CHAPTER V

ISOLATION AND STUDY OF A NOVEL FLAVANOL GLYCOSIDE; TAXIFOLIN 3-O-β-D-GALACTOPYRANOSYL (1→6)-O-β-D-GLUCOPYRANOIDE FROM THE SEEDS OF CROTALARIA PROSTRATA ROTTL.
Crotalaria prostrata Kottl. \(^1\) (N.O. Leguminosae) is commonly found in the drier parts of India, Sri Lanka, Burma and Java.

The plant is said to be useful in derangements of the stomach and infantile diarrhoea. \(^2\)

The detailed description of the plant has already been given in Chapter II on page No. 25 of the thesis.

**ISOLATION OF THE FLAVANOL GLYCOSIDE (Cp)**:

Air-dried, powdered and defatted seeds were extracted exhaustively with 95\% ethanol and the extract was concentrated under reduced pressure to brown viscous mass and separated into water soluble and water insoluble part. The water soluble part was concentrated to a coloured syrupy mass and extracted successively with benzene, chloroform, ethyl acetate, acetone and methanol.

The detailed study of ethyl acetate soluble fraction has already been described in Chapter II of this thesis.

The benzene, chloroform, acetone soluble parts on removal of the solvent gave very negligible amount and hence could not provide for any substantive investigations.

The study of the methanol soluble fraction has been described in this Chapter.
STUDY OF THE METHANOL SOLUBLE FRACTION:

The methanol soluble fraction was concentrated under reduced pressure to yield a brown viscous mass. It gave two spots on TLC examination using ethyl acetate:methanol:water (12:7:2) and I₂ vapours as visualizing agent, which was subjected to column chromatography over silica-gel G (60-120 mesh) and eluted with ethylacetate:methanol in various proportions. On evaporation of the solvent it gave a cream coloured compound (CP) yield (0.056%).

It responded to all the colour reaction for flavanoids and positive Molisch test for glycoside.³,⁴

STUDY OF THE FLAVANOL GLYCOSIDE (CP):

It had molecular formula C₂₇H₃₂O₁₇, m.p. 200-201°C, [α]⁺ 028. It was soluble in ethanol, methanol and water. It crystallised from ethanol.

UV SPECTRUM OF THE GLYCOSIDE (CP):

The wave lengths of maximum absorbance with various shifts of reagents were found at:

(i) Λ max MeOH 280, 325 (sh);
(ii) Λ max NaOMe 240, 330, 375 (sh);
(iii) Λ max AlCl₃ 292, 312, 385 (sh);
(iv) Λ max NaOAc 298, 362 (sh);
(v) $\lambda_{\text{max}}^{\text{NaOAc + H}_3\text{BO}_3} 265, 353$ (sh);

(vi) $\lambda_{\text{max}}^{\text{AlCl}_3 + \text{HCl}} 280, 310, 340, 350$ (sh).

**Infrared Spectrum of the Glycoside (GP):**

In the infrared spectrum (Fig. 1) of the compound the prominent signals observed and structural units inferred with the help of available literature\(^5,6\) are given below:

<table>
<thead>
<tr>
<th>S.N.o.</th>
<th>Peaks cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3348</td>
<td>-OH group(s)</td>
</tr>
<tr>
<td>2</td>
<td>2901</td>
<td>-C-H stretching vibration</td>
</tr>
<tr>
<td>3</td>
<td>1680</td>
<td>C = O</td>
</tr>
<tr>
<td>4</td>
<td>1618</td>
<td>Aromatic Ring system</td>
</tr>
<tr>
<td>5</td>
<td>1282</td>
<td>C-O-C vibration</td>
</tr>
<tr>
<td>6</td>
<td>820</td>
<td>Two adjacent hydrogen atom</td>
</tr>
</tbody>
</table>

**Presence of Hydroxyl Group(s):**

In infrared signals at $\lambda_{\text{max}}^{\text{KBr}} 3348$ cm\(^{-1}\) indicated the presence of hydroxyl groups. On acetylation with $\text{Ac}_2\text{O/Py}$ it gave an acetyl derivative, molecular formula $C_{49}H_{54}O_{28}$, m.p. 184-85°C, $[\text{M}]^+ 1090$. The percentage of acetyl product (30.45%) was estimated by Wiesenberger method\(^7\) as described.
IR SPECTRUM OF THE GLYCOSIDE
by Belcher and Godbert\textsuperscript{8} suggested the presence of eleven hydroxyl group(s) present in the glycoside.

