CHAPTER IV

*ISOLATION AND STUDY OF A NOVEL FLAVONOIDAL GLYCOSIDE;
[LUTEOLIN-5-O-α-L-RHAMNOPYRANOSYL (1→4)-O-β-D-GLUCOPYRANOSIDE]
FROM THE STEMS OF IMPATIENS SCABRIDA D.C.

* This work has been communicated for publication in Journal of Institution of Chemists.
Impatiens scabrida D.C. belongs to the natural order Balsaminaceae and is distributed in throughout India. It is an annual herb with sessile and narrow leaves. The Ayurvedic system of medicine describes the oil of the plant to be used as a semidrying oil.

In absence of adequate phytochemical investigations it was thought worthwhile to investigate it phytochemically.

ISOLATION OF THE FLAVONOIDAL GLYCOSIDE

Impatiens scabrida D.C. (N.O. Balsaminaceae) was supplied by United Chemical and Allied Products, Calcutta and authenticated by the Botany Department of this University.

Powdered stems (2.5 Kg.) of I. scabrida D.C. were extracted with 95% ethanol and concentrated under reduced pressure and the concentrated extract was poured into excess of distilled water. The water soluble part was concentrated to a cream coloured syrupy mass. It was successively extracted with petroleum ether, ethylacetate and methanol.

The residue obtained after the removal of the petroleum ether and ethylacetate were of inadequate amount for the substantive investigations.
STUDY OF THE METHANOL SOLUBLE PART

The methanol soluble part on concentration under reduced pressure yielded a viscous mass, which on TLC examination gave two spots, thereby showing the presence of two compounds, which were separated by column chromatography over silica-gel and eluated with ethylacetate : methanol in the ratio of 2:2, 2:4 and 2:6.

The study of the eluates from ethylacetate : methanol, 2:6 has been described in chapter V of the thesis.

STUDY OF THE ELUATES FROM ETHYLACETATE : METHANOL (2:2)

Eluates from ethylacetate : methanol (2:2) were of same $R_f$ value and so combined and on evaporation of the solvent gave a light yellow compound (LS, 0.096%), which was found to be homogeneous on TLC (Ethylacetate: Methanol:Water (12:7:2) $R_f$ value 0.72). It analysed for molecular formula, $C_{27}H_{30}O_{15}$, m.p. 227$^\circ$ C, molecular weight, $M^+$ 594. It was found to be soluble in ethylacetate and ethanol.

It gave positive Molisch test for glycoside and positive colour reactions for flavonoidal glycoside$^2,^3$. 
UV SPECTRUM OF FLAVONOIDAL GLYCOSIDE (LS)

The wavelengths of maximum absorbance with various shift reagents were at:

1. $\lambda_{\text{max}}^{\text{MeOH}}$ 343, 275, 254 nm
2. $\lambda_{\text{max}}^{\text{NaOMe}}$ 405, 268, 233 nm
3. $\lambda_{\text{max}}^{\text{AlCl}_3}$ 421, 354, 243 nm
4. $\lambda_{\text{max}}^{\text{AlCl}_3/\text{HCl}}$ 376, 357, 298, 258 nm
5. $\lambda_{\text{max}}^{\text{NaOAC}}$ 400, 300, 256 nm
6. $\lambda_{\text{max}}^{\text{NaOAC/H}_3\text{BO}_3}$ 393, 263 nm

IR SPECTRUM OF THE GLYCOSIDE (LS)

The important peaks obtained in the I.R. spectrum (Fig. I) and the structural units inferred with the help of available literature\(^5,6\) are recorded in table - I.
TABLE - I

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peaks cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3334</td>
<td>-OH grouping</td>
</tr>
<tr>
<td>2.</td>
<td>2892</td>
<td>-C-H stretching</td>
</tr>
<tr>
<td>3.</td>
<td>1728</td>
<td>Acetyl group.</td>
</tr>
<tr>
<td>4.</td>
<td>1677</td>
<td>&gt;C=O</td>
</tr>
<tr>
<td>5.</td>
<td>1614</td>
<td>Aromatic ring system</td>
</tr>
<tr>
<td>6.</td>
<td>1280</td>
<td>C-O-C vibration</td>
</tr>
<tr>
<td>7.</td>
<td>1152</td>
<td>C-O vibrating vibration</td>
</tr>
</tbody>
</table>

PRESENCE OF OH GROUP(S)

A peak at \(\text{KBr}_{\text{max}} 3334\) cm\(^{-1}\) in the IR spectrum of the glycoside suggested the presence of hydroxyl group(s) in it.

The glycoside was acetylated with \(\text{Ac}_2\text{O/pyridine}\) yielded an acetyl derivative, molecular formula, \(\text{C}_{45}\text{H}_{48}\text{O}_{24}\), m.p. 166° and \(M^+ 972\) (EIMS). The percentage of the acetyl groups (38.86%) in the acetylated product was estimated by Wiesenberger method\(^7\) as described by Belcher and Godbert\(^8\) indicated that there were nine acetylable hydroxyl groups in the glycoside.

Appearance of peak in IR spectrum of acetyl derivative at \(\text{KBr}_{\text{max}} 1728\) cm\(^{-1}\) with disappearance of
peak at $\nu_{\text{max}}^{\text{KBr}} 3334 \text{ cm}^{-1}$ indicated acetylation of all the hydroxyl groups present in the glycoside.

The structure of glycoside was elucidated by its acid hydrolysis.

**ACID HYDROLYSIS OF THE GLYCOSIDE (LS₁)**

The glycoside on hydrolysis with 7% ethanolic $\text{H}_2\text{SO}_4$ yielded aglycone (LS₁) and sugar moiety(ies) which were studied separately.

**STUDY OF THE AGLYCONES (LS)**

It was found to be homogeneous on TLC (CHCl₃:MeOH: Water (75:22:3), $R_f$ value 0.83). The aglycone analysed for molecular formula, $C_{15}H_{10}O_{6}$, m.p. 330° and $M^+$ 286 (EIMS). The aglycone was yellow coloured compound which was soluble in ethanol. It gave all characteristic colour reactions of flavonoids⁹,¹⁰.

**UV SPECTRUM OF THE AGLYCONES¹¹ (LS₁)**

The wave lengths of maximum absorbance in the UV spectrum of the aglycone were at:

1. $\lambda_{\text{max}}^{\text{MeOH}}$ 347, 293, 265, 235 nm
2. $\lambda_{\text{max}}^{\text{NaOMe}}$ 403, 328, 265 nm
3. $\lambda_{\text{max}}^{\text{AlCl₃}}$ 428, 327, 297, 290 nm
4. \(\lambda_{\text{max}}^{\text{AlCl}_3/\text{HCl}}\) 386, 251, 296, 273, 268 nm

5. \(\lambda_{\text{max}}^{\text{NaOAC}}\) 388, 283, 265 nm

6. \(\lambda_{\text{max}}^{\text{NaOAC/H}_3\text{BO}_3}\) 435, 373, 259 nm

**IR SPECTRUM OF THE AGLYCONE (LS1)**

The significant peaks as observed in the IR spectrum (Fig. II) and the structural units inferred with the help of available literature\textsuperscript{12,13} are recorded in table - II.

**TABLE - II**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Wave number cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3346</td>
<td>-OH</td>
</tr>
<tr>
<td>2.</td>
<td>2882</td>
<td>-C-H stretching</td>
</tr>
<tr>
<td>3.</td>
<td>1678</td>
<td>&gt;C=O</td>
</tr>
<tr>
<td>4.</td>
<td>1600</td>
<td>Ring system</td>
</tr>
<tr>
<td>5.</td>
<td>1294</td>
<td>C-O-C vibration</td>
</tr>
<tr>
<td>6.</td>
<td>1166</td>
<td>C-O vending vibration</td>
</tr>
</tbody>
</table>

**PRESENCE OF OH GROUPS**

A peak at a \(\lambda_{\text{max}}^{\text{KBr}}\) 3346 cm\(^{-1}\) suggested the presence of free hydroxyl group(s) in it. On acetylation (\(\text{Ac}_2\text{O/pyridine}\)), it formed an acetyl derivative (LS\(_2\)), m.p. 138°, molecular formula, \(\text{C}_{23}\text{H}_{18}\text{O}_{10}\) and \(M^+\) 454
IR SPECTRUM OF THE AGLYCONE
(EIMS). The percentage of acetyl group (36.91%) in the acetylated product was determined by Wiesenberger method\(^7\) as described by Belcher and Godbert\(^8\) which showed the presence of four hydroxyl groups in the aglycone.

**ALKALINE DEGRADATION OF THE AGLYCONE (Ls\(_1\))**

The aglycone on fusion with 50% ethanolic KOH\(^{14}\) gave two compounds identified as phloroglucinol molecular formula, \(\text{C}_6\text{H}_6\text{O}_3\), m.p. 118°, \(M^+\) 126 and protocatecheuic acid, m.p. 198°, molecular formula, \(\text{C}_7\text{H}_6\text{O}_4\), \(M^+\) 154.

On the basis of the above facts a tentative structure to the aglycone was assigned as (I):

![Structure](image)

**POSITION OF HYDROXYL GROUPS:**

1. OH GROUPS AT C-3', C-4'
   
   (i) Formation of 4:5 dihydroxy benzoic acid (protocatecheuic acid IIa) on alkaline degradation of
the aglycone confirmed the presence of OH groups at C-3' and C-4' in the aglycone. The presence of hydroxyl group at C-4' was further confirmed as it gave positive Shinoda test\textsuperscript{15}.

(ii) A bathochromic shift of 24 nm of band I in presence of NaOAC/H\textsubscript{3}BO\textsubscript{3} relative to band I in MeOH confirmed the presence of -OH group at C-3' and C-4',\textsuperscript{16}

2. OH GROUP AT C-5, C-7

(i) Formation of phloroglucinol (II\textsubscript{b}) on alkaline degradation showed the presence of two hydroxy groups at C-5 and C-7 respectively.

(ii) A bathochromic shift of 55 nm in band I with AlCl\textsubscript{3} (relative to MeOH) and 18 nm in band II with NaOAC (relative to MeOH) further confirmed the presence of -OH groups at C\textsubscript{5} and C\textsubscript{7} respectively\textsuperscript{17,18}.

Formation of above degradation products can be explained by assuming the structure of aglycone as; 5, 7, 3', 4' tetrahydroxy flavone (II) which is a well known compound Luteolin\textsuperscript{19}. 
The above proposed structure of the aglycone was further supported by its \textsuperscript{1}HNMR and mass spectral studies.

\textbf{\textsuperscript{1}HNMR SPECTRUM OF TETRAACETYL DERIVATIVE OF AGLYCONE (LS\textsubscript{2})}

The important chemical shifts obtained in the \textsuperscript{1}HNMR (Fig. III) of acetylated derivative and structural units inferred with the help of literature\textsuperscript{20,21} are recorded in table - III and were in complete conformity with the structure (II).

\begin{table}[h]
\centering
\begin{tabular}{lllll}
\hline
S. No. & Value & Pattern & J value (Hz) & No. of protons & Structural assignment \\
\hline
1. & 7.42 & d & 2.5 & 1 & H-2' \\
2. & 7.03 & d & 2.5 & 1 & H-5' \\
3. & 7.66 & d,d & 2.5,9 & 1 & H-6' \\
4. & 6.62 & s & - & 1 & H-3 \\
5. & 6.52 & d & 2.4 & 1 & H-6 \\
6. & 6.58 & d & 2.0 & 1 & H-8 \\
7. & 2.41 & s & - & 3 & 3'-OAC \\
8. & 2.35 & s & - & 3 & 4'-OAC \\
9. & 2.45 & s & - & 3 & 5-OAC \\
10. & 2.46 & s & - & 3 & 7-OAC \\
\hline
\end{tabular}
\caption{TABLE - III}
\end{table}
$^1H$ NMR SPECTRUM OF THE ACETYLATED AGLYGONE
MASS SPECTRUM OF AGLYCONE\textsuperscript{22} (LS\textsubscript{1})

The prominent fragmentation ion peaks observed in EIMS of the aglycone were as follows:

EIMS, \textit{M}^+ 286, m/z 258, 257, 153, 152, 134, 132, 124.

The various species obtained during its fragmentation are shown in scheme I and further confirmed its identity as 5', 7, 3', 4' tetrahydroxy flavone.

STUDY OF THE SUGAR MOIETY (IES)

The aqueous hydrolysate obtained by the hydrolysis of the glycoside was neutralised with BaCO\textsubscript{3} and BaSO\textsubscript{4} filtered off. The filtrate on concentration yielded a yellow syrupy mass. Paper chromatographic study of concentrated hydrolysate revealed the presence of L-rhamnose and D-glucose.

QUANTITATIVE ESTIMATION OF SUGARS

Quantitative estimation of the sugars in the glycoside was carried out by the procedure of Mishra and Rao\textsuperscript{23} which indicated that two sugars were present in equimolecular ratio (1:1).

PERIODATE OXIDATION OF THE GLYCOSIDE (LS)

The sodium metaperiodate oxidation\textsuperscript{24} of the glycoside consumed 3.01 molecule of periodate and
SCHEME - I
liberated 1.14 molecule of formic acid indicating the presence of one molecule of L-rhamnose and one molecule of D-glucose attached to the molecule of aglycone and also confirmed that both the sugars were present in the pyranose form. 25

POSITION OF ATTACHMENT OF SUGARS TO THE AGLYCONE

Both the sugars must be attached on the same C-atom in the form of a disaccharide, and this was confirmed by the fact that the glycoside during periodate oxidation consumed 3.01 molecule of sodium metaperiodate liberating 1.14 molecule of formic acid. These values were in accordance with the disaccharide nature of sugars.

By comparing the U.V. spectral data of aglycone and the glycoside, the position of sugar moiety to the aglycone was fixed at position 5 on the basis of following grounds;

(i) UV absorption data 16, and spectral shifts upon addition of AlCl₃ and H₃BO₃ indicated the presence of OH groups at C-3' and C-4' positions in the aglycone.

(ii) The glycoside was completely hydrolysable with 7% ethanolic sulphuric acid.
Methylation of the glycoside with $\text{Me}_2\text{SO}_4/\text{K}_2\text{CO}_3$ in acetone gave the methyl ester which upon hydrolysis with methanolic sulphuric acid gave 7,3',4' tri-O-methyl Luteolin (confirmed by Co-Pc and Co-Tlc) thereby confirming that the hydroxyl group at position C-5 was involved in glycosylation.

Keeping together all the above facts the structure to the glycoside was assigned as (III):

![Chemical Structure](image)

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

The sequence of sugar residue in the glycoside was determined by graded hydrolysis with Kiliani mixture\(^{26}\) which liberated L-rhamnose first followed by D-glucose suggesting that L-rhamnose was the terminal sugar and D-glucose was linked to the aglycone.

It was further supported by the isolation and study of two proaglycones designated as; LSA-1 and LSA-2 which were produced by partial hydrolysis of the
glycoside by Kiliani mixture and separated by column chromatography.

**STUDY OF THE PROAGLYCONE (LSA-1)**

The proaglycone LSA-1 analysed for the molecular formula, $C_{21}H_{20}O_{11}$, m.p. 288°, $M^+$ 448. On hydrolysis with 7% $H_2SO_4$ it yielded the aglycone Luteolin (m.m.p. and Co-TLC) and D-glucose.

The proaglycone LSA-1 was hydrolysable by the enzyme emulsin thereby indicating the presence of $\beta$-linkage between D-glucose and the aglycone.

**PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (LSA-1)**

The proaglycone (LSA-1) on permethylation by Khun's procedure\(^{27}\) yielded aglycone and 2,3,4,6-tetra-O-methyl glucose (Co-Pc) thereby indicating that C$_1$ of D-glucose was involved in the formation of glycosidic linkage and also suggested that D-glucose was present in the pyranose form.

Thus the proaglycone (LSA-1) was assigned the structure (IV) as; Luteolin-5-O-$\beta$-D-glucose;
STUDY OF THE PROAGLYCONE (LSA-2)

The proaglycone (LSA-2) analysed for $C_{27}H_{30}O_{15}'$ m.p. 227° and $M^+$ 594. On acid hydrolysis with 7% alc. sulphuric acid it yielded Luteolin (m.m.p. and Co-TLC) and D-glucose and L-rhamnose (Co-pc and Co-TLC).

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (LSA-2)

The proaglycone (LSA-2) on permethylation by Khun procedure followed by acid hydrolysis yielded the aglycone and 2,3,4,tri-O-methyl-L-rhamnose and 2,3,6-tri-O-methyl-glucose (by Co-pc and Co-TLC) which confirmed their presence in pyranose form and also showed that $C_4$-OH group of D-glucose was linked with $C_1$-OH of L-rhamnose.
ENZYMATIC HYDROLYSIS OF GLYCOSIDE

The glycoside when hydrolysed by enzyme tokadiastase\(^{28}\) yielded proaglycone (LSA-1) and L-rhamnose (Co-pc) indicating \(\alpha\)-linkage between LSA-1 and L-rhamnose.

The proaglycone (LSA-1) on its hydrolysis with emulsin solution yielded Luteolin and D-glucose, confirming \(\beta\)-linkage between Luteolin and D-glucose.

Keeping all the above facts together it was concluded that the 5-OH of the aglycone was linked with C-1 of the D-glucose via \(\beta\)-linkage and C-4 of the D-glucose was attached to the C-1 of L-rhamnose via \(\alpha\)-linkage.

Thus the structure to the glycoside was assigned (V) as; Luteolin-5-O-\(\alpha\)-L-rhamnopyranosyl (1\(\rightarrow\)4)-O-\(\beta\)-D-glucopyranoside.
$^{1}\text{HNMR}$ spectrum of nona acetyl derivative of the glycoside further supported the above structure (V). The significant peaks obtained in the $^{1}\text{HNMR}$ spectrum (Fig.IV) of nona acetyl derivative of the glycoside and structural units inferred with the help of available literature$^{29}$ are given in table - IV.

**TABLE - IV**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value</th>
<th>Pattern</th>
<th>J value (Hz)</th>
<th>No. of protons</th>
<th>Structural assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.42</td>
<td>d</td>
<td>9</td>
<td>2</td>
<td>H-2', 6'</td>
</tr>
<tr>
<td>2.</td>
<td>7.03</td>
<td>d</td>
<td>2.5</td>
<td>1</td>
<td>H-5'</td>
</tr>
<tr>
<td>3.</td>
<td>6.62</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td>4.</td>
<td>6.52</td>
<td>d</td>
<td>2.4</td>
<td>1</td>
<td>H-6</td>
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<td>5.</td>
<td>6.58</td>
<td>d</td>
<td>2.0</td>
<td>1</td>
<td>H-8</td>
</tr>
<tr>
<td>6.</td>
<td>2.41</td>
<td>s</td>
<td>-</td>
<td>3</td>
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</tr>
<tr>
<td>7.</td>
<td>2.35</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>4'-OAC</td>
</tr>
<tr>
<td>8.</td>
<td>2.44</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>7-OAC</td>
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<tr>
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<td>1</td>
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<tr>
<td>10.</td>
<td>4.40</td>
<td>d</td>
<td>7.6</td>
<td>1</td>
<td>1'' anomic proton</td>
</tr>
<tr>
<td>11.</td>
<td>3.00</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>2''-OAC</td>
</tr>
<tr>
<td>12.</td>
<td>2.98</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>3''-OAC</td>
</tr>
<tr>
<td>13.</td>
<td>2.92</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6''-OAC</td>
</tr>
<tr>
<td>14.</td>
<td>2.09</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>2'''-OAC</td>
</tr>
<tr>
<td>15.</td>
<td>3.02</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>3'''-OAC</td>
</tr>
<tr>
<td>S. No.</td>
<td>Value</td>
<td>Pattern</td>
<td>J value (Hz)</td>
<td>No. of protons</td>
<td>Structural assignment</td>
</tr>
<tr>
<td>-------</td>
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<td>--------------</td>
<td>----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>16.</td>
<td>2.04</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>4'''-OAC</td>
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<tr>
<td>17.</td>
<td>0.76</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6'''-CH₃</td>
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<tr>
<td>18.</td>
<td>4.64-4.80</td>
<td>m</td>
<td>-</td>
<td>4</td>
<td>Protons of rhamnose unit</td>
</tr>
<tr>
<td>19.</td>
<td>5.46-5.62</td>
<td>m</td>
<td>-</td>
<td>6</td>
<td>Protons of glucose unit</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM OF THE GLYCOSIDE$^{30}$ (LS)**

The important fragment ion peaks obtained in the EIMS are given below;

$M^+ 594, m/z = 448, 432, 286, 258, 257, 153, 152, 134, 132, 124.$

The various species assigned to the fragments are shown in scheme-II and further confirmed its identity as; Luteolin-5-O-α-L-rhamnopyranosyl (1→4)-O-B-D-glucopyranoside (V).
$^1$H NMR SPECTRUM OF THE ACETYLATED GLYCOSIDE
EXPERIMENTAL

Impatiens scabrida D.C. (N.O. Balsaminaceae) was supplied by United Chemical and Allied Products, Calcutta.

The air dried and powdered stems (2.5 Kg) of Impatiens scabrida were extracted with 95% ethanol and concentrated under reduced pressure and the concentrated extract was poured into excess of distilled water. The water soluble part was concentrated to a cream coloured mass. It was successively extracted with petroleum ether, ethylacetate and methanol.

The residue obtained after the removal of the petroleum ether and ethylacetate were of inadequate amount for any substantive investigations.

ISOLATION OF THE FLAVONOIDAL GLYCOSIDE

The concentrated cream coloured mass (8.2 g) obtained from the fractionation of water soluble part of alcoholic extract of stems, on its examination on TLC using solvent system ethylacetate:methanol:water (16:10:13 v/v) and 10% sulphuric acid as spraying reagent gave two spots (Rf 0.69, 0.58) thereby showing it to be a mixture of two compounds.
COLUMN CHROMATOGRAPHY

Length of the column 50 cm.
Diameter of the column 3.0 cm.
Weight of the crude extract 8.2 g.
Weight of silica gel 210 g.

TABLE - V

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction No.</th>
<th>Eluants</th>
<th>TLC</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1 - 6</td>
<td>Ethylacetate:Methanol (2:2)</td>
<td>One</td>
<td>LS</td>
</tr>
<tr>
<td>2.</td>
<td>7 - 12</td>
<td>Ethylacetate:Methanol (2:4)</td>
<td>Two</td>
<td>Rejected</td>
</tr>
<tr>
<td>3.</td>
<td>13 - 18</td>
<td>Ethylacetate:Methanol (2:6)</td>
<td>One</td>
<td>GS</td>
</tr>
</tbody>
</table>

On evaporation of the solvent it gave a light yellow compound which gave single spot on TLC (ethylacetate : methanol : water (12:7:2, $R_f = 0.72$) and crystallised from methanol.

STUDY OF FLAVONOIDAL GLYCOSIDE (LS)

The compound crystallised from methanol as light yellow compound (2.4 gm.) and was found to be soluble in methanol. It analysed for molecular formula, $C_{27}H_{30}O_{15}$, m.p. 227° and $M^+$ 594 (EIMS). It responded to positive Molisch test and following colour reactions -
1. Red colour with sodium amalgam and hydrochloric acid.
2. Deep yellow colour with liquid ammonia.

**ELEMENTAL ANALYSIS**

**Found**

C = 2.52%  
H = 5.02%  
Molecular weight = 594  
(By mass spectroscopy)

**Calculated for C_{27}H_{30}O_{15}**

C = 54.54%  
H = 5.05%  
Molecular weight = 594  
(By mass spectroscopy)

**ACETYLATION OF THE GLYCOSIDE (LS)**

100 mg of the compound, 6 ml acetic anhydride and 2 ml pyridine were taken in 50 ml. RB flask fitted with air condenser. The acetyl derivative was prepared in the same way as described on page 72 of the thesis which analysed for C_{45}H_{48}O_{24}, m.p. 166°, M+ 972.

**ELEMENTAL ANALYSIS**

**Found**

C = 54.09%  
H = 4.90%  
Percentage of acetyl group = 38.86%  
Molecular weight = 972  
(By mass spectroscopy)

**Calculated for C_{45}H_{48}O_{24}**

C = 55.55%  
H = 4.9382%  
Percentage of acetyl group = 38.88%  
Molecular weight = 972  
(By mass spectroscopy)

**ACID HYROLYSIS OF THE GLYCOSIDE (LS)**

The glycoside was hydrolysed in the same way as
described on page 72 of the thesis. Removal of alcohol from it, yielded a yellow coloured compound (482 mg), m.p. 330° which was filtered off. The separated aglycone and hydrolysate were examined separately.

**STUDY OF THE AGLYCOME (LS1)**

The aglycone was of yellow colour and soluble in acetone and ethanol. It showed single spot on TLC ($R_f = 0.83$, CHCl₃ : MeOH : Water, 75:22:3 using I₂ vapours as visualizing agent. It had m.p. 330° and analysed for $C_{15}H_{10}O_6$, $M^+ 286$ (EIMS). It gave:

1. Green colour with ethanolic FeCl₃.
2. A yellowish orange colour with characteristic fluorescence with concentrated sulphuric acid.

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for $C_{15}H_{10}O_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 62.90%</td>
<td>C = 62.93%</td>
</tr>
<tr>
<td>H = 3.39%</td>
<td>H = 3.49%</td>
</tr>
<tr>
<td>Molecular weight = 286</td>
<td>Molecular weight = 286</td>
</tr>
<tr>
<td>(By mass spectroscopy)</td>
<td>(By mass spectroscopy)</td>
</tr>
</tbody>
</table>

**ACETYLATION OF THE AGLYCOME**

The acetylation of the aglycone was done in a similar way as described on page No. 72 of the thesis.
The acetyl derivative (LS₂) was analysed for 
C₂₃H₁₈O₁₀, m.p. 268°, M⁺ 454 (EIMS).

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₂₃H₁₈O₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 60.71%</td>
<td>C = 60.79%</td>
</tr>
<tr>
<td>H = 3.92%</td>
<td>H = 3.96%</td>
</tr>
<tr>
<td>Percentage of acetyl group = 36.91%</td>
<td>Percentage of acetyl group = 37.004%</td>
</tr>
<tr>
<td>Molecular weight = 454</td>
<td>Molecular weight = 454</td>
</tr>
<tr>
<td>(By mass spectroscopy)</td>
<td>(By mass spectroscopy)</td>
</tr>
</tbody>
</table>

DEGRADATION OF THE AGLYCON WITH KOH

The degradation of the aglycone was done in the same way as described on page 74 of the thesis.

The etheral layer was washed with water and separated into two portions.

1. The first part of the etheral layer when treated with 50% sodium bicarbonate solution (30 ml) and the aqueous part on acidification yielded a compound, m.p. 198°, molecular formula, C₇H₆O₄ and M⁺ 154 (EIMS). The compound was identified as protocatecheueic acid (confirmed by m.m.p., Co-Pc and Co-TLC with authentic sample).

2. The second part of the etheral layer when treated with small quantity of 10% solution of
NaOH and the aqueous part on acidification yielded another compound m.p. 118°, molecular formula, \( \text{C}_6\text{H}_6\text{O}_3\), \( M^+ 126 \). The compound was identified as phloroglucinol (confirmed by m.m.p., Co-Pc and Co-TLC with authentic sample).

**PARTIAL HYDROLYSIS OF THE GLYCOSIDE (LS)**

The glycoside (210 mg) was hydrolysed with 80 ml. of Kilianni mixture (HCl : CH\(_3\)COOH : H\(_2\)O 15:35:50) and the reaction mixture worked up in the usual manner as described in chapter III, page 75 of the thesis. The ethanol soluble part on examination by P.C. showed two spots (\( R_f \) 0.38 and 0.53 in BAW).

The ethanol soluble part was concentrated and chromatographed over silica gel column. The column was eluated with CCl\(_4\):CH\(_3\)OH in various proportions. The details of the column chromatography is as follows:

- Weight of ethanol soluble part: 150 mg
- Length of the column: 50 cm.
- Diameter of the column: 3.0 cm.
- Weight of the silica-gel: 15 gm.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Spot on TLC</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1 - 8</td>
<td>CCl₄:CH₃OH(1:1) One</td>
<td></td>
<td>LSA - 1</td>
</tr>
<tr>
<td>2.</td>
<td>9 - 14</td>
<td>CCl₄:CH₃OH(1:2) Two</td>
<td></td>
<td>Mixture of two compounds</td>
</tr>
<tr>
<td>3.</td>
<td>15 - 21</td>
<td>CCl₄:CH₃OH(1:3) One</td>
<td></td>
<td>LSA - 2</td>
</tr>
</tbody>
</table>

The fraction (1-8) were of some $R_f$ value and so mixed together. The removal of solvent from combined fraction yielded a compound LSA-1 which crystallised from ethanol, m.p. 288°, molecular formula, $C_{21}H_{20}O_{11}$ and $M^+$ 448.

**ELEMENTAL ANALYSIS OF PROAGLYCONE (LSA - 1)**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for $C_{21}H_{20}O_{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 56.21%</td>
<td>C = 56.25%</td>
</tr>
<tr>
<td>H = 4.42%</td>
<td>H = 4.46%</td>
</tr>
<tr>
<td>Molecular weight = 448</td>
<td>Molecular weight = 448</td>
</tr>
</tbody>
</table>

(By mass spectroscopy)  (By mass spectroscopy)

**PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (LSA-1)**

The permethylation and hydrolysis of proaglycone (LSA-1) was done in the similar manner as described on page 76 of the thesis.
The sugar was identified as 2,3,4,6-tetra-O-methyl glucose.

**STUDY OF FRACTIONS 15-21**

The fractions were of same $R_f$ value and so were mixed together and on evaporation of the solvent gave a compound which crystallised from ethanol, m.p. 227°, molecular formula, $C_{27}H_{30}O_{15}$ and $M^+$ 594 (EIMS).

**ELEMENTAL ANALYSIS OF THE PROAGLYCONE (LSA-2)**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for $C_{27}H_{30}O_{15}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 52.52%</td>
<td>C = 54.56%</td>
</tr>
<tr>
<td>H = 5.02%</td>
<td>H = 5.05%</td>
</tr>
<tr>
<td>Molecular weight = 594</td>
<td>Molecular weight = 594</td>
</tr>
<tr>
<td>(By mass spectroscopy)</td>
<td>(By mass spectroscopy)</td>
</tr>
</tbody>
</table>

**PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (LSA-2)**

The permethylation and hydrolysis of the proaglycone LSA-2 was carried out in the same way as described on page 76 of the thesis. The hydrolysate revealed the presence of 2,3,4-tri-O-methyl-rhamnose and 2,3,4,6-tetra-O-methyl glucose (by Co-PC and Co-TLC with authentic sample).

**IDENTIFICATION OF SUGARS AFTER HYDROLYSIS**

The identification of the sugars was done in the same way as described on page 78 of the thesis. The results are recorded in table - VII.
TABLE - VII

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent system</th>
<th>R_f value</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>0.18</td>
<td>0.16 D-glucose</td>
</tr>
<tr>
<td>2.</td>
<td>n-butanol and 1% NH_4OH</td>
<td>0.08</td>
<td>0.07 D-glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.21</td>
<td>0.20 L-rhamnose</td>
</tr>
</tbody>
</table>

PERIODATE OXIDATION OF GLYCOSIDE

Periodate oxidation of glycoside was carried out in the similar manner as described on page 79 of the thesis.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE

The glycoside (45 mg) was dissolved in ethanol (28 ml) and mixed with tokadiastase solution (30 ml) in a conical flask (100 ml) fitted with stopper. The reaction mixture was left for 48 hrs. at room temperature and then filtered. The aglycone and hydrolysate were examined separately.

The hydrolysate on concentration was examined for sugar moiety by paper chromatography using W. atman no. 1 filter paper and BAW (4:1:5) solvent system. The sugar was identified as L-rhamnose.
The aglycone was identified as proaglycone LSA-1, m.p. 288° (by Co-TLC, m.m.p.). The proaglycone LSA-1 (28 mg) was dissolved in ethanol (12 mg) and mixed with equal volume of solution of emulsin in a conical flask.

The contents were left for 8 hrs and filtered. The aglycone was identified as Luteolin, m.p. 330° (by m.m.p. and Co-TLC). The hydrc1 when examined on Pca was found to contain D-glucose.
REFERENCES


