CHAPTER V

ISOLATION AND STUDY OF A TRI-TERPEHOIDAL SAPONIN (LUPANOL-3-O-β-D-GLUCOPYRANOSYL (1→5)-O-B-D-XYLOPYRANOSIDE) FROM THE ROOTS OF STREBLUS ASPER (LOUR).
Streblus asper (lour)* belongs to natural order Moraceae and is commonly known as 'Siora' in hindi.

The plant is distributed in the Himalaya from Himachal Pradesh to West Bengal and in the hills and plains of Assam and Tripura.

A detailed description of the plant and its medicinal values have been given in Chapter II.

The roots of Streblus asper (lour) were extracted with petroleum ether and the defatted roots extracted with 50% aqueous ethanol followed by 95% ethanol. (The study of 50% ethanolic extract has been reported in Chapter II of the thesis.)

ISOLATION OF THE SAPONIN:

The 95% aqueous ethanolic extract was concentrated under reduced pressure to a dark brown viscous mass, and successively extracted with benzene, chloroform, acetone and methanol and the solvents removed under reduced pressure. The benzene, chloroform and acetone soluble parts on removal of the solvent resulted in very small amount of viscous residues which were insufficient for any substantiative study.

* References about this plant have already been given on page 93 Chapter II of the thesis.
The methanol soluble part on concentration under reduced pressure yielded a brown viscous mass which on addition of excess of solvent ether gave a precipitate which on TLC examination over silica gel G using solvent system n-butanol:acetic acid:water (4:1:5) showed two spots indicating it to be a mixture of two compounds.

The precipitate was therefore subjected to column chromatography over silica gel G and eluted with acetone : methanol in different proportions.

The fraction (12-20) of acetone:methanol (4:1) obtained (as described in the Chapter IV, page 142 of the thesis) were of same Rf values and so mixed and on removal of the solvent yielded an amorphous mass (0.09%). The homogeneous nature of the mass was confirmed by TLC examination when only one spot was noticed. It was crystallised from pyridine, m.p. 170-72° and analysed for molecular formula C_{41}H_{70}O_{10}. M^+ = 722.

It was soluble in methanol, and absolute alcohol, but insoluble in petroleum ether, and benzene and responded to following characteristic tests of saponin^1-3.

(i)  Honey comb foam test
(ii) Haemolyses of the mammal blood,
(iii) Pink colour with acetic anhydride and pyridine.
IR SPECTRUM OF THE SAPONIN:

The significant peaks obtained in the IR spectrum (Fig. 1) of the saponin and the structural units inferred with the help of available literature\(^4\) is recorded in Table I.

**Table I**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3340</td>
<td>-OH</td>
</tr>
<tr>
<td>2</td>
<td>2966-2856</td>
<td>-CH(_2)-CH(_2) stretching</td>
</tr>
<tr>
<td>3</td>
<td>1445</td>
<td>-CH(_3)</td>
</tr>
<tr>
<td>4</td>
<td>1375,1195, 1110</td>
<td>Triterpenoidal nature</td>
</tr>
<tr>
<td>5</td>
<td>860</td>
<td>Cyclohexane ring</td>
</tr>
</tbody>
</table>

A peak at \[^\text{KBr}\]_{\text{max}} 2966 cm\(^{-1}\) in IR spectrum of the saponin indicated the presence of -CH\(_3\) group(s). The number of -CH\(_3\) groups were estimated by Zeisel method (10.38%) when it showed the presence of five -CH\(_3\) groups.

Another peak at \[^\text{KBr}\]_{\text{max}} 3340 cm\(^{-1}\) in the IR spectrum of the saponin indicated the presence of -OH group(s). The number of -OH group(s) were estimated by acetylation with Ac\(_2\)O/pyridine to yield an acetylated product, (m.p 195-96°C)
and molecular formula $\text{C}_{53}\text{H}_{82}\text{O}_{16}$. The percentage of the acetyl group in the acetylated product was estimated by the procedure of Weisenberger, as described by Belcher and Godbert, which showed the presence of $\sim$Six $\sim$OH groups.