\textbf{ACID HYDROLYSIS OF THE GLYCOSIDE (CP)}:

On acid hydrolysis (7\text\% H\textsubscript{2}SO\textsubscript{4}), of the glycoside gave an aglycone and sugar moiety(ies) which was separated by filtration and studied separately.

\textbf{STUDY OF THE AGLYCON}:

The aglycone crystallized from ethanol gave a white micro needles, which was found to be homogeneous on TLC, gave a single spot (Rf 0.69) using chloroform:methanol:water (6:3:1) and I\textsubscript{2} vapour as visualizing reagent. It had molecular formula \( \text{C}_{15}\text{H}_{12}\text{O}_{7} \), m.p. 238-39\textdegree C, \([M]^+ 304\).

It gave all the characteristic colour reaction of the flavanoids.

\textbf{UV SPECTRUM OF THE AGLYCON}:

The wavelength of maximum absorbance in UV spectrum of the aglycone were found at:

(i) \( \lambda_{\text{max}} \text{MeOH} \) 287, 300 (sh);

(ii) \( \lambda_{\text{max}} \text{NaOAc} \) 305, 324 (sh);

(iii) \( \lambda_{\text{max}} \text{AlCl}_3 \) 274, 360 (sh);

(iv) \( \lambda_{\text{max}} \text{NaOMe} \) 260, 328, 350 (sh);
(v) \[ \text{NaOAc} + \text{H}_3\text{BO}_3 \quad \lambda_{\text{max}} \quad 306, 328 \text{ (sh)}; \]

(vi) \[ \text{AlCl}_3 + \text{HCl} \quad \lambda_{\text{max}} \quad 294, 302, 360 \text{ (sh)}. \]

**In Spectrum of the Aglycone:**

The important signals observed in the In spectrum of the aglycone and structural assignment inferred with the help of available literature\(^9,10\) are recorded in Table-2.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peaks cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3315</td>
<td>(-\text{OH group(s)})</td>
</tr>
<tr>
<td>2</td>
<td>2904</td>
<td>(-\text{C-H-stretching})</td>
</tr>
<tr>
<td>3</td>
<td>1684</td>
<td>(\text{C = O})</td>
</tr>
<tr>
<td>4</td>
<td>1604</td>
<td>(\text{Aromatic ring system})</td>
</tr>
<tr>
<td>5</td>
<td>1282</td>
<td>(\text{C-O-C vibration})</td>
</tr>
<tr>
<td>6</td>
<td>820</td>
<td>(\text{Two adjacent hydrogen atom})</td>
</tr>
</tbody>
</table>

**Presence of Hydroxyl Group(s):**

In In at \(\nu_{\text{KBr}}^{\text{max}} \quad 3315 \text{ cm}^{-1}\) showed the presence of free \(-\text{OH} \) groups in it. On acetylation it gave a acetyl derivative (41.70%). The percentage of acetyl group was estimated by Wiesenerger\(^7\) method as described by Belcher and Godbert\(^8\), indicated the presence of five hydroxyl group(s) in the aglycone. It analysed for molecular formula.
IR SPECTRUM OF THE AGLYCONE
$C_{25}H_{22}O_{12}$, m.p. 225°C, $[M]^+ 514$.

**POSITION OF HYDROXYL GROUP(S):**

On alkaline degradation it gave two compounds identified as phlorogluconol (IIa) (m.m.p. Co-PC and Co-TLC), molecular formula $C_6H_6O_3$, m.p. 117-18°C and $[M]^+ 126$ and another compound was identified as protocatechuic acid (m.m.p. Co-PC and Co-TLC), molecular formula $C_7H_6O_4$, m.p. 198-99°C, $[M]^+ 154$.

![Chemical structure](image)

**POSITION OF THE -OH GROUP AT C-3' AND C-4':**

On KOH degradation the formation of protocatechuic acid indicated the presence of -OH group(s) at C-3' and C-4' in the aglycone.

