CHAPTER III

ISOLATION AND STUDY OF A NOVEL FLAVONE GLYCOSIDE: KAEMPFEROL 7-O-β-D-GLUCOPYRANOSE (1→4) O-β-D-XYLOPYRANOSIDE FROM THE SEEDS OF CROTALARIA LABURNIFOLIA LINN.
Crotalaria laburnifolia Linn. is commonly known as 'Muna' and belongs to N.O. Leguminosae. It occurs in Western Peninsula, Northern Andhra, Mysore, Travancore, Sri Lanka, Malaya and Phillipines.

The plant is erect, perineal and often cultivated in gardens for its large yellow flowers. It has been tried as a green manure for tea plantations in India, Sri Lanka and Dutch Indies. It produces plenty of leaves and it readily becomes woody which is generally not affected by pests.

The plant is said to be used as gargle to cure sore throat inflammation of the mouth.2

ISOLATION OF THE FLAVONE GLYCOSIDE (CL):

Crotalaria laburnifolia Linn. was supplied from the United Chemicals and Allied Products, Calcutta and authenticated by the Department of Botany of this University.

Air dried, powdered and defatted seeds of Crotalaria laburnifolia Linn. were subjected to extraction with 95% ethanol. The extract so obtained, on removal of the solvent yielded a brown viscous mass which was separated into water soluble and water insoluble part. The water soluble part was concentrated to viscous mass which was successively extracted with benzene, chloroform, ethylacetate, acetone and methanol.
The benzene, ethylacetate, acetone soluble parts were obtained in very small amount and hence discarded.

The detailed study of methanol soluble fraction has been described in Chapter IV of this thesis.

The study of the chloroform soluble fraction has been described in this chapter.

**STUDY OF CHLOROFORM SOLUBLE FRACTION:**

The chloroform soluble fraction was concentrated under reduced pressure yielded a dark brown syrupy mass, on TLC examination it gave two spots using chloroform:methanol (6:4) and iodine vapours as spraying reagent which was purified by Column chromatography over silica gel G (60-120 mesh) and eluted with chloroform:methanol:water in various proportions. On removal of the solvent gave a yellow compound yield (CL) 0.040%.

It gave positive Molisch test for glycoside and characteristic colour reactions for flavanoids.3,4

**STUDY OF THE FLAVONE GLYCOSIDE (CL):**

The flavone glycoside (CL) was soluble in chloroform, ethanol and water, and crystallised with solvent ether, it analysed for molecular formula \( C_{26}H_{28}O_{15} \), m.p. 237-38°C, and \([M]^+\) 580 (EIMS).
**UV SPECTRUM OF THE FLAVONE GLYCOSIDE (CL):**

The wave lengths of maximum absorption as recorded with various shift reagents is given below:

\[ \lambda_{max}^{\text{MeOH}} 290, 350 \text{ (sh);} \]

\[ \lambda_{max}^{\text{NaOMe}} 272, 377, 390 \text{ (sh);} \]

\[ \lambda_{max}^{\text{NaOAc}} 358, 384 \text{ (sh);} \]

\[ \lambda_{max}^{\text{AlCl}_3} 274, 330, 396 \text{ (sh);} \]

\[ \lambda_{max}^{\text{AlCl}_3/HCl} 267, 342, 352 \text{ (sh).} \]

**IR SPECTRUM OF THE FLAVONE GLYCOSIDE (CL):**

The prominent IR peaks (Fig. 1) of the (CL) as observed and assignments of the functional groups in the molecule made with the help or available literature.\(^5\),\(^6\) are given below:
**PRESENCE OF HYDROXYL GROUP(S):**

IR peak at $\nu_{\text{max}}^{\text{KBr}} 3340 \text{ cm}^{-1}$ confirmed the presence of $\text{-OH}$ groups in it. On acetylation with ($\text{Ac}_2\text{O/py}$), it gave an acetyl derivative, molecular formula $C_{44}H_{46}O_{24}$, m.p. 212-13°C, $[\mathcal{M}]^+ 958$ (LMG) and acetyl percentage (38.40%) was estimated by Wiesenberger's method as described by Belcher and Godbert. It suggested the presence of nine hydroxyl groups in the glycoside.

IR absorbance of the acetyl derivative at $\nu_{\text{max}}^{\text{KBr}} 1716 \text{ cm}^{-1}$ and disappearance of the peak at $\nu_{\text{max}}^{\text{KBr}} 3340 \text{ cm}^{-1}$ suggested that all the nine hydroxyl group(s) in the glycoside have acetylated.