The structure of the saponin was elucidated by its hydrolysis and studying separately the sapogenin and sugar hydrolysate. The saponin was therefore hydrolysed by 7\% sulphuric acid when the sapogenin precipitated out which was separated by filtration.

**STRUCTURAL STUDY OF SAPOGENIN:**

It analysed for molecular formula $\text{C}_{50}\text{H}_{52}\text{O}_{3}$, m.p. 201-102°C ($\times$)$_2$ -17.8° (in chloroform), $M^+ = 428$.

It responded the following colour reactions, characteristic to triterpenoids; e.g.

(i) A yellow colour changing to red in Salkowski reaction.
(ii) A red violet colour in Liebermann Burchard reaction.
(iii) A violet colour in Tschugajew reaction.

**UV SPECTRUM OF THE SAPOGENIN:**

The wave length of maximum absorbance in the UV spectrum of the sapogenin was at $\lambda_{\text{max}}^{\text{MeOH}} = 208 \text{ nm (Fig.II)}$. 
**IR spectrum of the sapogenin**

The important peaks obtained in the IR spectrum (Fig. iii) of the sapogenin and the structural units inferred with the help of available literature\(^ {11,12} \) is recorded in the Table. II

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak cm(^{-1} )</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3310</td>
<td>-OH</td>
</tr>
<tr>
<td>2</td>
<td>2958-2850</td>
<td>-CH(_3)–CH(_3)</td>
</tr>
<tr>
<td>3</td>
<td>1455</td>
<td>-CH(_3)</td>
</tr>
<tr>
<td>4</td>
<td>1375, 1355, 1335</td>
<td>triterpenoidal</td>
</tr>
<tr>
<td></td>
<td>1300, 1195, 1148</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1110, 1095, 1015</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>984, 875, 860</td>
<td>Cyclohexane ring</td>
</tr>
</tbody>
</table>

A peak at 2958 cm\(^{-1} \) in the IR spectrum of sapogenin showed the presence of -CH\(_3\) group(s). The estimation of methyl group(s) by Seisel method revealed the presence of five angular methyl groups in the sapogenin (17.52%).

Another peak at 3310 cm\(^{-1} \) in the IR spectrum of the sapogenin showed the presence of -OH group(s). The number of -OH group(s) determined by acetylation with Ac\(_2\)O/
pyridine to yield an acetylated product (m.p. 425-26°, molecular formula, C₃₂H₅₄O₂). The percentage of the acetyl group(s) (9.12%) in the acetylated product estimated by the method of Wiesenbergger and described by Belcher and Godbert when it showed the presence of only one \(-\text{OH}\) group in the sapogenin.

The formation of acetyl derivative was further supported by the appearance of the acetyl absorption at 1720 cm\(^{-1}\) and disappearance of the hydroxy absorption in the IR spectrum of the acetylated sapogenin.

The presence of one acetoxy group was also confirmed by the signal at \(\delta=2.09\) in the \(^1\)HNMR spectrum (Fig. 17).

The signal for the methylene proton at \(\delta=4.54\) in the \(^1\)HNMR spectrum further confirmed the nature of the hydroxyl group as secondary.

The sapogenin was oxidized with chromic acid when an oxidation product (m.p. 204-5°, molecular formula C₃₀H₅₀O) was formed which gave positive Zimmermann test for the presence of \(3\)-Keto group\(^{13}\).

This observation confirmed that \(-\text{SH}\) group must be at C-3 position and further indicated that it must be secondary.
The mass spectrum of the sapogenin showed fragments at m/z 414, 371 (loss of $\text{C}_2\text{H}_7$), 257, and 189 which are characteristic of lupane series and confirmed it to be a pentacyclic triterpene, belonging to lupane series$^{14}$.

The fragmentation pattern of major ions at 428($M^+$), 413, 398, 395, 315, 271, 257, 255, 189, 175 further established that -OH group was located in the ring 'A' and hence the structure of the sapogenin was assigned as; I which is a well known compound lupanol$^{15,16}$. 

(I)
The proposed structure of sapogenin was further confirmed by its $^1$H-NMR and mass spectral study.