A bathochromic shift of 50 nm in band I with NaOAc relative to band I with MeOH, a bathochromic shift of 28 nm in band I with NaOAc/H$_3$BO$_3$ relative to band I in MeOH and hypsochromic shift of 35 nm in band I with AlCl$_3$/HCl relative to band I with AlCl$_3$ in the UV spectrum of the
aglycone confirmed the presence of -OH group at C-3' and C-4'.

POSITION OF THE -OH GROUP AT C-5 AND C-7:

(a) Formation of phloroglucinol confirmed the presence of -OH at C-5 and C-7.

(b) A bathochromic shift of 18 nm in band II with NaOH (relative to MeOH) confirmed the presence of -OH group at C-7.

POSITION OF -OH GROUP AT C-3:

A characteristic colour reaction with Zn/HCl and zirconium oxychloride in citric acid further confirmed the presence of -OH group at C-3.

Yellow fluorescence of the aglycone in UV light and the spectral shift with AlCl₃ of 60 nm relative to band I in MeOH suggested a free -OH group at C-3 in the aglycone.

All the above observation indicates that the following structure II could be assigned the aglycone as;

![Diagram](image-url)
Thus the above structure of the aglycone was confirmed by its $^1$H NMR of its acetyl derivative and mass spectral studies.

$^1$H NMR SPECTRUM OF THE ACETYL DERIVATIVE OF THE AGLYCON:

$^1$H NMR of the acetyl derivative of the aglycone and structural units inferred with the help of available literature\textsuperscript{15,16} are tabulated below:

**TABLE: 3**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>$\delta$ Value</th>
<th>Pattern</th>
<th>J Value Hz</th>
<th>No. of Protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.20</td>
<td>d</td>
<td>9.8</td>
<td>1</td>
<td>C-2</td>
</tr>
<tr>
<td>2</td>
<td>4.60</td>
<td>d</td>
<td>9.8</td>
<td>1</td>
<td>C-3</td>
</tr>
<tr>
<td>3</td>
<td>6.74</td>
<td>d</td>
<td>7.2</td>
<td>2</td>
<td>C-2', C-6'</td>
</tr>
<tr>
<td>4</td>
<td>6.82</td>
<td>d</td>
<td>6.8</td>
<td>1</td>
<td>C-5'</td>
</tr>
<tr>
<td>5</td>
<td>2.36</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C3'-OAc</td>
</tr>
<tr>
<td>6</td>
<td>2.34</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C4'-OAc</td>
</tr>
<tr>
<td>7</td>
<td>2.42</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C5-OAc</td>
</tr>
<tr>
<td>8</td>
<td>5.84</td>
<td>d</td>
<td>2.5</td>
<td>1</td>
<td>C-6</td>
</tr>
<tr>
<td>9</td>
<td>4.28</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C7-OAc</td>
</tr>
<tr>
<td>10</td>
<td>5.92</td>
<td>d</td>
<td>2.5</td>
<td>3</td>
<td>C-8</td>
</tr>
<tr>
<td>11</td>
<td>2.40</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C3-OAc</td>
</tr>
</tbody>
</table>

MASS SPECTRUM OF THE AGLYCON:

The different species formed during fragments obtained in the EIMS of the aglycone are:

$[M]^+$ 304 m/e 303, 276, 153, 152, 150, 124.
1H NMR SPECTRUM OF ACETYLATED AGLYCONE

FIG-3
SCHEME I
STUDY OF THE SUGAR MOIETY (IES):

The glycoside was hydrolysed with (7% ethanolic $H_2SO_4$) neutralised with $BaCO_3$ & $BaSO_4$ and was filtered off, and the filtrate was concentrated under vacuum. On iC examination (n-BuOH-HOAc-$H_2O$) (4:1:5) it showed the presence of D-glucose and D-galactose (Co-Pc and Co-TLC) and aniline hydrogen phthalate as visualizing agent.

QUANTITATIVE ESTIMATION OF THE SUGAR:

Quantitative estimation of sugar indicated that both the sugars were present in equimolar ratio in the glycoside.

