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# Triterpenoid and steroidal esters from the roots of *Operculina turpethum* (L.) Silva Manso

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**ABSTRACT**

*Operculina turpethum (Linn.) Silva Manso, syn. Ipomoea turpethum (L.) R.Br. (Convolvulaceae) is a perennial herb with tuberous roots used as purgative, anthelmintic and febrifuge and to treat abdominal disorders, ascite, leucoderma, itches and joint pain. Phytochemical investigation of methanolic extract of the roots led to isolation of new lanostenoids and steroids characterised as 3α,7α-epoxy lanost-5,25-dien-3β-ol* ***(1),*** *lanost-5,25-dien-3α-ol* ***(2),*** *4β-hydroxy-3α,7α-epoxy stigmast-(Z)-5,22-dien-3β-tetradecanoate* ***(3)****, 3α,7α-epoxy stigmast-5,20-dien-3β-hexadecanoate* ***(4),*** *12β-hydroxy-3α,7α-epoxy lanost-(Z)-5,20,22-trien-26-oic acid-3β-tetradecanoate* ***(5)*** *and 3α,7α-epoxy stigmast-(Z)-5,20,22-trien-28-oic acid-3β-hexadecanoate* ***(6)*** *along with known compounds D-glucuronic acid* ***(7)****, D-galacturonic acid* ***(8)****, sucrose* ***(9)*** *and D-β-rhamnose* ***(10).*** *The strictures of these phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.*

**Keywords:** *Operculina turpethum*, Convolvulaceae, roots, lanostenoids, steroids, isolation.

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## INTRODUCTION

*Operculina turpethum* (Linn.) Silva Manso, syn. *Ipomoea turpethum* (L.) R.Br. (Convolvulaceae), commonly known as turpeth, nisod or Indian jalap, is a large perennial herbaceous vine with milky juice and fleshy branched roots distributed in south eastern Asia including India, Sri Lanka, China, Africa countries, Pacific Islands and Australia [1,2]. The drug occurs in two forms, white turpeth (Safed nisoth) and black turpeth (Krishna nisoth) [3]. It consists of cylindrical pieces of root and stem. White turpeth is preferred to black turpeth as cathartic, the later produces drastic purgation and causes vomiting, fainting and giddiness. The drug is antihelmintic, bitter, febrifuge and purgative and prescribed to treat ascite, leucoderma, itches, ulcers, abdominal troubles, anaemia, fevers, piles, tumors, liver, heart and eye diseases and joint pain [1,3]. The active principle is a brownish yellow and odourless resin with a bitter pungent taste. Turpethinic acids A-E, glycosidic acid [4], *N-p*-coumaryl tyramine, daucosterol, salicylic acid [5], 22,23-dihydro-α-spinosteryl glucoside [6], acrylamide derivatives [7], operculinosides [8], α- and β-turpethein [3], betulin, lupeol and β-sitosterol [9] have been reported from *O. turpethum*. The present paper describes the isolation and characterization of lanostenoids and steroids from the roots of white variety of the plant procured from Delhi.

## MATERIALS AND METHODS

### General

Melting points were determined on a Perfit melting apparatus (Ambala, 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 Hong) spectrophotometer using KBr pellets; $\gamma_{max}$ values are given in $cm^{-1}$. $^{1}H$ and $^{13}C$ NMR spectra were screened on advance DRY 300, Bruker spectrospin 300 and 100 MHz in 5 mm spinning tubes at 27 ºC, respectively instruments (Karlesruthe, Germany) using $CDCl_3$ and 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 solution.

**Plant material**

The crude drug was procured from a commercial source of Darya Ganj, New Delhi. The sample was identified on the basis of exomorphic characters and reviews of literature by Dr. H.B. Singh, taxonomist, NISCAIR, CSIR, New Delhi. A voucher specimen of the sample (No. NR/C/2007/08/842/26) was deposited in the NISCAIR, RHM Division, Dr. K.S. Krishnan Marg (Near Pusa Gate), New Delhi-110012.

**Extraction and Isolation**

The dried roots of *O. turpethum* (3 kg) were coarsely powdered and extracted in a Soxhlet apparatus with methanol for 72 hours. The methanolic extract was then concentrated on a steam bath and dried under reduced pressure to get 250 g (8.33% yield) of dark brown mass, packed with silica gel (for column chromatography, 60-120 mesh). The dried plant extract slurry was loaded over the column and then eluted successively with different solvents. The development and elution of the column were carried out with successive series of different solvents in increasing order of polarity in various combinations, such as petroleum ether, petroleum ether-chloroform (3:1, 1:1, 1:3), chloroform, chloroform- methanol (99:1, 97:3, 19:1, 93:7, 9:1, 17:3, 3:1, 3:2, 2:3, 1:4) and methanol. The following compounds have been isolated:

**Turpetholanostenyl epoxide (1)**

Elution of the column with petroleum ether-chloroform (1:3) afforded creamish amorphous powder of **1**, recrystallized from methanol-acetone (1:1), 91 mg (0.0049% yield); $R_f$: 0.55 (petroleum ether-chloroform; 1:3); m.p.: 158-159°C; UV $\lambda_{max}$ ($CH_3OH$): 222, 228 nm (log ε 5.3, 2.9); IR $\upsilon_{max}$ (KBr): 3401, 2932, 2868, 1640, 1447, 1378, 1260, 1216, 1080, 765 $cm^{-1}$;$^{1}H$ NMR ($CDCl_3$): δ 5.11 (1H, d, *J*=5.2 Hz, H-6), 4.64 (1H, brs, $H_2$-26a), 4.59 (1H, brs, $H_2$-26b), 4.27 (1H, dd, *J*=5.5, 5.2 Hz, H-7β), 1.53 (3H, brs, Me-27), 1.21 (3H, brs, Me-28), 1.18 (3H, brs. Me-19), 0.96 (3H, d, *J*=6.3 Hz, Me-20), 0.89 (3H, brs, Me-29), 0.82 (3H, brs, Me-30), 0.73 (3H, brs, Me-18), 2.16-1.28 (22H, m, 9×$CH_2$, 4×CH); $^{13}C$ NMR ($CDCl_3$): Table 1; +ve ion FAB MS *m/z* (*rel. int.*): 440 $[M]^+$ ($C_{30}H_{48}O_2$) (11.3), 425 (25.5), 410 (37.2), 407 (23.6), 329 (10.4), 311 (22.8), 287 (23.1), 266 (22.1), 234 (7.5), 220 (45.6), 206 (25.5), 188 (72.5), 180 (31.6), 162 (75.5), 153 (21.5), 138 (42.7), 135 (77.1), 95 (100).

