CHAPTER - V

*ISOLATION AND CHARACTERISATION OF A NOVEL FLAVANONE GLYCOSIDE: 6-METHYL, 7-METHOXY FLAVANONE-5-O-α-L-RHAMNOPYRA NOSYL (1 → 4) -O-β-D-GLUCOPYRANOSIDE, FROM THE AERIAL PARTS OF COCCINIA INDICA W & A.

* This work has been communicated for publication in journal of INDIAN CHEMICAL SOCIETY, CALCUTTA.
Coccinia indica W&A\(^1,2\) is commonly known as 'Kundru' in Hindi and belongs to natural order Cucurbitaceae. It is distributed throughout India, Ceylon and tropical Africa. It is a prostrate creeping herb with long tapering tuberous roots. Its leaves are simple, 5-10 cm long and broad, stem grooved, flowers white or yellow, dioecious. Seeds are compressed and yellowish grey.

The Ayurvedic system of medicine describes that the roots of this plant are used in the treatment of jaundice, leprosy, bronchitis, asthma and blood deseases. Fresh juice extracted from the leaves and roots of this plant is useful for diabetic patients\(^3-5\). The antidiabetic property of the roots have been studied by De and Mukerji\(^6\).

A number of bio-active constituents have been isolated by earlier workers\(^7\).

This chapter deals with the isolation and identification of a novel flavanone glycoside from the aerial parts of Coccinia indica W&A.

**ISOLATION OF THE FLAVANONE GLYCOSIDE CS**

The plant material was procured by M/s United Chemicals and Allied Products, Calcutta andidentified
by the Department of Botany of this University.

The air dried and crushed aerial parts of *Coccinia indica* W&A were extracted with 95% methanol. The total methanolic extract was concentrated to a yellow viscous mass, which was poured into distilled water. The water soluble part was concentrated to viscous mass and successively extracted with petroleum ether, benzene, chloroform, ethyl acetate, acetone and methanol.

The petroleum ether, chloroform, benzene, acetone and methanol soluble parts on removal of the solvent gave very small amount of residue and hence discarded.

The study of ethyl acetate soluble fraction has been described in the chapter.

**STUDY OF ETHYL ACETATE SOLUBLE FRACTION**

The ethyl acetate soluble fraction was concentrated under reduced pressure to yellow syrupy mass. TLC examination using EtOAc-MeOH-H$_2$O (12:5:3) solvent system and I$_2$ vapours as visualising agent, gave a single spot. It was therefore chromatographed over a si-gel G column and eluted with EtOAc-Methanol in various proportions. Eluates obtained from fractions (12-17) were found to have same Rf value
and so combined. On evaporation of the solvent and crystallisation from methanol, yielded a yellowish coloured needles, CS (0.056%). It was found to be homogenous on TLC examination.

**STUDY OF THE FLAVANONE GLYCOSIDE CS**

It's elemental analysis showed, molecular formula C_{29}H_{36}O_{13} m.p. 300°C and [M]^+ 592 (EIMS).

It gave positive Molisch test for glycoside and responded to all colour reactions for flavanoids^8,9.

**UV SPECTRUM OF THE FLAVANONE GLYCOSIDE CS**

The wave length of maximum absorption with various shift reagents were recorded below:

\[\text{MeOH} \quad \text{max} \quad 259, 316 \text{ nm};\]

\[\text{NaOMe} \quad \text{max} \quad 261, 316 \text{ nm};\]

\[\text{NaOAc} \quad \text{max} \quad 260, 316 \text{ nm};\]

\[\text{AlCl}_3 \quad \text{max} \quad 260, 317 \text{ nm}\]

**IR SPECTRUM OF THE GLYCOSIDE CS**

The important peaks obtained in the IR spectrum (Fig. 1) and the structural units inferred with the help of available literature^{10,11} are recorded in 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>3452</td>
<td>-OH group(s)</td>
</tr>
<tr>
<td>2.</td>
<td>2873</td>
<td>-OCH(_3) group(s)</td>
</tr>
<tr>
<td>3.</td>
<td>2920</td>
<td>-CH(_3) group(s)</td>
</tr>
<tr>
<td>4.</td>
<td>1655</td>
<td>(\alpha,\beta)-unsaturated C=O</td>
</tr>
<tr>
<td>5.</td>
<td>1525</td>
<td>Aromatic ring system</td>
</tr>
<tr>
<td>6.</td>
<td>1210</td>
<td>C-O-C-bending vibration</td>
</tr>
<tr>
<td>7.</td>
<td>1140</td>
<td>C-O-C-stretching vibration</td>
</tr>
<tr>
<td>8.</td>
<td>820</td>
<td>Two adjacent H atoms.</td>
</tr>
</tbody>
</table>