**ACID HYDROLYSIS OF THE GLYCOSIDE (CL):**

On acid hydrolysis (CL) (7% alc. $\text{H}_2\text{SO}_4$) it gave an aglycone and sugar moieties which were separated by filtration and studied separately.

**STUDY OF THE AGLYCONE:**

The aglycone was crystallized from ethanol, to give a light yellow crystalline compound which was found to be homogenous on TLC examination using benzene:methanol (8:2), which gave a single spot.

It analysed for molecular formula $C_{15}H_{10}O_{6}$, m.p. 280°C $[\mathcal{M}]^+ 286$ (LMG), and responded to all the characteristic colour reactions of flavanoids.
UV SPECTRUM OF THE AGLYCONE:

\[ \lambda_{\text{max}} \]
\[ \text{MeOH} \quad 254, 330 \text{ (sh)}; \]
\[ \text{NaOAc} \quad 271, 340, 370 \text{ (sh)}; \]
\[ \text{NaOAc} \quad 286, 326 \text{ (sh)}; \]
\[ \text{AlCl}_3 \quad 270, 334, 376 \text{ (sh)}; \]
\[ \text{AlCl}_3/\text{HCl} \quad 260, 304, 360 \text{ (sh)}. \]

IR SPECTRUM OF THE AGLYCONE:

The significant IR signals (Fig. 2) and functional groups assigned to the molecule with the help of available literature\(^9,10\) are recorded below:
**Presence of Hydroxyl Group(s):**

Appearance of peak in IR at $\nu_{\text{max}}^{\text{KBr}} 3330 \text{ cm}^{-1}$ suggested the presence of free $-\text{OH}$ group(s) in it. On acetylation ($\text{Ac}_2\text{O/py}$) it yielded a tetra acetyl derivative, molecular formula $\text{C}_{23}\text{H}_{18}\text{O}_{10}$, m.p. 265$^\circ\text{C}$, $[M]^+ 454$ (EIMS). The acetyl group percentage (47.64%) was estimated by Wiesenerber$^7$ as described by Belcher and Godert$^8$. They showed the presence of four $-\text{OH}$ groups in the aglycone.

**Alkaline Degradation:**

On alkaline degradation with 50% ethanolic KOH, the aglycone yielded two compounds which were identified as phloroglucinol by (Co-Pc and Co-TLC) [IIA], molecular formula $\text{C}_6\text{H}_6\text{O}_3$, m.p. 117-118$^\circ\text{C}$, $[M]^+ 126$, and p-hydroxy benzoic acid [IIA] by (Co-Pc and Co-TLC) molecular formula $\text{C}_7\text{H}_6\text{O}_3$, m.p. 215-17$^\circ\text{C}$, $[M]^+ 138$. 

![Diagram](image)

- **(II)**
- **(II-A)**
- **(II-B)**

\[ \text{EtOH} \xrightarrow{\text{KOH}} \text{HO} \]

\[ \text{HO} \]

\[ \text{COOH} \]
POSITION OF HYDROXYL GROUP AT C-3:

A characteristic colour reaction with Zn/HCl\textsuperscript{11} and zirconium oxychloride in citric acid further confirmed the presence of $\text{-OH}$ groups at C-3.\textsuperscript{12}

A bathochromic shift of 46 nm with AlCl\textsubscript{3} (relative to MeOH) in band I further indicated the presence of $\text{-OH}$ group at C-3.\textsuperscript{13}

POSITION OF HYDROXYL GROUP AT C-5 AND C-7:

On fussion with KOH formation of phloroglucinol indicated the presence of $\text{-OH}$ groups at C-5 and C-7.

A bathochromic shift of 8 nm of band II on addition with NaOAc (relative to MeOH) showed the presence of free $\text{-OH}$ group at C-7.\textsuperscript{14}

POSITION OF $\text{-OH}$ GROUP AT C-4:\n
The position of third $\text{-OH}$ group was fixed at C-4' because the pink coloured solution obtained in Shinoda reaction\textsuperscript{15} became blue on addition of NaHCO\textsubscript{3} which indicated the presence of $\text{-OH}$ group at C-4' in the ring B, which was further supported by the formation of p-hydroxy benzoic acid on alkaline fussion and a bathochromic shift of 40 nm with NaOMe in band I (relative to MeOH) in band I showed the presence of $\text{-OH}$ group at C-4'.
Thus the above structure was assigned as:

(II)

$^1$H NMR spectrum of the aglycone:

The chemical shifts obtained in the $^1$H NMR spectrum of the acetylated aglycone and structural units inferred with the help of available literature$^{16,17}$ are tabulated below:
MASS SPECTRUM OF THE AGLYCONE:

EIMS of the aglycone showed the fragment ion peaks at:

\[ [M]^+ \text{ 286 m/e 285, 257, 153, 152, 136, 124}. \]

The various species assigned to the fragment are described in Scheme - I.