**Turpetholanostenol (2)**

Further elution of the column with petroleum ether-chloroform (1:3) gave creamish white flakes of **2**, recrystallized from methanol-acetone (9:1), 34 mg (0.0018% yield); $R_f$: 0.52 (petroleum ether-chloroform; 1:3); m.p.: 150-152°C; UV $\lambda_{max}$ ($CH_3OH$): 282, 207 nm (log ε 5.7, 2.8); IR $\upsilon_{max}$ (KBr): 3450, 2935, 2870, 1640, 1447, 1378, 1216, 1046, 761 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$): δ 5.18 (1H, dd, *J*=5.3, 5.1, H-6), 4.72 (1H, brs, $H_2$-26a), 4.66 (1H, brs, $H_2$-26b), 3.28 (1H, dd, *J*=5.8, 4.5 Hz, H-3β), 1.62 (3H, brs, Me-27), 1.28 (3H, brs, Me-28), 1.26 (3H, brs, Me-19), 0.97 (3H, d, *J*=6.3 Hz, Me-21), 0.88 (3H, brs, Me-29), 0.81 (3H, brs, Me-30), 0.68 (3H, brs, Me-18), 2.21-1.23 (24H, m, 10×$CH_2$, 4×CH); $^{13}C$ NMR ($CDCl_3$): Table 1; +ve ion FAB MS *m/z* (*rel. int.*): 426 $[M]^+$ ($C_{30}H_{50}O$) (22.3), 411 (14.5), 408 (78.1), 393 (61.7), 326 (11.1), 313 (21.2), 298 (17.3), 283 (19.3), 271 (12.3), 220 (10.8), 215 (9.8), 206 (11.2), 192 (12.1), 187 (21.9), 174 (39.1), 155 (6.1), 109 (53.8), 99 (11.5), 95 (92.1).

**Turpethosterylepoxy myristate (3)**

Elution of the column with chloroform furnished brown crystalline powder of **3**, recrystallized with methanol-acetone (1:1), 76 mg (0.00 41% yield); $R_f$: 0.50 (chloroform-methanol; 99:1); m.p.: 83-84°C; IR $\upsilon_{max}$ (KBr): 3410, 3396, 2930, 2857, 1723, 1636, 1463, 1378, 1263, 1216, 1162, 1047, 722 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$): δ 5.21 (1H, d, *J*=5.2 Hz, H-6), 5.10 (1H, dd, *J*=6.3, 5.8 Hz, H-22), 5.04 (1H, dd, *J*=5.8, 5.2 Hz, H-23), 4.21 (1H, dd, *J*=5.2, 5.9 Hz, H-7β), 4.01 (1H, brs, H-4α), 1.01 (3H, brs, Me-19), 0.93 (3H, d, *J*=6.1 Hz, Me-21), 0.87 (3H, t, *J*=6.0 Hz, Me-29), 0.84 (3H, t, *J*=6.2 Hz, Me-14'), 0.82 (3H, d, *J*=6.3 Hz, Me-26), 0.77 (3H, d, *J*=6.1 Hz, Me-27), 0.61 (3H, brs, Me-18), 2.33-1.30 (21H, m, 7×$CH_2$, 7×CH), 2.24 (2H, t, *J*=7.9 Hz, $H_2$-2'), 1.21 (12H, brs, 6×$CH_2$), 1.18 (12H, brs, 6×$CH_2$); $^{13}C$ NMR ($CDCl_3$): Table 1; +ve ion FAB MS *m/z* (*rel. int.*): 652 $[M]^+$ ($C_{43}H_{72}O_4$) (1.5), 441 (2.3), 425 (15.3), 285 (21.6) , 270 (21.7), 255 (15.3), 227 (24.5), 213 (28.2), 211 (23.7), 199 (33.1), 190 (22.5), 176 (32.5), 167 (25.2),149 (39.1), 132 (73.8).

**Turpethosterylepoxy palmitate A (4)**

Elution of the column with chloroform yielded a yellow amorphous powder of **4**, recrystallized from methanol-acetone (9.1), 150 mg (0.0081% yield); $R_f$: 0.62 (chloroform 100%); m.p.: 242-244°C; UV $\lambda_{max}$ ($CH_3OH$): 223 nm (log ε 4.2); IR $\upsilon_{max}$ (KBr): 2929, 2851, 1721, 1601, 1429, 1378, 1216, 1076, 721 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 5.31 (1H, m, H-6), 5.09 (1H, brs, $H_2$-21a), 4.99 (1H, brs, $H_2$-21b), 4.33 (1H, dd, *J*=5.3, 5.6 Hz, H-7β), 1.01 (3H, brs, Me-19), 0.92 (3H, d, *J*=6.6 Hz, Me-26), 0.88 (3H, d, *J*=6.5 Hz, Me-27), 0.77 (3H, t, *J*=6.3 Hz, Me-29), 0.67 (3H, brs, Me-18), 2.48 (2H, t, *J*=7.2 Hz, H2-2'), 2.19-1.31 (26H, m, 10×$CH_2$, 6×CH), 1.29 (12H, brs, 6×$CH_2$), 1.27 (14H, brs, 7×$CH_2$), 0.84 (3H, t, *J*=6.2 Hz, Me-16'); $^{13}C$ NMR ($CDCl_3$): Table 1; +ve ion FAB MS *m/z* (*rel. int.*): 664 $[M]^+$ ($C_{45}H_{76}O_3$) (1.5), 425 (39.1), 409 (50.5), 274 (11.5), 255 (43.4), 239 (15.4), 234 (6.5), 220 (5.6), 216 (26.7), 202 (6.7), 193 (8.9), 189 (18.6), 188 (26.5), 175 (21.1), 174 (48.4), 161 (35.1), 139 (15.3), 135 (61.5), 109 (2.5), 95 (100).