PERIODATE OXIDATION OF THE GLYCOSIDE (CP):

When treated with sodium meta-periodate, it consumed 3.02 mole of periodate$^{17}$ with liberation of 1.02 mole of formic acid which showed the presence of one molecule of glucose and one molecule of galactose attached to the aglycone, which indicated the presence of sugar as diast-accaside nature and also both the sugar were present in pyranose form.$^{18}$

POSITION OF ATTACHMENT OF THE SUGARS TO THE AGLYcone:

The position of attachment of the sugar to the aglycone was confirmed at C-3 on the basis of the following facts:

1. On KOH degradation and UV spectral data, a bathochromic shift of $AlCl_3$ and $H_3BO_3$ showed the presence of -OH groups at C-3' and C-4' in the aglycone.
2. The presence of \(-\text{OH}\) groups at C-5 and C-7, in both the glycoside and the aglycone was confirmed by UV spectrum data and various shifts as described on page 124 of the thesis.

3. A characteristic colour reaction with Zn/HCl and zirconium oxychloride in citric acid further confirmed the presence of \(-\text{OH}\) group at C-3. Yellow fluorescence of the aglycone in UV light and the spectral shift with AlCl\(_3\) of 60 nm relative to band I in MeOH suggested a free \(-\text{OH}\) group at C-3 in the aglycone. Of course the glycoside did not show this shift thereby concluding that C-3 of the glycoside was involved in the glycosidic linkage.

4. The glycoside was completely hydrolysed with 7% ethanolic sulphuric acid.

On methylation of the glycoside with Me\(_2\)SO\(_4\)/K\(_2\)CO\(_3\) in acetone gave the methyl ester which on hydrolysis with methanolic sulphuric acid gave 7, 5, 3', 4' tetra-O-methyl taxifolin confirmed (Co-PC, Co-TLC) indicated that the hydroxyl group at position C-3 was involved in glycosylation.

**SEQUENCE OF THE SUGAR RESIDUE(S):**

The glycoside on partial hydrolysis with Killiani mixture liberated D-galactose first followed by D-glucose indicating that the terminal sugar D-galactose and D-glucose was attached to the C-3 position of the aglycone.
Partial hydrolysis by Killiani mixture afforded proaglycone (CP-1) and (CP-2) which were separated by column chromatography.

**STUDY OF PROAGLYCONE (CP-1):**

The proaglycone (CP-1) on acid hydrolysis with 7% \( \text{H}_2\text{SO}_4 \) gave an aglycone and D-glucose (by Co-PC and Co-TLC). It analysed for molecular formula \( \text{C}_{21}\text{H}_{22}\text{O}_{12} \), m.p. 220-22°C, [M]\(^+\) 466.

The proaglycone on enzymatic emulsin hydrolysis gave D-glucose and aglycone thereby showed the presence of \( \beta \)-linkage between D-glucose and the aglycone.

**PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE:**

On permethylation followed by acid hydrolysis by Khun\(^{19}\) method gave 2,3,4,6 tetra-0-methyl-D-glucose (by Co-PC and Co-TLC) which confirmed that C-1 of the glucose was attached at C-3 of the aglycone indicating that D-glucose was present in the pyranose form.

Thus the structure of the proaglycone was assigned as; Taxifolin 3-0-\( \beta \)-0-glucopyranoside.
STUDY OF THE PROAGLYCONE (CP-2):

It had molecular formula $C_{27}H_{32}O_{17}$, m.p. 200-201°C, $[\text{M}]^+ 628$. On acid hydrolysis 7% $H_2SO_4$ gave an aglycone (m.m.p. Co-Pc and Co-TLC) and D-glucose and D-galactose (Co-Pc and Co-TLC).

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (CP-2):

Proaglycone (CP-2) on permethylation followed by hydrolysis it yielded 2,3,4,tri-O-methyl-D-glucose and 2,3,4,6 tetra-O-methyl-D-galactose. Both the sugars were present in the pyranose form therefore it showed C6-OH group of the glucose was linked C1-OH of the D-galactose.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE:

The emulsin enzymatic $^2\text{C}$ hydrolysis confirmed the presence of a $\beta$-linkage between the sugars and aglycone.