STUDY OF THE SUGAR MOIETY(IES):

The hydrolysate of the glycoside was neutralised with BaCO₃ and BaSO₄ and filtered off. The filtrate was concentrated under reduced pressure to yield a yellowish syrupy mass which was examined by (Co-PC and Co-ILC) with authentic of sample, on Whatmann filter paper No. 1 and aniline-hydrogen phthalate was used as visualizing agent, revealed the presence of D-glucose and D-xylose.

QUANTITATIVE ESTIMATION OF THE SUGAR:

Quantitative estimation of the sugars was done by the procedure of Mishra and Rao \(^{18}\) which indicated that both the sugars were present in an equimolar ratio.

PERIODATE OXIDATION OF THE GLYCOSIDE:

The glycoside, on oxidation \(^{19}\) with sodium meta-periodate consumed 3.02 moles of periodate and liberated 1.02 moles of formic acid, suggested the presence of one molecule of glucose and one molecule of xylose was present in the pyranose form. \(^{20}\)
PERMETHYLATION AND HYDROLYSIS OF PRO-AGLYCONE (CL-1):

Permethylation of the proaglycone (CL-1) by Khun’s procedure\textsuperscript{21} followed by hydrolysis yielded 2,3,4 tri-O-methyl-D-xylose (Co-PC) and the aglycone, thereby indicating that C1 of D-xylose was involved in the formation of glycosidic linkage and also xylose was present in the pyranose form.

Thus the proaglycone (CL-1) was identified as; kaempferol 7-O-\(\beta\)-D-xylopyranoside (III).

\[
\text{(III)}
\]

STUDY OF THE PROAGLYCONE (CL-2):

The proaglycone (CL-2) on hydrolysis with 7\% \(\text{H}_2\text{SO}_4\) yielded the aglycone and D-xylose and D-glucose (m.m.p and Co-TLC). It analysed for molecular formula \(\text{C}_{26}\text{H}_{28}\text{O}_{15}\), m.p. 237-38\(^\circ\text{C}\), \([\text{M}]^+\) 580 (\text{EIMS}).

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

The proaglycone (CL-2) on permethylation followed by hydrolysis, yielded aglycone and 2,3,4,6 tetra-O-methyl-D-
glucose and 2,3, di-O-methyl-D-xylose (by Co-Pc and Co-TLC), suggested that C4-OH group of the xylose was linked with Cl-OH group of D-glucose and both sugars were present in the pyranose form.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE (CL):

The enzymatic emulsin\textsuperscript{22} hydrolysis confirmed the presence of β-linkage between the sugars and the aglycone.

Thus the structure to the glycoside was assigned as; Kaempferol 7-O-β-D-glucopyranosyl (1 → 4)-O-β-D-xylo-pyranoside \textsuperscript{[IV]}.

The above structure to the glycoside was confirmed by \textsuperscript{1}HNMR and Mass spectral studies.

\textsuperscript{1}HNMR SPECTRUM OF THE ACETYL DERIVATIVE OF THE GLYCOSIDE (CL):

The significant signals obtained in \textsuperscript{1}HNMR (Fig. IV) and structural units inferred with the help of available literature\textsuperscript{23,24} are recorded in Table - IV.
Scheme II
EXPERIMENTAL

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

About (2 Kg) of defatted, airdried powdered seeds of *Crotalaria laburnifolia* Linn. was subjected to extraction with hot 95% ethanol in a 5 litre round bottomed flask fitted with a condenser for a month. The total extract was concentrated under reduced pressure to yield a brown viscous mass (120 gm). It was separated into water soluble and water insoluble part by adding into distilled water with constant stirring. The water soluble portion was then subjected to successively extraction with benzene, chloroform, ethyl acetate, acetone and methanol.

Removal of the solvent from various fractions (except chloroform) produced negligible amount hence any substantive study on these parts was not possible.

