CHAPTER - 4

EXTRACTION, ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENTS FROM ROOTS OF C. INTYBUS LINN.
EXTRACTION, ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENTS FROM ROOTS OF C. INTYBUS LINN.

Materials and methods

Apparatus, chemicals and instruments

1. All the chemicals and reagents were obtained from s.d. fine chemicals and were of analytical (AR) grade.
2. Sodium sulfate was used as drying agent for various solvents used to run the column.
3. All the weighing was done on a Single Pan Mettler balance.
4. Melting points were determined on Perfit melting apparatus, generally melting point were uncorrected.
5. Ultraviolet spectra were recorded on Lambda Bio 20 Spectrophotometer in methanol.
6. Infrared spectra were recorded on Bio-Red FTIR Spectrophotometer using KBr pellets; $\nu_{\text{max}}$ values are given in cm$^{-1}$.
7. $^1$H NMR spectra were screened on Bruker spectrospin 300 MHz instrument using CDCl$_3$ as solvent and TMS as an internal standard. Chemical shift values are given in ppm scale and coupling constants ($J$) in Hz. Notations used throughout as $s =$ singlet, $d =$ doublet, $dd =$ double-doublet, $t =$ triplet, $m =$ multiplet, and brs = unresolved broad singlet.
8. $^{13}$C NMR spectra were recorded on Bruker Spectrospin 300 MHz in 5 mm spinning tubes at 27 $^\circ$C.
9. Mass spectra were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 instrument equipped with direct inlet probe system. The $m/z$ values of the more intense peaks were mentioned and the figures in parenthesis to each $m/z$ value indicate relative intensities with respect to the base peak.
10. Silica gel (Qualigens), 60-120 mesh for column packing; silica gels G (Qualigens) for TLC, spots were visualized by exposure to iodine vapours and UV radiation.
Extraction

The dried roots (2 kg) were coarsely powdered and extracted exhaustively with methanol. The methanolic extract was then concentrated on the water bath and dried under reduced pressure to get 155 g (7.75% yields) of dark brown mass.

Preparation of slurry

The concentrated extract of the drug was taken in a china dish and heated continuously on a water bath by gradually adding methanol in small portions with constant stirring, till desired consistency was obtained. A weighed quantity of silica gel for column chromatography was then added slowly with continuous mixing with a steel spatula until the whole methanolic solution of plant extract gets adsorbed on silica gel particles. It was dried in the air; the larger lumps were broken by rubbing between hands and finally passed through a sieve (No. 8) to get uniform particle size.

Packing of column

A column of 5.0 feet, height and 16 mm internal diameter was taken, cleaned properly and dried. The lower end of the column was plugged with non-absorbent cotton wool. The column was clamped and fitted in a vertical position on a stand. The column was then half filled with petroleum ether (b.p. 60-80 °C). Silica gel (for column, 60-120 mesh) was then poured in small portions and allowed to settle down and the dried plant extract slurry was located over the column and then eluted successively with different solvents, in their order of increasing polarity. The developments and elution of the column was carried out with successive series of different solvents in various combinations, such as petroleum ether, petroleum ether : chloroform, chloroform, chloroform: methanol and methanol.

Homogeneity of the fractions

The fractions collected were subjected to thin layer chromatography (TLC) to check homogeneity of various fractions. Chromatographically identical fractions (having same Rf values) were combined together and concentrated. They were then crystallized with suitable solvent system.
The following compounds were isolated from methanolic extract of *C. inybus* roots

**Lupeonyl arabinosyl palmitate (CI-1)**

Elution of the column with petroleum ether : chloroform (3 : 1) furnished colourless crystals of compound CI-1, recrystallized from acetone, 214.40 mg (0.01072 % yield).

R<sub>f</sub>: 0.42 [Petroleum ether : chloroform :1:1]

m.p.: 64-65 °C

UV <i>λ</i><sub>max</sub> (MeOH): 205 nm (log ε 5.7)

IR <i>υ</i><sub>max</sub> (KBr): 3459, 2922, 2852, 1732, 1650, 1457, 1373, 1246, 1174, 1095, 1022, 803 cm<sup>-1</sup>

<i>H</i> NMR (CDCl<sub>3</sub>): δ 5.27 (1H, d, J = 5.9Hz, H-12), 5.19 (1H, d, J = 7.1 Hz , H-1), 5.04 (2H, brs, H<sub>2</sub>-29), 4.53 (1H, m, H-4), 4.41 (1H, dd, J = 6.5, 7.1 Hz, H-2'), 4.39 (1H, m, H-3'), 3.98 (1H, dd, J = 5.5, 9.3 Hz, H-3β), 3.21 (2H, brs, H<sub>2</sub>-5'), 2.71 (1H, m, H-18), 2.23 (1H, d, J = 7.2 Hz, H<sub>2</sub>-2'a), 2.21 (1H, d, J =7.2 Hz, H<sub>2</sub>-2'b), 1.97 (2H, m, H<sub>2</sub>-11), 1.93 (1H, m, H-19), 1.55 (3H, brs, Me -30), 1.18 (26H, brs, 13 × CH<sub>2</sub>), 1.06 (3H, brs, Me-23), 0.95 (3H, brs, Me-27), 0.87 (3H, brs, Me-24), 0.84 (6H, brs, Me-25, Me- 16'), 0.81 (3H, brs, Me-26), 0.78 (3H, brs, Me-28).

<i>13</i>C NMR (CDCl<sub>3</sub>): Values reported in Table 4.1

<i>+</i>ve ion FAB MS <i>m/z</i> (rel. int.): 794 [M]<sup>+</sup> (<i>C</i><sub>51</sub>H<sub>80</sub>O<sub>6</sub>) (1.2), 423 (28.3), 409 (100), 406 (47.8), 394 (31.2), 379 (11.6), 376 (10.3), 364 (15.9), 255 (39.9), 239 (18.7), 211 (13.2), 206 (31.2), 203 (63.7), 190 (42.1), 188 (53.5), 175 (39.2), 173 (27.3), 169 (21.3), 160 (43.6), 158 (42.1), 155 (32.6), 148 (69.3), 141 (21.4), 132 (71.6), 127 (29.2).

**Alkaline hydrolysis of CI-1:** Compound CI-1 (35 mg), heated with alcoholic IN KOH solution for 2 h on a steam bath. It was extracted with CHCl<sub>3</sub> (3 × 5 ml), in the separating funnel; the organic phase was separated and washed with H<sub>2</sub>O (2 × 5 ml), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to get lup-12, 20 (29)-dien-3β-ol. The mother liquor was acidified to congoed, re-extracted with CHCl<sub>3</sub> (3 × 5 ml), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to get palmitic acid, m.p. 63-64 °C, Co-TLC comparable. The mother liquor after separation of the fatty acid was chromatographed over TLC along with a standard sample of α-D-arabinose.
Lupeolyl palmitate (CI-2)
Elution of the column with petroleum ether : chloroform (1 : 1) afforded colourless crystals of compound CI-2, recrystallized from acetone, 348.50 mg (0.01742 % yield).

Rf: 0.65 [Ethylacetate:glacial acetic acid:formic acid::10:1:1]
m.p.: 205-207 °C
UV $\lambda_{max}$ (MeOH): 205 nm (log $\varepsilon$ 4.9)
IR $\nu_{max}$ (KBr): 2930, 2855, 1726, 1459, 1374, 1253, 1090, 1034, 803 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): δ 5.47 (1H, d, J = 5.3 Hz, H-12), 5.27(1H, brs, H$_2$-29a), 5.18 (1H, brs, H$_2$-29b), 3.12 (1H, dd, J = 5.2, 9.5 Hz, H-3α), 2.23 (1H, d, J = 12.6 Hz, H-18β), 1.97 (1H, d, J = 7.2 Hz, H$_2$ -2a), 1.95 (1H, d, J =7.2 Hz, H$_2$-2b), 1.88 (2H, brs, H$_2$-7), 1.83 (2H, brs, H$_2$-11), 1.55 (3H, brs, Me- 30), 1.40 (2H, brs, CH$_2$), 1.31 (2H, brs, CH$_2$), 1.19 (20H, brs, 10 × CH$_2$), 1.01 (2H, brs, CH$_2$), 0.98 (3H, brs, Me-23), 0.96 (3H, brs, Me-27), 0.90 (3H, brs, Me-24), 0.88 (3H, brs, Me-25), 0.85 (3H, t, J = 6.0 Hz, Me-16"), 0.79 (3H, brs, Me-26), 0.67 (3H, brs, Me-28).

$^{13}$C NMR (CDCl$_3$): Values reported in Table 4.2

$^+$ve ion FAB MS m/z (rel. int.): 662 [M]$^+$ (C$_{46}$H$_{78}$O$_2$) (1.3), 424 (11.3), 409 (22.6), 394 (15.3), 256 (21.5), 239 (10.8), 205 (24.5), 203 (42.6), 190 (31.38), 188 (23.5), 176 (27.8), 174 (23.2).

Alkaline hydrolysis of CI-2: Compound CI-2 (45 mg) dissolved in ethanol (5 ml) and 1N NaOH solution (1 ml) was added, the reaction mixture was heated on the steam bath for 1 h. It was dried under reduced pressure and the residue was extracted with CHCl$_3$ (3 × 5 ml). The CHCl$_3$ layer was washed with H$_2$O (2 × 5 ml), dried over anhydrous Na$_2$SO$_4$ and evaporated to get lupeol, m.p. 213-214 °C. The residue was dissolved in H$_2$O (5 ml) acidified with dil. HCl and re-extracted with CHCl$_3$ which on drying afforded palmitic acid, m.p. 63-64 °C, Co-TLC comparable.
Intybusteryl palmitoleate (CI-3)

Elution of the column with petroleum ether : chloroform (1 : 3) yielded colourless crystals of compound CI-3, recrystallized from methanol, 580 mg (0.029% yield).

Rf: 0.5 [Toluene:chloroform:ethanol:: 6:3:1]

m.p.: 89-90 °C

UV \( \lambda_{\text{max}} \) (MeOH): 204, 239 (log e 5.7, 1.1)

IR \( v_{\text{max}} \) (KBr): 3397, 2924, 2855, 1740, 1652, 1462, 1373, 1248, 1189, 1039 cm\(^{-1}\).

