyceryl-*n*-1-tetracontanoate (3)

Elution of the column with chloroform-methanol (97:3) gave a pale yellow mass of **3**, recrystallized from methanol-acetone (1:1), 170 mg (0.0068% yield); R_f: 0.50 (chloroform-methanol, 17:3); m.p.: 68- 69°C; UV λ_{max} (MeOH): 212 nm (log ε 5.6); IR ν_{max} (KBr): 3344, 3265, 2922, 2858, 1725, 1638, 1448, 1381, 1256, 1069, 721 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.32 (2H, d. J=7.5 Hz, H_2-1′), 3.58 (1H, m, H-2′), 3.27 (2H, d, J=7.3 Hz, H_2-3′), 2.49 (2H, t, J=7.1 Hz, H_2-2), 1.95 (4H, brs, 2×CH_2), 1.86 (2H, brs,CH_2), 1.42 (2H,brs, CH_2), 1.25 (66H, brs, 33×CH_2), 0.83 (3H, t, *J*=6.1 Hz, Me-40); ^{13}C NMR (DMSO-d_6): δ 171.22 (C-1), 81.13 (C-2), 68.17 (C-1), 62.73 (C-3), 48.81 (C-2), 31.51 (CH_2), 29.40 (31×CH_2), 28.99 (CH_2), 25.36 (CH_2), 22.25 (CH_2), 21.22 (CH_2), 20.62 (CH_2), 14.06 (Me-40′); +ve ion FAB MS *m/z (rel. int.)*: 666 $[M]^+$($C_{43}H_{86}O_4$) (38.7), 575 (15.3), 91 (71.3).

Docosanyl triglucoside (4)

Elution of the column with chloroform-methanol (93:7) afforded a light brown mass of **4**, recrystallized from methanol-acetone (1:1), 1.18 g (0.0472% yield); R_f: 0.41 (chloroform-methanol; 93:7); m.p.: 80-82°C; UV λ_{max}

(MeOH): 211 nm (log ε 4.9); IR ν_{max} (KBr): 3415, 3380, 2928, 2850, 1739, 1725, 1441, 1377, 1217, 1040, 719 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.31 (1H, d, *J*=7.1 Hz, H-1′), 5.12 (1H, d, *J*=7.0 Hz, H-1″), 5.03 (1H, d, *J*=7.1 Hz, H-1‴), 4.58 (2H, m, H-5′, H-5″), 4.52 (1H, m, H-5‴), 4.31 (2H, m, H-2‴, H-3‴), 4.22 (1H, m, H-4‴), 4.10 (2H, m, H-2′, H-2″), 3.67 (2H, m, H-4′, H-4‴), 3.56 (1H, m, H-3′), 3.50 (1H, m, H-3″), 3.37 (2H, t, J=6.8 Hz, H_2-1), 3.30 (4H, brs, H_2-6′, H_2-6″), 3.28 (2H, brs, H_2-6‴), 2.07 (3H, brs, $COCH_3$), 2.05 (6H, brs, 2×$COCH_3$), 1.69 (2H, m, CH_2), 1.16 (38H, brs, 19×CH_2), 0.79 (3H, t, *J*=6.3 Hz, Me-22); ^{13}C NMR ($CDCl_3$): δ 172.52 ($COCH_3$), 172.08 ($COCH_3$), 170.88 ($COCH_3$), 103.43 (C-1′), 101.21 (C-1″), 98.47 (C-1‴), 81.43 (C-2′), 81.22 (C-2″), 81.19 (C-2‴), 74.60 (C-5′), 73.16 (C-5″, C-5‴), 71.99 (C-3′, C-3″), 70.57 (C-3‴, C-4′), 68.72 (C-4″), 66.59 (C-4‴), 63.38 (C-1), 62.16 (C-6′), 62.37 (C-6″), 60.13 (C-6‴), 31.54 (CH_2), 30.44 (CH_2), 28.35 (12×CH_2), 28.54 (3×CH_2), 25.30 (CH_2), 24.15 (CH_2), 22.28 (CH_2), 20.56 ($COCH_3$), 19.54 ($COCH_3$), 19.49 ($COCH_3$), 13.69 (CH_3-22); +ve ion FAB MS *m/z (rel. int.)*: 938 $[M]^+$ ($C_{46}H_{82}O_{19}$) (1.3), 649 (6.8), 487 (12.2), 325 (20.1), 289 (22.3), 163 (21.9).