The detailed study of methanol soluble part has been described in Chapter IV of this thesis.
STUDY OF THE CHLOROFORM SOLUBLE FRACTION:

The chloroform soluble fraction was concentrated under reduced pressure to yield a yellow coloured substance (2.8 gm). On TLC examination, it gave two spots using solvent system chloroform:methanol (6:4) and I₂ vapours as visualizing agent, which purified by column chromatography over si-gel u and eluting by chloroform:methanol:water in various proportions.

COLUMN CHROMATOGRAPHY:

Length of the column - 160 cm
Diameter of the column - 5.0 cm
Weight of the crude extract - 2.9 gm
Weight of si-gel G - 250 g.
(60-120 mesh)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fraction No.</th>
<th>Eluant</th>
<th>Spot on TLC</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - 5</td>
<td>Chloroform:methanol:</td>
<td>Nil</td>
<td>No solid substance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>water 13:7:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6 - 10</td>
<td>Chloroform:methanol:</td>
<td>One</td>
<td>Compound (CL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>water 13:7:2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11 - 17</td>
<td>Chloroform:methanol:</td>
<td>Nil</td>
<td>No crystalline material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>water 13:7:3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
STUDY OF FRACTION (6-10):

The fractions collected (6-10) were of same Rf values and so mixed together and crystallised to give a yellow coloured compound (2.05 gm). It gave single spot on TLC examination over si-gel G using chloroform:methanol:water (8:2:1).

STUDY OF THE FLAVONE GLYCOSIDE (CL):

The flavone glycoside (CL) (1.90 gm) was a light yellow crystalline compound soluble in ethanol, methanol and water. It analysed for molecular formula \( C_{26}H_{28}O_{15} \), m.p. 237-38° C and \([M]^+ 580 (EIMS)\).

It gave a positive Molish test for glycoside and characteristic colour reactions of flavanoids.

(i) Intense green colour with \( \text{Fe}^2+\text{Cl}_3 \).
(ii) Pink colour with \( \text{Na}^+\text{Hg/HCl} \).
(iii) Yellow colour with \( \text{Mg/HCl} \).

ELEMENTAL ANALYSIS:

<table>
<thead>
<tr>
<th>FOUND</th>
<th>CALCULATED ( C_{26}H_{28}O_{15} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 53.40%</td>
<td>C - 53.44%</td>
</tr>
<tr>
<td>H - 4.80%</td>
<td>H - 4.82%</td>
</tr>
</tbody>
</table>

Molecular weight 580 (by mass spectroscopy)
ACETYLATION OF THE COMPOUND (CL):

Compound (CL) (400 mg), fused pyridine (800 mg) and acetic anhydride (6 ml) was taken in a 250 ml round bottomed flask fitted with an air condenser and the contents heated on an oil bath for about 10 hrs at 130°C. After cooling, the mixture was poured into cold water, when thick white precipitate was obtained. This was extracted with solvent ether and separated by separating funnel. The ethereal layer after washing with aqueous NaHCO₃, and ether was removed by evaporation. The residue was crystallised from chloroform as white needles (70 mg) and analysed for molecular formula C₄₄H₄₆O₂₄, m.p. 212-13°C, [α]⁺ 968 (EIMS).

ELEMENTAL ANALYSIS:

<table>
<thead>
<tr>
<th>FOUND</th>
<th>CALCULATED C₄₄H₄₆O₂₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 55.10%</td>
<td>C - 55.11%</td>
</tr>
<tr>
<td>H - 4.78%</td>
<td>H - 4.79%</td>
</tr>
</tbody>
</table>

Molecular formula 968 (By mass spectroscopy)

ACID HYDROLYSIS OF THE GLYCOSIDE (CL):

(1.90 gm) of the glycoside along with 20% ethanolic H₂SO₄ was taken in a 250 ml round bottomed flask and refluxed for 16 hrs. The contents were added in (100 ml) of water and transferred to a separating funnel. The aqueous solution was neutralized with BaCO₃ and BaSO₄ and was filtered off. Removal of the solvent yielded an aglycone.
molecular formula \( \text{C}_{15}\text{H}_{10}\text{O}_6 \), m.p. 280°C, \([\text{M}]^+ 286\) (EIMS). The aglycone and hydrolysate were studied separately.