\(^1\)H NMR and \(^{13}\)C NMR (CDCl\(_3\)): Values reported in Table 4.3

-ve ion FAB MS \( m/z \) (rel. int.): 664 [M]+ (C\(_{45}\)H\(_{76}\)O\(_3\)) (21.6), 428 (22.8), 413 (100), 410 (87.2), 398 (42.5), 395 (36.1), 392 (12.7), 383 (25.9), 380 (13.1), 271 (32.6), 256 (42.3), 254 (21.2), 241 (16.3), 180 (43.6), 165 (23.4), 162 (63.5), 123 (64.8), 108 (73.4).

**Alkaline Hydrolysis of CI-3**: Compound CI-3 (40 mg) dissolved in ethanol (5 ml) and IN NaOH solution (1 ml) was added, the reaction mixture was heated on a steam bath for 1 h. It was dried under reduced pressure and the residue was extracted with CHCl\(_3\) (3 x 5 ml). The CHCl\(_3\) layer was washed with H\(_2\)O (2 x 5 ml), dried over anhydrous Na\(_2\)SO\(_4\) and evaporated to get intybusteral, IR \( v_{\text{max}} \): 3450 cm\(^{-1}\). The residue after separation with CHCl\(_3\) was dissolved in H\(_2\)O (5 ml) acidified to pH 3 and re-extracted with CHCl\(_3\) to isolate palmitoleic acid (Co-TLC comparable).

Intybusteroic acid A (CI-4)

Elution of column with petroleum ether : chloroform (1 : 3) gave colourless crystals of CI-4, recrystallized from methanol, 564 mg (0.0282% yield).

Rf: 0.62 [Toluene:ethyl acetate:glacial aceic acid:: 7:3:1]

m.p.: 98-100 °C

UV \( \lambda_{\text{max}} \) (MeOH): 205, 239 nm (log e 5.7, 1.1)

IR \( v_{\text{max}} \) (KBr): 3442, 2922, 2850, 1703, 1650, 1459, 1243, 1189, 1054, 964 cm\(^{-1}\).

\(^1\)H NMR and \(^{13}\)C NMR (CDCl\(_3\)): Values reported in Table 4.4

+ve ion FAB MS \( m/z \) (rel. int.): 442 [M]+ (C\(_{29}\)H\(_{46}\)O\(_3\)) (2.3), 427 (9.6), 412 (59.7), 398 (100), 397 (71.6), 383 (37.5), 382 (13.6), 369 (10.1), 301 (9.5), 273 (23.8), 255 (39.5), 240 (10.5), 213 (22.7), 201 (18.3), 199 (11.5), 192 (8.3), 178 (11.1), 174 (21.6), 169 (15.7), 164 (13.2), 159 (48.3), 149 (21.3), 146 (36.2), 145 (42.8), 144 (28.1), 138 (19.3), 135 (23.6), 131 (28.2), 130 (21.3), 124 (18.7), 120 (22.8), 109 (39.8), 106 (42.3), 105 (48.5), 95 (46.8), 91 (41.2).
Intybuspolic acid B (CI-5)
Elution of the column with chloroform afforded colourless crystals of compound CI-5, recrystallized from methanol, 319.60 mg (0.01598% yield).

R<sub>f</sub>: 0.72 [Petroleum ether:toluene:chloroform::2:2:6]

m.p.: 60-62 °C

UV <i>λ<sub>max</sub></i> (MeOH): 204 nm (log ε 5.6)

IR <i>ν<sub>max</sub></i> (KBr): 3427, 2913, 2844, 1701, 1646, 1461, 1369, 1297, 1055 cm<sup>-1</sup>.

<sup>1</sup>H NMR and <sup>13</sup>C NMR (CDCl<sub>3</sub>): Values reported in Table 4.5

+VE ion FAB- MS <i>m/z</i> (rel. int.) : 442 [M]+ (C<sub>29</sub>H<sub>46</sub>O<sub>3</sub>) (4.3), 427 (12.6), 412 (47.8), 398 (44.2), 397 (48.5), 383 (25.1), 382 (19.8), 273 (41.2), 255 (56.9), 240 (18.3), 213 (38.2), 199 (26.6), 192 (18.7), 178 (18.5), 174 (36.8), 164 (35.2), 160 (63.8), 146 (66.3), 143 (51.9), 138 (35.3), 124 (33.6), 120 (69.1), 106 (75.5), 83 (100).

Stigmasterol glycoside (CI-6)
Elution of the column with chloroform : methanol (99 : 1) mixture yielded colourless amorphous powder of CI-6, recrystallised from methanol, 78 mg (0.0039% yield).

R<sub>f</sub>: 0.53 [Benzene: chloroform:methanol::5:4:1]

m.p.: 270-272 °C

UV <i>λ<sub>max</sub></i> (MeOH): 221 nm (log ε 4.3)

IR <i>ν<sub>max</sub></i> (KBr): 3421, 3390, 2923, 2850, 1640, 1458, 1379, 1257, 1166, 1074, 1021, 797 cm<sup>-1</sup>.

<sup>1</sup>H NMR (DMSO): δ 5.32 (1H, m, H-6), 5.29 (1H, m, H-22), 5.02 (1H, m, H-23), 4.88 (1H, brs, H-1'), 4.44 (1H, m, H-5'), 4.23 (1H, d, J = 7.2 Hz, H-2'), 3.63 (1H, brm, <i>ω</i><sub>1/2</sub> = 16.5 Hz, H-3), 3.39 (1H, m, H-4'), 3.11 (1H, m, H-3'), 3.04 (2H, m, H-6'), 1.22 (3H, brs, Me-19), 0.95 (3H, d, J = 6.1 Hz, Me-21), 0.89 (3H, d, J = 6.3 Hz, Me-26), 0.80 (3H, d, J = 6.3 Hz, Me-29), 0.78 (3H, d, J = 6.2 Hz, Me-27), 0.65 (2H, brs, Me-18).

<sup>13</sup>C NMR (DMSO): δ 36.84 (C-1), 29.06 (C-2), 73.48 (C-3), 40.32 (C-4), 140.41 (C-5), 121.21 (C-6), 31.41 (C-7), 33.36 (C-8), 49.63 (C-9), 36.23 (C-10), 22.62 (C-11), 35.51 (C-12), 40.05 (C-13), 56.20 (C-14), 25.47 (C-15), 27.81 (C-16), 55.43 (C-17), 11.79 (C-18), 19.11 (C-19), 36.08 (C-20), 18.36 (C-21), 138.3 (C-22), 128.84 (C-23), 45.16 (C-24), 28.72 (C-25), 19.73 (C-26), 20.61 (C-27), 23.89 (C-28), 11.73 (C-29), 100.83 (C-1'), 71.62 (C-2'), 70.11 (C-3'), 70.11 (C-4'), 76.77 (C-5'), 61.10 (C-6').
Acid hydrolysis of compound CI-6: compound CI-6 (15 mg) refluxed with 2N HCl in 80% methanol (1:1, 15 ml) for 1 h after cooling, the reaction mixture was poured on to crushed ice and the hydrolysate was then extracted with EtOAc to give the aglycone; m.p. 168-170 °C, Co-TLC comparable. The neutralized and concentrated aqueous hydrolysate showed the presence of glucose on comparison with authentic sugar on silica gel TLC, Rf 0.4 (EtOAc:HOAc:H2O:MeOH::6:1:1:2).

Triglyceride phosphate (CI-7)

Elution of the column with chloroform: methanol (99 : 1) yielded colourless amorphous mass of CI-7, recrystallized from methanol, 239.60 mg (0.01198% yield).

Rf: 0.58 (Toluene:chloroform:ethylacetate:: 5:2:3

m.p.: 58-60 °C

IR vmax (KBr): 3444, 2915, 2847, 1731, 1629, 1459, 1371, 1264, 1163, 1031, 817, 720 cm⁻¹.

¹H NMR (CDCl₃): δ 5.34 (2H, m, H-10”, H-12”), 5.13 (2H, m, H-9”, H-13”), 4.26 (1H, m, H-2), 3.91 (1H, d, J = 6.6 Hz, H₂-3a), 3.89 (1H, d, J = 6.6 Hz, H-3b), 3.71 (1H, d, J = 12.9 Hz, H₂-1a), 3.67 (1H, d, J = 12.9 Hz, H₂-1b), 2.33 (1H, d, J = 7.8 Hz, H₂-2’a), 2.30 (1H, d, J = 7.8 Hz, H₂-2’b), 2.19 (2H, brs, H₂-2”), 2.03 (2H, brs, H₂-11”), 1.66 (2H, brs, H₂-8”), 1.64 (2H, brs, H₂-14”), 1.26 (46H, brs, 23 × CH₂), 0.88 (3H, t, J = 6.2 Hz, Me-18”), 0.85 (3H, t, J = 6.1 Hz, Me-18”).

¹³C NMR (CDCl₃): δ 176.81 (C-1’), 175.32 (C-1”), 130.37 (C-9”), 129.97 (C-10”), 128.86 (C-12”), 115.45 (C-13”), 69.43 (C-3), 65.01 (C-2), 62.07 (C-1), 55.95 (C-2”), 50.77 (C-2”), 34.06 (C-8”), 33.93 (C-5”), 31.87 (C-11”), 29.65 (19 × CH₂), 27.17 (CH₂), 25.95 (CH₂), 24.84 (CH₂), 22.63 (CH₂), 21.05 (CH₂), 18.29 (Me-18”), 14.05 (Me-18”).

+ve ion FAB MS m/z (rel. int.): 699 [M]+ (C₃₉H₇₃O₈P) (10.5), 619 (21.8), 267 (12.6), 263 (15.2), 239 (18.9).

Alkaline Hydrolysis of CI-7: Compound CI-7 (35 mg) refluxed with ethanolic 1N KOH solution for thirty min on a steam bath. The reaction mixture was acidified, extracted with CHCl₃ (3 × 10 ml), chloroform layer was washed with H₂O (2 × 10 ml) and dried over anhydrous Na₂SO₄. After evaporation of the solvent, a mixture of stearic and linoleic acids was obtained (TLC - comparable).
Diglyceride phosphate (CI-8)

Elution of the column with chloroform : methanol (49 : 1) furnished colourless amorphous mass of CI-8, recrystallized from methanol, 564 mg (0.0282% yield). 


m.p.: 88-90 °C 

UV \( \lambda_{\text{max}} \) (MeOH): 206, 284 nm (log e 4.9, 1.1) 

IR \( \nu_{\text{max}} \) (KBr): 3399, 2909, 2843, 1721, 1718, 1627, 1489, 1443, 1377, 1250, 1163, 715 cm\(^{-1}\). 