$^1$H-NMR SPECTRUM OF MONO ACETYL DERIVATIVE OF THE SAPOGENIN:

The $^1$H-NMR spectrum of the mono acetyl derivative of the sapogenin was found to be in complete conformity with the above structure. The significant signals obtained in the $^1$H-NMR spectrum (Fig. iv) of the sapogenin and the structural units inferred with the help of available literature are given below in the Table III.

### Table III

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value (δ)</th>
<th>Nature</th>
<th>$J$ (Hz)</th>
<th>No. of Protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.76</td>
<td>s</td>
<td></td>
<td>3</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>2</td>
<td>0.84</td>
<td>s</td>
<td></td>
<td>3</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>3</td>
<td>0.94</td>
<td>s</td>
<td></td>
<td>3</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>4</td>
<td>0.78</td>
<td>s</td>
<td></td>
<td>6</td>
<td>2 x CH$_3$</td>
</tr>
<tr>
<td>5</td>
<td>1.04</td>
<td>s</td>
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<td>3</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>6</td>
<td>2.09</td>
<td>s</td>
<td></td>
<td>3</td>
<td>3-OAc</td>
</tr>
<tr>
<td>7</td>
<td>4.54</td>
<td>a</td>
<td></td>
<td>1</td>
<td>CHOCH</td>
</tr>
</tbody>
</table>
MASS SPECTRUM OF THE SAPOGENIN:

The important fragmentation pattern obtained in the electron impact mass spectrum of the sapogenin is given below and further confirmed the structure assigned to it.

\[428, 413, 398, 395, 315, 271, 257, 255, 242, 220, 216, 207, 205, 189, 175, 161, 147, 135, 121, 109, 107, 95, 93, 81, 69, 65.\]

STUDY OF THE SAPOGENIN HYDROLYSATE:

The aqueous hydrolysate obtained after separating sapogenin after hydrolysis of saponin was neutralized with BaCO₃ and BaSO₄ filtered off. The filtrate was concentrated to get a yellow viscous mass which was found to reduce Fehling’s solution. It also gave colour with aniline hydrogen phthalate.

The concentrated hydrolysate was therefore, subjected to paper chromatography with authentic sugar samples on Whatmann No. 1 filter paper using aniline hydrogen phthalate as spraying reagent. The analysis revealed the presence of D-xylose and D-glucose as sugar moiety (confirmed by CoPc and Co TLC with authentic sugars).
QUANTITATIVE ESTIMATION OF SUGARS:

Quantitative estimation of sugars present in the saponin by procedure of Mishra and Rao\textsuperscript{20} revealed that two sugars were present in equimolecular ratio (1:1).

Thus it was concluded that one molecule of the saponin was made up of one molecule of sapogenin and one molecule each of D-xylose and D-glucose.

POSITION OF ATTACHMENT OF SUGARS TO THE SAPOGENIN:

Since there is only one hydroxyl group available for glycoside formation in the sapogenin, naturally sugar must be attached on it. Thus concluding that sugar must be attached at C-3.

Thus a tentative structure to the sapogenin was assigned as (II).

\[ R = \text{Sugars (D-xylose, D-glucose)} \]
SEQUENCE OF SUGARS IN THE SAPONIN:

The sequence of sugars was determined by partial hydrolysis of the saponin.

The graded hydrolysis of the saponin with 0.02 N \( \text{H}_2\text{SO}_4 \) for one hour at room temperature liberated first D-glucose followed by D-xylose thereby confirming D-glucose as terminal sugar.

The contents were subjected to column chromatography over silica gel G using chloroform:methanol (1:1) as eluant when a mixture of two prosapogenins were isolated designated as \( \text{SC}_1 \) (m.p. 220-22\(^\circ\) molecular formula \( \text{C}_{35}\text{H}_{55}\text{O}_{5} \)) and \( \text{SC}_2 \) (m.p. 170-72\(^\circ\)C molecular formula \( \text{C}_{41}\text{H}_{70}\text{O}_{10} \)).