**STUDY OF THE AGLYCON**

The aglycone was crystallised from ethanol, it gave yellow crystalline solid (1.05 gm) and was found to be homogenous in nature on TLC examination which gave a single spot using solvent system benzene:methanol (8:2) and I2 vapours as visualizing agent. It was soluble in chloroform, ethanol and water. It analysed for molecular formula \( \text{C}_{15}\text{H}_{10}\text{O}_6 \), m.p. 280°C, \([\text{M}]^+ 286\) (EIMS).

It also showed the following colour reactions.

(i) Green colour with \( \text{Fe-Cl}_3 \).
(ii) Pink colour with \( \text{Na-Hg/HCl} \).
(iii) Yellow colour with \( \text{Mg/HCl} \).

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>FOUND</th>
<th>CALCULATED ( \text{C}<em>{15}\text{H}</em>{10}\text{O}_6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 62.90%</td>
<td>C = 62.93%</td>
</tr>
<tr>
<td>H = 3.40%</td>
<td>H = 3.42%</td>
</tr>
</tbody>
</table>

Molecular weight 286 (By mass spectroscopy)

**ACEYLATION OF THE AGLYCON**

The acetylation of the aglycone was done in a similar procedure to that described on page No. 73 of this thesis. It analysed for molecular formula \( \text{C}_{23}\text{H}_{12}\text{O}_{16} \), m.p. 265°C, \([\text{M}]^+ 454\) (EIMS).
**Elemental Analysis:**

**Found**

C - 66.78%
H - 3.50%

**Calculated**

C₂₃H₁₈O₁₀
C - 60.79%
H - 3.52%

Molecular weight 454 (by mass spectroscopy)

**Methylation of the Aglycone:**

The aglycone (160 mg), (2.5 ml) anhydrous K₂CO₃ and (100 ml) of dry acetone, (80 ml) dimethyl sulphate was taken in 250 ml round bottomed flask and attached with air condensor and heated on water bath for 9 hrs. On addition of water, the reaction mixture filtered to removed the inorganic salt and acetone evaporated off. A yellow coloured solution was obtained which was purified by column chromatography on si-gel G using solvent ether.

On removal of the solvent, it gave colourless mass (40 mg). It had molecular formula C₁₉H₁₈O₆, m.p. 150-52°C, [M]⁺ 342.

**Elemental Analysis:**

**Found**

C - 66.64%
H - 5.24%

**Calculated**

C₁₉H₁₈O₆
C - 66.66%
H - 5.26%

Molecular weight 342 (by mass spectroscopy)
ALKALINE DEGRADATION OF THE AGLYCONE:

About (200 mg) of the aglycone was treated with 15 ml of ethanol and 50% ethanolic KOH in a 250 ml B-14 ground joint flask. The content were cooled and acidified by dil HCl. The degraded products were separated with solvent ether in a separating funnel. The ethereal layer after washing with water and separated into two parts:

1. The first part when treated with 50% NaHCO₃ solution and the aqueous part on acidification gave a compound [II]B which was identified as p-hydroxy benzoic acid (Co-PC and Co-TLC). It was analysed for molecular formula C₇H₆O₃, m.p. 215-17°C, [M]+ 138.

2. The second part was treated with 10% NaOH solution and after subsequent acidification gave another compound [II]A which was identified as phloroglucinol by (Co-PC and Co-TLC). It was analysed for molecular formula C₆H₆O₃, m.p. 117-18°C, [M]+ 140 (EIMS).
PARTIAL HYDROLYSIS OF THE GLYCOSIDE (CL)

The glycoside (200 mg) was treated with Killiani mixture (HCl:CH₃COOH:H₂O) in 250 ml round bottomed flask and attached with air condensor and heated for about 8 days at room temperature. Thereafter the content were extracted with n-butanol. The butanol solution part on TLC examination gave two spots (Rf 0.32 and 0.50) in n-butanol:acetic acid:water (4:1:5) as solvent system and aniline hydrogen pthalate as visualizing agent. It was concentrated and purified by column chromatography using si-gel G (60-120 mesh) column and eluted with chloroform:methanol:water in different proportions.

COLUMN CHROMATOGRAPHY:

<table>
<thead>
<tr>
<th>Weight of butanol soluble part</th>
<th>310 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of column</td>
<td>100 cm</td>
</tr>
<tr>
<td>Diameter of column</td>
<td>5.0 cm</td>
</tr>
<tr>
<td>Weight of si-gel G</td>
<td>250 g</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE - 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. No.</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
STUDY OF THE FRACTION (1-6):

The fraction (1-6) were of same Rf values, therefore combined and on evaporation of the solvent gave the pro-aglycone (CL-1), which was crystallised from ethanol. It analysed for molecular formula \( \text{C}_{20}\text{H}_{18}\text{O}_{10} \), m.p. 186-82°C, \([M]^+ 418\).