Thus identified as:

Taxifolin 3-O-$\beta$-D-galactopyranosyl (1→6)-O-$\beta$-D-glucopyranoside.
Thus the above structure was confirmed by $^1$HNMR as spectrum of its acetyl derivative and mass spectral studies.

$^1$HNMR SPECTRUM OF THE ACETYLATED GLYCOSIDE:

In $^1$HNMR spectrum the significant peaks obtained and structural units inferred with the help of available literature are given below:

**TABLE 4**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>$\delta$ value</th>
<th>Pattern</th>
<th>J value Hz</th>
<th>No. of protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.18</td>
<td>d</td>
<td>9.8</td>
<td>1</td>
<td>C-2</td>
</tr>
<tr>
<td>2</td>
<td>4.65</td>
<td>d</td>
<td>9.8</td>
<td>1</td>
<td>C-3</td>
</tr>
<tr>
<td>3</td>
<td>6.74</td>
<td>d</td>
<td>9.0</td>
<td>2</td>
<td>C-2', C-6'</td>
</tr>
<tr>
<td>4</td>
<td>6.82</td>
<td>d</td>
<td>6.8</td>
<td>1</td>
<td>C-5'</td>
</tr>
<tr>
<td>5</td>
<td>2.35</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C3'-OAc</td>
</tr>
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<td>6</td>
<td>2.34</td>
<td>s</td>
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<td>2.40</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C5'-OAc</td>
</tr>
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<td>5.75</td>
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<td>2.5</td>
<td>1</td>
<td>C-6</td>
</tr>
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<td>d</td>
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<td>1</td>
<td>C-8</td>
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<tr>
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<td>s</td>
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<td>3</td>
<td>C3'-OAc</td>
</tr>
<tr>
<td>11</td>
<td>4.28</td>
<td>d</td>
<td>7.0</td>
<td>1</td>
<td>C1'' anomic proton</td>
</tr>
<tr>
<td>12</td>
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<td>-</td>
<td>3</td>
<td>C2''-OAc</td>
</tr>
<tr>
<td>13</td>
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<td>3</td>
<td>C3''-OAc</td>
</tr>
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<td>2.05</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C4''-OAc</td>
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<tr>
<td>15</td>
<td>4.30-4.60</td>
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<td>-</td>
<td>6</td>
<td>Protons of glucose</td>
</tr>
<tr>
<td>16</td>
<td>4.70</td>
<td>d</td>
<td>7.0</td>
<td>1</td>
<td>C1''' anomic proton</td>
</tr>
<tr>
<td>17</td>
<td>2.04</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C2'''-OAc</td>
</tr>
<tr>
<td>18</td>
<td>2.12</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C3'''-OAc</td>
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<td>19</td>
<td>2.07</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C4'''-OAc</td>
</tr>
<tr>
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<td>2.10</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>C6'''-OAc</td>
</tr>
<tr>
<td>21</td>
<td>4.84-5.00</td>
<td>m</td>
<td>-</td>
<td>6</td>
<td>Protons of galactose</td>
</tr>
</tbody>
</table>
MASS SPECTRUM OF THE GLYCOSIDE:

The different species obtained during fragmentation are given below as:

\[ [M]^+ \text{ 628, m/e 465, 449, 304, 303, 276, 153, 152, 124}. \]

The significant species obtained during the fragmentation peaks is shown in scheme-2, and further supported the structure- (IV) as:

Taxifolin 3-O-β-D-galactopyranosyl (1 → 6)-O-β-D-glucopyranoside.
SCHEME II
EXPERIMENTAL

*Crotalaria prostrata* Rottl. was supplied by M/s. United Chemicals and Allied Products, Calcutta (India), and authenticated by the Department of Botany, Doctor Hari Singh Gour University, Sagar. An herbarium specimen (No. XVIII) has been deposited in the room No. 40 of the Chemistry department.

(2 Kg) Air dried, powdered and defatted seeds of *Crotalaria prostrata* Rottl. was extracted exhaustively with 95% EtOH in a 5 litre round bottomed flask attached with water condensor. The extract was concentrated under reduced pressure to a brown viscous mass (130 gm) and separated into water soluble and insoluble part. The water soluble part was successively extracted with benzene, chloroform, ethylacetate acetone and methanol.