STUDY OF THE PROSAPOGENIN \( \text{SC}_1 \):

HYDROLYSIS:

The prosapogenin, \( \text{SC}_1 \), molecular formula \( \text{C}_{35}\text{H}_{55}\text{O}_{5} \) on hydrolysis with 7% \( \text{H}_2\text{SO}_4 \) yielded a sapogenin and D-xylose (confirmed by Co-PC and Co-TLC). The sapogenin was identified as lupanol.

PERMETHYLATION HYDROLYSIS OF \( \text{SC}_1 \):

Permethylation was done by the procedure of Khan et al. The permethylated prosapogenin \( \text{SC}_1 \) on hydrolysis
yielded 2:3:5 tri-o-methyl-D-xylose (confirmed by Co-PC and Co-TLC) thereby indicating that C₁ of D-xylose was involved in the glycosidic linkage and also suggested that D-xylose was present in the furanose form. Thus SC₁ was assigned as Lapanol-3-0-β-D-xylofuranoside (III).

(III)

**STUDY OF PROSAPOGENIN SC₂:**

**HYDROLYSIS:**

The prosapogenin SC₂ molecular formula C₄₁H₇₀O₁₀ on hydrolysis with 75% H₂SO₄ yielded a sapogenin identified as Lapanol and sugars identified as D-glucose and D-xylose (confirmed by Co-PC and Co-TLC).
PERMETHYLATION AND HYDROLYSIS OF $SO_2$

The permethylation of prossapogenin $SO_2$ followed by hydrolysis and chromatographic examination of the hydrolysate showed the presence of 2:3:4:6, tetra-0-methyl-D-glucose and 2:3:di-0-methyl-D-xylose (by Co-PC and Co-THO) indicating that D-xylose was present as furanoside form and D-glucose as D-glucose as pyranoside form.

NATURE OF THE GLYCOSIDIC LINKAGE

Hydrolysis of the saponin with emulsion liberated D-glucose and D-xylose indicating that linkage between sapogenin and D-xylose, as well as between D-xylose and D-glucose was $\beta$.

Thus structure of saponin was assigned as: Lupanol-3-O-$\beta$-D-glucopyranosyl (1→5)-O-$\beta$-D-xylofuranoside. (IV)
Finally the proposed structure for saponin was confirmed by $^1$H-NMR and mass spectral study.

$^1$H-NMR SPECTRUM OF OCTA ACETYL DERIVATIVE OF SAPONIN:

The $^1$H-NMR spectrum of the octa acetyl derivative of the saponin was found to be in complete conformity with above structure. The significant signals obtained in the $^1$H-NMR spectrum (Fig. v) of the saponin and structural units inferred with the help of available literature 25,26 is given below in the table IV.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value (δ)</th>
<th>J (Hz)</th>
<th>Nature</th>
<th>No. of Protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.76</td>
<td>s</td>
<td></td>
<td>3</td>
<td>CH₃</td>
</tr>
<tr>
<td>2</td>
<td>0.78</td>
<td>s</td>
<td></td>
<td>3</td>
<td>CH₃</td>
</tr>
<tr>
<td>3</td>
<td>0.84</td>
<td>s</td>
<td></td>
<td>6</td>
<td>2 x CH₃</td>
</tr>
<tr>
<td>4</td>
<td>0.96</td>
<td>s</td>
<td></td>
<td>3</td>
<td>CH₃</td>
</tr>
<tr>
<td>5</td>
<td>1.05</td>
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<td></td>
<td>3</td>
<td>CH₅</td>
</tr>
<tr>
<td>6</td>
<td>4.54</td>
<td>a</td>
<td></td>
<td>1</td>
<td>1H, CHOH</td>
</tr>
<tr>
<td>7</td>
<td>5.05</td>
<td>a</td>
<td></td>
<td>2</td>
<td>H-1’, H-2’</td>
</tr>
<tr>
<td>8</td>
<td>5.32</td>
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<td>2</td>
<td>H-3’, H-4’</td>
</tr>
<tr>
<td>9</td>
<td>4.53</td>
<td>a</td>
<td></td>
<td>2</td>
<td>H-5’</td>
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<td>3</td>
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<td>11</td>
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</tr>
<tr>
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<td></td>
<td>2</td>
<td>H-6”</td>
</tr>
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<td></td>
<td>6</td>
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</tr>
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<td>6</td>
<td>OAc-4””, 6”</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM OF THE SAPONIN**