21-Keto-octacosanyl triglucoside (5)

Elution of the column with chloroform-methanol (17:3) furnished a light brown mass of **5**, recrystallized from methanol-acetone (9:1), 780 mg (0.0312% yield); R_f: 0.38 (chloroform-methanol; 17:3); m.p.: 83-84°C; UV λ_{max} (MeOH): 212 nm (log ε 5.1); IR ν_{max} (KBr): 3430, 3390, 3260, 2930, 2851, 1743, 1725, 1702, 1445, 1377, 1218, 1085, 1035, 720 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.33 (1H, d, *J*=7.2 Hz, H-1′), 5.10 (1H, d, *J*=7.0 Hz, H-1″), 5.06 (1H, d, *J*=7.1 Hz, H-1‴), 4.81 (2H, m, H-5′, H-5″), 4.73 (1H, m, H-5‴), 4.57 (1H, dd, *J*=7.1, 6.5 Hz, H-2‴), 4.51 (H, m, H-3‴), 4.30 (H, m, H), 3.97 (2H, m, H, H), 3.55 (H, m), 3.53 (H, m), 3.37 (2H, brs, H_2-1), 3.14 (2H, brs, H_2-6′), 3.03 (4H, brs, H_2-6″, H_2-6‴), 2.11 (3H, brs, $COCH_3$), 2.05 (3H, brs, $COCH_3$), 1.99 (3H, brs, $COCH_3$), 1.91 (2H, brs, H_2-22), 1.87 (2H, brs, H_2-20), 1.69 (6H, brs, 3×CH_2), 1.60 (2H, brs, CH_2), 1.52 (4H, brs, 2×CH_2), 1.19 (32H, brs, 16×CH_2), 1.04 (2H, brs, CH_2), 0.81 (3H, t, *J*=6.3 Hz, Me-28); ^{13}C NMR (DMSO-d_6): δ 206.65 (C-21), 171.93 ($COCH_3$), 170.21 ($COCH_3$), 169.93 ($COCH_3$), 104.80 (C-1′), 104.35 (C-1″), 103.07 (C-1‴), 80.59 (C-2′), 79.23 (C-2″, C-2‴), 76.74 (C-5′), 76.70 (C-5″), 75.82 (C-5‴), 74.51 (C-3′), 73.55 (C-3″), 72.19 (C-3‴), 71.70 (C-4′), 70.72 (C-4″), 68.71 (C-4‴), 63.78 (C-1), 62.08 (C-6′), 60.94 (C-6″), 60.75 (C-6‴), 48.75 (C-20, C-22), 31.46 (CH_2), 30.75 (CH_2), 29.24 (13×CH_2), 28.88 (4×CH_2), 25.57 (CH_2), 24.88 (CH_2), 23.35 (CH_2), 22.25 (CH_2), 14.05 (CH_3-28); +ve ion FAB MS *m/z (rel. int.)*: 1036 $[M]^+$ ($C_{52}H_{92}O_{20}$) (1.3), 585 (7.2), 451 (20.3), 423 (31.5), 324 (12.6), 296 (18.3), 289 (38.1),127 (78.4), 99 (28.2).