**Turpetholanostenyl myristate (5)**

Elution of the column with chloroform-methanol (99:1) produced yellow amorphous powder of **5**, recrystallized from methanol-acetone (1:1), 92 mg (0.0050% yield); $R_f$: 0.38 (chloroform 100%); m.p.: 123-125°C; UV $\lambda_{max}$ ($CH_3OH$): 228 nm (log ε 5.8); IR $\upsilon_{max}$ (KBr): 3219, 2922, 2852, 1722, 1708, 1602, 1463, 1215, 1044, 717 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 5.34 (1H, d, *J*=5.1 Hz, H-6), 5.30 (1H, d, *J*=6.8 Hz, H-22), 5.26 (1H, m, H-23), 4.71 (1H, brs, $H_2$-21a), 4.66 (1H, $H_2$-21b), 4.25 (1H, dd, *J*=5.1, 5.4 Hz, H-7β), 3.30 (1H, dd, *J*=5.2, 8.5 Hz, H-12α), 1.04 (3H, brs, Me-19), 1.01 (3H, d, *J*=7.5 Hz, Me-27), 0.96 (3H, brs, Me-28), 0.89 (3H, brs, Me-30), 0.84 (3H, t, *J*=6.2 Hz, Me-14'), 0.82 (3H, brs, Me-29), 0.68 (3H, brs, Me-18), 2.33 (2H, t, *J*=7.2 Hz, $H_2$-2'), 2.21-1.57 (18H, m, 7×$CH_2$, 4×CH), 1.25 (20H, brs, 10×$CH_2$); $^{13}C$ NMR ($CDCl_3$): Table 1; +ve ion FAB MS *m/z* (*rel. int.*): 694 $[M]^+$ ($C_{44}H_{70}O_6$) (2.5), 555 (12.3), 483 (15.6), 467 (18.1), 460 (11.3), 430 (22.2), 264 (10.5), 234 (23.1), 227 (18.6), 211 (14.2), 139 (32.6).

**Turpethosterylepoxy palmitate B (6)**

Further elution of the column with chloroform-methanol (99:1) gave yellow amorphous powder of **6**, recrystallized from acetone-methanol (1:1), 148 mg (0.0080% yield); $R_f$: 0.35 (chloroform-methanol; 99:1); m.p.: 165-167°C; UV $\lambda_{max}$ ($CH_3OH$): 229 nm (log ε 5.3); IR $\upsilon_{max}$ (KBr): 3360, 2928, 2857, 1721, 1701, 1645, 1461, 1378, 1216, 1044, 723 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 5.35 (1H, d, *J*=5.2 Hz, H-6), 5.31 (1H, d, *J*=7.1 Hz, H-22), 5.22 (1H, dd, *J*=7.1, 6.3 Hz, H-23), 4.71 (1H, brs, $H_2$-21a), 4.66 (1H, brs, $H_2$-21b), 4.17 (1H, dd, *J*=5.2, 5.6 Hz, H-7β), 1.03 (3H, d, *J*=6.7 Hz, Me-27), 1.01(3H, brs, Me-19), 0.88 (3H, t, *J*=6.8 Hz, Me-16'), 0.85 (3H, d, *J*=7.2 Hz, Me-29), 0.68 (3H, brs, Me-18), 2.36 (2H, t, *J*=7.5 Hz, $H_2$-2'), 2.77-1.30 (20H, m, 7×$CH_2$, 6×CH), 1.25 (10H, brs, 5×$CH_2$), 1.22 (16H, brs, 8×$CH_2$); $^{13}C$ NMR ($CDCl_3$): Table 1; +ve ion FAB MS *m/z* (*rel. int.*): 692 $[M]^+$ ($C_{45}H_{72}O_5$) (2.3), 453 (1.5), 437 (9.1), 286 (37.2), 270 (23.6), 271 (25.1), 255 (21.0), 239 (19.8), 213 (22.5), 189 (18.3), 175 (25.6), 167 (22.5), 161 (44.3), 135 (53.7), 123 (37.3), 122 (54.7), 95 (100).

**D-Glucuronic acid (7)**

Further elution of the column with chloroform-methanol (19:1) afforded colourless amorphous powder of **7**, recrystallized from methanol-acetone (9:1), 59 mg (0.0032 % yield); $R_f$: 0.21 (*n*-butanol-ethanol-water; 4:1: 2.2); m.p.: 163-165 °C; IR $\upsilon_{max}$ (KBr): 3353, 3020, 2922, 2853, 1690, 1600, 1523, 1422, 1215, 1044 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): 4.73 (1H, brs, H-1), 4.08 (1H, m, H-5), 3.92 (1H, m, H-2), 3.68 (2H, brs, H-3, H-4); $^{13}C$ NMR (DMSO-$d_6$): 176.83 (C-6), 75.28 (C-1), 74.35 (C-5), 72.84 (C-2), 71.93 (C-3, C-4); +ve ion FAB MS *m/z* (*rel. int.*): 194 $[M]^+$($C_6H_{10}O_7$) (22.8).

**D-Galacturonic acid (8)**

Elution of the column with chloroform-methanol (3:1) yielded colourless flakes of **8**, recrystallized from methanol, 87 mg (0.0047% yield); $R_f$: 0.19 (*n*-butanol-ethanol-water; 4:1: 2.2); m.p.: 156-157 °C; IR $\upsilon_{max}$ (KBr): 3456, 2925, 1702, 1600, 1430, 1215, 1043 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 4.15 (1H, brs, H-1), 4.01 (1H, m, H-5), 3.75 (1H, m, H-2), 3.41 (1H, m, H-3), 3.35 (1H, m, H-4); +ve ion FAB MS *m/z* (*rel. int.*): 194 $[M]^+$ ($C_6H_{10}O_7$) (21.2).

**Sucrose (9)**

Elution of the column with chloroform-methanol (3:2) furnished colourless crystalline powder of **9**, recrystallized from methanol, 91 mg (0.0049% yield); $R_f$: 0.96 (chloroform-methanol; 3:2); m.p.: 161-163 °C; IR $\upsilon_{max}$ (KBr): 3463, 3360, 2920, 2853, 1460, 1216 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 5.18 (1H, d, *J* = 6.3 Hz, H-1′), 5.14 (1H, d, *J*=5.7 Hz, H-1), 5.04 (2H, m, H-4, H-5′), 4.78 (1H, m, H-2), 4.50 (1H, m, H-2′), 4.38 (1H, m, H-3), 3.85 (1H, m, H-3′), 3.74 (1H, m, H-4′), 3.53 (2H, brs, $H_2$-5), 3.43 (2H, brs, $H_2$-6), 3.37 (2H, brs, $H_2$-6′); $^{13}C$ NMR (DMSO-$d_6$) δ 104.12 (C-1′), 91.86 (C-1), 82.63 (C-4), 77.11 (C-5′), 74.37 (C-2), 72.94 (C-2′), 71.76 (C-3), 71.73 (C-3′), 69.93 (C-4′), 62.21 (C-5, C-6), 60.59 (C-6′); +ve ion FAB MS *m/z* (*rel. int.*): 365$[M + Na]^+$ ($C_{12}H_{22}O_{11}$+ Na) (100).