\(^1\)H NMR (CDCl\(_3\)): \( \delta 5.34 \) (3H, m, H-10', H-13', H-15'), 5.31 (3H, m, H-9', H-12', H-16'), 5.11 (2H, m, H-9'', H-10''), 4.17 (1H, m, H-2), 3.92 (1H, d, \( J = 12.6 \) Hz, H\(_2\)-3a), 3.90 (1H, d, \( J = 12.6 \) Hz, H\(_2\)-3b), 3.71 (1H, d, \( J = 12.6 \) Hz, H\(_2\)-1a), 3.69 (1H, d, \( J = 12.5 \) Hz, H\(_2\)-1b), 2.77 (2H, m, H\(_2\)-14'), 2.50 (2H, m, H\(_2\)-11'), 2.36 (1H, d, \( J = 7.2 \) Hz, H\(_2\)-2'a), 2.34 (1H, d, \( J = 7.2 \) Hz, H\(_2\)-2'b), 2.32 (1H, d, \( J = 6.6 \) Hz, H\(_2\)-2'a), 2.30 (1H, d, \( J = 6.6 \) Hz, H\(_2\)-2'b), 2.03 (2H, m, H\(_2\)-8'), 1.75 (2H, m, H\(_2\)-17'), 1.67 (2H, m, H\(_2\)-8''), 1.62 (2H, m, H\(_2\)-11''), 1.25 (32H, brs, 16 x CH\(_2\)), 0.87 (3H, t, \( J = 6.1 \) Hz, Me-18'), 0.85 (3H, t, \( J = 6.2 \) Hz, Me-18''). 

\(^{13}\)C NMR (CDCl\(_3\)): \( \delta 173.26 \) (C-1'), 171.68 (C-1''), 144.68 (C-12'), 129.99 (C-13'), 127.79 (C-15'), 127.76 (C-16'), 125.01 (C-10'), 122.11 (C-9'), 115.55 (C-9''), 113.94 (C-10''), 70.26 (C-2), 65.11 (C-3), 62.09 (C-1), 55.96 (C-2'), 50.73 (C-2''), 40.75 (C-11'), 34.10 (C-14'), 34.02 (C-8''), 32.16 (C-11''), 31.88 (C-17'), 29.65 (11 x CH\(_2\)), 27.17 (CH\(_2\)), 25.94 (CH\(_2\)), 24.86 (CH\(_2\)), 23.64 (CH\(_2\)), 21.02 (CH\(_2\)), 19.72 (CH\(_2\)), 18.49 (CH\(_2\)), 14.06 (Me-18''), 14.05 (Me-18'').

+ve ion FAB MS \( m/z \) (rel. int.): 695 [M]+ (C\(_{30}\)H\(_{69}\)O\(_8\)P) (6.8), 665 (24.3), 650 (18.7), 350 (31.6), 265 (15.6), 261 (14.2).

Alkaline hydrolysis of CI-8: Compound CI-8 (35 mg) refluxed with ethanolic 1N KOH solution for thirty min on a steam bath. The reaction mixture was acidified, extracted with CHCl\(_3\) (3 x 10 ml), the chloroform layer washed with H\(_2\)O (2 x 10 ml) and dried over anhydrous Na\(_2\)SO\(_4\). After evaporation of the solvent, a mixture of oleic acid and linolenic acid was obtained (TLC - comparable).
Oleoyl diglucoside (CI-9)

Further elution of column with chloroform: methanol (48:2) yielded colourless crystals of compound CI-9, recrystallized from methanol, 548.30 mg (0.02741% yield).

Rf: 0.73 [Benzene:ethyl acetate:formic acid::7:3:1]

m.p.: 56-58 °C

UV $\lambda_{\text{max}}$ (MeOH): 201 nm (log $\varepsilon$ 3.2)

IR $\nu_{\text{max}}$ (KBr): 3334, 3245, 2914, 2846, 1730, 1618, 1462, 1370, 1255, 1166, 1071, 1021, 721 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): δ 5.36 (2H, m, H-9, H-10), 5.11 (1H, d, $J = 7.1$ Hz, H-1), 5.03 (1H, d, $J = 7.2$ Hz, H-1"), 4.78 (2H, m, H-5', H-5") , 4.36 (1H, d, $J = 6.5$ Hz, H-2'), 4.33 (1H, d, $J = 6.5$ Hz, H-2"), 4.10 (1H, m, H-3'), 4.07 (1H, m, H-3"), 3.78 (1H, m, H-4'), 3.70 (1H, m, H-4"), 3.47 (4H, brs, H$_2$-6', H$_2$-6") , 2.33 (1H, d, $J = 7.2$ Hz, H$_2$-2a), 2.31 (1H, d, $J = 7.2$ Hz, H$_2$-2b), 2.01 (2H, m, H$_2$-8), 1.83 (2H, m, H$_2$-11), 1.47 (2H, m, H$_2$-3), 1.25 (20H, brs, 10 $\times$ CH$_2$), 0.85 (3H, t, $J = 6.6$ Hz, Me-18).

$^{13}$C NMR (CDCl$_3$): δ 175.61 (C-1), 129.64 (C-9), 121.42 (C-10), 101.04 (C-1', C-1"), 79.01 (C-5', C-5"), 75.41 (C-2'), 73.47 (C-2"), 73.17 (C-3'), 71.78 (C-3"), 71.76 (C-4'), 70.27 (C-4"), 63.47 (C-6'), 61.06 (C-6"), 51.59 (C-2), 49.61 (CH$_2$), 34.23 (CH$_2$), 33.93 (CH$_2$), 32.48 (CH$_2$), 32.21 (CH$_2$), 31.47 (CH$_2$), 30.50 (CH$_2$), 29.26 (CH$_2$), 28.91 (CH$_2$), 25.58 (CH$_2$), 24.91 (CH$_2$), 24.59 (CH$_2$), 22.24 (CH$_2$), 13.71 (CH$_3$).

$^+$ve ion FAB MS m/$z$ (rel. int.): 606 [M]$^+$ (C$_{30}$H$_{54}$O$_{12}$) (5.3), 281 (16.2), 265 (21.6), 162 (37.8).

Acid hydrolysis of CI-9: Compound CI-9 (35 mg) dissolved in ethanol (5 ml), dil HCl (3 ml) added and the reaction mixture refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue was dissolved in CHCl$_3$. It was chromatographed over silica gel TLC (petroleum ether : chloroform:: 1:1) with a standard solution of oleic acid. The residue after separating the fatty acid fraction was dissolved in water and chromatographed over paper with a standard solution of D-glucose (n-BuOH:H$_2$O:AcOH::4:1:5) top layers, Rf 0.12.
Oleoyl glucoside (CI-10)

Elution of the column with chloroform: methanol (48:2) gave colourless crystals of CI-10, recrystallized from methanol, 187.20 mg (0.00936% yield).

Rf: 0.68 [Benzene:ethyl acetate:formic acid::7:3:1]

m.p.: 113-115 °C

UV λ_max (MeOH): 206 nm (log ε 4.2)

IR ν_max (KBr): 3403, 3355, 2913, 2845, 1736, 1647, 1464, 1369, 1257, 717 cm⁻¹.

\(^1^H\) NMR (CDCl₃): δ 5.39 (1H, m, H-9), 5.33 (1H, m, H-10), 4.28 (1H, d, J = 7.1 Hz, H-1'), 4.15 (1H, m, H-5'), 3.88 (1H, dd, J = 7.2 Hz, H-2'), 3.77 (1H, dd, J = 6.5, 7.0 Hz, H-3'), 3.65 (1H, m, H-4'), 3.16 (2H, brs, H₂-6'), 2.44 (1H, d, J = 7.1Hz, H₂-2a), 2.33 (1H, d, J = 7.1Hz, H₂-2b), 2.02 (2H, m, H₂-8), 1.82 (2H, m, H₂-11), 1.61 (2H, m, H₂-3), 1.26 (20H, brs, 10 × CH₂), 0.88 (3H, t, J = 6.5 Hz, Me-18).

\(^1^C\) NMR (CDCl₃): δ 176.63 (C-1), 129.65 (C-9), 127.83 (C-10), 75.59 (C-1'), 74.12 (C-5'), 72.68 (C-2'), 71.93 (C-3'), 69.89 (C-4'), 61.12 (C-6'), 51.95 (C-2), 34.39 (C-8), 34.02 (C-11), 32.17 (C-3), 32.17 (CH₂), 31.44 (CH₂), 29.21 (3 × CH₂), 25.52 (CH₂), 25.18 (CH₂), 24.84 (CH₂), 24.57 (CH₂), 22.18 (CH₂), 13.61 (Me-18).

+ve ion FAB MS m/z (rel. int.): 444 [M]^+ (C₂₄H₄₄O₇) 2.3, 163 (27.8).

Acid hydrolysis of CI-10: Compound CI-10 (35 mg) was dissolved in ethanol (5 ml), dil HCl (3 ml) added and the reaction mixture refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue was dissolved in CHCl₃. It was chromatographed over silica gel TLC (petroleum ether : chloroform:: 1:1) with a standard solution of oleic acid fraction dissolved in water and chromatographed over paper with a standard solution of D-glucose (n-BuOH : H₂O : ACOH : 4:1:5) top layers, Rf 0.12.

Cichoriumone (CI-11)

Elution of the column with chloroform: methanol (19:1) furnished colourless crystals of CI-11, recrystallized from methanol, 104.90 mg (0.0052% yield).

Rf: 0.54 [Toluene:ethyl acetate:formic acid::9:4:1]

m.p.: 119-120 °C

IR λ_max (KBr): 2915, 2847, 1739, 1621, 1463, 1422, 1370, 1244, 1075, 717 cm⁻¹.
Chapter-4 Isolation & Characterization

^1H NMR (CDCl₃): δ 7.10 (1H, dd, J = 5.6, 6.8, H-3), 6.79 (1H, d, J = 6.8 Hz, H-2), 3.72 (2H, brs, H2-5), 2.71 (1H, brm, w_1/2=14.5 Hz, H-4), 2.30 (2H, brs, CH₂), 2.03 (4H, brs, 2 x CH₂), 1.61 (6H, brs, 3 x CH₂), 1.25 (56H, brs, 28 x CH₂), 0.87 (3H, t, J = 6.5 Hz, Me-41).