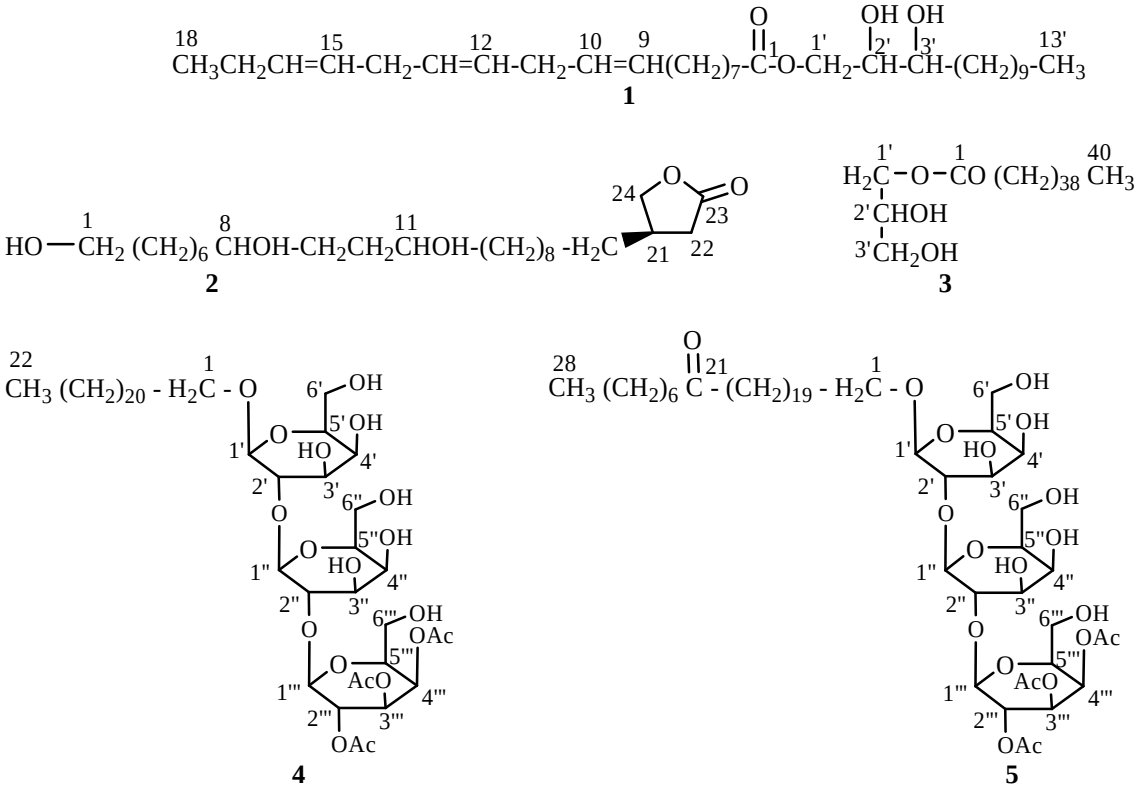

Figure 1: Structures of compound 1-5

RESULTS AND DISCUSSION

Compound **1**, named dihydroxytridecanyl linolenate, was obtained as a pale yellow mass from petroleum ether-chloroform (9:1) eluants. Its IR spectrum exhibited important absorption bands for hydroxyl groups (3313 cm^{-1}), ester function (1746 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its +ve FAB mass displayed a molecular ion peak at *m/z* 492 corresponding to the molecular formula of a fatty acid ester, $C_{31}H_{56}O_4$. The diagnostic ion peaks arising at *m/z* 261 $[CH_3(CH_2CH=CH)_3(CH_2)_7CO]^+$, 231 [M-261, OCH_2CHOH-$CHOH(CH_2)_9CH_3]^+$, 215 [O-C_1' fission, $CH_2CHOHCHOH(CH_2)_9CH_3]^+$, 201 [$C_{1'}$-$C_{2'}$ fission, $CHOHCHOH(CH_2)_9CH_3]^+$ and 171 [$C_{2'}$-$C_{3'}$ fission, $CHOH(CH_2)_9CH_3]^+$ indicated the presence of hydroxyl groups at C-2′ and C-3′ of a tetradecanyl unit linked to linolenic acid. The ^{1}H NMR spectrum of **1** exhibited six one-proton downfield multiplets at δ 5.46, 5.43, 5.26, 5.24, 5.08 and 4.87 assigned correspondingly to vinylic H-13, H-10, H-15, H-9, H-16 and H-12 protons, respectively. Two one-proton broad multiplets at δ 3.14 ($w_{1/2}$=16.2 Hz) and 3.39 ($w_{1/2}$=18.7 Hz) were ascribed to H-2′α and H-3′α carbinol protons, respectively. A two-proton doublet at δ 4.49 (J=8.5 Hz) was ascribed to oxygenated methylene H_2-1′ protons. A two-proton triplet at δ 2.04 (*J*=8.7 Hz) was attributed to H_2-2 methylene protons adjacent to the ester group. Four two-proton multiplets at δ 1.94, 1.90, 1.88 and 1.70 were ascribed correspondingly to methylene H_2-11, H_2-14, H_2-17 and H_2-8 protons adjacent to vinylic linkages. The remaining methylene protons resonated as multiplets at δ 1.54 (2H), 1.35 (2H) and as a broad singlet at δ 1.25 (24H). Two three-proton triplets at δ 0.82 (*J*=6.5 Hz) and 0.73 (*J*=6.1 Hz) were assigned to primary C-18 and C-13′ methyl protons, respectively. The ^{13}C NMR spectrum of **1** exhibited important signals for ester carbon at δ 171.61 (C-1) and vinylic carbons from δ 136.32 to 120.49, carbinol carbons at δ 68.16 (C-2′) and 66.28 (C-3′), oxygenated methylene carbon at δ 62.13 (C-1′), methylene carbons in the range of δ 42.76 to 20.02 and the primary methyl carbons at δ 15.29 (C-18) and 14.04 (C-13′). Alkaline hydrolysis of **1** yielded linolenic acid (co-TLC comparable). On the basis of above discussion the structure of the new aliphatic ester **1** has been elucidated as 2′β, 3′β-dihydroxy-tridecanyl *n*-octadec-9,12,15-trien-1-oate (Figure 1).