**D-β-Rhamnose (10)**

Elution of the column with chloroform-methanol (2:3) produced colourless powder of **10**, recrystallized from methanol-acetone (9:1), 60 mg (0.0032% yield); $R_f$: 0.37 (*n*-butanol-ethanol-water; 4:1: 2.2); m.p.: 122-125 °C; IR $\upsilon_{max}$ (KBr): 3619, 3427, 2916, 2850, 1526, 1436, 1215, 1040 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 4.35 (1H, brs, H-1), 3.76 (1H, m, H-5), 3.45 (1H, m, H-3), 3.16 (1H, m, H-4), 1.24 (1H, d, *J* = 7.1 Hz, Me-6); +ve ion FAB Ms *m/z (rel. int.)* : 164 $[M]^+$ ($C_6H_{12}O_5$) (11.5).

## RESULTS AND DISCUSSION

Compound **1,** designated as turpetholanostenyl epoxide, was obtained as a creamish amorphous powder from petroleum ether-chloroform (1:3) eluants. It responded positively to Liebermann-Burchard test for triterpenoids. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3401 $cm^{-1}$) and unsaturation (1640 $cm^{-1}$). On the basis of FAB mass and $^{13}C$ NMR spectra, its molecular ion peak was determined at *m/z* 440 consistent with the molecular formula $C_{30}H_{48}O_2$ of a triterpenoid epoxide. The diagnostic fragment ion peaks arising at *m/z* 425 $[M\text{-}Me]^+$, 410 $[425\text{-}Me]^+$, 407 $[425\text{-}H_2O]^+$, 153 $[C_{1,10}\text{-}C_{5,10}\text{-}C_7,C_8$ fission$]^+$, 180 $[C_{9,10}$ –$C_{7,8}$ fission$]^+$, 135 $[153\text{-}H_2O]^+$ and 162 $[180\text{-}H_2O]^+$ supported the presence of two hydroxyl groups in ring A/B, one of which is involved in an epoxy bridge. The important ion peaks generating at *m/z* 206, 234 $[C_{9,11}\text{-}C_{8,14}$ fission$]^+$, 220 $[C_{8,14}\text{-}C_{11,12}$ fission$]^+$, 329 $[M\text{-}C_8H_{15}$, side chain$]^+$, 266 $[329\text{-}3\times Me]^+$ and 287 $[329\text{-ring D}]^+$ suggested the saturated nature of rings C and D and a monounsaturated $C_8$-side chain in the compound. The $^1H$ NMR spectrum of **1** exhibited a downfield doublet at δ 5.11 (*J* = 5.2 Hz) and two broad singlet at δ 4.64 and 4.59, each integrating for one-proton, assigned correspondingly to vinylic H-6 and unsaturation methylene $H_2$-26 protons. A one-proton double doublet at 4.27 (*J*=5.5, 5.2 Hz) was attributed to oxygenated methine H-7 proton bonded to oxygen of the epoxy bridge. A three-proton broad singlet at δ 1.53 was accounted to the methyl C-27 protons attached to the vinylic carbon. The tertiary methyl protons resonated as five three-proton broad singlets at δ 0.73, 1.18, 1.21, 0.89 and 0.82 ascribed correspondingly to tertiary C-18, C-19, C-28, C-29 and C-30 methyl protons. A three-proton doublet at δ 0.96 (*J*=6.3 Hz) was attributed to secondary C-21 methyl protons. The remaining methine and methylene protons appeared between δ 2.16-1.28. The $^{13}C$ NMR spectrum of **1** displayed signals for 30 carbons. The important signals appeared for vinylic carbons at δ 138.98 (C-5), 125.20 (C-6), 130.79 (C-25), 117.45 (C-26) and methyl carbons between δ 25.67 - 13.93. The signal typical of C-3 carbinol carbon and C-7 methine carbons shifted downfield to δ 105.91 and δ 71.81, respectively, supporting the presence of epoxy bridge between C-3 and C-7. The $^1H$ NMR and $^{13}C$ NMR spectral values of **1** were compared with the reported data of lanostenoids [10-12]. On the basis of above discussion the structure of the new lanostane-type triterpenoid **1** has been elucidated as 3α,7α-epoxy lanost-5,25-dien-3β-ol (Figure 1).

