CHAPTER 2

LYCHNIS CORONARIA L.
2.1 PLANT DESCRIPTION

2.1.1 Family: Caryophyllaceae

2.1.2 Common Name

   English – Mulline pink or Rose champion; Kashmiri – Chatjal.

2.1.3 Part investigated

   Whole plant.

2.1.4 Distribution

   *Lychnis coronaria* Linn. a genus of herbs distributed in the north temperate and
   arctic zones and the mountains of South-America. About 15 species are found in India. *Lychnis coronaria* is a herb and grows abundantly in Kashmir at Gadsar road and dry
   places, Dachigam Rahk, below Gulmarg and wooded hill side at 8000 ft. It is also known
   by the common name of “Rose champion” or “Mullein pink” (Chopra et al, 1986; Bamber, 1916).
2.1.5. Morphology

*Lychnis coronaria* is a white wooly herb, 30 to 75 cm high, with spathulate to oblong-lanceolate leaves. Purplish, flowers on long stalk, calyx 2 to 2.5 cm long conical, inerved. Teeth twisted to the left. Petals 2.5 cm long and more red purple, broadly, inversely heart shaped, with stiff 2 toothed scales at the claw. Capsule almost stalkless, included in the calyx, consisting of five values. Seeds are many, doubly convex, striate and warded (Bamber, 1916; Walthen *et al*., 1992; Anonymous, 1962).

2.1.6. Medicinal Properties

Decoction of the roots has been used in the Spain for liver lung complaints and for infraction of the lymph glands and the mesentery (Chopra *et al*., 1986). The plant extract was found to possess anti-inflammatory properties (Georgieva *et al*., 1982). Hot aqueous extract from the ariel parts of the plant has been used for the treatment of hemorrhoids (Butoescu *et al*., 1987).

2.2. BIOLOGICAL ACTIVITIES

Anti-inflammatory activity

Anti-inflammatory activity has been carried out to study the effect of plant extract of *Lychnis coronaria* L. on inflammatory swellings of the hind paws of white rats (Georgieva *et al*., 1982).

Hemorrhoid treatment

Hot aqueous extract from the ariel parts of *Lychnis coronaria* has been used for the treatment of hemorrhoids. The drug, prepared is patented under Patent no. RO91250, it contains dry matter 25, saponins 3.875, free sugars 4.667 and flavones 0.13 weight/volume (Butoescu *et al*., 1987).

2.3. CHEMICAL CONSTITUENTS

Three compounds have been isolated from the leaves of *L. coronaria* extracted with Butanol. These compound were obtained after separation by thin-layer and 2-dimensional paper chromatography. The three compounds were identified as pinitol,
isoscoparin, and feruloyl glucose by spectral data, hydrolysis, and acetylation. The last two substances were isolated from the plant for the first time (Balabanova et al, 1982).

The presence of two glycosylflavones has been detected by spectral and chemical methods. The structure of glycosyl flavones that have been detected are O-α-L-rhamnosyl derivative and β-D-glucopyranosyl (Balabanova et al, 1981).

Ilert et al. (1972), has reported the presence of 2-methyl butyl amine in Lychnis coronaria for the first time using chromatographic technique as described by Schwartz.

Eleven compounds have been isolated from ethanolic extract of Lychnis coronaria. The eleven known compounds are: tricin 7-O-glucopyranoside, (+) isoscoparin, epoxyactinidionoside, 20R-hydroxyecdysone, ecdysterone, polypoding B, ecdysterone 22-O-β-D-glucopyranoside, stigmast-5-ene-3-one, taraxerol, α-tocopherol and dehydrodiconiferyl alcoholic 4-O-β-D-glucopyranoside (Dai Haofu et al, 2002).

A detailed study was carried out related to the analysis of anthocyanidins and anthocyanins in flower petals of Lychnis senno and its related species in Caryophyllaceae. Petal anthocyanidins were analyzed by high-performance liquid chromatography (HPLC) in Lychnis senno, a traditional ornamental plant conserved in Japan, and its related species. However, L. coronaria, flower color was vivid reddish purple (JHS 9207), and the relative level of peonidin in petals was much higher than cyanidin (Kuwayama et al, 2005).

Qualitative analysis of alcoholic extract of Lychnis coronaria leaves yielded coumarins, saponins and tannins. Coumarins and saponins were obtained in the chloroform and butanol extracts and tannins were separated by polymide sorbent. Heterogeneity of the obtained groups of substances was proven by thin-layer chromatography. Coumarins were separated into 7 fractions on Kieselgel G, the saponins into 3 fractions on cellulose paste, and tannins into 3 fractions on silica gel HF 254. By acid hydrolysis with HCl and paper chromatography glucose was found in saponins and tannins (Balabanova et al, 1973).