The important fragmentation pattern obtained in the electron impact mass spectrum of the saponin is given below:

722 (M⁺), 559, 428, 413, 398, 395, 315, 271, 257, 255, 242, 220, 218, 207, 203, 189, 175, 161, 147, 135, 121, 109, 107, 93, 91, 69, 65

and further supported its structure as luponol-3-O-B-D-glucopyranosyl (1→5)-O-B-D-xylofuranoside (IV)
EXPERIMENTAL

ISOLATION OF SAPONIN:

Eluates (12-20) each of 15 ml of acetone:methanol (4:1) had same Rf values and so were obtained as (described on page No. 192 Chapter IV of the thesis) and on removal of the solvent yielded light cream coloured compound (2.76 gm). The homogeneous nature of the compound was confirmed by TLC examination. It analysed for molecular formula $C_{41}H_{70}O_{10}$, $M^+$ 722, m.p. 170-72°. It responded to following characteristic reactions of saponin.

FOAM TEST:

20 mg. of the crude compound was taken along with 20 ml. of distilled water in a measuring cylinder and shaken well. The formation of one inch soapy layer resulted which remained as such for a very long time.

HAEMOLYSIS TEST:

A suspension of 5.0 gm of gelatin in 100 ml of (0.9%) saline solution was made in a measuring flask and 3.0 ml of blood was taken separately in a conical flask and stirred with a small amount of the above gelatin solution.

This blood was spread on a glass plate and a drop of the extract to be tested for the presence of saponin was
applied with the help of a micropipette.

The red blood gelatin became transparent after about one hour at the place of application of the extract, thus confirming the presence of saponin in the extract.

PREPARATIVE THIN LAYER CHROMATOGRAPHY:

A small amount of the crude saponin was dissolved in methanol and used for TLC purpose. The examination was carried out on (10x10cm) glass plates using silica gel G. as stationary phase and n-butanol:acetic acid:water (4:1:5 v/v) as mobile phase. The chromatographic chamber was saturated with solvent system before starting the experiment. A small quantity of the saponin was placed on the plate one cm above the base, with the help of a micropipette and it was kept in saturated chamber containing the solvent system. When the solvent reached about 3/4 height of the plate it was removed and dried at room temperature and sprayed with 10% sulphuric acid and heated in the oven at about 100°C for two minutes when only one spot was noticed.

STUDY OF SAPONIN:

A light cream coloured compound, m.p. 170-72°, analysed for molecular formula C_{41}H_{70}O_{10}, M^+ = 722
ELE MENTAL ANALYSIS OF THE SAPONIN:

**Found**  
C = 68.10%  
H = 9.66%

**Calculated for C₄₁H₇₀O₁₀**  
C = 68.14%  
H = 9.69%

Molecular weight = 722  
(M⁺) = 722

Molecular formula = C₄₁H₇₀O₁₀

HYDROLYSIS OF THE SAPONIN:

460 mg of the saponin was taken in a 500 ml. of B-14 quick fit round bottomed flask having a reflux condenser attached to it. 100 ml. of 75% H₂SO₄ was added to the flask. The flask was heated on a water bath for two hours and cooled when a crystalline sapogenin precipitated out which was separated by filtration. The aqueous part was separately extracted with solvent ether in a separatory funnel. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Removal of the solvent ether, yielded sapogenin which was crystallised from absolute alcohol, m.p. 201-2°C, (α)D²⁵ = -17.8° (in CHCl₃).

The aqueous layer was neutralised, concentrated and worked up separately for identification of sugars.
ELEMENTAL ANALYSIS OF THE SAPOGENIN:

FIND

CALCULATED FOR \( \text{C}_{30}\text{H}_{52}O \)

\[
\begin{align*}
\text{C} & = 85.98\% \\
\text{H} & = 12.12\% \\
\text{Molecular weight} & = 428 \\
(\text{by mass spectroscopy})
\end{align*}
\]

Molecular formula = \( \text{C}_{30}\text{H}_{52}O \)

COLOUR REACTIONS OF SAPOGENIN:

1. **SAKOWSKI REACTION**:

    Concentrated sulphuric acid when added to a solution of the compound in chloroform, produced a yellow colour changing to red.