^13C NMR (CDCl₃): δ 171.52 (C-1), 127.58 (C-2), 113.96 (C-3), 68.72 (C-5), 50.06 (C-4), 45.98 (CH₂), 33.18 (CH₂), 29.56 (29 x CH₂), 22.37 (CH₂), 21.13 (CH₂), 19.29 (CH₂), 14.24 (Me-41).

+ve ion FAB MS m/z (rel int.): 602 [M]^+ (C₄₁H₇₈O₂) (33.1), 97(53.8).

Ricinooleoyl glucoside (Cl-12)

Elution of the column with chloroform : methanol (23 : 2) mixture gave colourless crystals of Cl-12, recrystallized from methanol, 286.40 mg (0.0143% yield).

R_f: 0.63 [Toluene:ethyl acetate:formic acid::5:4:1]

m.p.: 83-85 °C

UV λ_max (MeOH): 207 nm (log ε 4.3)

IR ν_max (KBr): 3445, 2921, 2852, 1741, 1651, 1462, 1418, 1368, 1251, 1077 cm⁻¹.

^1H NMR (CDCl₃): δ 5.33 (2H, m, H-9, H-10), 4.36 (1H, m, H-1'), 4.10 (1H, m, H-5'), 3.77 (1H, dd, J = 6.5, 7.1 Hz, H-3'), 3.69 (1H, m, H-12), 3.35 (1H, d, J = 9.5 Hz, H₂-6'a), 3.33 (1H, d, J = 9.5 Hz, H₂-6'b), 2.30 (1H, d, J = 7.1 Hz, H₂-2'a), 2.28 (1H, d, J = 7.1 Hz, H₂-2'b), 2.01 (2H, m, H₂-10), 1.99 (2H, m, H₂-11), 1.52 (2H, m, H₂-3), 1.26 (16 H, brs, 8 x CH₂), 0.85 (3H, t, J = 6.6 Hz, Me-18).

^13C NMR (CDCl₃): δ 176.35 (C-1), 130.62 (C-9), 129.87 (C-10), 103.69 (C-1'), 73.52 (C-5'), 72.43 (C-2'), 71.81 (C-3'), 69.89 (C-12), 63.67 (C-4'), 61.40 (C-6'), 56.19 (C-2), 34.72 (C-8), 32.68 (C-11), 31.92 (C-13), 29.76 (3 x CH₂), 27.76 (CH₂), 25.46 (CH₂), 22.66 (CH₂), 14.02 (C-18).

+ve ion FAB MS m/z (rel int.): 460 [M]^+ (C₂₄H₄₄O₈) (5.3), 281 (48.6), 163 (15.3).
Acid hydrolysis of CI-12: Compound CI-12 (35 mg) dissolved in ethanol (5 ml), dil HCl (3 ml) added and the reaction mixture refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue was dissolved in CHCl₃. It was chromatographed over silica gel TLC (petroleum ether:chloroform::1:1) with a standard solution of ricinoleic acid fraction dissolved in water and chromatographed over paper with a standard solution of D-glucose (n-BuOH:H₂O:AcOH:: 4:1:5) top layers Rₚ 0.12.

Results and Discussion

Compound CI-1 designated as lupeolyl arabinosyl palmitate, was obtained as colourless crystalline mass from petroleum ether : chloroform (3:1) eluants. It responded positively to triterpenic glycoside tests and exhibited characteristic absorption bands for hydroxyl group (3459 cm⁻¹), ester group (1732 cm⁻¹) and unsaturation (1650 cm⁻¹). Its mass spectrum exhibited a molecular ion peak at m/z 794 corresponding to a triterpenic glycoside C₃₁H₆₆O₉. It indicated nine double bond equivalents; five of them were adjusted in the pentacyclic carbon framework of the triterpene, two in the vinylic linkages and one each in the sugar moiety and ester group. The prominent ion peaks generated at m/z 239 [CH₃(CH₂)i₄CO]+, 255 [CH₃(CH₂)₁₄COO]+, 132 [C₅H₅O₃]+, 148 [C₅H₅O₃]+, 424 [M – CH₃(CH₂)₁₄ COOC₅H₅O₃]+ and 406 [M – glycoside]+ suggested that a C-5 sugar unit esterified with C-16 saturated fatty acid was attached to the triterpenic molecule. The ion peaks arising at m/z 391[406 – Me]+, 376 [391 – Me]+, 409 [424 – Me]+, 394 [409 – Me]+, 379 [394 – Me]+, 364 [379 – Me]+, 383 [424 – C₃H₅]+ and 365 [383 – H₂O]+ indicated a lupene type triterpene. The ion peaks at m/z 206 [C₁₄H₂₄O]+ and 203 [C₁₅H₂₄]+ were produced due to retro-Diels Alder fragmentation. The ion peaks at m/z 190 [206 – OH]+, 175 [190 – Me]+, 160 [175 – Me]+, 188 [203 – Me]+, 173 [188 – Me]+ and 158 [173 – Me]⁺ supported the triterpenic nature of the molecule (Scheme 4.1) (Ali, 2001).

The ¹H NMR spectrum of CI-1 showed a one- proton doublet at δ 5.27 with coupling interaction of 5.9 Hz ascribed to vinylic H-12 proton. A one-proton doublet at δ 5.19 with coupling interaction of 7.1 Hz assigned to anomeric H-1’ proton. A two-proton broad signal at δ 5.04 was accounted to vinylic H₂-29 protons. A one-proton multiplet at δ 4.53 was assigned to carbinol H-4’ proton. A one-proton double-doublet at δ 4.4 (J = 6.5 and 7.1 Hz) was ascribed to H-2’ carbinol proton. A one-proton multiplet at δ 4.39 was
assigned to H-3' carbinol proton. A one-proton double-doublet at δ 3.98 (J = 5.5 and 9.3 Hz) was ascribed to α-oriented H-3 carbinol proton. A two-proton broad signal at δ 3.21 was attributed to H2-5' oxygenated methylene protons of the sugar. Two one-proton doublets at δ 2.23 (J = 7.2 Hz) and 2.21 (J = 7.2 Hz) were due to methylene H2- 2" adjacent to the ester group. A three-proton broad signal at δ 1.55 was assigned to Me-30 methyl group attached to the vinylic carbon. Five three-proton broad signals at δ 1.06, 0.95, 0.87, 0.81 and 0.78 all integrated for three proton each, were associated with Me-23, Me-27, Me-24, Me-26 and Me-28 tertiary methyl protons, respectively. The remaining methylene and methine protons resonated between δ 1.97-1.18. A six-proton broad signal at δ 0.84 was accounted to C-25 tertiary and C-16" primary methyl functionalities.

The 13C NMR spectrum of CI-1 showed important signals for C-1" ester carbon at δ 173.66, vinylic carbons at δ 122.57 (C-12), 138.31 (C-13), 154.64 (C-20) and 109.35 (C-29), C-3 carbinol carbon at δ 73.69, C-1' anomeric carbon at δ 107.13 and sugar carbons between δ 81.01-64.40. The 1H and 13C values were compared with the related lupene type triterpenic molecules (Mahato and Kundu, 1994; Ali, 2001).

Alkaline hydrolysis of CI-1, yielded lup-12, 20(29)-dien-3α-ol, palmitic acid and α-D-arabinose. On the basis of spectral data analysis and chemical reactions the structure of CI-1 has been formulated as lup-12,20(29)-dien-3α-ol-3α-D-arabinofuranosyl-5'-hexadecanoate. This is a new lupene type triterpenic glycoside isolated from a natural or synthetic source for the first time.
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Coupling constants in Hertz were provided in parenthesis.
Spectrum 4.1. $^1$H NMR spectrum of lupeolyl arabinosyl palmitate (CI-1).

Spectrum 4.2. $^{13}$C NMR spectrum of lupeolyl arabinosyl palmitate (CI-1).
Scheme 4.1. Mass fragmentation pattern of lupeolyl arabinosyl palmitate (CI-1).
Compound CI-2 named lupeoly palmitate, was obtained as a colourless crystals from petroleum ether : chloroform (1:1) eluants. It responded positively to Liebermann-Burchard test indicating triterpene nature of the compound. Its IR spectrum showed characteristic absorption bands for ester group (1726 cm\(^{-1}\)) and unsaturation (1649 cm\(^{-1}\)).

The +ve ion FAB mass spectrum of compound CI-2 displayed a molecular peak at \(m/z\) 662 corresponding to a lupeoly ester, \(C_{46}H_{78}O_2\). It indicated eight double bond equivalents; five of them were adjusted in the pentacyclic carbon framework, two in the vinylic linkages and the remaining one in the ester group. The retro-Diels-Alder fragmentation pattern of the triterpenic moiety yielded ion fragments at \(m/z\) 205 \([C_{14}H_{21}O]^+\) and 203 \([C_{15}H_{21}]^+\). The ion peaks at \(m/z\) 190 \([205 - Me]^+\) and 188 \([203 - Me]^+\) suggested the presence of one of the vinylic linkage in ring C. The ion fragments arose at \(m/z\) 256 \([C_{16}H_{32}O_2]^+\) and 239 \([C_{16}H_{31}O]^+\) indicated the esterification of the triterpenic residue with hexadecanoic acid (Scheme 4.2).

The \(^1\)H NMR spectrum of compound CI-2 exhibited one-proton doublet at \(\delta\) 5.47 with coupling interaction of 5.3 Hz that was assigned to H-12 vinylic proton. Two one-proton broad signals at \(\delta\) 5.27 and 5.18 were ascribed to methylene \(H_2\)-29a and \(H_2\)-29b protons, respectively. A one-proton double-doublet at \(\delta\) 3.12 with coupling interactions of 5.2 and 9.5 Hz was accounted to α-oriented H-3 carbino1 proton. Two one-proton doublets at \(\delta\) 1.97 and 1.95 (\(J = 7.2\) Hz each) were attributed to \(H_2\)-2'a and \(H_2\)-2'b methylene protons adjacent to ester group, respectively. A three-proton broad signal at \(\delta\) 1.55 was due to C-30 methyl protons located on C-20 vinylic carbon. Six three-proton broad signals at \(\delta\) 0.98, 0.96, 0.90, 0.88, 0.79 and 0.67 were assigned correspondingly to tertiary C-23, C-27, C-24, C-25, C-26 and C-28 methyl proton. A three-proton triplet at \(\delta\) 0.85 with coupling constant of 6.0 Hz was associated with C-16" primary methyl proton. The remaining methylene and methine proton signals resonated between \(\delta\) 1.40-1.01.

The \(^{13}\)C NMR spectrum of CI-2 exhibited signals for ester carbon at \(\delta\) 168.73 (C-1'), vinylic carbons at \(\delta\) 118.35 (C-12), 143.86 (C-13), 156.61 (C-20), 107.51 (C-29) and oxygenated methine carbon at 79.05 (C-3). The \(^1\)H and \(^{13}\)C values of the triterpenic moiety were compared with the related lupeol-type molecules (Tchivounda \textit{et al.}, 1990; Savona \textit{et al.}, 1987; Ngassapa \textit{et al.}, 1991; Mahato and Kundu, 1994; Ali, 2001).

\textit{Jamia Hamdard} 135
Alkaline hydrolysis of CI-2 yielded lupeol and palmitic acid. On the basis of the foregoing discussion the structure of CI-2 has been elucidated as $\text{Lup-12, 20(29')-dien-3P-olyl hexadecanoate.}$

**Table 4.2. $^{13}$C NMR values of lupeoyl palmitate (CI-2)**

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Coupling constants in Hertz were provided in parenthesis.
Spectrum 4.3. $^1$H NMR spectrum of lupeolyl palmitate (CI-2).

Spectrum 4.4. $^{13}$C NMR spectrum of lupeolyl palmitate (CI-2).
Compound CI-3 designated as intyburneryl palmitoleate, was obtained as a colourless crystalline mass from petroleum ether : chloroform (1:3) eluants. It gave positive test for steroids and its IR spectrum exhibited characteristic absorption band for hydroxyl group (3397 cm\(^{-1}\)), ester group (1740 cm\(^{-1}\)) and unsaturation (1652 cm\(^{-1}\)). Its mass spectrum displayed a molecular ion peak at \(m/z\) 664 corresponding to a steroidal ester \(C_{45}H_{76}O_3\). It indicated eight degrees of unsaturation; four of them were adjusted in the tetracyclic carbon framework of the sterol, three in the vinylic linkages and the remaining one in the ester linkage. The prominent ion peaks generated at \(m/z\) 254 \(\[\text{CH}_3(\text{CH}_2)\text{CH}=\text{CH}(\text{CH}_2)\text{COOH}\]\(^+\), 410 \([\text{M} - 254]\(^+\), 395 \([410 - \text{Me}]^+\), 380 \([395 - \text{Me}]^+\), 392 \([410 - \text{H}_2\text{O}]^+\), 271 \([410 - \text{C}_{10}H_{19}, \text{side chain}]^+\), 256 \([271 - \text{Me}]^+\) and 241 \([256 - \text{Me}]^+\) suggested the esterification of the sterol with palmitoleic acid, existence of C-10
unsaturated side chain and two hydroxyl groups in the steroidal skeleton. The ion fragments arising at \(m/z\) 428 \([M - \text{CH}_3(\text{CH}_2)\text{CH}=\text{CH}(\text{CH}_2)\text{C}=\text{O}]^+\), 413 \([428 - \text{Me}]^+\), 398 \([413 - \text{Me}]^+\) and 383 \([398 - \text{Me}]^+\) also suggested the location of two hydroxyl functions in the steroidal nucleus. The ion peaks formed at \(m/z\) 123 \([\text{C}_7 - \text{C}_{10} \text{ fission}]^+\), 108 \([123 - \text{Me}]^+\), 180 \([\text{C}_8,14 - \text{C}_{9,11} \text{ fission}]^+\), 162 \([180 - \text{H}_2\text{O}]^+\) and 165 \([180 - \text{Me}]^+\) supported the location of the hydroxyl group in ring A which was placed at C-3 on the basis of biogenetic consideration and another hydroxyl group at C-7 (Schemes 4.3).

The \(^1\text{H}\) NMR spectrum of compound CI-3 showed a one-proton doublet signal at \(\delta 5.34\) (\(J = 5.2\) Hz) assigned to vinylic H-6 proton. Four one-proton multiplets at \(\delta 5.23, 5.18, 5.13\) and 5.07 were accounted to vinylic C-22 and C-23 protons of the steroidal side chain and to C-7' and C-8' protons of the fatty acid chain, respectively. A one-proton broad multiplet at \(\delta 3.51\) with half width of 18.5 Hz was attributed to carbinol 3\(\alpha\)-proton. Two three-proton broad signals at \(\delta 0.68\) and 1.01 were ascribed to tertiary C-18 and C-19 methyl protons. Four three-proton doublets at \(\delta 0.95\) (\(J = 6.2\)), 0.86 (\(J = 6.0\)), 0.87 (\(J = 6.1\)) and 0.81 (\(J = 6.2\)) were accounted to C-21, C-26, C-27 secondary and C-29 primary methyl protons, respectively. A three-proton triplet at \(\delta 0.83\) (\(J = 6.2\) Hz) were ascribed to the C-16' primary methyl group of the fatty acid chain. The remaining methylene and methine protons appeared between \(\delta 2.35-1.26\). The presence of all methyl signals in the range \(\delta 1.01-0.68\) suggested that all these functionalities were located on the saturated carbons.

The \(^{13}\text{C}\) NMR spectrum of compound CI-3 exhibited signals for vinylic carbons at \(\delta 140.08\) (C-5), 121.71 (C-6), 138.29 (C-22), 130.11 (C-23), 123.72 (C-7') and 118.89 (C-8'), ester carbon at 178.15 (C-1'), carbinol carbons at \(\delta 79.05\) (C-3) and 71.82 (C-7) and methyl signals between \(\delta 19.39-12.22\). The remaining methylene and methine carbon resonated between 55.98-21.07.

The \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectral data compound CI-3 were compared with related steroidal constituents. On the basis of foregoing account the structure of CI-3 has been elucidated as stigmasta-5, 22-dien-3\(\beta\), 7-diol-3-O-hexadec-7-enoate. This is a new phytosterol ester isolated from the natural source for first time.
Table 4.3. $^1$H and $^{13}$C NMR values of intybusteryl palmitoleoate (CI-3)

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### Chapter-4

**Isolation & Characterization**

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Coupling constants in Hertz were given in parenthesis.

Spectrum 4.5. $^1$H NMR spectrum of intybusteryl palmitoleate (CI-3).

Spectrum 4.6. $^{13}$C NMR spectrum of intybusteryl palmitoleate (CI-3).
Scheme 4.3. Mass fragmentation pattern of intybusteryl palmitolate (CI-3).

Compound CI-4 designated as intybusteric acid A, was obtained as a colourless crystalline mass from petroleum ether : chloroform (1:3) eluants. It responded positively to Liebermann-Burchard test for sterols and yielded effervescences with NaHCO₃ solution. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3442 cm⁻¹), carboxyl group (1703 cm⁻¹) and unsaturation (1650 cm⁻¹). On the basis of $^{13}$C and +ve ion FAB mass spectra, the molecular weight at m/z 442 consistent with the steroidal carboxylic acid, C₂₉H₄₆O₃ was established. It had seven degrees of unsaturation;

The 1H NMR spectrum of compound CI-4 exhibited a one-proton doublet at δ 5.36 (J = 5.2 Hz) and two one-proton double-doublets at δ 5.14 (J = 6.8, 5.7 Hz) and 5.05 (J = 4.8, 6.8 Hz) assigned to vinylic H-6, H-22 and H-23 proton, respectively. A one-proton broad multiplet at δ 3.53 with half width of 18.3 Hz was ascribed to α-oriented H-3 carbinol proton. Two three-proton broad signals at δ 0.69 and 1.01 were attributed to tertiary C-18 and C-19 methyl protons. Two three-proton doublets at δ 0.93 (J = 6.1 Hz) and 0.84 (J = 6.3 Hz) were attributed to C-21 and C-27 secondary methyl protons. A one-proton triplet at δ 0.80 (J = 6.1 Hz) was assigned to the C-29 primary methyl protons. The remaining methylene and methine protons appeared between δ 2.71-1.18. The presence of all methyl signals in the range δ 1.01- 0.69 suggested that all these functionalities were located on the saturated carbons.

The 13C NMR spectrum of compound CI-4 exhibited signals for vinylic carbons at δ 140.88 (C-5), 121.67 (C-6), 138.24 (C-22) and 129.48 (C-23) and the carboxylic carbon at δ 177.03 (C-26). The C-3 carbinol signal appeared at δ 71.82 and carbon signal in the upfield region at 11.89, 12.09, 18.83, 19.38, 20.98 and 21.17 were associated with the methyl functionalities.

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The $^1$H and $^{13}$C NMR spectral data compound CI-4 were compared with related steroidal constituent β-sitosterol, stigme-4-en-3-one (Greca et al., 1990), stigmasterol (Akihisa et al., 1988), lawsaritol (Gupta et al., 1992) and other 24-ethylcholestene derivatives (Ali, 2001).

On the basis of spectral data analysis and chemical reactions the structure of compound CI-4 has been established as stigmaster-5, 22-dien-3β-ol-26-oic acid. This is a new phytosterol isolated from a natural or synthetic source for the first time.

Table 4.4. $^1$H and $^{13}$C NMR values of intybusterolic acid A (CI-4)

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Coupling constants in Hertz were provided in parenthesis.
Spectrum 4.7. $^1$H NMR spectrum of intybusteroic acid A (CI-4).