The benzene, chloroform, acetone, soluble parts gives small amounts and hence discarded.

The detailed study of ethyl acetate soluble fraction has already been described in Chapter II of this thesis.

STUDY OF THE METHANOL SOLUBLE FRACTION:

The methanol soluble fraction was concentrated under reduced pressure to yield a brown syrupy mass (3.2 gm). On TLC examination showed two spots using ethylacetate:
methanol:water (12:7:2) and I₂ vapours as visualizing agent, which was subjected to column chromatography over silica gel G and eluted with ethyl acetate:methanol in different proportions.

**COLUMN CHROMATOGRAPHY**

Length of column             - 150 cm  
Diameter of the column       - 5.0 cm  
Weight of the crude extract  - 3.2 gm  
Weight of silica gel G (60-120 mesh) - 200 gm.

<table>
<thead>
<tr>
<th>S.No No.</th>
<th>Fraction No.</th>
<th>Eluant</th>
<th>TLC</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - 8</td>
<td>ethylacetate:methanol</td>
<td>Nil</td>
<td>No sticky mass (7 : 1)</td>
</tr>
<tr>
<td>2</td>
<td>9 - 18</td>
<td>ethylacetate:methanol</td>
<td>One Compound (CF) (7 : 3)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19 - 28</td>
<td>ethylacetate:methanol</td>
<td>Nil</td>
<td>No crystalline material</td>
</tr>
</tbody>
</table>

**STUDY OF THE FRACTIONS (9-18):**

The fractions (9-18) were of same RF value and therefore mixed together, and crystallized from ethanol to gave a cream coloured compound (3.0 gm). It showed a single spot on TLC examination using solvent system ethylacetate: methanol:water (7:3:1). It analysed for molecular formula C₂₇H₃₂O₁₇, m.p. 200-210°C, [M]⁺ 628.
STUDY OF THE FLAVANOL GLYCOSIDE:

The flavanol glycoside (CP) (2.6 gm) was a cream colour crystalline compound, soluble in ethanol, and water. It analysed for molecular formula $C_{27}H_{32}O_{17}$, m.p. 200-201°C, $[M]^+ 628$ (EIMS). It responded positive melish test for glycoside and all the colour test for flavanoids.

(i) It gave green colour with FeCl$_3$.
(ii) Pink colour with Na-Hg/HCl.
(iii) Yellow colour with Mg/HCl.

ELEMENTAL ANALYSIS:

<table>
<thead>
<tr>
<th>FOUND</th>
<th>CALCULATED $C_{27}H_{32}O_{17}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 59.53%</td>
<td>C - 59.59%</td>
</tr>
<tr>
<td>H - 5.02%</td>
<td>H - 5.09%</td>
</tr>
</tbody>
</table>

Molecular weight 628 (By mass spectroscopy)

ACETYLATION OF THE COMPOUND:

(250 mg) of the compound, 5 ml acetic anhydride solution and 4 ml of pyridine were taken in 250 ml of round bottomed flask attached with air condensor. The mixture was refluxed on sand bath at 125°C for 8 hrs. After cooling the contents, a white precipitate was obtained, which was separated by solvent ether. The filtrate was washed with sodium bicarbonate. The residue was crystallized from ethanol as a white crystalline needles (70 mg), molecular formula $C_{49}H_{54}O_{28}$, m.p. 184-85°C, $[M]^+ 1090$ (EIMS).
ELEMENTAL ANALYSIS:

FOUND

CALCULATED $C_{49}H_{54}O_{28}$

C - 53.90%  
H - 4.90%

C - 53.94%  
H - 4.95%

Molecular weight 1090 (By mass spectroscopy)

ACID HYDROLYSIS OF THE GLYCOSIDE (GP):

(2.6 gm) of the glycoside with 7% $H_2SO_4$ was taken in 250 ml B-14 ground joint flask and refluxed for 9 hrs. The contents were cooled and transferred to separating funnel and the aqueous part was separated and neutralized with $BaCO_3$, and $BaSO_4$ filtered off.

The non aqueous part was taken in methanol and on removal of the solvent it yielded an aglycone, molecular formula $C_{15}H_{12}O_{7}$, m.p. 238-39°C, [$M]^+$ 304 (EIIMS).