Compound **2**, named myrrhatetracosanyl lactone, was obtained as a pale yellow mass from petroleum ether-chloroform (3:1) eluants. Its IR spectrum exhibited absorption bands for hydroxyl groups (3434, 3390 cm^{-1}), γ-lactone ring [1761 cm^{-1}) and long chain aliphatic moiety (725 cm^{-1}). Its molecular formula was determined at m/z 414 on the basis of its +ve FAB mass and ^{13}C NMR spectra corresponding to a trihydroxy aliphatic lactone $C_{24}H_{46}O_5$. The ion fragments arising at *m/z* 115 [C_7-C_8 fission, $HOCH_2$ $(CH_2)_6]^+$, 299 [M-115]$^+$, 85 [C_{20}-C_{21} fission, $C_4H_5O_2]^+$ and 329 [M-85]$^+$ supported the presence of primary hydroxyl group at one terminal and a lactone ring at another terminal carbon in the compound. The other diagnostic ion peaks generating at *m/z* 145 [C_8-C_9 fission, $HOCH_2(CH_2)_6CHOH]^+$, 173, 241 [C_{10}-C_{11} fission]$^+$ and 203, 211 [C_{11}-C_{12} fission]$^+$ indicated the location of the other hydroxyl groups at C-8 and C-11. The ^{1}H NMR spectrum of **2** exhibited a downfield two-proton doublet at δ 4.18 (J=7.5 Hz) assignable to oxygenated methylene H_2-24 protons. Two one-proton multiplets at δ 3.85 ($w_{1/2}$=10.3 Hz) and 3.73 ($w_{1/2}$=10.6 Hz) were ascribed to α-oriented carbinol H-8 and H-11 protons, respectively. A two-proton triplet at δ 3.11 (J=9.5 Hz) was accounted to hydroxymethylene H_2-1 protons. A one-proton multiplet at δ 1.81 ($w_{1/2=}$11.2 Hz) was accounted to α-oriented H-21 methine proton of the lactone ring. A two- proton doublet at δ 2.11 (*J=8.*5 Hz) was assigned to methylene H_2-22 protons supporting the presence of a γ-lactone ring. The remaining methylene protons resonated from δ 1.65 to 1.21. The ^{13}C NMR spectrum of **2** displayed signals for a lactone carbon at δ 173.16 (C-23), carbinol carbons at δ 71.28 (C-8) and 69.05 (C-11), oxygenated methylene carbon at δ 63.87 (C-24), hydroxymethylene carbon at δ 61.22 (C-1) and methylene carbons between δ 54.17-22.10. The absence of any ^{1}H NMR signal beyond δ 4.18 and ^{13}C NMR signal between δ 173.16-71.28 ruled out the presence of olefinic protons in the compound. On the basis of above discussion the structure of the new γ-lactone **2** has been elucidated as 1,8β,11β-trihydroxy tetracosan-23,24-olide (Figure 1). The lactone containing aliphatic constituents have been isolated earlier from the seeds of *Althea officinalis* [13], hulls of *Oriza sativa* [14] and roots of *Streblus aspera* [15].