2. **LIMBERMANN-BURCHARD REACTION**:

    When few drops of concentrated sulphuric acid was added to the solution of compound in acetic anhydride it produced a red violet colour.

3. **TSCHUGAJEW REACTION**:

    When the solution of the compound in chloroform was mixed with acetyl chloride and boiled, it produced a violet red colour.
PREPARATION OF ACETYL DERIVATIVE OF SAPOGENIN:

70 mg. of the sapogenin was taken in a round bottomed flask and 4.0 ml of acetic anhydrid was added to it and the mixture refluxed on a water bath, cooled, when the crystalline acetyl product was obtained which separated by filtration. It crystallised from ethanol m.p. at 425-26°C, molecular formula C₃₂H₅₃O₂

OXIDATION OF THE SAPOGENIN WITH CHROMIC ACID:

The sapogenin (0.5g) was taken in 10 ml of acetic acid and 2.0 ml water and heated for one hour with (0.1 gm) of chromic acid in 2.0 ml of acetic acid and water (2.0 ml) added dropwise. The contents of the flask were kept at 50°C for 10 minutes, diluted with water and extracted with solvent ether. The ethereal layer on removal of the solvent gave a ketone m.p. 204-5°C, molecular formula C₃₀H₅₀O, and molecular weight M° 426.

IDENTIFICATION OF SUGARS AFTER HYDROLYSIS:

The aqueous layer obtained after the hydrolysis of the saponin was neutralised by the addition of BaCO₃ and BaSO₄ filtered off. The filtrate was subjected to paper chromatography for the identification of sugars and aniline hydrogenphthalate used as a spraying reagent. The observations are recorded in the Table V.
TABLE - V

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent system</th>
<th>Rf Values Recorded</th>
<th>Rf Values Found</th>
<th>Sugar identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Isopropanol:pyridine:water:acetic acid (8:8:4:1 v/v)</td>
<td>0.64</td>
<td>0.69</td>
<td>D-glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.73</td>
<td>0.72</td>
<td>D-xylose</td>
</tr>
<tr>
<td>2.</td>
<td>n-butanol:acetic acid:water (4:1:5 v/v)</td>
<td>0.18</td>
<td>0.17</td>
<td>D-glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.28</td>
<td>0.26</td>
<td>D-xylose</td>
</tr>
<tr>
<td>3.</td>
<td>Water saturated Plx phenol +13NH₄OH</td>
<td>0.39</td>
<td>0.40</td>
<td>D-glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.44</td>
<td>0.41</td>
<td>D-xylose</td>
</tr>
</tbody>
</table>

PARTIAL HYDROLYSIS OF THE SAPONIN:

500 mg. of the saponin was treated with 0.02 NH₂SO₄ (20ml) in 250 ml. round bottomed flask and the reaction mixture kept at room temperature for 6 days and extracted with n-butanol. Thin layer chromatographic examination of butanol extract showed the presence of two compounds. The butanol extract was therefore concentrated and chromatographed over a column of silica gel G. and methanol:chloroform used as eluant, when two substances designated as SC₁, m.p. 220-22° molecular formula C₅₅H₃₅O₁₅ and SC₂ m.p. 170-72°, molecular formula C₄₁H₄₀O₁₀ were obtained which crystallised from methanol.

The details of experiment are recorded in the Table VI and VII.
TABLE VI

<table>
<thead>
<tr>
<th>(i)</th>
<th>Length of the column</th>
<th>=</th>
<th>50.0 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ii)</td>
<td>Weight of the silica gel G</td>
<td>=</td>
<td>10.0 gm</td>
</tr>
<tr>
<td>(iii)</td>
<td>Diameter of the column</td>
<td>=</td>
<td>5.0 cm</td>
</tr>
<tr>
<td>(iv)</td>
<td>Weight of the compound</td>
<td>=</td>
<td>200.0 mg</td>
</tr>
</tbody>
</table>

TABLE VII

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fractions</th>
<th>Eluant</th>
<th>Spot on TLC Plate</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-8</td>
<td>methanol:chloroform (1:1)</td>
<td>One</td>
<td>SC₁</td>
</tr>
<tr>
<td>2</td>
<td>9-11</td>
<td>methanol:chloroform (1:1)</td>
<td>Two</td>
<td>Rejected</td>
</tr>
<tr>
<td>3</td>
<td>12-19</td>
<td>methanol:chloroform (1:3)</td>
<td>One</td>
<td>SC₂</td>
</tr>
</tbody>
</table>

STUDY OF THE PROSAPOGENIN SC₁:

HYDROLYSIS:

The prosapogenin SC₁ (100 mg) was hydrolysed with 7% sulphuric acid (15 ml) for five hours and the precipitate extracted with chloroform. The chloroform extract was concentrated and the amorphous mass crystallised from a mixture of benzene:methanol (2:3) in colourless crystals m.p 201-20°C and identified as Lupanol (by nmp, Co-PC and Co-TLC).
The hydrolysate on chromatographic examination revealed the presence of D-xylose.

**Elemental Analysis of Prosapogenin Sc₁**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for $C_{35}H_{55}O_{5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 75.58%</td>
<td>C = 75.67%</td>
</tr>
<tr>
<td>H = 9.98%</td>
<td>H = 9.99%</td>
</tr>
</tbody>
</table>

Molecular weight = 555 (by mass spectroscopy)

Molecular formula = $C_{35}H_{55}O_{5}$

**Permethylation and Hydrolysis of Prosapogenin Sc₁**

Permethylation was carried out by the procedure of Khun et al. Prosapogenin Sc₁ (30 mg) was treated with methyl iodide (1.0 ml), silver oxide (25 mg) in dimethyl formamide (2.0 ml) for two days at room temperature, and the contents filtered. The residue was taken in chloroform (15 ml), and the chloroform layer was washed with water. A syrupy mass was obtained on removal of solvent. The mass was hydrolysed with Hilliani mixture, when the sapogenin precipitated out on addition of excess of water. The aqueous part was neutralised and chromatographed on Whatmann No. 1 filter paper using n-butanol:acetic acid:water (4:1:5 v/v) as developing solvent for methylated sugars. The methylated sugars were identified as 2:3:5-tri-O-methyl-D-xylose by comparison with the spot of authentic methylated D-xylose on Co-PC and Co-TLC.
STUDY OF PROSAPOGENIN SG₂:

HYDROLYSIS:

Hydrolysis of prosapogenin SG₂ was carried out similarly as that of prosapogenin SG₁. It also showed the presence of Lupanol as sapogenin and the aqueous hydrolysate on paper chromatographic examination revealed the presence of D-xylose and D-glucose (by Co-PC and Co-TLC).

ELEMENTAL ANALYSIS:

FIND: CALCULATED FOR C₄₁H₇₀O₁₀

C = 68.10%  C = 68.14%
H = 9.28%    H = 9.69%

Molecular weight = 722  Molecular weight (M⁺) = 722
(by mass spectroscopy)

Molecular formula = C₄₁H₇₀O₁₀

PERMETHYLATION AND HYDROLYSIS OF PROSAPOGENIN SG₂:

It was done in the same manner as described for prosapogenin SG₁, when it showed the presence of two methylated sugars identified as 2:3:4:1-C-methyl-D-xylose and 2:3:4:1:6 tetra-O-methyl-D-glucose (by Co-PC and Co-TLC)
ENZYMATIC HYDROLYSIS:

The saponin (25 mg) was suspended in an almond emulsion solution (30 mg) and kept at 40°C for 30 hours. The hydrolysate was paper chromatographed over Whatmann No. 1 filter paper with authentic sugar samples. Aniline hydrogen phthalate was used as spraying reagent. Appearance of two spots which corresponding to D-xylose and D-glucose of authentic samples indicated that the nature of linkage was B between sapogenin and D-xylose as well as between D-xylose and D-glucose.
REFERENCES