STUDY OF THE AGLYCONE:

The aglycone was crystallized from ethanol. It gave a cream colour solid yield (1.90 gm). It was found to be amorphous in nature. It gave a single spot on TLC examination using chloroform:methanol:water (6:3:1) and $I_2$ vapours as visualizing agent. It was soluble in methanol and water. It had molecular formula $C_{15}H_{12}O_{7}$, m.p. 238-39°C, [$M]^+$ 304 (EIIMS). It gave positive Molisch test for glycoside and characteristic colour reaction for flavanoids.
(i) It gave green colour with FeCl₃.
(ii) Pink colour with Na₂Hg/HCl.
(iii) Yellow colour with Mg/HCl.

**ELEMENAL ANALYSIS:**

<table>
<thead>
<tr>
<th>FOUND</th>
<th>CALCULATED C₁₅H₁₂O₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 59.20%</td>
<td>C - 59.25%</td>
</tr>
<tr>
<td>H - 3.90%</td>
<td>H - 3.91%</td>
</tr>
</tbody>
</table>

Molecular weight 304 (By mass spectroscopy)

**ACETYLCATION OF THE AGLYCONE:**

A similar procedure was repeated of acetylation of aglycone as described on page No. 134 of the thesis.

An acetyl derivative was obtained, had molecular formula C₂₅H₂₂O₁₂, m.p. 225°C, [M⁺] 514.

**ELEMENAL ANALYSIS:**

<table>
<thead>
<tr>
<th>FOUND</th>
<th>CALCULATED C₂₅H₂₂O₁₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 53.90%</td>
<td>C - 53.94%</td>
</tr>
<tr>
<td>H - 4.90%</td>
<td>H - 4.95%</td>
</tr>
</tbody>
</table>

Molecular weight 514 (By mass spectroscopy)

**METHYLATION OF THE AGLYCONE:**

(160 mg) of the aglycone, 2.6 ml anhydrous K₂CO₃ and (100 ml) of dry acetone, 90 ml dimethyl sulphate was taken in a 250 ml round bottomed flask and attached with air condensor and heated on water bath for 8 hrs. On addition of water, the reaction mixture filtered to removed the inorganic salt and acetone evaporated off. A yellow colour solution was obtained which was purified by column chromatography on si-gel G using solvent ether.
On removal of the solvent, it yielded a colourless mass (50 mg). It had molecular formula $C_{20}H_{22}O_7$, m.p. 150-51°C $[\mu]^+ 374$ (EIMS).

**ELEMENTAL ANALYSIS:**

<table>
<thead>
<tr>
<th>FOUND</th>
<th>CALCULATED $C_{20}H_{22}O_7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 64.10%</td>
<td>C - 64.16%</td>
</tr>
<tr>
<td>H - 5.80%</td>
<td>H - 5.88%</td>
</tr>
</tbody>
</table>

Molecular weight 374 (By mass spectroscopy)

**ALKALINE FUSION OF THE AGLYCONES:**

The aglycone (120 mg), 10 ml of ethanol and 20 ml of KOH was taken in 250 ml ground joint flask attached with water condensor. The reaction mixture was acidified by dil HCl. The product were separated out and extracted with solvent ether. The etheral layer was washed with water and separated into two parts.

(a) The etheral layer was treated with 50% NaHCO$_3$ solution yielded protocatechuic acid (IIB), molecular formula $C_6H_6O_4$, m.p. 197-98°C, $[\text{M}]^+$ 154 (by Co-PC and Co-TLC).

(b) The second part on treatment with 10% NaOH yielded a phloroglucinol (IIA) molecular formula $C_6H_6O_3$, m.p. 117-18°C, $[\text{M}]^+$ 126 (by Co-PC and Co-TLC).
TABLE 6

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction</th>
<th>Eluant</th>
<th>Spot on TLC</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - 6</td>
<td>ethylacetate:methanol (6:1)</td>
<td>One</td>
<td>CP'-1</td>
</tr>
<tr>
<td>2</td>
<td>7 - 11</td>
<td>ethylacetate:methanol (6:2)</td>
<td>Two</td>
<td>CP'-1 + CP'-2</td>
</tr>
<tr>
<td>3</td>
<td>12 - 16</td>
<td>ethylacetate:methanol (6:3)</td>
<td>One</td>
<td>CP'-1</td>
</tr>
</tbody>
</table>

STUDY OF THE FRACTION (1-6):

The fraction (1-6) were of same n$_f$ value and so mixed together. On evaporation of the solvent yielded a compound CP'-1 which was crystallised from ethanol. It analysed for molecular formula C$_{21}$H$_{22}$O$_{12}$ m.p. 220-221ºC, [M]$^+$ 466.