Compound **3**, designated as glyceryl tetracontanoate, was obtained as a pale yellow mass chloroform-methanol (97:3) eluants. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3344, 3265 cm^{-1}), ester function (1725 cm^{-1}) and long aliphatic moiety (721 cm^{-1}). The +ve FAB mass spectrum of **3** exhibited a molecular ion peak at *m/z* 666 consistent with the molecular formula of an acyl glycerol $C_{43}H_{86}O_4$. The diagnostic ion peaks at *m/z* 575 [C_1-O fission, CO $(CH_2)_{38}CH_3$, $C_{40}H_{79}O]^+$ and 91 [M-575, $CH_2OCHOHCH_2OH]^+$ suggested that glycerol was esterified with a C_{40} fatty acid. The ^{1}H NMR spectrum of **3** exhibited a one-proton multiplet at δ 3.58 attributable to H-2′ carbinol proton. Two two-proton doublets at δ 4.32 (J=7.5 Hz) and 3.27 (J=7.3 Hz) were ascribed correspondingly to oxygenated methylene H_2-1′ and H_2-3′ protons, respectively. A two-proton triplet at δ 2.49 (J=7.1Hz) was accounted to methylene H_2-2 protons adjacent to the ester group. The remaining methylene protons resonated from δ 1.95 to 1.25. A three-proton triplet at δ 0.83 (*J*=6.1 Hz) was attributed to primary C-40 methyl protons. The ^{13}C NMR spectrum of **3** exhibited important signals for ester carbon at δ 171.22 (C-1), carbinol carbon at δ 81.3 (C-2), oxygenated methylene carbons at δ 68.17 (C-1) and 62.73 (C-3), methylene carbons between

δ 48.81- 20.62 and methyl carbon at δ 14.06 (C-40′). Acid hydrolysis of **3** yielded tetracontanoic acid (m.p. and co-TLC comparable). On the basis of above spectral data and chemical reactions the structure of the new ester **3** has been elucidated as glyceryl-*n*-1-tetracontanoate (Figure 1).

Compound **4**, designated as docosanyl triglycoside, was obtained as a light brown mass from chloroform-methanol (93:7) eluants. It responded positively to the tests of glycosides. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups (3415, 3380 cm^{-1}), ester functions (1739, 1725 cm^{-1}) and long chain aliphatic moiety (719 cm^{-1}). On the basis of +ve FAB mass and ^{13}C NMR spectra its molecular weight was established as *m/z* 938 consistent with a molecular formula of an aliphatic alcohol triglucoside, $C_{46}H_{82}O_{19}$. A prominent ion peak generating at *m/z* 325 $[CH_3(CH_2)_{20}CH_2O]^+$ due to glycosidic bond cleavage indicated the presence of C_{22}-alcohol as the aglycone part. The ion fragments arising at *m/z* 487 $[CH_3(CH_2)_{21}OC_6H_{10}O_5]^+$, 649 $[CH_3(CH_2)_{21}OC_6H_{10}O_5 - C_6H_{10}O_5]^+$ and 289 $[C_6H_8O_5(Ac)_3]^+$ supported the presence of a triacetylated hexose at the terminal of the sugar chain. The ^1H NMR spectrum of **4** exhibited three one-proton doublets at δ 5.31 (*J*=7.1 Hz), 5.13 (*J*=7.0 Hz) and 5.12 (*J*=7.1 Hz) assigned correspondingly to anomeric H-1′, H-1″ and H-1‴ protons. A two-proton multiplet at δ 4.58 and a one one-proton multiplet at δ 4.52 were ascribed to oxygenated H-5′, H-5″ and H-5‴ methine protons, respectively. A two-proton multiplet at δ 4.31 and a one-proton multiplet at δ 4.22 were due to oxygenated methine H-2‴, H-3‴ and to H-4‴, respectively, linked to acetoxyl groups. A four-proton broad signal at δ 3.30 and a two-proton broad signal at δ 3.28 were attributed to oxygenated methylene H_2-6′, H_2-6″ and H_2-6‴ protons. A two-proton triplet at δ 3.37 (J=6.8 Hz) was accounted to oxygenated methylene H_2-1 protons. The remaining methylene protons appeared as a multiplet at δ 1.69 (2H) and as a broad signal at δ 1.16 (38H). A three-proton broad singlet at δ 2.07 and a six-proton broad singlet at δ 2.05 were attributed to acetyl protons. A three-proton triplet at δ 0.79 (*J*=6.3 Hz) was assigned to primary C-22 methyl protons. The ^{13}C NMR spectrum of **4** displayed important signals for ester carbons at δ 172.52, 172.08 and 170.88 of the acetate groups, three anomeric carbons at δ 103.43 (C-1′), 101.21(C-1″) and 98.47 (C-1‴), other sugar and oxygenated methylene carbons from δ 81.43 to 60.13, remaining methylene carbons between δ 31.54-22.28, primary methyl carbon at δ 13.69 (C-22) and acetyl carbons at δ 20.56, 19.54 and 19.49. The ^{13}C NMR values of the sugar residues were compared with the chemical shift values of sugar parts [16,18]. The appearance of C-2′ at δ 81.43 and C-2″ at 81.22 in the deshielded region suggested (2→1) linkage at the sugar units. Acid hydrolysis of **4** yielded D-glucose (co-TLC-comparable). On the basis of above discussion, the structure of the new aliphatic alcoholic triglycoside **4** has been established as *n*-docosanyl-β-D-glucopyranosyl-(2′→1″)-β-D-glucopyranosyl-(2″→1‴)-β-D-(2‴,3‴,4‴-triacetyl)-glucopyranoside (Figure 1).

Compound **5**, designated as 21-keto-octacosanyl triglucoside, was obtained as a light brown mass from chloroform-methanol (17:3) eluants. It responded positively to tests for glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3430, 3390, 3260 cm^{-1}), ester groups (1743, 1725 cm^{-1}), carbonyl function (1702 cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of +ve FAB mass and ^{13}C NMR spectra its molecular weight was established as 1036 consistent with the molecular formula of an alkyl triglycosidic ester $C_{52}H_{92}O_{20}$. The prominent ion peak at *m/z* 423 $[CH_3(CH_2)_6CO(CH_2)_{19}CH_2O$, $C_{28}H_{55}O_2]^+$ arose due to C_1-O fission supported the presence of a C_{28}-ketoalcohol in the compound. The ion fragments generating at *m/z* 99 [C_{21}-C_{22} fission, $CH_3(CH_2)_6]^+$, 324 [423-99]$^+$, 127 [C_{20}-C_{21} fission, $CH_3(CH_2)_6CO]^+$ and 296 [423-127]$^+$ indicated the presence of the carbonyl group at C-20 in the C_{28}-aglycone unit. The other important ion peaks producing at *m/z* 289 $[C_6H_7O_5(Ac)_3]^+$, 451 $[C_6H_9O_5$-$C_6H_8O_5(Ac)_3]^+$ and 585 $[CH_3(CH_2)_6CO(CH_2)_{19}CH_2O$-$C_6H_{10}O_5]^+$ indicated the presence of a terminal triacetylated hexose and two other hexose units in the compound. The ^1H NMR spectrum of **5** exhibited three one-proton doublets at δ 5.33 (*J*=7.2 Hz), 5.10 (*J*=7.0 Hz) and 5.06 [*J*=7.1 Hz) ascribed correspondingly to anomeric H-1′, H-1″ and H-1‴ protons. The other sugar and oxygenated methylene protons appeared from δ 4.81 to 3.03. The acetate protons appeared as three-proton broad singlets at δ 2.11, 2.05 and 1.99. The remaining methylene protons resonated between δ 1.91-1.04. A three-proton triplet at δ 0.81 (*J*=6.3 Hz) was attributed to C-28 primary methyl protons. The ^{13}C NMR spectrum of **5** displayed important signals for carbonyl carbon at δ 206.65 (C-21), acetyl carbons at δ 171.93, 170.21 and 169.93, anomeric carbons at δ 104.80 (C-1′), 104.35 (C-1″) and 103.07 (C-1‴), other sugar protons in the range of δ 80.59-60.75, oxygenated methylene carbon of the aliphatic chain at δ 63.78 (C-1), the remaining methylene carbons between δ 48.75-22.25 and the primary methyl carbon at δ 14.05 (C-28). The ^{13}C NMR values of the sugar residues were compared to the chemical shift values of sugars [15-18]. The presence of C-2′ at δ 80.59 and C-2″ at δ 79.23 in the desheilded region suggested (2→1) linkages of the sugar units. Acid hydrolysis of **5** yielded D-glucose (co-TLC-comparable). On the basis of above discussion the structure of the new ketoalkyl triglucoside **5** was elucidated as *n*-octacos-21-onyl-β-D-glucopyranosyl-(2′→1″)--β-D-glucopyranosyl-(2″→1‴)-)-β-D-(2‴,3‴,4‴-triacetyl)-glucopyranoside (Figure 1).

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