Compound **2,** designated as turpetholanostenol, was obtained as creamish white flakes from petroleum ether-chloroform (1:3) eluants. It responded positively to Liebermann-Burchard test for triterpenoids. Its IR spectrum displayed important absorption bands for hydroxyl group (3450 $cm^{-1}$) and unsaturation (1640 $cm^{-1}$). Its molecular ion peak was determined on the basis of +ve FAB mass and $^{13}C$ NMR spectra at *m/z* 426 ($C_{30}H_{50}O$). The ion fragments arising at *m/z* 408 $[M\text{-}H_2O]^+$, 99, 326 $[C_{1,10}\text{-}C_{4,5}$ fission$]^+$, 155, 271 $[C_{6,7}\text{-}C_{9,10}$ fission], 192 $[C_{8,14}\text{-}C_{9,11}$ fission$]^+$ and 174 $[192\text{-}H_2O]^+$ indicated the location of hydroxyl group in ring A which was placed at C-3 on the basis of biogenetic considerations and the existence of the vinylic linkage at $C_5$ in ring B. The ion peaks produced at *m/z* 206 $[C_{11,12}\text{-}C_{8,14}$ fission, $C_{14}H_{22}O]^+$ and 220 $[M\text{-}206$, $C_{16}H_{28}]$ supported the presence of vinylic linkage in ring B and saturated nature of rings C and D. The prominent ion peaks formed at *m/z* 109 $[220\text{-}C_8H_{15}$, side chain$]^+$, 313 $[M\text{-}C_8H_{15}$ side chain$]^+$, 155 $[271\text{-side chain}]^+$ and 215 $[326\text{-side chain}]^+$ suggested the presence of vinylic linkage in the side chain. The $^1H$ NMR spectrum of **2** exhibited a one-proton double doublet at δ 5.18 (*J*=5.3, 5.1 Hz) assignable to vinylic H-6 proton. Two one-proton broad singlets at δ 4.72 and 4.66 were due to unsaturated methylene $H_2$-26 protons. A one-proton double doublet at δ 3.28 was ascribed to carbinol H-3 proton which was placed in α-orientation on the basis of its coupling constants (*J*=5.8, 4.5 Hz). A three-proton broad singlet at δ 1.62 was attributed to methyl C-27 protons located on the vinylic carbon. A three-proton doublet at δ 0.97 (*J*=6.3 Hz) was accounted to secondary C-21 methyl protons. The tertiary methyl protons resonated as five three-proton broad singlets at δ 1.28 (Me-28), 1.26 (Me-19), 0.88 (Me-29), 0.81 (Me-30) and 0.68 (Me-18). The $^{13}C$ NMR spectrum of **2** displayed signals for vinylic carbons at δ 141.27 (C-5), 121.91 (C-6), 131.08 (C-25) and 112.26 (C-26), carbinol carbon at δ 78.45 (C-3) and methyl carbons between δ 24.14-14.51. The $^1H$ NMR and $^{13}C$ NMR spectral values of **2** were compared with the reported data of lanosteroids [10-12]. On the basis of spectral data analysis the structure of the new lanostenol-type triterpenoid **2** has been characterized as lanost-5,25-dien-3α-ol (Figure 1).

Compound **3**, designated as turpethosterylepoxy myristate, was obtained as a brown crystalline powder from chloroform eluants. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3410 $cm^{-1}$), ester group (1723 $cm^{-1}$), unsaturation (1636 $cm^{-1}$) and long aliphatic moiety (722 $cm^{-1}$). On the basis of +ve FAB mass and $^{13}C$ NMR spectra, its molecular ion peak was determined at *m/z* 652 consistent with the molecular formula

$C_{43}H_{72}O_4$ of a steryl ester. The diagnostic ion peaks arising at *m/z* 211 $[CH_3(CH_2)_{12}CO]^+$, 441 $[M-21]^+$, 227 $[CH_3(CH_2)_{12}COO]^+$ and 425 $[M-227]^+$ arising due to ester linkage fission supported the presence of a $C_{29}$ sterol esterified with myristic acid. The fragment ion peaks generating at *m/z* 167 [$C_{7,8}$-$C_{9,10}$ fission]$^+$, 149 $[167-H_2O]^+$ and 132 $[149-OH]^+$ indicated the presence of olefinic linkage in ring B and two hydroxy groups in ring A/B, one of which was involved in epoxy bridge in ring A/B. Other fragment ion peaks produced at 285 [425-$C_{10}H_{19,}$ side chain]$^+$, 270 [285-Me]$^+$, 255 [270-Me]$^+$ and 213 [255-ring D] suggested the presence of a $C_{10}$-side chain with one olefinic linkage and saturated nature of rings C and D. The $^1$H NMR spectrum of **3** exhibited a downfield one-proton doublet at δ 5.21 (*J*=5.2 Hz) and two double doublets at δ 5.10 (*J*=6.3, 5.8 Hz) and 5.04 (*J*=5.8, 5.2 Hz), each integrating for one-proton, assigned correspondingly to *cis*-oriented vinylic H-6, H-22 and H-23 protons. A one-proton double-doublet at δ 4.21 (*J*=5.2, 5.9 Hz) was attributed to β-oriented oxygenated methine proton on C-7 bonded to oxygen of the epoxy bridge. A one-proton broad singlet at δ 4.01 was ascribed to carbinol H-4 proton. Two three-proton broad singlets at δ 0.61 and 1.01 were assigned to tertiary C-18 and C-19 methyl protons, respectively. Three doublets at δ 0.93 (*J*=6.1 Hz), 0.82 (*J*=6.3 Hz) and 0.77 (*J*=6.1 Hz), and two triplets at δ 0.84 (*J*=6 Hz) and 0.87 (*J*=6.2 Hz) were accounted to secondary C-21, C-26 and C-27 and primary C-29 and C-14′ methyl protons, respectively, all of them attached to saturated carbons. A two-proton triplet at δ 2.24 (*J*=7.9 Hz) was attributed to C-2′ methylene protons adjacent to the ester linkage. The remaining methine and methylene protons appeared from 2.33 to 1.18. The $^{13}$C NMR spectrum of **3** displayed important signals for ester carbon at δ 173.12 (C-1′), vinylic carbons at δ 140.71 (C-5), 117.47 (C-6), 130.86 (C-22), 129.97 (C-23) and oxygenated methine carbons at δ 78.83 (C-4) and 71.76 (C-7). The signal typical of tertiary C-3 oxygenated carbon and C-7 methylene carbon shifted downfield to δ 105.92 and 71.76 respectively, supporting the presence of epoxy bridge between C-3 and C-7. The $^1$H NMR and $^{13}$C NMR spectral values of **3** were compared with the reported data of steroids [10,13,14]. The acid hydrolysis of **3** yielded myristic acid (m.p. and co-TLC comparable). On the basis of these evidences the structure of the new phytosterol **3** was elucidated as 4β-hydroxy-3α,7α-epoxy stigmast-(Z)-5,22-dien-3β-tetradecanoate (Figure 1).