ELEMENTAL ANALYSIS OF THE PROAGLYCONE (CP'-1):

```
FOUND        CALCULATED C$_{21}$H$_{22}$O$_{12}$
C - 54.02%    C - 54.07%
H - 4.70%     H - 4.72%
```

Molecular weight 466 (By mass spectroscopy)

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE CP'-1:

(130 mg) of CP'-1 was treated with methyl iodide (40 ml) and Ag$_2$O (130 mg) in dimethyl formamide (5.0 ml) in a 250 ml of conical flask and heated at room temperature.
The contents were filtered and residue was washed with dimethyl formamide. On evaporation of the filtrate, the residue was concentration under reduced pressure and dissolved in acetone (20 ml). On removal of the solvent yielded a syrupy viscous mass, which on hydrolysis with 20% H₂SO₄ gave an aglycone and methylated sugar. The aqueous part was neutralized by BaCO₃ and BaSO₄ and filtered off. The filtrate was concentrated and examined by paper chromatography using n-butanol:acetic acid:water (4:1:5) and aniline hydrogen phthalate as spraying reagent. The methylated sugar was identified as 2,3,4, tri-O-methyl-D-glucose (Co-PC and Co-TLC).

STUDY OF THE FRACTION (12-16):

The fraction (12-16) were of same Rf value and so mixed together. Removal of the solvent yielded a compound. It had molecular formula C₂₇H₄₂O₁₇, m.p. 200-201°C, [M]+ 628.

ELEMENTAL ANALYSIS OF THE PROAGLYCONE (CP-2):

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 59.53%</td>
<td>C - 51.59%</td>
</tr>
<tr>
<td>H - 5.02%</td>
<td>H - 5.09%</td>
</tr>
</tbody>
</table>

Molecular weight 628 (by mass spectroscopy)

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (CP-2):

Permethylation and hydrolysis of proaglycone (CP-2) was carried out by a similar procedure as described on page
The methylated sugars were identified as 2,3,4 tri-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-galactose confirmed by (Co-PC and Co-TLC).

IDENTIFICATION OF THE SUGARS AFTER HYDROLYSIS:

The aqueous hydrolysate obtained by acid hydrolysis (7% H₂SO₄) of the glycoside was neutralized with BaCO₃ and BaSO₄ and filtered off. The filtrate was concentrated and examined by paper chromatography on Whatmann filter paper using solvent system n-butanol:acetic acid:water (4:1:5) and aniline hydrogen phthalate as spraying agent.

The results are described below:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent system</th>
<th>kₜ value 23</th>
<th>Sugars identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reported</td>
<td>Found</td>
</tr>
<tr>
<td>1</td>
<td>n-butanol:acetic acid:water (4:1:5)</td>
<td>0.17</td>
<td>0.16 D-galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.18</td>
<td>0.19 D-glucose</td>
</tr>
</tbody>
</table>

PERIODATE OXIDATION OF THE GLYCOSIDE (CP):

200 mg of the glycoside was dissolved in (100 ml) ethanol and sodium metaperiodate was taken in 250 ml of
round bottomed flask attached with air condensor. The reaction mixture was reacted with sodium metaperiodate (10 ml of 0.1M). The contents left for 4 days at room temperature. The liberation of HCOOH and periodate consumed estimated by Jones et al. method.

**ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE (CP):**

The glycoside (40 mg) was hydrolysed with almond emulsin (40 ml) at room temperature for about 60 hrs. and the hydrolysate on chromatography over Whatmann No. 1 filter paper revealed the presence of D-galactose and D-glucose and also indicated β-linkage between both the sugar residues and between sugar and the aglycone.
REFERENCES:


