CHAPTER - III

*ISOLATION AND CHARACTERISATION OF A NOVEL FLAVONE GLYCOSIDE: 7-HYDROXY-6-METHOXY-5-O-L-RHAMNOPYRANOSIDE FROM THE SEEDS OF TRICHOSANTHES ANGUINA LINN.*

*This work has been accepted for publication in Journal of FITOTERAPIA, ITALY.*
Trichosanthes anguina Linn. belongs to natural order Cucurbitaceae. It is commonly known as "Chachinda" in hindi and "Snakegaurd" in English. It is cultivated throughout the hotter parts of India and China. The plant is used as tonic, to cure cough, biliousness and is purgative and anthelmintic. Its seeds are used as antidiarrhoeal and also used in the treatment of syphilis\textsuperscript{2,3}.

The detailed description of this plant has been given in page no. 26 of chapter II of the thesis.

ISOLATION OF THE FLAVONE GLYCOSIDE TB

The plant material was procured by M/s United Chemicals and Allied Products, Calcutta, India and authenticated by the Department of Botany of this University. Air dried and powdered seeds of \textit{T.anguina} (3 kg) were extracted exhaustively with hot 95\% ethanol. The total extract was concentrated under reduced pressure to yield a pale yellow syrupy mass, which was separated into water soluble and insoluble parts. The water soluble part was concentrated to viscous mass (125 g) and successively extracted with petroleum ether, benzene, chloroform, ethyl
acetate, acetone and methanol.

STUDY OF ETHYL ACETATE SOLUBLE PART

The ethyl acetate soluble fraction was concentrated under reduced pressure to gave yellow syrupy mass. It gave two spots on TLC examination, using EtOAc-ACOH-H₂O (9:3:1) and I₂ vapours as visualising agent, which were separated by column chromatography over si-gel G, and eluted with CHCl₃-MeOH in the ratio of 6:1, 6:2, 6:4 and 6:8.

STUDY OF THE ELUATES FROM CHLOROFORM-METHANOL (6:4)

Eluates from chloroform-methanol (6:4) were collected and found to have the same Rf value, and so combined together. On evaporation of the solvent it gave a brownish yellow coloured compound (0.068%), which was found to be homogenous on TLC examination.

It analysed for C_{22}H_{22}O_{9}, m.p. 327°C and [M]+ 430 (EIMS). It was found to be soluble in ethyl acetate. It gave positive Molisch test for the glycoside and responded to characteristic colour reactions for flavonoidal glycosides⁴,⁵.

UV SPECTRUM OF THE FLAVONE GLYCOSIDE TB

The wave lengths of maximum absorption with various shift reagents is recorded below:
\( \lambda_{\text{max}} \text{MeOH} \) 248, 270, 317 nm;  \
\( \lambda_{\text{max}} \text{NaOAc} \) 285, 295 nm;  \
\( \lambda_{\text{max}} \text{AlCl}_3 \) 250, 270, 320 nm;  \
\( \lambda_{\text{max}} \text{AlCl}_3/\text{HCl} \) 252, 272, 322 nm;  \
\( \lambda_{\text{max}} \text{NaOMe} \) 250, 272, 318 nm.

**IR SPECTRUM OF THE GLYCOSIDE TB**

The significant peaks obtained in the IR spectrum (Fig.1) and structural units inferred with the help of available literature\(^6,7\) are given in the Table-I.

**TABLE - I**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Wave number cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3450</td>
<td>-OH group(s)</td>
</tr>
<tr>
<td>2.</td>
<td>2872</td>
<td>-OCH(_3) group(s)</td>
</tr>
<tr>
<td>3.</td>
<td>1650</td>
<td>C=O</td>
</tr>
<tr>
<td>4.</td>
<td>1520</td>
<td>Aromatic ring system</td>
</tr>
<tr>
<td>5.</td>
<td>1215</td>
<td>C-O-C-stretching vibration</td>
</tr>
<tr>
<td>6.</td>
<td>1125</td>
<td>C-O-C-bending vibration</td>
</tr>
<tr>
<td>7.</td>
<td>2920</td>
<td>-CH-stretching</td>
</tr>
<tr>
<td>8.</td>
<td>840</td>
<td>Two adjacent H-atom in ring system</td>
</tr>
</tbody>
</table>
IR SPECTRUM OF THE FLAVONE GLYCOSIDE TB
PRESENCE OF \(-\text{OH}\) GROUP(S) IN TB

In the IR spectrum a peak at \(\nu_{\text{max}}^{\text{KBr}} 3450 \text{ cm}^{-1}\) suggested the presence of hydroxyl group(s) in it. On acetylation of the glycoside with \(\text{Ac}_2\text{O}/\text{pyridine}\) yielded an acetyl derivative, molecular formula \(\text{C}_{30}\text{H}_{30}\text{O}_{13}\), m.p. 196°C and \([\text{M}]^+ 598\) (EIMS). Estimation of acetyl derivative (28.76%) was done by Wiesenberger's method\(^8\) as described by Belcher and Godbert\(^9\), suggested the presence of four hydroxyl group in the glycoside (TB). The acetylated product did not show any peak at 3450 \text{ cm}^{-1} for the presence of \(-\text{OH}\) group, thereby indicating that all the \(-\text{OH}\) group(s) had undergone acetylation.

PRESENCE OF \(-\text{OCH}_3\) group(s) in TB

A peak at \(\nu_{\text{KBr}} 2872 \text{ cm}^{-1}\) in the IR spectrum of the glycoside, indicated, the presence of \(-\text{OCH}_3\) group(s) in it. Estimation of methoxyl group was done by Zeisel's method\(^10\) (7.20%), which confirmed the presence of only one methoxyl group in it.

ACID HYDROLYSIS OF THE GLYCOSIDE TB

On acid hydrolysis with 7% alcoholic \(\text{H}_2\text{SO}_4\), it yielded aglycone (TB-1) and sugar moiety(ies), which were separated by filtration and studied separately.
STUDY OF THE AGLYCONE TB-1

The aglycone TB-1 crystallised from methanol as a light yellow coloured compound. It was found to be homogenous on TLC examination (Ethyl acetate-methanol-water (8:2:1)). It analysed for molecular formula C_{16}H_{12}O_{5}, m.p. 202°C and [M]^+ 284 (EIMS) and gave all the characteristic colour reactions of flavonoids.\(^4\),\(^5\).

UV SPECTRUM OF THE AGLYCONE TB-1

The UV spectrum of the aglycone exhibited absorption maxima with various shift reagents were as follows:

\[ \text{MeOH} \]
\[ \lambda \text{max} \quad 247, 270, 317 \text{ nm}; \]

\[ \text{NaOMe} \]
\[ \lambda \text{max} \quad 243, 270, 365 \text{ nm}; \]

\[ \text{NaOAc} \]
\[ \lambda \text{max} \quad 245, 286, 365 \text{ nm}; \]

\[ \text{AlCl}_3 \]
\[ \lambda \text{max} \quad 253, 282, 339 \text{ nm}; \]

\[ \text{AlCl}_3/\text{HCl} \]
\[ \lambda \text{max} \quad 253, 282, 339 \text{ nm}. \]

IR SPECTRUM OF THE AGLYCONE TB-1

The prominent peaks observed in the IR spectrum (Fig.2) and the structural units inferred with the help of available literature\(^11\),\(^12\) are recorded in 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>3420</td>
<td>-OH group(s)</td>
</tr>
<tr>
<td>2.</td>
<td>2870</td>
<td>-OCH₃ group(s)</td>
</tr>
<tr>
<td>3.</td>
<td>1680</td>
<td>C=O</td>
</tr>
<tr>
<td>4.</td>
<td>1525, 1540</td>
<td>Aromatic ring system</td>
</tr>
<tr>
<td>5.</td>
<td>2910</td>
<td>-CH stretching vibration</td>
</tr>
<tr>
<td>6.</td>
<td>1152</td>
<td>-C=O-bending vibration</td>
</tr>
<tr>
<td>7.</td>
<td>1230</td>
<td>C-O-C stretching vibration</td>
</tr>
<tr>
<td>8.</td>
<td>825</td>
<td>Two adjacent hydrogen atom in ring system</td>
</tr>
</tbody>
</table>

**PRESENCE OF -OH GROUP(S) IN TB-1**

In the IR spectrum a peak at $\nu_{\text{KBr max}} = 3420$ cm⁻¹, suggested the presence of free hydroxyl group(s) in it. On its acetylation (Ac₂O/Pyridine), it formed an acetyl derivative, m.p. 152°C, molecular formula C₂₀H₁₆O₇ and [M]⁺ 368 (EIMS). The percentage of acetyl group (23.36%) was determined by Wiesenberger method¹³ as described by Belcher and Godbert¹⁴, which confirmed the presence of two hydroxyl groups in the aglycone.

**PRESENCE OF -OCH₃ GROUP(S) IN TB-1**

In the IR spectrum a peak at $\nu_{\text{KBr max}} = 2870$ cm⁻¹ indicated the presence of -OCH₃ group(s) in the
IR SPECTRUM OF THE AGLYCONE TB-1
aglycone. Estimation of methoxy group (10.9%) by Zeisel's method\textsuperscript{15} suggested the presence of one methoxyl group in it.

**ALKALINE DEGRADATION OF THE AGLYCONES TB-1**

When aglycone was fused with 50% ethanolic KOH, gave two compounds, which were identified as (i) 2-methoxy phloroglucinol ([IA]) m.p. 154°C, m.f. C\textsubscript{7}H\textsubscript{8}O\textsubscript{4}, [M]\textsuperscript{+} 156 and (ii) benzoic acid ([IB]), m.p. 162°C, m.f. C\textsubscript{7}H\textsubscript{6}O\textsubscript{2} [M]\textsuperscript{+} 122.

 POSITION OF -OH GROUP(S) AT C-5 AND C-7 POSITION

The UV spectrum of the aglycone (TB-1) showed bathochromic shifts of 22 nm in band I with AlCl\textsubscript{3}.
(relative to MeOH) and 16 nm in band II with NaOAc (relative to MeOH), confirming the presence of -OH group(s) at C-5 and C-7 respectively\textsuperscript{16}.

**POSITION OF METHOXYL GROUP(S) IN TB-1**

The presence of one methoxyl group at position 6 in ring A, was confirmed by the \textsuperscript{1}H NMR spectrum of acetylated derivative of aglycone which showed a sharp singlet at $\delta$ 3.84 integrating for three protons\textsuperscript{17}.

Thus the structure of aglycone was assigned as; 5,7-dihydroxy, 6-methoxy flavone (I).

![Chemical Structure of I]

\textsuperscript{1}HNMR SPECTRUM OF THE DIACETYL DERIVATIVE OF THE AGLYCONEN TB-1

The chemical shifts observed in \textsuperscript{1}HNMR spectrum (Fig.3) of the acetylated derivative of the aglycone and
structural units inferred with the help of available literature are given in Table-III, which supported the identity of the compound as 5,7-dihydroxy, 6-methoxy flavone.

**TABLE - III**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value ($\delta$)</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>6.55</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td>2.</td>
<td>7.20</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-8</td>
</tr>
<tr>
<td>3.</td>
<td>7.45-7.54</td>
<td>m</td>
<td>-</td>
<td>3</td>
<td>H-3',4',5'</td>
</tr>
<tr>
<td>4.</td>
<td>7.80-7.91</td>
<td>m</td>
<td>-</td>
<td>2</td>
<td>H-2',6'</td>
</tr>
<tr>
<td>5.</td>
<td>3.84</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>H-6-OME</td>
</tr>
<tr>
<td>6.</td>
<td>2.35</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>H-5-OAc</td>
</tr>
<tr>
<td>7.</td>
<td>2.50</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>H-7-OAc</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM** \(^{20}\) **OF THE AGLYCONE TB-1**

The significant fragment ion peaks observed in EIMS of the aglycone were as follows;

\[ [M]^+ 284, m/z 256, 183, 182, 154, 102, 101. \]

The various species assigned to the fragments are shown in Scheme-I, which further confirmed its identity as 5,7-dihydroxy, 6-methoxy flavone.
$^1$H NMR SPECTRUM OF THE ACETYL DERIVATIVE OF THE AGLYCONE TB-1
Scheme I

$C_{16}H_{12}O_{5}$

$[M]^+ 284$

- CO

Pathway I

Pathway II

Without H⁺ transfer

$C_{15}H_{12}O_{4}$

$M/z 256$

$C_{8}H_{5}O_{5}$

$M/z 183$

$C_{8}H_{6}O_{5}$

$M/z 182$

$C_{8}H_{6}$

$M/z 102$

$C_{8}H_{5}$

$M/z 101$

$C_{7}H_{6}O_{4}$

$M/z 154$
STUDY OF THE SUGAR MOIETY (IES)

The aqueous hydrolysate obtained by the hydrolysis of the glycoside was neutralised with BaCO$_3$ and BaSO$_4$ filtered off. The filtrate on concentration under reduced pressure yielded a syrupy mass. On paper chromatography examination the sugar was identified as L-rhamnose (Rf = 0.38).

QUANTITATIVE ESTIMATION OF SUGAR

Quantitative estimation of the sugar in the glycoside was carried out by the procedure of Mishra and Rao$^{21}$, which revealed that the glycoside contains aglycone, and L-rhamnose in an equimolar ratio of one molecule each.

PERIODATE OXIDATION OF THE GLYCOSIDE

The glycoside on treatment with sodium meta periodate$^{22}$ consumed 2.03 moles of periodate and liberated 1.06 moles of formic acid, revealing the presence of one molecule of L-rhamnose attached to aglycone and also indicating that the sugar was in the pyranose form$^{23}$.

POSITION OF ATTACHMENT OF SUGAR TO THE AGLYCON

The position of sugar moiety at C-5 -OH of the aglycone was fixed by comparing the UV spectra of algycone and the glycoside;
1. The UV spectrum of the aglycone displayed a bathochromic shift of 22 nm in band-I with AlCl₃ (relative to MeOH) indicating the presence of free 5-OH and another bathochromic shift of 16 nm in band-II with NaOAc is for 7-OH.

2. The UV spectrum of glycoside displayed a bathochromic shift of 15 nm in band II with NaOAc (relative to MeOH), but no markable shift observed with AlCl₃ (relative to MeOH), suggested that -OH group at C-5 was free in aglycone while substituted in the glycoside.

PERMETHYLATION AND HYDROLYSIS OF THE GLYOSIDE TB

On permethylation of the glycoside by Kuhns procedure²⁴ followed by the acid hydrolysis of permethylated glycoside gave the aglycone (mmp, Co-PC) and 2,3,4-tri-O-methyl-rhamnose (Co-PC and Co-TLC), indicating that C-1 -OH group of rhamnose was involved in the glycosylation.

ENAYMATIC HYDROLYSIS OF GLYOSIDE TB

The glycoside on hydrolysis with enzyme to Kadiastase²⁵ yielded aglycone and L-rhamnose, confirming the presence of α-linkage between aglycone and L-rhamnose.
Keeping all the facts together, it was concluded that the 5-OH of aglycone was linked with C-1 of the L-rhamnose via \( \alpha \)-linkage.

Thus the glycoside was assigned the structure II as; 7-hydroxy 6-methoxy-5-O- \( \alpha \)-L-rhamnopyranoside which was finally established by its \( ^1 \)HNMR and mass spectral analysis.

\[ \text{(II)} \]

\( ^1 \)H NMR SPECTRUM OF THE TETRA ACETYL DERIVATIVE OF THE GLYCOSIDE TB

The significant peaks obtained in the \( ^1 \)H NMR spectrum of the tetra acetyl derivative of the glycoside; (Fig. 4) and structural units inferred with the help of available literature\(^ {26,27} \) are given in Table-IV;
### TABLE - IV

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value (δ)</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>6.62</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td>2.</td>
<td>6.85</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-8</td>
</tr>
<tr>
<td>3.</td>
<td>7.45-7.60</td>
<td>m</td>
<td>-</td>
<td>3</td>
<td>H-3'4'5'</td>
</tr>
<tr>
<td>4.</td>
<td>7.85-7.97</td>
<td>m</td>
<td>-</td>
<td>3</td>
<td>H-2'6'</td>
</tr>
<tr>
<td>5.</td>
<td>3.90</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6-OMe</td>
</tr>
<tr>
<td>6.</td>
<td>2.36</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>7-OAc</td>
</tr>
<tr>
<td>7.</td>
<td>4.9</td>
<td>d</td>
<td>2.0</td>
<td>1</td>
<td>H-1'-anomeric proton</td>
</tr>
<tr>
<td>8.</td>
<td>1.78-1.98</td>
<td>m</td>
<td>-</td>
<td>9</td>
<td>2',3',4'-OAc</td>
</tr>
<tr>
<td>9.</td>
<td>4.5-5.50</td>
<td>m</td>
<td>-</td>
<td>5</td>
<td>Sugar's protons</td>
</tr>
<tr>
<td>10.</td>
<td>1.02</td>
<td>d</td>
<td>6.0</td>
<td>3</td>
<td>Rham-CH₃</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM²⁰ OF THE GLYCOSIDE TB**

The important fragment ion peaks observed in the EIMS are given below:

\[ [M]^+ \text{ 430 m/z 284, 256, 183, 182, 154, 102, 101.} \]

The different species assigned to the fragments are shown in scheme-II which further confirmed its identity as; 7-hydroxy, 6-methoxy-5-O-α-L-rhamnopyranoside (II).
$^1$H NMR SPECTRUM OF THE ACETYL DERIVATIVE OF THE GLYCOSIDE TB
Scheme II

Pathway I: Without H⁺ transfer

Pathway II: With H⁺ transfer

M/z 256 → C₁₅H₁₂O₄

M/z 183 → C₈H₇O₅

M/z 182 → C₈H₆O₅

M/z 154 → C₈H₆O₄

M/z 101 → C₈H₅
EXPERIMENTAL

**Trichosanthes anguina** Linn. (N.O. cucurbitaceae) was procured by M/s United Chemicals and Allied Products, Calcutta and identified by the Botany Department of Dr. Hari Singh Gour University, Sagar. A herbarium specimen has been deposited in the room no. 36 of the Chemistry department.

The air-dried and powdered seeds (3.0 kg) of **T. anguina** Linn. were extracted with 95% ethanol in a 5 litre round bottom flask fitted with a condenser. The extract was then concentrated under reduced pressure to a yellow syrupy mass (125 gm) and resolved into the water soluble and insoluble part. The water soluble part was extracted successively with Pet. ether, benzene, chloroform, ethyl acetate, acetone and methanol.

The detailed study of acetone soluble fraction has been described in chapter IV of the thesis.

The study of ethyl acetate soluble fraction has been dealt with in this chapter.

**STUDY OF ETHYL ACETATE SOLUBLE PART**

The ethyl acetate soluble fraction of the water soluble part of the ethanolic extract was concentrated under reduced pressure to yield a yellow
syrupy mass (3.4 gm). It gave two spots on TLC examination, using solvent system EtOAc-Acetone-H₂O (9:3:1) and I₂ vapours as visualising agent, which were separated by column chromatography over si-gel G and eluted with chloroform-methanol in various proportions, (6:1), (6:2), (6:4) and (6:8).

COLUMN CHROMATOGRAPHY

Length of the column - 150 cm.
Diameter of the column - 4.0 cm.
Weight of the crude extract - 3.4 gm.
Weight of the si-gel G - 250 gm.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction No.</th>
<th>Eluants</th>
<th>Spot on TLC</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1 - 6</td>
<td>Chloroform-methanol (6:1)</td>
<td>Nil</td>
<td>No solid mass</td>
</tr>
<tr>
<td>2.</td>
<td>7 - 14</td>
<td>Chloroform-methanol (6:2)</td>
<td>One</td>
<td>Compound TA</td>
</tr>
<tr>
<td>3.</td>
<td>15 - 24</td>
<td>Chloroform-methanol (6:4)</td>
<td>One</td>
<td>Compound TB</td>
</tr>
<tr>
<td>4.</td>
<td>25 - 36</td>
<td>Chloroform-methanol (6:8)</td>
<td>Nil</td>
<td>Sticky mass</td>
</tr>
</tbody>
</table>

The detailed study of the fraction 7-14 (compound TA) has been described in chapter II of the thesis.

STUDY OF FRACTION (15-24)

Eluates obtained from chloroform-methanol (6:4) were found to have same Rf value hence mixed together.
On removal of the solvent, it was crystallised from methanol yielded a brownish yellow coloured compound (2.06 gm). It gave single spot on TLC examination using solvent system, BuOH-HOAc-H₂O, (4:1:5) and I₂ vapours as visualising agent.

STUDY OF THE FLAVONE GLYCOSIDE TB

The flavone glycoside (TB) was a brownish yellow crystalline compound and soluble in ethyl acetate and water. It analysed for molecular formula C₂₂H₂₂O₉, m.p. 327°C and [M]+ 430 (EIMS).

It responded positive Molisch fest for glycoside and following characteristic colour reactions of flavonoids:

(i) Red colour with Mg-HCl.
(ii) Intense green colour with FeCl₃.

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₂₂H₂₂O₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>C  61.38%</td>
<td>C  61.39%</td>
</tr>
<tr>
<td>H  5.10%</td>
<td>H  5.11%</td>
</tr>
</tbody>
</table>

Molecular weight - 430
(By EIMS)

ACETYLATION OF THE GLYCOSIDE TB

The acetylation of the glycoside was done in a similar way as described on page no. 49 of chapter II of the thesis.
The acetyl derivative was crystallised from methanol as white needles (45 mg) m.p. 196°C and analysed for $C_{30}H_{30}O_{13}$, [M]$^+$ 598 (EIMS).

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for $C_{30}H_{30}O_{13}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 60.18%</td>
<td>C 60.20%</td>
</tr>
<tr>
<td>H 5.00%</td>
<td>H 5.01%</td>
</tr>
</tbody>
</table>

Molecular weight = 598 (By EIMS)

**ACID HYDROLYSIS OF THE GLYCOSIDE TB**

The glycoside (350 mg) was refluxed with 7% ethanolic $H_2SO_4$ in a 250 ml RB flask for about 10 hours. The contents were allowed to cool and after filtration, a yellow coloured compound (TB-1) was obtained.

The aglycone and hydrolysate were studied separately.

**STUDY OF THE AGLYCONE TB-1**

The aglycone was yellow, crystalline compound and soluble in ethyl acetate. It showed single spot on TLC examination, using solvent system EtOAc-CHCl$_3$-H$_2$O (98:1:1) $R_f = 0.74$, $I_2$ vapours used as visualizing agent. It had m.p. 201-202°C and analysed for m.f. $C_{16}O_{12}O_5$, [M]$^+$ 284 (EIMS). It gave
following characteristic colour reactions for flavonoids:

(i) Green colour with ethanolic FeCl₃
(ii) Red colour with Na-Hg/HCl.

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₁₆H₁₂O₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 67.62%</td>
<td>C 67.60%</td>
</tr>
<tr>
<td>H 4.24%</td>
<td>H 4.22%</td>
</tr>
</tbody>
</table>

Molecular weight - 284
(By EIMS)

ACETYLATION OF THE AGLYCON TB-1

The acetylation of the aglycone (TB) was carried out in a similar procedure as described on page no. 49 of chapter II of the thesis.