ELEMENTAL ANALYSIS OF PROAGLYCONE (CL-1):

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated ( \text{C}<em>{20}\text{H}</em>{18}\text{O}_{10} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 57.40%</td>
<td>C - 57.41%</td>
</tr>
<tr>
<td>H - 4.30%</td>
<td>H - 4.30%</td>
</tr>
</tbody>
</table>

Molecular weight 418 (by mass spectroscopy)

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (CL-1):

About (140 mg) of (CL-1) was treated with methyl iodide (50 ml) and \( \text{Ag}_2\text{CO}_3 \) (200 mg) in dimethyl formamide (6.0 ml) in a 250 ml conical flask and heated on room temperature for four days. The contents after filtration were washed with dimethyl formamide. On evaporation of the filtrate yielded a residue was concentrated under reduced pressure which was treated with acetone. On removal of acetone, a syrupy mass was obtained. On hydrolysis with 20% \( \text{H}_2\text{SO}_4 \), it gave the aglycone and methylated sugar.

The aqueous hydrolysate was neutralised with \( \text{BaCO}_3 \) and \( \text{BaSO}_4 \) and filtered off, and the filtrate was
concentrated under reduced pressure. The methylated sugar was identified as 2,3,di-O-methyl-D-xylose (by Co-PC and Co-TLC).

STUDY OF FRACTIONS (10-12):

The fraction (10-12) were of same Rf values, and therefore they were mixed together and on the removal of the solvent yielded a compound which was crystallised from ethanol, and analysed for molecular formula $\text{C}_{26}\text{H}_{28}\text{O}_{15}$, m.p. 237-38°C, [M]$^+\text{ 580 (EIMS)}$.

ELEMENTAL ANALYSIS OF THE PROAGLYCONE (CL-2):

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated $\text{C}<em>{26}\text{H}</em>{28}\text{O}_{15}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - 53.40%</td>
<td>C - 53.44%</td>
</tr>
<tr>
<td>H - 4.80%</td>
<td>H - 4.82%</td>
</tr>
</tbody>
</table>

Molecular weight 580 (By mass spectroscopy)

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

The process of permethylation and hydrolysis of the proaglycone (CL-2) was carried out in the similar way as described on page No. 83 of the thesis. The hydrolysate was found to contain 2,3,di-O-methyl-D-xylose (by Co-PC and Co-TLC) and 2,3,4,6 tetra-O-methyl-D-glucose (by Co-PC and Co-TLC) and authentic sample.
IDENTIFICATION OF THE SUGARS AFTER HYDROLYSIS:

The aqueous hydrolysate (50 ml) obtained as a result of hydrolysis (7% H₂SO₄) of the glycoside was neutralised with BaCO₃ and BaSO₄ and filtered off. The filtrate was concentrated under reduced pressure, to yield a yellow syrupy mass, which was identified as D-glucose and D-xylose by paper chromatography using solvent system n-butanol:acetic acid:water (4:1:5) on Whatmann filter paper No. 1 using aniline hydrogen phthalate as detecting agent.

The results are recorded below in Table - 7.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent system</th>
<th>Reported²⁵</th>
<th>Found</th>
<th>Sugar identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-butanol:acetic acid: water (4:1:5)</td>
<td>0.28</td>
<td>0.26</td>
<td>D-xylose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.18</td>
<td>0.19</td>
<td>L-glucose</td>
</tr>
</tbody>
</table>

PERIODATE OXIDATION OF THE GLYCOSIDE (CL):

The glycoside (150 mg) was dissolved in ethanol (40 ml) in a 250 ml B-14 ground joint conical flask attached with a reflux condensor. The contents treated with sodium metaperiodate (20 ml of 0.1 M) and allowed to stand at room temperature for two days. The amount of formic acid liberated and periodate molecule consumed was estimated by the method as described by Jones et al.
ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE (CL):

(50 mg) of the glycoside was hydrolysed with almond emulsin (50 ml) at room temperature for about 60 hrs and the hydrolysate on chromatography over Whatmann No. 1 filter paper revealed the presence of D-glucose and D-xylose and also indicated β-linkage between both the sugar residues and between sugar and the aglycone.