Compound **4,** named turpethosterylepoxy palmitate A, was obtained as a yellow amorphous powder from chloroform eluants. It responded positively to Liebermann-Burchard test for steroids. Its IR spectrum displayed characteristic absorption bands for ester group (1721 cm$^{-1}$), unsaturation (1601 cm$^{-1}$) and long aliphatic moiety (721 cm$^{-1}$). On the basis of FAB mass and $^{13}$C NMR spectra, its molecular weight was established at *m/z* 664 consistent with the molecular formula $C_{45}H_{76}O_3$ of a sterol ester. The diagnostic ion fragments arising at *m/z* 239 $[CH_3(CH_2)_{14}CO]^+$, 425 $[M-239]^+$, 255 $[CH_3(CH_2)_{14}COO]^+$ and 409 $[M-255]^+$ due to ester linkage fission supported the presence of $C_{29}$ sterol esterified to palmitic acid. The ion peaks generating at *m/z* 135 [$C_{7,8}$-$C_{9,10}$ fission]$^+$ and 161 [$C_{8,14}$-$C_{9,11}$ fission]$^+$ indicated the presence of olefinic linkage in ring B and an epoxy bridge in ring A/B. Other ion peaks formed at *m/z* 234, 175 [$C_{11,12}$-$C_{8,14}$ fission]$^+$, 189 [$C_{12,13}$-$C_{8,14}$ fission]$^+$, 174 [$C_{9,11}$-$C_{13,14}$-$C_{14,15}$-fission]$^+$, 188 [$C_{11,12}$-$C_{13,14}$-$C_{14,15}$ fission]$^+$, 202 [$C_{12,13}$-$C_{13,14}$- $C_{14,15}$ fission]$^+$ and 216, 193 [$C_{13,17}$-$C_{15,16}$ fission]$^+$ suggested the saturated nature of rings C and D. The ion peak produced at *m/z* 139 [$C_{10}H_{19}$, side chain]$^+$ and the base peak at 95 [234-side chain]$^+$ supported the presence of a $C_{10}$ unsaturated side chain in the compound. The $^1$H NMR spectrum of **4** displayed three signals, one-proton each, at δ 5.31 (m) and 5.09 (brs), 4.99 (brs) assigned correspondingly to vinylic H-6 and methylene $H_2$-21 protons. A one-proton double doublet at δ 4.33 (*J*=5.3, 5.6 Hz) was attributed to oxygenated methine H-7 proton bonded to oxygen of the epoxy bridge. Two broad singlets at δ 0.67 and 1.01, two-doublets at δ 0.92 (*J*=6.6 Hz), 0.88 (*J*=6.5 Hz), and two triplets at 0.77 (*J*=6.3 Hz) and 0.84 (*J*=6.5 Hz) were accounted correspondingly to secondary C-26, C-27 and primary C-29 and C-16′ methyl protons, all attached to saturated carbons. A two-proton triplet signal at δ 2.48 (*J*=7.2 Hz) was ascribed to methylene C-2′ protons adjacent to the ester group. The remaining methylene and methine protons appeared between δ 2.19-1.22. The $^{13}$C NMR spectrum of **4** displayed important signals for ester carbon at δ 173.16 (C-1′) and vinylic carbons at δ 141.21 (C-5), 122.35 (C-6), 155.60 (C-20) and 117.35 (C-21). The signal typical of C-3 carbinol carbon and C-7 methylene carbon shifted downfield to δ 105.91 and 71.38, respectively, suggesting the epoxy bridge between C-3 and C-7. The $^1$H NMR and $^{13}$C NMR spectral values of **2** were compared with the reported data of steroids [10,13,14]. The alkaline hydrolysis of **4** yielded palmitic acid (mmp and co-TLC comparable). On the basis of the foregoing account the structure of the new steroidal ester **4** was elucidated as 3α,7α-epoxy stigmast-5,20-dien-3β-hexadecanoate (Figure 1).

Compound **5,** designated as turpetholanostenyl myristate, was obtained as a yellow amorphous powder from chloroform-methanol (99:1) eluants. It responded positively to tests for triterpenoids. Its IR spectrum displayed distinctive absorption bands for ester group (1722 cm$^{-1}$), carboxyl function (3219, 1708 cm$^{-1}$), unsaturation (1602 cm$^{-1}$) and long aliphatic chain (717 cm$^{-1}$). On the basis of +ve FAB mass and $^{13}$C NMR spectra, its molecular weight was established at *m/z* 694 consistent with the molecular formula $C_{44}H_{70}O_6$ of a triterpenoid ester. The diagnositc ion peaks arising at *m/z* 227 $[CH_3(CH_2)_{12}COO]^+$, 211 $[CH_3(CH_2)_{12}CO]^+$, 467 $[M-227]^+$ and 483 [M-

211]$^+$arising due to ester linkage fission supported the presence of a $C_{30}$ triterpene esterified to myristic acid. The ion peaks generating at m/z 139 [$C_8H_{11}O_2$, side chain]$^+$, 555 [M-139]$^+$, 430, 264 [$C_{8,14}$-$C_{11,12}$ fission]$^+$ and 460, 234 [$C_{8,14}$-$C_{12,13}$ fission]$^+$ suggested the location of $C_8$ side chain containing a carboxylic acid and two vinylic linkages and a hydroxyl group in ring C at C-12. The $^1$H NMR spectrum of **5** displayed two one-proton doublets at δ 5.34 (*J*=5.1 Hz), 5.30 (*J*=6.8 Hz) and as a one-proton multiplet at 5.26 assigned correspondingly to *cis*-oriented vinylic H-6, H-22 nad H-23 protons. Two one-proton broad singlets at δ 4.71 and 4.66 were attributed to unsaturated methylene $H_2$-21 protons. A one-proton double doublet at δ 4.25 (J=5.1, 5.4 Hz) was accounted to β-oriented methine H-7 proton. A one-proton double doublet at δ 3.30 (*J* = 5.2, 8.5 Hz) was ascribed to H-12α carbinol carbon. Five three-proton broad singlets at δ 0.68, 1.04, 0.96, 0.82 and 0.89 were assigned correspondingly to tertiary C-18, C-19, C-28, C-29 and C-30 methyl protons. A doublet at δ 1.01 (*J*=7.5 Hz) and a triplet at δ 0.84 (*J*=6.2 Hz), both intergrating three protons each, were assigned to secondary C-27 and to primary C-14′ methyl protons, respectively. A two-proton triplet at δ 2.33 (*J*=7.2 Hz) was ascribed to methylene $H_2$-2′a protons adjacent to the ester linkage. The remaining methylene and methine protons resonated from δ 2.21 to δ 1.25. The appearance of all methyl signals between δ 1.01 to 0.68 suggested their location on saturated carbons. The $^{13}$C NMR spectrum of **5** displayed signals for carboxyl carbon at δ 178.59 (C-26), ester carbon at δ 173.16 (C-1′), vinylic carbons at δ 140.69 (C-5), 117.44 (C-6), 156.76 (C-20), 117.42 (C-21), 130.82 (C-22) and 129.95 (C-23) and oxygenated carbons at δ 105.44 (C-3) and 72.2 (C-7). The $^1$H NMR and $^{13}$C NMR spectral values of **5** were compared with the reported data of lanosteroids [10-12]. The acid hydrolysis of **5** yielded myristic acid (m.p. and co-TLC comparable). On the basis of above discussion the structure of new lanostenyl ester **5** was elucidated as 12β-hydroxy-3α,7α-epoxy lanost-(Z)-5,20,22-trien-26-oic acid-3β-tetradecanoate (Figure 1).