The acetyl derivative was crystallised from methanol as a white needles (40 mg), m.p. 190°C and analysed for C₂₀H₁₆O₇, [M]⁺ 368 (EIMS).

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₂₀H₁₆O₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 65.20%</td>
<td>C 65.21%</td>
</tr>
<tr>
<td>H 4.35%</td>
<td>H 4.34%</td>
</tr>
</tbody>
</table>

Molecular weight - 368
(By EIMS)
ESTIMATION OF METHOXY GROUP(S) IN THE AGLYCON TB

The estimation of methoxy group was carried out by Zeisel's method as described on page no. 51 of the thesis.

DEGRADATION OF THE AGLYCON WITH KOH

(150 mg) of the aglycone, 10 ml. of ethanol and 20 ml. of KOH solution were refluxed in 250 ml. of RB flask. The reaction mixture was cooled and acidified by dil HCl. The contents were extracted with solvent ether. The ethereal layer was washed with water and separated into two parts:

1. The ethereal layer was treated with 50% sodium bicarbonate solution and the aqueous part, on acidification gave a compound, molecular formula C₇H₆O₂, m.p. 162°C, [M]⁺ 122, which was identified as benzoic acid (IB) (by Co-Pc and Co-TLC).

2. The second part on treatment with 12% solution of NaOH and on acidification gave another compound, molecular formula C₇H₈O₄, m.p. 154°C [M]⁺ 156 identified as 2-methoxy phloroglucinol (IA), (by Co-Pc and Co-TLC).
STUDY OF THE SUGAR HYDROLYSATE

The sugar hydrolysate obtained by the hydrolysis of the glycoside was neutralised with BaCO₃ and BaSO₄ filtered off. The filtrate on concentration yielded a syrupy mass which was subjected to paper chromatographic examination using various solvent system and aniline hydrogen phthalate as detecting agent. The results are as follows:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent system</th>
<th>Rf value</th>
<th>Sugar identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethyl acetate-pyridine-water (2:1:2)</td>
<td>0.49</td>
<td>L-rhamnose</td>
</tr>
<tr>
<td>2.</td>
<td>Butanol-acetic acid-water (4:1:5)</td>
<td>0.39</td>
<td>L-rhamnose</td>
</tr>
</tbody>
</table>

PERMETHYLATION AND HYDROLYSIS OF THE GLYCOSIDE

The glycoside (45 mg) was treated with MeI (5 ml) and Ag₂O (110 mg) in DMF (6 ml) in a 50 ml. conical flask and left for 48 hours at room temperature. The contents were filtered and residue washed with dimethyl formamide (DMF). After concentration, the filtrate was hydrolysed with 7% H₂SO₄ to give the aglycone and methylated sugar. After removal of the aglycone the aqueous part was neutralised with BaCO₃ and BaSO₄ filtered off. The filtrate
was concentrated and examined by paper chromatography on Whatman paper no. 1 using n-butanol-acetic acid-water (4:1:5) as solvent system and aniline hydrogen phthalate as spraying reagent, the sugar was identified as 2,3,4-tri-O-methyl-rhamnose.

PERIODATE OXIDATION OF THE GLYCOSIDE

The periodate oxidation of the glycoside was carried out in the same way as described on page no. 56 of the thesis.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE

The glycoside (55 mg) was dissolved in ethanol (25 ml) and mixed with enzyme tokadiastase (25 ml) in a 100 ml. conical flask attached with a stopper. The reaction mixture was allowed to stay at room temperature and filtered. The aglycone and hydrolysate were studied separately.

The hydrolysate was concentrated under reduced pressure and examined by PC using Whatman filter paper no. 1 and BAW (4:1:5) as solvent system, and aniline hydrogen phthalate as spraying reagent. The sugar was identified as L-rhamnose (Rf = 0.38).
REFERENCES