The presence of free lysine, arginine, aspartic acid, alanine, proline, tyrosine, valine, serine, glycine, cysteine and glutamic acid, was detected by paper chromatography. Also, glucose, galactose, mannose, xylose, arabinose and uronic acids were found (Balabanova et al. 1971).
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Lychnis coronaria

Chemical constituents of Lychnis coronaria

Isoscoparin

Dehydrodiconiferyl alcohol 4-O-beta-D-glucopyranoside

Epoxyactinidionoside
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Polypodine B

Taraxerol

Pinitol

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Tricin 7-O-glucopyranoside

\[
\text{I} = (R = H; O-\alpha-L\text{-rhamnosyl glycosyl flavone})
\]

\[
\text{II} = (R = \beta-D\text{-glucopyranosyl glycosyl flavone})
\]
Ecdysterone-22-O-β-D-glucopyranoside

Ecdysterone
2.4. EXPERIMENTAL
2.4.1. Materials and Methods

General

- All chemicals and reagents were obtained from s.d fine chemicals Ltd. Mumbai and were of analytical reagents (AR) grade.
- Silica gel (60-120) mesh and silica gel-G, obtained from s.d fine chemicals Ltd. Mumbai were used for column chromatography and thin layer chromatography respectively.
- M.P was determined on Perfit m.p. apparatus and were uncorrected.
- Ultra violet (UV) spectra were recorded on Beckman DU-64 Spectrophotometer in Methanol and Chloroform.
- Infra red (IR) spectra were recorded on Hitachi-270.
- $^1$H NMR spectra were recorded on Bruker DRX-400 (400 MHz FT-NMR) using, CDCl$_3$ and DMSO-$d_6$ as solvent and TMS as internal standard. Chemical shifts are given in $\delta$ (ppm) scale with tetramethylsilane (TMS) and coupling constant ($J$ values) are expressed in Hz. The spin coupled pattern is designed as: s-singlet, d- doublet, dd- doublet, ddd- triple doublet, t- triplet, q- quatret, m- multiplet and brs- unresolved broad singlet.
- $^{13}$C NMR spectra were recorded on DRX-400 (400 MHz FT-NMR) with TMS as internal standard in 5mm spinning tubes at 27°C.
- Mass spectra (MS) were scanned by effecting Electron Impact (EI) ionization at 70 eV on a JEOL-JMS-DX 300 and FAB on JEOL SX 120/DA-6000 instrument equipped with direct inlet probe system. The m/z values of only intense peaks have been mentioned.
- Spots were visualized by exposure to iodine vapours and UV radiations.

2.4.2. Collection of material

The whole plant of *Lychnis coronaria* L. was collected from local areas of Aharbal, Srinagar (J&K) and authenticated by taxonomist Prof. A. R. Naqshi (Dept. of...
Botany, University of Kashmir, Srinagar, India). The voucher specimen (LC-FP-17) of the plant has been kept in the herbarium of Jamia Hamdard for future reference.

2.4.3. Preparation of plant material for extraction

The plant material (3.5 kg) was dried and crushed to coarse powder and then successively extracted with petroleum ether (60-80 °C), chloroform and methanol using cold percolation method till completely exhausted. The different extracts were dried under reduced pressure to get the crude dried extracts of petroleum ether, chloroform and methanol 65.0, 90.0, and 180.0 g respectively.

2.4.4. Column chromatography of Methanol fraction.

The methanol fraction (170 gm) obtained from the plant was dissolved in little methanol and adsorbed on the silica gel (60-120 mesh) for the preparation of slurry. It was then dried, packed on the top of silica gel column packed in petroleum ether. The column was then eluted with petroleum ether, chloroform and methanol successively in the order of increasing polarity to isolate the following compounds (Table No. 4).

Table No. 4: Compounds isolated from the whole plant of *Lychnis coronaria*.

<table>
<thead>
<tr>
<th>Code</th>
<th>Compound Name</th>
<th>m.p. (°C)</th>
<th>Mol. Formula</th>
<th>Mol. Wt.</th>
<th>IUPAC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-1</td>
<td>β-Stigmasterol glucoside</td>
<td>255-257</td>
<td>C_{35}H_{59}O_{6}</td>
<td>574</td>
<td>Stigmast-5-en-3-O-β-D-glucopyranoside</td>
</tr>
<tr>
<td>LC-2</td>
<td>Aliphatic alcohol</td>
<td>56-58</td>
<td>C_{28}H_{58}O</td>
<td>413</td>
<td>n-Octacosanol</td>
</tr>
<tr>
<td>LC-3</td>
<td>Aliphatic alcohol</td>
<td>70-72</td>
<td>C_{19}H_{34}O_{2}</td>
<td>294</td>
<td>Nonadecan-4,10-diene, 6-one, 1-ol.</td>
</tr>
<tr>
<td>LC-4</td>
<td>Aliphatic alcohol</td>
<td>62-64</td>
<td>C_{29}H_{60}O</td>
<td>423</td>
<td>n-Nonacosanol</td>
</tr>
<tr>
<td>LC-5</td>
<td>Aliphatic alcohol</td>
<td>67-69</td>
<td>C_{40}H_{82}O</td>
<td>579</td>
<td>n-Tetracontanol</td>
</tr>
</tbody>
</table>
Compound LC-1

Elution of column with Petroleum ether-CHCl₃ (60:40) yielded compound LC-1 as white crystals recrystallised from CHCl₃-MeOH (1:1), 30mg.

R<sub>f</sub>: 0.53 (CHCl₃-Pet. Ether, 6:4)

m.p: 255-257°C.

IR (KBr): ν<sub>max</sub> 3433 (OH), 2960 (CH₃), 2850 (CH₂), 1463, 1062 (C=O), 810 (C=C), 728, 719 cm<sup>-1</sup>

1D and 2D NMR (CDCl₃): Table No. 5

UV: λ<sub>max</sub> 244, 204 (sh) nm.

EIMS (probe) 70 eV, m/z % (rel. int): 574 (M<sup>+</sup> C₃₅H₆₉O₆) (30), 301 (20), 367 (22), 255 (25), 255 (11), 177 (15), 163 (26), 159 (18).

β-Stigmasterol glucoside (LC-1)
# Table No. 5: 1D and 2D NMR spectral data of Compound LC-1.

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H NMR</th>
<th>$^1$C NMR / HMOC</th>
<th>$^1$H$-^1$H COSY</th>
<th>DEPT</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.93 $dd$ (16.0, 9.5, 5.3)</td>
<td>36.6 $t$</td>
<td>H-1b, H-2</td>
<td>CH &lt;sub&gt;2&lt;/sub&gt;</td>
<td>C-2, C-10</td>
</tr>
<tr>
<td>1b</td>
<td>1.63 $dd$ (16.0, 8.5, 4.5)</td>
<td>-</td>
<td>H-1b, H-2</td>
<td>-</td>
<td>C-2, C-10</td>
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<tr>
<td>2a</td>
<td>1.43 $dd$ (15.0, 8.5, 4.5)</td>
<td>29.6 $t$</td>
<td>H-2b, H-3, H-&lt;sub&gt;2&lt;/sub&gt;1</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C-1, C-3</td>
</tr>
<tr>
<td>2b</td>
<td>1.75 $m$</td>
<td>-</td>
<td>H-2a, H-3, H-&lt;sub&gt;2&lt;/sub&gt;1</td>
<td>-</td>
<td>C-1, C-3</td>
</tr>
<tr>
<td>3a</td>
<td>3.08 $ddd$ (5.2, 4.4, 5.6, 8.8) W/ω=16Hz</td>
<td>78.2 $d$</td>
<td>H-2-2, H-4</td>
<td>CH</td>
<td>C-2, C-4</td>
</tr>
<tr>
<td>4a</td>
<td>2.02 $d$ (11.5, 12.5)</td>
<td>35.9 $t$</td>
<td>H-4b, H-3</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C-3, C-5</td>
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<tr>
<td>4b</td>
<td>2.07 $ddd$ (12.5, 6.5, 5.0)</td>
<td>-</td>
<td>H-4a, H-3</td>
<td>-</td>
<td>C-3, C-5</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>140.8 $s$</td>
<td>-</td>
<td>C</td>
<td>-</td>
</tr>
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<td>6</td>
<td>5.31 $t$ (6.0)</td>
<td>121.7 $d$</td>
<td>H-&lt;sub&gt;2&lt;/sub&gt;7</td>
<td>CH</td>
<td>C-5, C-7</td>
</tr>
<tr>
<td>7a</td>
<td>1.62 $m$</td>
<td>28.2 $t$</td>
<td>H-7b, H-6</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C-6, C-8</td>
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<tr>
<td>7b</td>
<td>1.05 $m$</td>
<td>-</td>
<td>H-7a, H-8</td>
<td>-</td>
<td>C-6, C-8</td>
</tr>
<tr>
<td>8</td>
<td>0.95 $m$</td>
<td>56.6 $d$</td>
<td>H-7a, H-9</td>
<td>CH</td>
<td>C-9, C-7</td>
</tr>
<tr>
<td>9</td>
<td>0.73 $m$</td>
<td>45.5 $d$</td>
<td>H-8, H-&lt;sub&gt;2&lt;/sub&gt;11</td>
<td>CH</td>
<td>C-8, C-10, C-11</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>35.9 $s$</td>
<td>-</td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>11a</td>
<td>1.26 $ddd$ (15.0, 9.5, 4.0)</td>
<td>21.06 $t$</td>
<td>H-9, H-11b, H-&lt;sub&gt;12&lt;/sub&gt;12</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C-9, C-12</td>
</tr>
<tr>
<td>11b</td>
<td>1.27 $ddd$ (15.0, 6.5, 5.0)</td>
<td>-</td>
<td>H-9, H-11a, H-&lt;sub&gt;12&lt;/sub&gt;12</td>
<td>-</td>
<td>C-9, C-12</td>
</tr>
<tr>
<td>12a</td>
<td>1.78 $m$</td>
<td>31.7 $t$</td>
<td>H-&lt;sub&gt;2&lt;/sub&gt;11</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C-11, C-13</td>
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<tr>
<td>12b</td>
<td>1.82 $m$</td>
<td>-</td>
<td>H-&lt;sub&gt;2&lt;/sub&gt;11</td>
<td>-</td>
<td>C-11, C-13</td>
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<tr>
<td>13</td>
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<td>42.3 $s$</td>
<td>-</td>
<td>C</td>
<td>-</td>
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<td>14</td>
<td>0.84 $m$</td>
<td>55.7 $d$</td>
<td>H-8, H-&lt;sub&gt;15&lt;/sub&gt;</td>
<td>CH</td>
<td>C-8, C-15, C-13</td>
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<tr>
<td>15a</td>
<td>1.79 m</td>
<td>33.7 t</td>
<td>H₂-16, H-14, H-15a</td>
<td>CH₂</td>
<td>C-14, C-16</td>
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<tr>
<td>15b</td>
<td>1.82 m</td>
<td>-</td>
<td>H-14, H-15a, H₂-16</td>
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<td>C-14, C-17</td>
</tr>
<tr>
<td>16a</td>
<td>0.94 dddd (13.0, 6.5, 3.5)</td>
<td>39.9 t</td>
<td>H-16b, H-17, H₂-15</td>
<td>CH₂</td>
<td>C-15, C-17</td>
</tr>
<tr>
<td>16b</td>
<td>1.82 m</td>
<td>-</td>
<td>H-16b, H-17</td>
<td>-</td>
<td>C-15, C-17</td>
</tr>
<tr>
<td>17</td>
<td>2.09 dddd (9.0, 8.5)</td>
<td>39.4 d</td>
<td>H₂-16, H-20</td>
<td>CH</td>
<td>C-20, C-13</td>
</tr>
<tr>
<td>18</td>
<td>0.65 s</td>
<td>12.13 q</td>
<td>-</td>
<td>CH₃</td>
<td>C-13, C-17</td>
</tr>
<tr>
<td>19</td>
<td>0.98 s</td>
<td>19.0 q</td>
<td>-</td>
<td>CH₃</td>
<td>C-10, C-9</td>
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<tr>
<td>20</td>
<td>1.13 m</td>
<td>29.7 d</td>
<td>H-17, Me-21, H₂-22</td>
<td>CH</td>
<td>Me-21, C-17, C-22</td>
</tr>
<tr>
<td>21</td>
<td>0.90 d (5.2)</td>
<td>19.3 q</td>
<td>H-20</td>
<td>CH₃</td>
<td>C-20</td>
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<tr>
<td>22</td>
<td>5.075 dd (8.8, 8.4)</td>
<td>117.2 d</td>
<td>H-23, H-20</td>
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<td>C-20, C-23</td>
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<tr>
<td>23</td>
<td>4.93 dd (8.8, 8.4)</td>
<td>128.2 d</td>
<td>H-22, H-23</td>
<td>CH</td>
<td>C-22, C-24</td>
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<tr>
<td>24</td>
<td>0.76 m</td>
<td>45.9 d</td>
<td>H₂-25, H₂-23, H₂-28</td>
<td>CH</td>
<td>C-25, C-28</td>
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<tr>
<td>25</td>
<td>1.46 septet (W½=24.5)</td>
<td>29.1 d</td>
<td>H-24, Me-26, Me-27</td>
<td>CH</td>
<td>C-24, Me-26, Me-27</td>
</tr>
<tr>
<td>26</td>
<td>0.78 d (6.0)</td>
<td>20.1 q</td>
<td>H-25</td>
<td>CH₃</td>
<td>C-25</td>
</tr>
<tr>
<td>27</td>
<td>0.82 d (6.0)</td>
<td>19.5 q</td>
<td>H-25</td>
<td>CH₃</td>
<td>C-25</td>
</tr>
<tr>
<td>28a</td>
<td>1.03 dddd (14.0, 10.5, 8.0)</td>
<td>23.0 t</td>
<td>H-24, Me-29, Me-24b</td>
<td>CH₂</td>
<td>C-24, C-29</td>
</tr>
<tr>
<td>28b</td>
<td>1.08 dddd (14.0, 7.5, 10.5)</td>
<td>-</td>
<td>H-24, Me-29</td>
<td>-</td>
<td>C-24, C-29</td>
</tr>
<tr>
<td>29</td>
<td>0.99 d (6.0)</td>
<td>19.5 q</td>
<td>H-28</td>
<td>CH₃</td>
<td>C-28</td>
</tr>
<tr>
<td>1'</td>
<td>4.93 d (6.4) β-linkage anomic proton</td>
<td>101.2 d</td>
<td>H-2'</td>
<td>CH</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>4.206 dd (5.6)</td>
<td>73.91 d</td>
<td>H-3'</td>
<td>CH</td>
<td>H-3', H-1'</td>
</tr>
<tr>
<td>3'</td>
<td>4.48 dd (5.2, 5.1)</td>
<td>77.19 d</td>
<td>H-4'</td>
<td>CH</td>
<td>-</td>
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<tr>
<td>4'</td>
<td>4.90 dd (5.6, 5.2)</td>
<td>77.37 d</td>
<td>H-5'</td>
<td>CH</td>
<td>H-3'</td>
</tr>
<tr>
<td>5'</td>
<td>3.64 brm</td>
<td>70.52 d</td>
<td>H-4'</td>
<td>CH</td>
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<tr>
<td>6a'</td>
<td>2.89 dd (8.4, 4.8)</td>
<td>61.52 t</td>
<td>H-6'b</td>
<td>CH₂</td>
<td>-</td>
</tr>
<tr>
<td>6b'</td>
<td>2.91 dd (8.4, 4.8)</td>
<td>-</td>
<td>H-6'b</td>
<td>-</td>
<td>-</td>
</tr>
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</table>
Result and Discussion

Compound LC-1

Compound LC-1, stigmasterol-β-glucoside, was obtained as white crystals exhibiting a molecular formula C_{35}H_{59}O_{6} as established on the basis of mass spectrum (M+574), $^{13}$C NMR and DEPT spectra. It gave a positive Libermann-Burchard test and Molisch's test indicating it to be a steroidal glycoside. The IR spectrum indicated the presence of hydroxyl group (3388 cm$^{-1}$), ether linkage (1164 cm$^{-1}$) and a double bond (1637, 970 cm$^{-1}$). The $^{13}$C NMR and DEPT spectra (Pegg et al., 1982) showed 35 carbon atoms for the molecule consisting of six methyls, ten methylenes, eleven methines, one olefinic, three quaternary, one anomeric and one carbinolic carbon atoms (in total C_{35}H_{59}). The assignments of proton and carbon atoms were made with the help of $^1$H-$^1$H COSY and HMQC experiments (Nakanishi et al., 1990) starting with easily distinguishable olefinic and carbinolic protons. The carbinolic proton in the $^1$H NMR spectrum appeared at $\delta_H = 3.08$ (dddd, J = 5.1, 4.4, 5.6, 8.8 Hz, $1/2$ w = 16, $\delta_C = 78.2$) attributable to position-3 biogenetically, and on the basis of fragmentation pattern of its mass spectrum displaying prominent peaks at m/z 163, 177 and 231 (Scheme 2a). The small coupling constants of the carbinolic proton indicated the α-orientation of the proton and β-orientation of the hydroxyl oxygen atom (Silverstein et al., 1981). The carbinolic proton exhibited correlation in $^1$H-$^1$H COSY spectrum with two methylene groups at $\delta_H = 1.43, 1.75$ ($\delta_C = 29.6$) assignable at position-2 and $\delta_H = 2.02, 2.09$ ($\delta_C = 35.8$) assignable at position-4. The CH$_2$-2, in turn, correlated with other methylene protons at $\delta_H = 0.93, 1.63$ ($\delta_C = 36.5$) that could be assigned to position-1. These positions were further substantiated by long range couplings in HMBC spectrum, wherein H-3 correlated with C-2, C-4, C-5 ($\delta_C = 140.8$) and C-1' ($\delta_C = 101.2$, anomeric carbon atom of glucose unit); CH$_2$-2 correlated with C-1 and C-3; CH$_2$-1 with C-2, C-3, C-10 ($\delta_C = 35.9$), and Me-19 ($\delta_C = 19.08$) substantiating the structure of the ring-A. The olefinic proton at $\delta_H = 5.31$ ($\delta, J = 6.0$Hz, $\delta_C = 121.7$) assignable to position-6 showed correlations in $^1$H-$^1$H COSY spectrum with a methylene group at $\delta_H = 1.62, 1.05$ ($\delta_C = 28.2$), which was attributable to position 7, which in turn, correlated with a methine proton at position-8 ($\delta_H = 0.95, \delta_C = 56.6$). H-8 displayed correlations with H-9 ($\delta_H = 0.73, \delta_C = 45.5$); and consequently H-9
Fig. 1. Significant heteronuclear multiple bond correlations (HMBC) for β-Stigmasterol glucoside (LC-1).
Mass scheme 2a for compound LC-1: C_{35}H_{59}O_{6} (M^{+} 574)
with a methylene group $\delta_H = 1.26, 1.27$ ($\delta_C = 21.0$) that could be assigned at position-11. The long range coupling in HMBC spectrum further confirmed the above assignments (Table No. 5). The methylene protons at position-11 showed correlations in the $^1$H-$^1$H COSY spectrum with other methylene protons at $\delta_H = 1.78, 1.82$ ($\delta_C = 31.7$), which were attributable to position-12. The HMBC spectrum showed long range correlations of $H_{2-11}$ with C-9, C-12, C-8 and C-13, while $H_{2-12}$ displayed with C-11, C-13 ($\delta_C = 42.3$), C-14 ($\delta_C = 55.7$) and Me-18 ($\delta_C = 12.13$). In the same way, CH-14 showed correlations in the $^1$H-$^1$H COSY spectrum with CH$_2$-15 with ($\delta_H = 1.78, 1.82$, $\delta_C = 33.8$); CH$_2$-15 with CH$_2$-16 ($\delta_H = 0.94, 1.82$, $\delta_C = 39.9$); while CH$_2$-16 showed correlations with CH-17 ($\delta_H = 2.09$, $\delta_C = 39.4$). The methine proton at position-17 displayed correlations in the $^1$H-$^1$H COSY spectrum with another methine proton at ($\delta_H = 1.13$, $\delta_C = 29.7$) attributable to position-20, which in turn, showed correlation with a methyl group at $\delta_H = 0.90$ ($d, J = 5.2$Hz, $\delta_C = 19.3$) that could be assigned at position-21.

Hence the presence of 5-ethyl-6-methyl-hept-3-ene-y 1 side chain was assigned at C-17 and was further confirmed with the help of the $^1$H-$^1$H COSY and HMBC spectra. The CH-20 showed a correlation with an olefinic proton at $\delta_H = 4.93$, ($\delta_C = 117.2$) in $^1$H-$^1$H COSY spectrum attributable to position-22, which in turn, displayed correlation with another olefinic proton at $\delta_H = 5.07$, $\delta_C = 128.2$ assignable at position-23. The CH-23 exhibited correlation with a methine proton at $\delta_H = 0.76$, ($\delta_C = 45.9$) to be placed at position-24. The long coupling in HMBC spectrum showed correlation of H-20 with Me-21, C-22 and C-17; H-22 with C-20, C-17 and C-23; while H-23 showed with C-24, C-25, C-28 and C-22, which substantiate further the proposed assignments of the side chain. The $^1$H-$^1$H COSY spectrum displayed correlations of H-24 with a methylene proton at $\delta_H = 1.03, 1.08$ ($\delta_C = 23.0$) assignable to proton-28; and with a methine proton at $\delta_H = 1.49$ ($\delta_C = 29.1$) attributable to position-25. The H$_2$-28, in turn, showed correlations with a methyl group at $\delta_H = 0.99$ ($\delta_C = 19.5$) placeable at position-29; whereas, methine -25 displayed correlations with two methyl groups at $\delta_H = 0.78$ ($\delta_C = 20.1$) and $\delta_H = 0.82$ ($\delta_C = 19.5$), which could be placed at positions 26 and 27 respectively. The coupling pattern and coupling constants of CH-25 (septet, $w \frac{1}{2} 24.5$), CH$_3$-26 ($d, J = 6.0$Hz) and CH$_3$-27 ($d, J = 6.0$Hz) indicated the presence of an isopropyl group. The long-range coupling in HMBC spectrum showed correlation of H-24 with C-23, C-28, C-29 and C-25; H-25 with
C-24, C-28, C-26 and C-27; while CH$_3$-29 displayed correlation with C-28 and C-24. In the same way CH$_3$-26 showed long-range correlation with C-25 and C-27; whereas CH$_3$-27 displayed with C-25 and C-26.

The carbinolic proton at position-3 of the sterol moiety displayed long-range coupling in HMBC spectrum with another carbinolic anomeric proton at $\delta_H = 4.93$ (d, $J = 6.4$Hz, $\delta_C = 101.2$) assignable at position-1' of the glucose unit. The high coupling constant of H-1' indicated that the sugar was $\beta$-glucose. The sequential assignments of the resonances for the glucose residue were made with COSY and HMQC experiments (Fig. 1), whereupon the HMBC spectrum showed correlation of H-3 of the aglycone with C-1' of glucose unit indicating it to be linked with the hydroxyl group at position-3 and linked through an ether linkage ($\nu_{max} 1165$ cm$^{-1}$). Other correlations in COSY and HMBC spectra were also clearly indicative of the proposed structure. In (Table No. 5; Fig. 1), wherein H-1' ($\delta_H = 4.93, \delta_C = 101.2$) showed correlation in COSY spectrum with H-2' ($\delta_H = 4.20, \delta_C = 73.9$); H-2' with H-3' ($\delta_H = 4.48, \delta_C = 77.1$); H-3' with H-4' ($\delta_H = 4.90, \delta_C = 77.3$); H-4' with H-5' ($\delta_H = 3.64, \delta_C = 70.52$); H-5' with a methylene group assignable at position H-6' ($\delta_H = 2.89, 2.91, \delta_C = 61.52$) of the glucose unit. The long-range coupling in HMBC spectrum also supported the structure of glucose moiety which displayed correlation of H-1' with C-3 and C-2'; H-2' with C-1', C-3' and C-4'; whereas H-3' exhibited coupling with C-4', C-2' and C-5'; while H-5' with C-4' and C-6'. The methylene group at position-6' showed also correlations with C-5' and C-4' substantiating the structure of glucose unit.

The high value of coupling constant ($J = 16$Hz) of the carbinolic proton at position-3 indicated its $\alpha$-orientation and, consequently $\beta$-orientation of the oxygen atom of the ether linkage. The chemical-shift of proton and carbon atoms were compared with literature (Agarwal, 1992; Seo et al, 1978) indicating it to be a $\beta$-glucose, which was further substantiated by co-paper chromatography of the sugar obtained on hydrolysis of LC-1 with an authentic sample of glucose using aniline-phthalate as visualizing reagent.

The structure of compound LC-1 was also supported by the fragmentation pattern of its mass spectrum, which displayed prominent peaks at $m/z$ 574 due to molecular ion peak, $m/z$ 412 due to elimination of glucose unit, $m/z$ 397 due to elimination of CH$_3$. $m/z$
369 due to elimination of isopropyl group splitting at 24(25), m/z 301 due to splitting at 22(23), m/z 231 (frag.-a) due to an ion produced by rupture of ring-C by a-a', m/z 177 (frag.-c) due to an ion produced by rupture of ring-C by c-c', m/z 163 due to an ion produced by rupture of ring-C by b-b', m/z 255 due to ion produced by elimination of side chain at position-20. Other peaks in the spectrum were also supportive of the proposed structure of the compound LC-1.

Based on the above chemical and spectral studies the compound LC-1 was characterized as stigmast-5-en-3-O-β-D-glucopyranoside, and identified as β-stigmasterol.
Compound LC-2

Elution of column with Petroleum ether-CHCl₃ (60:40) yielded white flakes of 1-Octacosanol (LC-2), 20mg.

Rₚ: 0.67 (CHCl₃-MeOH : 8:2)
m.p: 56-58°C.

IR (KBr): νmax 3398 (OH), 2916 (CH₃), 2848 (CH₂), 1473, 1060 (C-O) 728, 719 cm⁻¹

¹H NMR (CDCl₃): δ 0.82 (3 H, m, CH₃-28), 1.18 (5 H, brs, 26 x CH₂-), 3.56 (2 H, dd, J = 6.0H, -CH₂-OH).

UV: λmax 241, 265, 276 (sh) nm.

EIMS (probe) 70 eV, m/z % (rel. int): 413(M)+ (C₂₈H₅₀O), 10%, 393.2 (100%), (71.9), 103.1 (100).

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\begin{align*}
\text{CH}_3(\text{CH}_2)_{26}\text{CH}_2\text{OH} \\
\text{Aliphatic alcohol (LC-2)}
\end{align*}
\]
Compound LC-2

Compound LC-2, an aliphatic alcohol, was obtained as white flakes from CHCl₃ eluants. It did not give color test for steroids and terpenes indicating it to be a fatty alcohol. Its IR showed absorption bands for hydroxyl group (3398 cm⁻¹), methyl group (2916 cm⁻¹) and long aliphatic chain (782, 719 cm⁻¹). It did not respond to bromine water test for unsaturation. Its Mass spectrum displayed a molecular ion peak at m/z 413 consistent with molecular formula of an aliphatic alcohol C₂₈H₅₈O.

The ¹H NMR spectrum of LC-2 showed two one-proton doublet at δ 3.56 (J=6.0Hz) assigned to oxygenated methylene protons of C-1. The methylene protons appeared as 52-proton broad signal at δ 1.18. A three-proton multiplet at δ 0.82 was assigned to Me-28 terminal primary methyl protons. A single proton of hydroxyl group of C-1 appeared at δ 7.2. The ¹³C NMR spectrum of LC-2 exhibited important signals for oxygenated methylene carbons at C-1 at δ 63.11 and a primary methyl carbon C-28 at δ 14.12. All the methylene carbons resonate between δ 32.83-22.70. On the basis of above discussion the structure of LC-2 has been elucidated as n-octacosanol.
Compound LC-3

Elution of column with Petroleum ether-CHCl₃ (95:05) yielded (LC-3), a white amorphous powder, 84mg

Rf: 0.61 (Pet. Ether-CHCl₃, 8:2)

m.p: 70-72°C.

IR (KBr): vₘₐₓ 3469 (OH), 2919 (CH₃), 2850 (CH₂), 1734 (C=O), 802 (C-C), 1022 (C-O), 861, 720 cm⁻¹.

¹H NMR (CDCl₃): δ 0.81 (3 H, m, J = 6.0 Hz, CH₃-19), 1.18 (24 H, brs, 7 x CH₂-12-18, 3 x CH₂-7-9, 2 x CH₂-2-3 ), 3.55 (2 H, m, J = 6.0 Hz, -CH₂-OH), 5.28 (1 H, ddd, J = 6.0 Hz, =CH-4), 5.09 (1 H, ddd, J = 6.0 Hz, =CH-5). 4.24 (1 H, ddd, J = 6.0 Hz, =CH-10). 4.09 (1 H, ddd, J = 6.0 Hz, =CH-11).

UV: λₘₐₓ 233, 236nm.

EIMS (probe) 70 eV, m/z % (rel. int): 294 (M⁺, C₁₉H₃₄O₂) (10), 274 (26), 181 (16), 155 (27), 113 (11).

Aliphatic alcohol

\[
\text{CH}_3-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_2-\text{CH}=\text{CH}-(\text{CH}_2)_2-\text{CH}_2\text{OH}
\]
Result and discussion

Compound LC-3

The compound LC-3 obtained as white amorphous powder, had molecular formula $C_{19}H_{34}O_2$ as determined on the basis of Mass spectra ($M^+ 294$) and $^{13}$C NMR spectrum. It did not give color test for steroids and terpenes indicating it to be a fatty alcohol. The IR spectrum exhibited absorption bands of hydroxyl group (3469 cm$^{-1}$), methyl group (2919 cm$^{-1}$), methylene groups (2850 cm$^{-1}$), carbonyl group (1743 cm$^{-1}$), olefinic linkage (820 cm$^{-1}$) and C-O alcoholic groups (1022 cm$^{-1}$). The $^1$H NMR spectrum exhibited peaks at $\delta$ 0.81 (3 H, $d$, $J = 6.0$ Hz, $CH_3$-19) indicating one methyl group in the molecule. The $^1$H NMR spectrum also displayed a broad peak at $\delta$ 1.18 (24 H, brs, 7 x $CH_2$-12-18, 3 x $CH_2$-7-9, 2 x $CH_2$-2-3) due to twelve methylene groups, which could be assigned at C-12 to C-18, C-7 to C-9, C-2 to 3. The peaks at $\delta$ 4.09 (1 H, ddd, $J = 6.0$ Hz, $>CH$-11) and $\delta$ 4.24 (1 H, ddd, $J = 6.0$ Hz, $>CH$-10) indicated the presence of an olefinic linkage between C-10 and C-11. The peaks at $\delta$ 5.09 (1 H, ddd, $J = 6.0$ Hz, $>CH$-5) and $\delta$ 5.28 (1 H, ddd, $J = 6.0$ Hz, $>CH$-4) indicated the presence of another olefinic linkage between C-5 and C-4. The peak at $\delta$ 3.55 (2 H, $m$, $J = 6.0$ Hz, $-CH_2$-OH-1) was assigned to the alcoholic methylene group at positioned C-1. The structure of the compound was also further confirmed on the basis of mass fragmentation pattern, which exhibited prominent peaks at $m/z$ 294 due to molecular ion peak, $m/z$ 279 due to elimination methyl group, $m/z$ 181 due to further elimination of seven $CH_2$ groups, $m/z$ 155 due to further elimination of olefinic linkage at C-10, $m/z$ 113 due to further elimination of three $CH_2$ groups. Peaks at $m/z$ 85 due to elimination of carbonyl group, $m/z$ 59 due to further elimination of olefinic linkage, $m/z$ 31 due to elimination of two methyl groups. The peak at $m/z$ 31 due to elimination of terminal $-CH_2$-OH was also obtained. The position of one hydroxyl group was assigned at C-1 on the basis of above mass spectrum fragmentation pattern. Thus on the basis of these findings the structure of LC-3 was established as nonadecan-4,10-diene, 6-one, 1-ol.
Mass scheme 2b of Compound LC-3: $\text{C}_{19}\text{H}_{34}\text{O}_2 (M^+ 294)$
Compound LC-4

Elution of column with Petroleum ether-CHCl₃ (65:35) yielded compound LC-4, a yellow buff coloured amorphous solid, 38mg.

R<sub>r</sub>: 0.60 (CHCl₃-pet. Ether, 7:3)
m.p: 62-64°C.

IR (KBr): ν<sub>max</sub> 3398 (OH), 2916 (CH₃), 2848 (CH₂), 1470, 1462 (C-O), 1061, 729, 719 cm<sup>-1</sup>

¹H NMR (CDCl₃): δ 0.80 (3 H, m, CH₃-29), 1.25 (54 H, brs, 27 x CH₂-28), 3.32 (2 H, dd, J = 6.0 Hz, -CH₂-OH).

UV: λ<sub>max</sub> 243, 275 (sh) nm.

EIMS (probe) 70 eV, m/z % (rel. int): 423[M]<sup>+</sup> (C<sub>29</sub>H₆₀O), (36.6%).

\[
\text{CH}_3(\text{CH}_2)_{27}\text{CH}_2\text{OH}
\]

Aliphatic alcohol
Results and Discussion

Compound LC-4

Compound LC-4, an aliphatic alcohol, was obtained as buff yellow amorphous powder from CHCl₃ eluants. Its IR showed absorption bands at 3398 (OH), 2916 (CH₃), 2848 (CH₂) and long aliphatic chain at 729, 719 cm⁻¹. It did not respond to bromine water test for unsaturation. Its Mass spectrum displayed a molecular ion peak at m/z 423 consistent with molecular formula of an aliphatic alcohol C₂₉H₆₀O.

The ¹H NMR spectrum of LC-4 showed one-proton doublet at δ 3.32 (J=6.0 Hz) assigned to oxygenated methylene protons of C-1. The methylene protons appeared as 54-proton broad signal at δ 1.25. A three-proton multiplet at δ 0.82 was assigned to Me-29 terminal primary methyl protons. A single proton of hydroxyl group of C-1 appeared at δ 7.1. The ¹³C NMR spectrum of LC-4 exhibited important signals for oxygenated methylene carbons at C-1 at δ 63.10 and a primary methyl carbon C-29 at δ 14.12. All the methylene carbons resonate between δ 32.81-22.69. On the basis of above discussion the structure of LC-4 has been elucidated as n-nonacosanol.
Compound LC-5
Elution of column with Petroleum ether-CHCl₃ (75:25) yielded compound LC-8, a yellow buff coloured amorphous powder, 42mg.

Rₚ: 0.59 (CHCl₃-Pet.ether, 8:2)
m.p: 67-69°C.

IR (KBr): vₘₐₓ 3450 (OH), 2917 (CH₃), 2849 (CH₂), 1470, 956 (C-O), 729, 720 cm⁻¹

¹H NMR (CDCl₃): δ 1.10 (3 H, m, CH₃), 1.16 (76 H, brs, 38 x CH₂-2-39), 3.82 (2 H, dd, J = 6.0 Hz, -CH₂-OH).

UV: λₘₐₓ 275 (sh), 242 nm.

EIMS (probe) 70 eV, m/z % (rel. int): 579 [M]+ (C₄₀H₈₂O), (36.6%).

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\begin{align*}
\text{CH}_3(\text{CH}_2)_{38}\text{CH}_2\text{OH}
\end{align*}
\]

Aliphatic alcohol
Compound LC-S, an aliphatic alcohol, was obtained as buff yellow amorphous powder from CHCl₃ eluants. Its IR showed absorption bands at 3450 (OH), 2917 (CH₃), 2849 (CH₂) and long aliphatic chain at 729, 720 cm⁻¹. It did not respond to bromine water test for unsaturation. Its Mass spectrum displayed a molecular ion peak at m/z 579 consistent with molecular formula of an aliphatic alcohol C₄₀H₈₂O.

The ¹H NMR spectrum of LC-S showed one-proton doublet at δ 3.82 (J=6.0 Hz) assigned to oxygenated methylene protons of C-1. The methylene protons appeared as 76-proton broad signal at δ 1.16. A three-proton multiplet at δ 1.1 was assigned to Me-40 terminal primary methyl protons. A single proton of hydroxyl group of C-1 appeared at δ 8.5. The ¹³C NMR spectrum of LC-S exhibited important signals for oxygenated methylene carbons at C-1 at δ 65.73 and a primary methyl carbon C-40 at δ 15.45. All the methylene carbons resonate between δ 35.73-24.01. On the basis of above discussion the structure of LC-S has been elucidated as n-tetracontanol.