Compound **6,** designated as turpethosterylepoxy palmitate B, was obtained as a yellow amorphous powder from chloroform-methanol (99:1) eluants. Its IR spectrum displayed characteristic absorption bands for ester group (1721 cm$^{-1}$), for carboxyl function (3360, 1701 cm$^{-1}$), unsaturation (1645 cm$^{-1}$) and long aliphatic moiety (723 cm$^{-1}$). On the basis of +ve FAB mass and $^{13}$C NMR spectra of **6** its molecular ion peak was determined at *m/z* 692 consistent with the molecular formula $C_{45}H_{72}O_5$ of a steroid ersterified to a $C_{16}$-fatty acid. The fragment ion peaks generating at *m/z* 167 [$C_{10}H_{15}O_2$, side chain]$^+$, 123 [167-$CO_2$]$^+$ and 122 [167-COOH]$^+$ suggested the presence of a di-unsaturated $C_{10}$-side chain containing a carboxylic group. The fragment ions arising at *m/z* 239 [$CH_3(CH_2)_{14}CO$]$^+$, 453 [M-239]$^+$, 286 [453-side chain]$^+$, 271 [286-Me]$^+$, 255 [$CH_3(CH_2)_{14}COO$]$^+$ and 437 [M-255]$^+$, arising due to ester-linkage fission supported the presence of $C_{29}$ sterol esterified to palmitic acid. The ion peaks forming at *m/z* 135 [$C_{7,8}$-$C_{9,10}$ fission]$^+$ and 161 [$C_{8,14}$-$C_{9,11}$ fission]$^+$ indicated the presence of vinylic linkage in ring B and an epoxy bridge in ring A/B. Other fragment ion peaks producing at *m/z* 175 [$C_{11,12}$-$C_{8,14}$ fission]$^+$, 189 [$C_{12,13}$-$C_{8,14}$ fission]$^+$, 270 [437-side chain ]$^+$, and 213 [255-ring D, -Me]$^+$ suggested the saturated nature of ring C and D. The $^1$H-NMR spectrum of **6** showed two one-proton doublets at δ 5.35 (*J*=5.2 Hz) and 5.31 (J=7.1 Hz) and a one-proton double doublet at δ 5.22 (J=7.1, 6.3 Hz) assigned to *cis*-oriented H-6, H-22 and H-23 vinylic protons, respectively. Two one-proton broad singlets at δ 4.71 and 4.66 were ascribed to unsaturated methylene $H_2$-21 protons. A one-proton double doublet at δ 4.17 (*J* = 5.2, 5.6 Hz) was attributed to β-oriented oxygenated methine H-7 proton bonded to oxygen of the epoxy bridge. Two three-proton broad singlets at δ 0.68 and 1.01 were assigned to tertiary C-18 and C-19 methyl protons, respectively. Two doublets at δ 1.03 (*J* = 6.7 Hz), 0.85 (*J* = 7.2 Hz) and a triplet 0.88 (*J*=6.8 Hz) integrated three-protons each were ascribed correspondingly to secondary C-27 and primary C-29 and C-16′ methyl protons. The presence of all methyl signals in the range of δ 1.03 to 0.68 suggested the location of these functionalities on the saturated carbons. A two-proton triplet at δ 2.36 (*J*=7.5 Hz) was accounted to C-2′ methylene protons adjacent to the ester group. The other methine and methylene resonated from δ 2.77-1.12. The $^{13}$C NMR spectrum of **6** showed important signals for carboxyl carbon at δ 179.61 (C-26), ester carbon at δ 173.34 (C-1′), vinylic carbons at δ 140.68 (C-5), 121.71 (C-6), 156.76 (C-20), 117.48 (C-21), 139.03 (C-22) and 129.98 (C-23), oxygenated carbons at δ 105.96 (C-3) and 71.83 (C-7) and methyl carbons between δ 18.81-11.84. The $^1$H NMR and $^{13}$C NMR spectral values of **6** were compared with the reported data of steroids [10,13,14]. Acid hydrolysis of **6** yielded palmitic acid (co-TLC comparable). On the basis of above discussion the structure of the new steroidal ester **6** was elucidated as 3α,7α-epoxy stigmast-(Z)-5,20,22-trien-28-oic acid-3β-hexadecanoate (Figure 1).