Spectrum 4.8. $^{13}$C NMR spectrum of intybusteroic acid A (CI-4).
Scheme 4.4a. Mass fragmentation pattern of intybusteroic acid A (CI-4)
Isolation & Characterization

Compound CI-5 designated as intybusteroic acid B, was obtained as a colourless crystalline mass from chloroform eluants. It responded positively to Liebermann-Burchard test for sterols and gave effervescences with sodium bicarbonate solution. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3427 cm$^{-1}$), carboxyl group (1701 cm$^{-1}$) and unsaturation (1646 cm$^{-1}$). On the basis of $^{13}$C NMR and mass spectra, the molecular weight of CI-5 was established at m/z 442 corresponding to a steroidal molecular formula, C$_{29}$H$_{46}$O$_{3}$. It had seven double bond equivalents; four of them were adjusted to tetracyclic carbon framework of steroid, two in the vinylic linkages and remaining one in the carboxylic function. The fragmentation pattern of CI-5 was
similar to intybusteroic acid A, supporting the presence of one hydroxyl group in ring A at C-3, two vinylic linkages one each in steroidal nucleus and one in the side chain and one carboxylic group in the side chain. The ion fragments at m/z 398 [M – CO₂]⁺, 383[398 – Me]⁺, 273[M – C₁₀H₁₇O₂, side chain]⁺ and base peak at m/z 83 [CH(C₂H₅)CHMe₂, C₆H₁₁]⁺ indicated the presence of the carboxylic group at C-21 (Scheme 4.5a and b).

¹H NMR spectrum of compound CI-5 exhibited a one-proton broad multiplet at δ 3.53 with half-width of 18.1 Hz was ascribed to α-oriented H-3 carbinol proton. A one-proton doublet at δ 5.35 (J = 5.2 Hz) and two one-proton double-doublet at δ 5.13 (J = 6.7, 5.5 Hz) and 5.04 (J = 4.9, 6.7 Hz) were assigned to vinylic H-6, H-22 and H-23 protons, respectively. Two three-proton broad signals at δ 0.69 and 1.01 were attributed to tertiary C-18 and C-19 methyl protons. Three doublets at δ 0.87 (J = 6.3 Hz), 0.85 (J = 6.2 Hz) and 0.81 (J = 6.1 Hz) were attributed to secondary C-26 and C-27 and primary C-29 methyl protons, respectively. The remaining methylene and methine protons appeared between δ 2.31-1.10. The presence of all methyl signals in the range 1.01-0.69 suggested that all these functionalities were located on the saturated carbons.

The ¹³C NMR spectrum of compound CI-5 exhibited signals for vinylic carbon at δ 140.12 (C-5), 121.73 (C-6), 138.30 (C-22), 129.30 (C-23) and for carboxylic carbon at δ 179.29 (C-21). The C-3 carbinol signal appeared at δ 71.85. The carbon signals in the upfield region at 12.04, 14.09, 18.99, 19.38 and 21.08 were associated with methyl functionalities.

The ¹H NMR and ¹³C NMR spectrum data compound CI-5 were compared with related steroidal constituent particularly β-sitosterol, stigmast-4-en-3-one (Greca et al., 1990), stigmasterol (Akihisa et al., 1988), lawsaritol (Gupta et al., 1992) and other 24-ethylcholestene derivatives (Ali, 2001). On the basis of these evidences, the structure of compound CI-5 has been established as stigmasta-5, 22-dien-3β-ol-21-oic acid. This is a new phytosterol isolated from a natural or synthetic source for the first time.
### Table 4.5. $^1$H NMR and $^{13}$C NMR values of intybuseroic acid B (CI-S)

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<tr>
<td>26</td>
<td>0.87 d (6.3)</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>0.85 d (6.2)</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>1.25 brs</td>
<td>1.29 brs</td>
</tr>
<tr>
<td>29</td>
<td>0.81 d (6.1)</td>
<td>-</td>
</tr>
</tbody>
</table>

Coupling constant in Hertz were given in parenthesis.
Spectrum 4.9. $^1$H NMR spectrum of intybsteroic acid B (CI-5).

Spectrum 4.10. $^{13}$C NMR spectrum of intybsteroic acid B (CI-5).
Scheme 4.5a. Mass fragmentation pattern of intybusteroic acid B (Cl-S).
Scheme 4.5b. Mass fragmentation pattern of intybusteroic acid B (CI-5).

Compound CI-6 named stigmasterol glycoside, was obtained as a colourless amorphous powder from chloroform : methanol (99:1) eluants. It responded positively to steroidal glycosides and its IR exhibited characteristic absorption bands for hydroxyl group (3421, 3390 cm\(^{-1}\)) and unsaturation (1640 cm\(^{-1}\)). The +ve FAB mass spectra of CI-6 displayed a molecular ion peak at m/z 574 consistent with the molecular formula C\(_{35}\)H\(_{58}\)O\(_6\), it indicated seven double bond equivalents; four of them were adjusted in the tetracyclic carbon framework of the sterol, two in the vinylic linkages and one in the sugar moiety.
The \(^1H\) NMR spectrum of CI-6 displayed three one-proton signals at \(\delta\) 5.32 (m), 5.29 (m) and 5.02 (m) assigned correspondingly to vinylic H-6, H-22 and H-23 protons. Another one-proton broad signal at \(\delta\) 4.88 was attributed to anomeric H-1' proton. A one-proton broad multiplet at \(\delta\) 3.63 with half-width of 16.5 Hz was accounted H-3α-carbinol proton. The remaining sugar proton resonated between \(\delta\) 4.44-3.04. Two three-proton broad signals at \(\delta\) 1.22 and 0.65 were attributed to C-19 and C-18 tertiary methyl protons, respectively. Four three-proton doublet at \(\delta\) 0.95 (J = 6.1 Hz), 0.89 (J = 6.3 Hz), 0.80 (J = 6.3 Hz) and 0.78 (J = 6.2 Hz) were assigned correspondingly to secondary Me-21, Me-26, Me-29 and primary Me-27 methyl protons. All the methyl protons resonated in the range \(\delta\) 1.22-0.65 indicating that these functionalities were present on saturated carbinols.

Further evidences in support of proposed structure were drawn from its \(^{13}C\) NMR spectrum that exhibited signals for 35 carbons. The important signal appeared at \(\delta\) 73.48 for carbinol C-3 carbon and at \(\delta\) 140.41, 121.21, 138.3 and 128.84 for vinylic C-5, C-6, C-22 and C-23 carbons. The anomeric C-1' carbon appeared at \(\delta\) 100.83, the remaining sugar carbons resonated between \(\delta\) 76.77-61.60. The \(^{13}C\) NMR data were compared with other stigmastene type molecules (Greca et al., 1990; Gupta et al., 1992; Ali, 2001).

Acid hydrolysis of CI-6 yielded D-glucose and stigmasterol (TLC comparable). On the basis of the spectral data analyses and chemical reactions, the structure of CI-6 has elucidated as stigmaster-5, 22-dien-3β-ol-3β-D-glucopyranoside.

![Stigmaster-5, 22-dien-3β-ol-3β-D-glucopyranoside (CI-6).](image)
Compound CI-7 a mixture triglyceride phosphate, was obtained as colourless mass from chloroform : methanol (99:1) eluants. It decolourised bromine water and formed soap on saponification. Its IR spectrum displayed characteristic absorption bands for ester group (1731 cm⁻¹), unsaturation (1629 cm⁻¹) and long aliphatic chain (720 cm⁻¹). The mass spectrum of CI-7 showed a molecular ion peak at m/z 699 corresponding to molecular formula of diglyceride phosphate, C₃₉H₇₃O₈P. The ion fragments were generated at m/z 619[M–PO₄H₂]⁺, 267[CO(CH₂)₉CH₃]⁺, 263[CO(CH₂)₉CH=CH–CH₂CH=CH(CH₂)₄CH₃], 604 [619 – Me]⁺ and 576 [604 – CH₂CH₂]⁺. The formation of intensified ion at m/z 263 in comparision to m/z 267 indicated location of linoleic function at C-2 (Scheme 4.6).

Its ^1H NMR spectrum exhibited two multiplet at δ 5.13 and 5.34 both integrated for two protons each, assigned to vinylic proton H-9", H-13", H-10" and H-12". The methylene proton adjacent to vinylic linkages H₂-8", H₂-11" and H₂-14" appeared at δ 1.66, 2.03 and 1.64 each integrated for two-protons, respectively. A one-proton multiplet at δ 4.26 was ascribed to oxygenated H-2 methine proton. Four one-proton doublets at δ 3.91 (J = 6.6 Hz), 3.89 (J = 6.6 Hz), 3.71 (J = 12.9 Hz) and 3.67 (J = 12.9 Hz) were attributed to oxygenated methylene protons H₂-3 and H₂-1, respectively. Two one-proton doublets at δ 2.33 (J = 7.2 Hz) and 2.30 (J = 7.8 Hz) and a two-proton broad signals at δ 2.19 were attributed to methylene H₂-2' and H₂-2" protons, adjacent to the ester groups. Two three-proton triplets at δ 0.88 (J = 6.2 Hz) and 0.85 (J = 6.1 Hz) were assigned to Me-18' and Me-18" terminal primary methyl protons, respectively. The remaining methylene protons appeared as a 46-proton broad signal at δ 1.26.

The ^13C NMR spectrum of compound CI-7 exhibited signals for vinylic carbon at δ 130.37 (C-9"), 129.97 (C-10"), 128.86 (C-12") and 115.45(C-13"). The C-1' and C-1" ester signals appeared at δ 176.81 and 175.32, respectively. The oxygenated methylene carbons and methine carbons resonated at δ 69.43 (C-3), 65.01 (C-2) and 62.07 (C-1), the remaining carbon signal appeared in the range at δ 55.95 to 21.05. The carbon signal in the upfield region at δ 18.29 (C-18") and 14.05 (C-18") were associated with methyl functionalities.

Alkaline hydrolysis of CI-7 yielded a mixture of stearic and linoleic acids. On the basis of above mentioned discussion the structure of CI-7 has been identified as glyceryl-1-octadecanoyl-2-octadec-9",12"-dienoyl-3-phosphate.
Chapter 4

Isolation & Characterization

Spectrum 4.11. $^1$H NMR spectrum of triglyceride phosphate (CI-7).

Spectrum 4.12. $^{13}$C NMR spectrum of triglyceride phosphate (CI-7).
Scheme 4.6. Mass fragmentation pattern of triglyceride phosphate (Cl-7).
Compound CI-8 a mixed diglyceride phosphate was obtained as colourless amorphous mass from chloroform : methanol (49:1) eluants. It decolourised bromine water and formed soap on saponification. Its IR spectrum displayed characteristic absorption bands for ester group (1721, 1718 cm\(^{-1}\)), unsaturation (1627 cm\(^{-1}\)) and long aliphatic chain (715 cm\(^{-1}\)). The mass spectrum of CI-8 showed a molecular ion peak at \(m/z\) 696 corresponding to a molecular formula diglyceride phosphate, \(C_{39}H_{69}O_{9}P\). The generation of an ion peak at \(m/z\) 265 \([\text{CO(CH}_2\text{)}_7\text{CH}=\text{CH(CH}_2\text{)}_7\text{CH}_3]\) in higher intensity in comparison to \(m/z\) 261 \([\text{CO(CH}_2\text{)}_7\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH-CH}_2\text{CH}_3]\) supported the location of oleoyl moiety at C-2 (Scheme 4.7).

Its \(^1\)H NMR spectrum of CI-8 exhibited three multiplets at \(\delta\) 5.34 (3H), 5.31(3H) and 5.11 (2H) assigned to eight vinylic protons. A one-proton multiplet at \(\delta\) 4.17 was ascribed to oxygenated H-2 methine proton. Four one-proton doublets at \(\delta\) 3.92 (J = 12.6 Hz), 3.90 (J = 12.6 Hz) and 3.71 (J = 12.6 Hz) and 3.69 (J = 12.5 Hz) were ascribed to oxygenated methylene H-2-3 and H-2-1 protons, respectively. Four one-proton doublets at \(\delta\) 2.36 (J = 7.2 Hz), 2.34 (J = 7.2 Hz), 2.32 (J = 6.6 Hz) and 2.30 (J = 6.6 Hz) were accounted to methylene H-2'-2'' and H-2-2'' adjacent to the ester groups. Five two-proton multiplets at \(\delta\) 2.77, 2.50, 2.03, 1.75, 1.67 and 1.62 were associated correspondingly with the methylene H-2-14', H-2-11', H-2-8', H-2-17', H-2-8'', and H-2-11'' protons adjacent to the vinylic linkages. Two three-proton triplets at \(\delta\) 0.87 (J = 6.1 Hz) and 0.85 (J = 6.2 Hz) were ascribed to the primary C-18' and C-18'' methyl protons. The remaining methylene protons appeared as a broad signal at \(\delta\) 1.25.

The \(^{13}\)C NMR spectrum of compound CI-8 exhibited signals for vinylic carbon at \(\delta\) 122.11 (C-9'), 125.01 (C-10'), 144.68 (C-12'), 129.99 (C-13'), 127.79 (C-15'), 127.76 (C-16'), 115.55 (C-9") and 113.94 (C-10"). The C-1' and C-1'' ester signals appeared at \(\delta\) 173.26 and 171.68, respectively. The oxygenated methylene carbons C-1, C-3 and methine carbon C-2 resonated correspondingly at \(\delta\) 62.09, 65.11 and 70.26. The signals at \(\delta\) 14.06 and 14.05 were assigned to C-18' and C-18'' primary methyl carbons. The remaining methylene carbons appeared between \(\delta\) 55.96-18.49.

Alkaline hydrolysis of CI-8 yielded oleic and linolenic acids. On the basis of the foregoing account the structure of CI-8 has been identified as glyceryl-1-linolenyl-2-oleoyl-3-phosphate or glyceryl-1-octadec-9',12',15'-trieneoyl-2-octadec-9''-enoyl-3-phosphate.
Chapter 4

Isolation & Characterization

Spectrum 4.13. $^1$H NMR spectrum of diglyceride phosphate (CI-8).

Spectrum 4.14. $^{13}$C NMR spectrum of diglyceride phosphate (CI-8).
Scheme 4.7. Mass fragmentation pattern of diglyceride phosphate (CI-8).

Jamia Hamdard
Compound CI-9 named oleoyl diglucoside, was obtained from chloroform : methanol (48:2) eluants as a colourless crystals. It gave positive tests for glycosides and decolourized bromine water. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3334, 3245 cm\(^{-1}\)), ester group (1730 cm\(^{-1}\)), unsaturation (1618 cm\(^{-1}\)) and long aliphatic chain (721 cm\(^{-1}\)). On the basis of mass and \(^{13}\)C NMR spectra, its molecular weight was established at \(m/z\) 606 consistent with a molecular formula \(C_{30}H_{54}O_{12}\) of a C-18 fatty acid diglycoside. A prominent ion peaks appearing at \(m/z\) 265 \([\text{C}_{17}\text{H}_{33}\text{CO}, \text{CO}–\text{O fission}]^+\) and 281 \([\text{C}_{17}\text{H}_{33}\text{COO}]^+\) indicated that oleic acid was attached to glycone moiety. The ion fragments arising at \(m/z\) 162 \([\text{C}_6\text{H}_{10}\text{O}_2]\) supported the location of the glucose moiety at the terminal side of the glycoside chain (Scheme 4.8).

The \(^1\)H NMR spectrum of CI-9 exhibited one two-proton multiplet at \(\delta\) 5.36 assigned to vinylic H-9 and H-10 protons. Two one-proton doublets at \(\delta\) 5.11 (\(J = 7.1\) Hz) and 5.03 (\(J = 7.2\) Hz) were ascribed to anomic protons H-1' and H-1". One two-proton multiplet at \(\delta\) 4.78 was accounted to carbinol H-5' and H-5" of sugar moieties. Two one-proton doublet at \(\delta\) 4.36 and 4.33, with coupling interaction 6.5 Hz each, were ascribed to carbinol H-2' and H-2" protons, respectively. The remaining sugar protons resonated between \(\delta\) 4.10 - 2.31. Two multiplets at \(\delta\) 2.01 and 1.83, both integrated for two-protons each, were attributed to H-2-8 and H-2-11 methylene protons adjacent to vinylic C-9 and C-10 carbons. A three-proton triplet at \(\delta\) 0.85 (\(J = 6.6\) Hz) was ascribed to terminal C-18 primary methyl protons.

The \(^{13}\)C NMR spectrum of CI-9 displayed important signals for ester carbon at \(\delta\) 175.61 (C-1), vinylic carbon at \(\delta\) 129.64 (C-9) and 121.42 (C-10), methyl carbon at \(\delta\) 13.71 (Me-18) and methylene carbons between \(\delta\) 51.59 - 22.24. The anomic carbons appeared at 101.04, carbinol signals of the sugar residue appeared between \(\delta\) 79.01 - 61.06. The appearance of C-6" in the deshielding region at \(\delta\) 63.47 indicated the location of another sugar at this carbon.

Acid hydrolysis of compound CI-9 yielded oleic acid and \(\beta\)-D glucose (TLC comparable). On the basis of the above mention discussion the structure of CI-9 has been established as octadec-9-enoyl-\(\beta\)-D-glucopyranosyl (1→6)-\(\beta\)-D-glucopyranoside.
Spectrum 4.15. \( ^1H \) NMR spectrum of oleoyl diglucoside (CI-9).

Spectrum 4.16. \( ^{13}C \) NMR spectrum of oleoyl diglucoside (CI-9).
Chapter-4

Isolation & Characterization

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} [M]^+ 606 (5.3)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[
\text{C}_{19}\text{H}_{34}\text{O}_{11} \quad \text{Mass Line} 444 (24.1)
\]

\[
\text{C}_{18}\text{H}_{32}\text{O}_{12} \quad \text{Mass Line} 444 (21.6)
\]

\[*

Scheme 4.8. Mass fragmentation pattern of oleoyl diglucoside (CI-9)

Compound CI-10 named oleoyl glucoside, was obtained from chloroform : methanol (48:2) eluants as a colourless crystals. It decolourized bromine water and gave positive tests for glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3403, 3355 cm\(^{-1}\)), ester group (1736 cm\(^{-1}\)), unsaturation (1647 cm\(^{-1}\)) and long aliphatic chain (717 cm\(^{-1}\)). On the basis of mass and \(^1\)C NMR spectrum its molecular weight was established at \(m/z\) 444 consistent with a molecular formula \(C_{24}H_{44}O_7\) of a C-18 fatty acid glycoside. The ion fragments arising at \(m/z\) 163 [C\(_6\)H\(_{11}\)O\(_3\)] supported the location of the glucose moiety at the terminal side of the oleic acid chain (Scheme 4.9).

The \(^1\)H NMR spectrum of CI-10 exhibited two one-proton multiplets at \(\delta\) 5.39 and 5.33 assigned to vinylc H-9 and H-10 protons. A one-proton doublet at \(\delta\) 4.28 (\(J = 7.1\) Hz) was ascribed to anomeric proton H-1' and a one-proton multiplet at \(\delta\) 4.15 was accounted to carbinol H-5'. The remaining sugar protons resonated between \(\delta\) 3.88 - 3.16. Two one-proton doublets at \(\delta\) 2.44 and 2.33 (\(J = 7.1\) Hz, each) were ascribed to H\(_2\)-2 methylene protons adjacent to ester group. Two multiplets at \(\delta\) 2.02 and 1.82, both integrating for two-protons each, were attributed to H\(_2\)-8 and H\(_2\)-11 methylene protons adjacent to...
vinyl linkage. The remaining methylene protons resonated as a broad singlet at δ 1.26 (20 H). A three-proton triplet at δ 0.88 (J = 6.5 Hz) was ascribed to C-18 primary methyl protons.

The $^1$H NMR spectrum of CI-10 showed important signals for ester carbon at δ 176.63, vinylic carbons at δ 129.65 (C-9) and 127.83 (C-10), methyl carbon at δ 13.61 (Me-18) and methylene carbons between δ 32.17 - 22.18. The hydroxyl methylene carbon appeared at δ 61.12 (C-6') and the remaining carbon of sugar resonated between δ 75.59 - 69.89. The carbon adjacent to ester group appeared at δ 51.95 (C-2), carbon adjacent to the vinylic linkages appeared at δ 34.39 (C-8) and 34.02 (C-11). The $^1$C NMR values of the sugar were compared with $^1$C NMR chemical shift of sugar parts (Agarwal et al., 1985).

Acid hydrolysis of compound CI-10 yielded oleic acid and β-D glucose (TLC comparable). On the basis of the above mention discussion the structure of CI-10 has been established as octadec-9-enoyl-β-D-glucopyranoside.

\[
\begin{align*}
\text{CH}_2\text{--(CH}_2\text{)}_7\text{CH=CH--(CH}_2\text{)}_7\text{CO--O} \\
\text{CH}_2 \quad \text{H} \\
18 \quad 10 \quad 9 \quad 1 \\
\end{align*}
\]

Spectrum 4.17. $^1$H NMR spectrum of oleoyl glucoside (CI-10).
Spectrum 4.18. $^{13}$C NMR spectrum of oleoyl glucoside (CI-10).

Scheme 4.9. Mass fragmentation pattern of oleoyl glucoside (CI-10).
Compound CI-11 named cichoriumone, was obtained as colourless crystalline mass from chloroform : methanol (19:1) eluants. Its IR spectrum displayed characteristic absorption bands for δ-lactone (1739 cm⁻¹), unsaturation (1621 cm⁻¹) and long aliphatic chain (717 cm⁻¹). The +ve FAB mass spectrum of CI-11, exhibited a molecular ion peak at m/z 602 consistent with the molecular formula C₄₁H₇₈O₂ . A prominent fragment ion peak at m/z 97 (C₅H₇O₂) suggested the presence of a δ-lactone in the aliphatic chain (Scheme 4.10).

The ¹H NMR spectrum of CI-11 displayed a one-proton double-doublet at δ 7.10 (J = 5.6, 6.8 Hz) and a doublet at δ 6.79 (J = 6.8 Hz) assigned to vinylic H-3 and H-2 protons, respectively. A two-proton broad signal at δ 3.72 assigned to oxygenated H₂-5 methylene proton and a one-proton multiplet at δ 2.71 with half-width of 14.5 Hz was ascribed to methine H-4 β proton. A three-proton triplet at δ 0.87 (J = 6.5 Hz) was ascribed to terminal C-41 primary methyl proton and the remaining methylene proton resonated between δ 2.30 - 1.25.

The ¹³C NMR spectrum of CI-11, exhibited important peaks for ester carbon at δ 171.52 (C-1), vinylic carbon at δ 127.58 (C-2) and 113.96 (C-3), oxygenated methylene carbon at δ 68.72 (C-5) and methine δ 50.06 (C-4). The remaining methylene carbons of the aliphatic chain resonated between δ 45.98 - 19.29 and the terminal primary methyl Me-41 carbon appeared at δ 14.24.

Based on the above spectral data the structure of CI-11 was elucidated as 4β -(pent-2-enyrolactone)-hexatriacontane. This is an unknown δ-lactone of a long chain aliphatic compound isolated from a natural source. Earlier malastrone, identified as 2-(pentyrolactone)- undecane was isolated from the leaves of Malvastrum coromandelianum Garcke (Alam et al., 1996).
Spectrum 4.19. $^1$H NMR spectrum of cichoriumone (CI-11).

Spectrum 4.20. $^{13}$C NMR spectrum of cichoriumone (CI-11).
Compound CI-12 named ricinoleoyl glucoside, was obtained from chloroform : methanol (23:2) as a colourless crystalline mass. It decolourized bromine water and gave positive test for glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3445 cm\(^{-1}\)), ester group (1741 cm\(^{-1}\)) and unsaturation (1651 cm\(^{-1}\)). On the basis of mass and \(^{13}\)C NMR spectra, its molecular weight was established at \(m/z\) 460 \([M]^+\) consistent with a molecular formula \(C_{24}H_{44}O_8\) of a C-18 fatty acid glycoside. A prominent ion peaks at \(m/z\) 281 \([\text{CO} - \text{O fission}]^+\) and \(m/z\) 163 \([\text{C}_6\text{H}_11\text{O}_3]\) supported the location of the glucose moiety at the terminal side of the ricinoleic acid chain (Scheme 4.11).

The \(^1\)H NMR spectrum of CI-12 exhibited a two-proton multiplet at \(\delta\) 5.33 assigned to vinylic H-9 and H-10 protons. Two one-proton multiplet at \(\delta\) 4.36 and \(\delta\) 4.10 were ascribed to anomeric H-1' and carbinol H-5' protons, respectively. The remaining sugar protons resonated at \(\delta\) 3.77, 3.35 and 3.33. A one-proton multiplet at \(\delta\) 3.69 assigned to H-12 carbinol proton. Two one-proton doublets at \(\delta\) 2.30 \((J = 7.1\ \text{Hz})\) and 2.28 \((J = 7.1\ \text{Hz})\) were attributed to C-2 methylene protons adjacent to the ester group. A three-proton

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Scheme 4.10. Mass fragmentation pattern of cichoriumone (CI-11)
triplet at $\delta$ 0.85 ($J = 6.6$ Hz) was ascribed to terminal C-18 primary methyl protons. The remaining methylene protons appeared between $\delta$ 2.01-1.26.

The $^{13}$C NMR spectrum of CI-12 displayed important signals for ester carbon at $\delta$ 176.35 (C-1), vinylic carbons at $\delta$ 130.69 (C-9), 129.87 (C-10); methyl carbon at $\delta$ 14.02 (Me-18) and sugar carbons between $\delta$ 73.52-61.40. The anomeric carbon appeared at $\delta$ 103.62 indicating the presence of one sugar moiety in the glycosidic chain. The carbinol carbon appeared at $\delta$ 69.89 (C-12). The $^{13}$C NMR values of the sugar were compared with $^{13}$C NMR chemical shifts of sugar parts (Agarwal et al., 1985).

Acid hydrolysis of compound CI-12 yielded ricinoleic acid and $\beta$-D-glucose (TLC comparable). On the basis of the above discussion the structure of CI-12 has been established as Octadec-9-en-12-ol-1-oyl- $\beta$-D-glucopyranoside.

![NMR spectrum of ricinoleoyl glucoside (CI-12).](image)
Spectrum 4.22. $^{13}$C NMR spectrum of ricinoleoyl glucoside (CI-12).

Scheme 4.11. Mass fragmentation pattern of ricinoleoyl glucoside (CI-12)
Table 4.6. Phytoconstituents isolated from roots of *C. intybus* Linn.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Common name</th>
<th>Polarity</th>
<th>m.p. (°C)</th>
<th>Mol. formula and mol. weight</th>
<th>IUPAC name</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-1</td>
<td>Lupeolyl arabinosyl palmitate*</td>
<td>A:B (3:1)</td>
<td>64-65</td>
<td><strong>C_{31}H_{50}O_{6} 794</strong></td>
<td>Lup-12,20(29)-dien-3 α ol-3α-D arabinofuranosyl-5’hexadecanoate.</td>
</tr>
<tr>
<td>CI-2</td>
<td>Lupeolyl palmitate*</td>
<td>A:B (1:1)</td>
<td>205-207</td>
<td><strong>C_{40}H_{78}O_{2} 662</strong></td>
<td>Lup-12,20(29)-dien-3βolyl hexadecanoate.</td>
</tr>
<tr>
<td>CI-3</td>
<td>Intybusteryl palmitoleate*</td>
<td>A:B (1:3)</td>
<td>89-90</td>
<td><strong>C_{35}H_{78}O_{3} 664</strong></td>
<td>Stigmasta-5,22-dien-3β,7-diol-3-O-hexadec-7enoate.</td>
</tr>
<tr>
<td>CI-4</td>
<td>Intybusteroic acid A*</td>
<td>A:B (1:3)</td>
<td>90-100</td>
<td><strong>C_{29}H_{46}O_{3} 442</strong></td>
<td>Stigmasta-5,22-dien-3β-ol-26-oic acid.</td>
</tr>
<tr>
<td>CI-5</td>
<td>Intybusteroic acid B*</td>
<td>B</td>
<td>60-62</td>
<td><strong>C_{29}H_{46}O_{3} 442</strong></td>
<td>Stigmasta-5,22-dien-3β-ol-21-oic acid.</td>
</tr>
<tr>
<td>CI-6</td>
<td>Stigmasterol glycoside</td>
<td>B:C (99:1)</td>
<td>270-272</td>
<td><strong>C_{35}H_{58}O_{6} 574</strong></td>
<td>Stigmasta-5,22-dien-3β-ol-3β-D-glucopyranoside.</td>
</tr>
<tr>
<td>CI-7</td>
<td>Triglyceride phosphate</td>
<td>B:C (99:1)</td>
<td>58-60</td>
<td><strong>C_{36}H_{73}O_{8} P 699</strong></td>
<td>Glyceril-1-octadecanoyl-2-octadec-9”,12”-dienoyl-3-phosphate.</td>
</tr>
<tr>
<td>CI-8</td>
<td>Diglyceride phosphate</td>
<td>B:C (49:1)</td>
<td>89-90</td>
<td><strong>C_{36}H_{69}O_{8} P 696</strong></td>
<td>Glyceril-1-linolenyl-2-oleoyl-3-phosphate.</td>
</tr>
<tr>
<td>CI-9</td>
<td>Oleoyl diglucoside</td>
<td>B:C (48:2)</td>
<td>56-58</td>
<td><strong>C_{36}H_{54}O_{12} 606</strong></td>
<td>Octadec-9-enoyl-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.</td>
</tr>
<tr>
<td>CI-10</td>
<td>Oleoyl glucoside</td>
<td>B:C (48:2)</td>
<td>113-115</td>
<td><strong>C_{24}H_{44}O_{7} 444</strong></td>
<td>Octadec-9-enoyl-β-D-glucopyranoside.</td>
</tr>
<tr>
<td>CI-11</td>
<td>Cichoriumone</td>
<td>B:C (19:1)</td>
<td>119-120</td>
<td><strong>C_{41}H_{78}O_{2} 602</strong></td>
<td>4β-(Pent-2-enyrolactone)-hexatriacontane.</td>
</tr>
<tr>
<td>CI-12</td>
<td>Ricinoleoyl glucoside</td>
<td>B:C (23:2)</td>
<td>83-85</td>
<td><strong>C_{22}H_{44}O_{6} 460</strong></td>
<td>Octadec-9-en-12-ol-1-oyl-β-D-glucopyranoside.</td>
</tr>
</tbody>
</table>

Where; A = Petroleum ether, B = Chloroform and C = Methanol.

* Reported for the first time.
Chapter-4 Isolation & Characterization

Lupeolyl palmitate (CI-1)

Lup-12,20(29)-dien-3β-ol hexadecanoate (CI-2)

Intybusteryl palmitoleate (CI-3)

Intybusteroic acid A (CI-4)

Intybusteroic acid B (CI-5)

Intybusteroic acid B (CI-5)

Triglyceride phosphate (CI-7)

Diglyceride phosphate (CI-8)

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Oleoyl diglucoside (CI-9)

Oleoyl glucoside (CI-10)

Cichoriumone (CI-11)

Ricinoleoyl glucoside (CI-12)
References