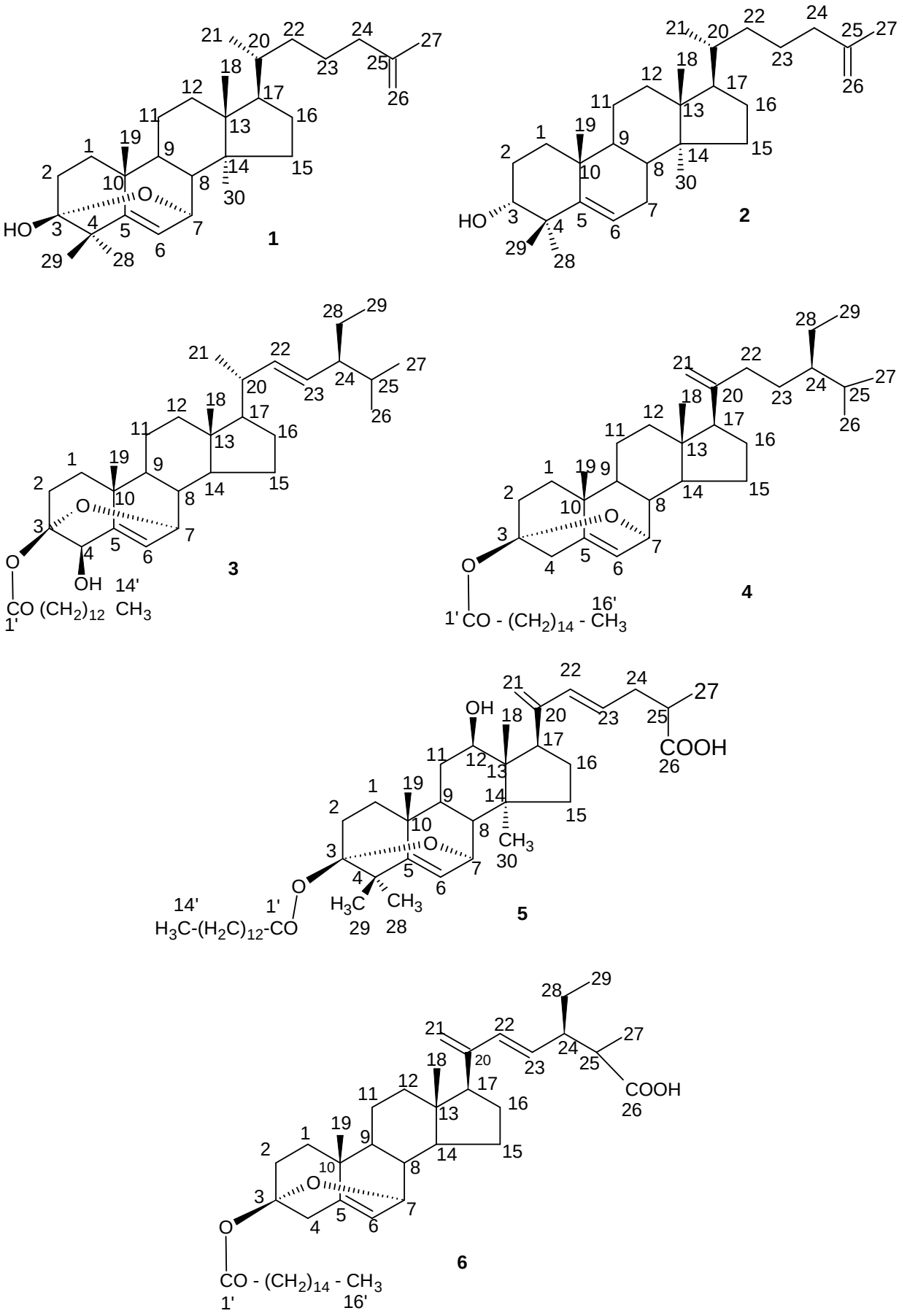


**Figure 1: The structures of Compound 1-6.**

**Table 1: $^{13}$C NMR spectral values of compounds 1-6.**

| S. No. | **1** $\delta_C$ | **2** $\delta_C$ | **3** $\delta_C$ | **4** $\delta_C$ | **5** $\delta_C$ | **6** $\delta_C$ |
|---|---|---|---|---|---|---|
| 1 | 36.22 | 35.83 | 36.46 | 36.30 | 39.72 | 36.48 |
| 2 | 31.89 | 30.06 | 27.15 | 27.21 | 31.87 | 27.18 |
| 3 | 105.91 | 78.45 | 105.92 | 105.91 | 105.44 | 105.96 |
| 4 | 40.14 | 41.08 | 78.83 | 42.59 | 40.16 | 42.30 |
| 5 | 138.98 | 141.27 | 140.71 | 141.21 | 140.69 | 140.68 |
| 6 | 125.20 | 121.91 | 117.47 | 122.35 | 117.44 | 121.71 |
| 7 | 78.73 | 27.68 | 71.76 | 71.38 | 78.21 | 71.83 |
| 8 | 35.51 | 42.76 | 31.87 | 31.10 | 36.12 | 31.51 |
| 9 | 52.21 | 50.63 | 50.09 | 50.43 | 52.24 | 50.12 |
| 10 | 35.81 | 37.14 | 37.21 | 38.67 | 36.45 | 37.01 |
| 11 | 21.05 | 20.95 | 21.69 | 21.26 | 21.04 | 21.06 |
| 12 | 39.47 | 38.37 | 36.12 | 35.77 | 71.76 | 35.61 |
| 13 | 45.20 | 45.08 | 42.23 | 41.56 | 45.79 | 40.15 |
| 14 | 49.55 | 51.92 | 56.73 | 55.26 | 49.58 | 56.75 |
| 15 | 33.73 | 33.60 | 24.82 | 25.12 | 33.01 | 25.4 |
| 16 | 28.07 | 28.13 | 28.20 | 28.48 | 28.18 | 28.21 |
| 17 | 48.72 | 49.89 | 55.98 | 53.89 | 48.75 | 55.98 |
| 18 | 17.97 | 14.51 | 11.84 | 11.53 | 17.98 | 11.84 |
| 19 | 19.24 | 19.57 | 19.34 | 18.96 | 11.81 | 18.81 |
| 20 | 40.40 | 33.45 | 39.71 | 155.60 | 156.76 | 156.76 |
| 21 | 25.97 | 19.13 | 21.82 | 117.35 | 117.42 | 117.48 |
| 22 | 34.74 | 36.52 | 130.86 | 33.43 | 130.82 | 139.03 |
| 23 | 25.37 | 24.69 | 129.97 | 26.59 | 129.95 | 129.98 |
| 24 | 46.58 | 46.73 | 45.79 | 45.37 | 46.61 | 45.81 |
| 25 | 130.79 | 131.08 | 31.03 | 31.03 | 31.03 | 38.61 |
| 26 | 117.45 | 112.26 | 21.82 | 18.62 | 178.59 | 179.61 |
| 27 | 25.67 | 24.14 | 18.74 | 18.24 | 26.01 | 18.22 |
| 28 | 22.84 | 23.62 | 25.39 | 24.85 | 22.64 | 24.29 |
| 29 | 13.93 | 14.15 | 11.82 | 11.56 | 18.77 | 11.86 |
| 30 | 17.57 | 17.67 | - | - | 17.57 | - |
| 1′ | - | | 173.12 | 173.16 | 173.16 | 173.34 |
| 2′ | - | | 34.59 | 34.49 | 55.97 | 54.93 |
| 3′-13′/15′ | - | - | 34.49-22.68 | 35.84-22.88 | 34.04-22.68 | 35.59-22.46 |
| 14′/16' | - | - | 13.96 | 14.07 | 14.11 | 14.07 |

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