PRESENCE OF -OH GROUP(s) IN THE CS

A peak at \(\nu_{\text{max}}^{\text{KBr}} \approx 3452 \text{ cm}^{-1}\) in the IR spectrum of the glycoside suggested the presence of hydroxy group(s) in it. The glycoside on acetylation with Ac\(_2\)O/Pyridine gave an acetyl derivative, molecular formula \(\text{C}_{41}\text{H}_{48}\text{O}_{19}\), m.p. 203°C and [M]\(^+\) 844 (EIMS). The percentage of acetyl groups (30.56%) was estimated by Weisenberger method\(^{12}\) as described by Belcher and Godbert\(^{13}\) suggesting the presence of six acetylable hydroxyl groups in the glycoside (CS).

Appearance of a peak in IR spectrum of the acetyl derivative at \(\nu_{\text{max}}^{\text{KBr}} \approx 1730 \text{ cm}^{-1}\) with the disappearance
of peak at $\nu_{\text{max}}^\text{KBr} 3452$ cm$^{-1}$ indicated acetylation of all the hydroxyl groups present in the glycoside.

**PRESENCE OF $-\text{CH}_3$ GROUP(s) IN THE CS**

In the IR spectrum, a peak at $\nu_{\text{max}}^\text{KBr} 2920$ cm$^{-1}$ indicated the presence of methyl group(s) in the glycoside. Estimation of methyl groups by the semi-micro method as mentioned by Belcher and Godbert$^{13}$ (2.53%) confirming the presence of one methyl group in it.

**PRESENCE OF $-\text{OCH}_3$ GROUP(s) IN THE CS**

In the IR spectrum of the glycoside another peak at $\nu_{\text{max}}^\text{KBr} 2873$ cm$^{-1}$, indicating the presence of $-\text{OCH}_3$ group(s) in it. Estimation of methoxy group was carried out by Zeisel's method$^{14}$ (5.23%), which confirmed the presence of only one methoxyl group in it.

The structure of the glycoside (CS) was established by its acid hydrolysis.

**ACID HYDROLYSIS OF THE GLYCOSIDE CS**

The glycoside (CS) on hydrolysis with 7% ethanolic $\text{H}_2\text{SO}_4$ gave an aglycone (CS-1) and sugar moiety(ies) which were separated and studied separately.
STUDY OF THE AGLYCONE CS-1

The aglycone (CS-1) crystallised from methanol as light yellow crystals and was found to be homogenous on TLC examination using chloroform-methanol-water (12:7:2). It analysed for molecular formula $\text{C}_{17}\text{H}_{16}\text{O}_4$, m.p. 278°C and $[\text{M}]^+$ 284 (EIMS).

It responded to all the characteristic colour reactions of the flavonoids$^8,^9$.

UV SPECTRUM OF THE AGLYCONE CS-1

The wavelengths of maximum absorbance with various shift reagent in UV spectrum of the aglycone were at:

$$\lambda_{\text{max}} \quad \text{MeOH} \quad 260, 314 \text{ nm};$$

$$\lambda_{\text{max}} \quad \text{MeOH+NaOAc} \quad 260, 318 \text{ nm};$$

$$\lambda_{\text{max}} \quad \text{MeOH+NaOMe} \quad 260, 316 \text{ nm};$$

$$\lambda_{\text{max}} \quad \text{MeOH+AlCl}_3 \quad 262, 349 \text{ nm}$$

IR SPECTRUM OF THE AGLYCONE CS-1

The significant IR peaks obtained in the IR spectrum (Fig. 2) and structural units inferred with the help of available literature$^{15,16}$ 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>3450</td>
<td>-OH group(s)</td>
</tr>
<tr>
<td>2.</td>
<td>2874</td>
<td>-OCH₃ group(s)</td>
</tr>
<tr>
<td>3.</td>
<td>2925</td>
<td>-CH₃ group(s)</td>
</tr>
<tr>
<td>4.</td>
<td>1652</td>
<td>α-β-unsaturated C=O</td>
</tr>
<tr>
<td>5.</td>
<td>1524</td>
<td>Aromatic ring system</td>
</tr>
<tr>
<td>6.</td>
<td>1211</td>
<td>C-O-C-bending vibration</td>
</tr>
<tr>
<td>7.</td>
<td>1136</td>
<td>C-O-C stretching vibration</td>
</tr>
<tr>
<td>8.</td>
<td>823</td>
<td>Two adjacent hydrogen atom</td>
</tr>
</tbody>
</table>

PRESENCE OF -OH GROUP(s) IN THE AGLYCONE CS-1

In the IR spectrum (Fig. 2) a peak at $\nu_{\text{max}}^{\text{KBr}}$ 3450 cm⁻¹ indicated the presence of free hydroxyl group(s) in the aglycone (CS-1). It formed an acetyl derivative, on acetylation with Ac₂O/Pyridine, molecular formula, C₁₉H₁₈O₅, m.p. 197°C and [M]⁺ 402 (EIMS). The percentage of acetyl group (10.6%) in the acetylated product was determined by Weisenberger method¹² as described by Belcher and Godbert¹³, suggested the presence of only one hydroxyl group in the aglycone.

PRESENCE OF -CH₃ GROUP(s) IN THE AGLYCONE CS-1

A peak at $\nu_{\text{max}}^{\text{KBr}}$ 2925 cm⁻¹ in the IR spectrum indicated the presence of methyl group(s) in the
IR SPECTRUM OF THE AGLYCONE CS-1
aglycone. Estimation of methyl group by the Semi-micro method as mentioned by Belcher and Godbert\textsuperscript{13} (5.28\%) confirming the presence of only one methyl group in it.

**PRESENCE OF -OCH\textsubscript{3} GROUP(s) IN THE AGLYCONES CS-1**

In the IR spectrum (Fig. 2) a peak at $\nu_{\text{max}}^{\text{KBr}}$ 2874 cm\textsuperscript{-1} showed the presence of -OCH\textsubscript{3} group(s) in the aglycone. Methoxyl group estimation was done by Zeisel's method\textsuperscript{14} (10.9\%), which revealed the presence of only one methoxyl group in it.

The $^{1}H$ NMR spectrum of the aglycone showed singlet at $\delta$ 3.93 integrating for three protons, suggesting the presence of one methoxyl group in the aglycone.

On the basis of above facts the tentative structure of the aglycone (CS-1) was assigned as:

![Structure of aglycone CS-1](image)

(I)
The position of the different groups in the aglycone was established by its alkaline degradation, various colour reactions and various shifts in the UV spectrum.

ALKALINE DEGRADATION OF THE AGLYCONE CS-1

The aglycone on fusion with 50% ethanolic KOH yielded two compounds identified as: 2-methyl, 3-methoxy resorcinol (IIA) (by Co-PC and Co-TLC), molecular formula \( \text{C}_8\text{H}_{10}\text{O}_3 \), m.p. 160°C \([M]^+ 154\) and cinnamic acid (IIB), (by Co-PC and Co-TLC), molecular formula \( \text{C}_9\text{H}_8\text{O}_2 \), m.p. 200°C, \([M]^+ 148\).
POSITION OF \(-\text{OH}\) GROUP AT C-5

(i) Formation of 2-methyl 3-methoxy resorcinol, on alkaline degradation of \(\text{CS-1}\) suggested the presence of \(-\text{OH}\) group at C-5 position.

(ii) A bathochromic shift of 35 nm in band I with \(\text{AlCl}_3\) (relative to MeOH) further confirmed the presence of \(-\text{OH}\) group at C-5\(^1\)7.

POSITION OF \(-\text{CH}_3\) GROUP

Formation of 2 methyl 3 methoxy resorcinol, on alkaline degradation of the aglycone \(\text{CS-1}\) indicating the presence of methyl group at C-6 position of ring A. The methyl group must be attached at C-6, was further confirmed by \(^1\)HNMR spectrum of the aglycone which showed sharp singlet at \(\delta 1.56\) integrating for three protons.

POSITION OF \(-\text{OCH}_3\) GROUP

The aglycone on alkaline degradation yielded 2-methyl, 3-methoxy resorcinol, suggesting the methoxyl group to be present at C-7 position of ring A.

The \(^1\)H NMR spectrum of the aglycone showed singlet at \(\delta 3.93\) integrating for three protons, further confirmed the presence of methoxyl group at C-7 position of ring A.
Thus the structure of the aglycone was finally assigned as; 5-hydroxy, 6-methyl, 7-methoxy flavanone (II).

\[
\begin{align*}
&\text{H}_3\text{C} \\
&\text{H}_3\text{C} \\
\end{align*}
\]

(II)

The above proposed structure was further confirmed by $^1$H NMR and mass spectral studies of the aglycone.

$^1$H NMR SPECTRUM OF THE AGLYCONE CS-1

The significant chemical shifts obtained in the $^1$H NMR of the aglycone and structural units inferred with the help of available literature$^{18,19}$ are recorded in Table-III (Fig. 3).
TABLE - III

<table>
<thead>
<tr>
<th>S. No.</th>
<th>δ Value</th>
<th>Pattern</th>
<th>J value</th>
<th>No. of Protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.68</td>
<td>dd</td>
<td>11.8</td>
<td>1</td>
<td>H-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>2.64</td>
<td>dd</td>
<td>17.0</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>3.21</td>
<td>dd</td>
<td>17.0</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>3.93</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>7-OMe</td>
</tr>
<tr>
<td>5.</td>
<td>1.56</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6-CH₃</td>
</tr>
<tr>
<td>6.</td>
<td>6.69</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-8</td>
</tr>
<tr>
<td>7.</td>
<td>7.90-8.12</td>
<td>m</td>
<td>-</td>
<td>2</td>
<td>H-2',6'</td>
</tr>
<tr>
<td>8.</td>
<td>7.60-7.77</td>
<td>m</td>
<td>-</td>
<td>3</td>
<td>H-3',4',5'</td>
</tr>
</tbody>
</table>

MASS SPECTRUM OF THE AGLYCONENE CS-1

The prominent fragments obtained in the EIMS of the aglycone were as follows:

[M]+ 284, m/e 256, 255, 181, 180, 152, 103, 102.

The various species obtained during its fragmentation are described in Scheme-I, which further confirmed its identity as 5-hydroxy, 6-methyl, 7-methoxy flavanone (II).

STUDY OF THE SUGAR MOIETY(IES)

The hydrolysate obtained after the acid hydrolysis of the glycoside, was neutralised with
$^1$H NMR SPECTRUM OF THE AGLYCONE CS-1
SCHEME I

C_{17}H_{16}O_4 [M]^+ 284

C_{16}H_{16}O_3 M/z 256

C_{16}H_{15}O_3 M/z 255

Pathway I without H transfer

Pathway II with H transfer

C_{9}H_{8}O_4 M/z 180

C_{9}H_{9}O_4 M/z 181

C_{8}H_{7} M/z 102

C_{8}H_{8}O_3 M/z 152
BaCO$_3$ and BaSO$_4$ filtered off. The filtrate was concentrated to yield a syrupy mass, which was subjected to paper chromatographic examination suggested the presence of L-rhamnose (Rf 0.38) and D-glucose (Rf 0.17) (mmp and CO-PC).

**QUANTITATIVE ESTIMATION OF SUGARS**

The quantity of sugars was estimated by the procedure of Mishra and Rao$^{20}$, which indicated that both the sugars were present in an equimolar ratio (1:1).

**PERIODATE OXIDATION OF THE GLYCOSIDE CS**

The glycoside on oxidation with sodium meta periodate$^{21}$, consumed 3.02 moles periodate and liberated 1.06 moles of formic acid, thereby revealing the presence of one molecule of glucose and one molecule of rhamnose attached to the aglycon and also confirmed that both the sugars were present in pyranose form$^{22}$.

**POSITION OF ATTACHMENT OF THE SUGARS TO THE AGLYCONE**

Periodate oxidation of the glycoside consumed 3.02 moles of periodate and liberated 1.06 moles of formic acid confirming that both the sugars must be linked on the same carbon atom in the form of discharide. These values were in accordance with the disaccharide nature of sugars.
By comparing the UV spectra of the aglycone and the glycoside, the position of sugar moiety was fixed at C-5 position, on the basis of following facts;

(i) The UV spectral data of the aglycone indicated a bathochromic shift of 35 nm in band I with \( \text{AlCl}_3 \) (absent in glycoside), confirming the presence of free -OH group at C-5 in the aglycone and not in the glycoside.

(ii) Permethylation of the glycoside followed by acid hydrolysis yielded 5 hydroxy, 6-methyl, 7-methoxy flavanone (confirmed by Co-PC and Co-TLC) thereby suggesting that -OH group at C-5 was involved in glycosylation.

Thus, on the basis of above facts a tentative structure to the flavanone glycoside was assigned as (III).

![Structural Diagram](attachment:structure.png)
SEQUENCE OF SUGAR RESIDUE

The sequence of sugar moiety in the glycoside was determined by graded hydrolysis with Kiliani mixture\(^{23}\), which liberated L-rhamnose first followed by D-glucose revealing that L-rhamnose was terminal sugar and D-glucose was linked to the aglycone.

Which was further supported by the isolation and study of two proaglycones CSP-1 and CSP-2 which were obtained by the partial hydrolysis of the glycoside with Kiliani mixture. The two proaglycones were separated by column chromatography and examined separately.

STUDY OF THE PROAGLYCONE CSP-1

The proaglycone (CSP-1) analysed for the molecular formula \(C_{23}H_{25}O_9\), m.p. 285\(^\circ\)C \([M]^+\) 445, on hydrolysis with 7\% \(H_2SO_4\) it yielded the aglycone and D-glucose. The proaglycone (CSP-1) on hydrolysis with the enzyme emulsin, indicated the presence of \(\beta\)-linkage between D-glucose and the aglycone.

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (CSP-1)

Permethylolation of the proaglycone (CSP-1) by Kuhn's method\(^{24}\) followed by acid hydrolysis gave the aglycone and 2,3,4,6-tetra-O-methylglucose (mmp, Co-PC, Co-tlc) thereby showing 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 (CSP-1) was assigned the structure IV as: 6-methyl,7-methoxy flavanone 5-O-β-D-glucopyranoside.

STUDY OF THE PROAGLYCONC CPS-2

The proaglycone (CSP-2) analysed for molecular formula C_{29}H_{36}O_{13}, m.p. 301°C [M]^+ 592 (EIMS). On acid hydrolysis with 7% acoholic H_2SO_4 gave the aglycone and D-glucose (Rf 0.25) and L-rhamnose (Rf 0.48) (Co-PC and Co-TLC).
PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE CSP-2

The proaglycone (CSP-2) was permethylated and subsequently on hydrolysis yielded the aglycone, 2,3,4-tri-O-methyl-L-rhamnose and 2,3,6-tri-O-methyl-glucose (by Co-PC and Co-TLC), which confirmed that both the sugars were present in pyranose form and also showed that C₄-OH group of D-glucose was linked with C₁-OH group of L-rhamnose.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE CS

The glycoside on hydrolysis with enzyme tokadiastase²⁵ gave proaglycone (CSP-1) and L-rhamnose (Co-PC) indicating α-linkage between proaglycone (CSP-1) and L-rhamnose.

The proaglycone (CSP-1) on hydrolysis with enzyme emulsin yielded aglycone and D-glucose (by Co-PC), confirming the presence of β-linkage between aglycone and D-glucose.

Therefore it was concluded that 5-OH of the aglycone was attached with C-1 of the glucose via β-linkage and C-4 of the D-glucose was linked to the C-1 of L-rhamnose via α-linkage.

Thus the structure to the glycoside was assigned (V) as; 6-methyl-7-methoxy flavanone, 5-O-α-L-rhamnopyranosyl (1→4)-O-β-D-glucopyranoside.
\[ ^1H \text{ NMR spectrum of hexa acetyl derivative of the glycoside CS} \]

The various chemical shifts in \(^1H\) NMR spectrum of hexa acetyl derivative of the glycoside and structural units inferred with the help of available literature\(^{26,27}\) are given in Table-IV (Fig. 4).

**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>5.67</td>
<td>dd</td>
<td>11.9, 3.6</td>
<td>1</td>
<td>H-2</td>
</tr>
<tr>
<td>2.</td>
<td>2.62</td>
<td>dd</td>
<td>17.3, 6</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td>3.</td>
<td>3.52</td>
<td>dd</td>
<td>17.4, 2</td>
<td>1</td>
<td>H-3</td>
</tr>
<tr>
<td>4.</td>
<td>3.92</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>7-OCH(_3)</td>
</tr>
<tr>
<td>5.</td>
<td>1.55</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6-OCH(_3)</td>
</tr>
<tr>
<td>6.</td>
<td>6.68</td>
<td>s</td>
<td>-</td>
<td>1</td>
<td>H-8</td>
</tr>
<tr>
<td>7.</td>
<td>7.80-8.0</td>
<td>m</td>
<td>-</td>
<td>2</td>
<td>H-2', 6'</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>
<td>-------</td>
<td>---------</td>
<td>-------------</td>
<td>----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>8.</td>
<td>7.50-7.70</td>
<td>m</td>
<td>-</td>
<td>3</td>
<td>H-3',4',5'</td>
</tr>
<tr>
<td>9.</td>
<td>4.25</td>
<td>d</td>
<td>2.0</td>
<td>1</td>
<td>H-1''-anomeric proton</td>
</tr>
<tr>
<td>10.</td>
<td>4.41</td>
<td>d</td>
<td>8.5</td>
<td>-</td>
<td>H-1'''-anomeric proton</td>
</tr>
<tr>
<td>11.</td>
<td>4.65-4.87</td>
<td>m</td>
<td>-</td>
<td>4</td>
<td>Protons of rhamnose unit</td>
</tr>
<tr>
<td>12.</td>
<td>5.35-5.50</td>
<td>m</td>
<td>-</td>
<td>6</td>
<td>Protons of glucose unit</td>
</tr>
<tr>
<td>13.</td>
<td>1.07-1.40</td>
<td>m</td>
<td>-</td>
<td>18</td>
<td>OAc of disaccharide</td>
</tr>
<tr>
<td>14.</td>
<td>1.03</td>
<td>d</td>
<td>6.0</td>
<td>3</td>
<td>Rham-CH₃</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM**²⁸ OF THE GLYCOSIDE CS

The various important fragment ion peaks obtained in the EIMS are displayed below:

\[ [M]^+ 592 \text{ (absent)} m/z 444, 445, 284, 256, 255, 181, 180, 152, 103, 102. \]

The various species obtained during the fragmentation are given in Scheme-II, which further confirmed the identity of the glycoside (CS) as; 6-methyl,7-methoxy flavanone-5-O-α-L-rhamnopyranosyl (1→4)-O-β-D-glucopyranoside (V).
$^1$H NMR spectrum of the acetyl derivative of the glycoside CS
Scheme II
EXPERIMENTAL

_Coccinia indica_ W&A belongs to family cucurbitaceae, and supplied by M/s United Chemicals and Allied Products, Calcutta, India and authenticated by the Department of Botany, of this University. A herbarium specimen has been deposited in the room no. 36 of the Chemistry department.

The air dried and powdered aerial parts (2.5 Kg) of _Coccinia indica_ W&A were extracted with 95% MeOH in a five litre RB flask fitted with a condensor. The total methanolic extract was concentrated to a yellow viscous mass (118 gm) which was poured into distilled water. The water soluble part was concentrated to a viscous mass and successively extracted with petroleum ether, benzene, chloroform, ethyl acetate, acetone and methanol.

The petroleum ether, benzene, chloroform, acetone and methanol soluble parts on removal of the solvent yielded very small amount of residue and therefore discarded.

STUDY OF ETHYL ACETATE SOLUBLE PART

The EtOAc soluble part of the water soluble part of the methanolic extract of the aerial parts of the plant, was concentrated under reduced pressure and obtained a dark yellow syrupy mass (2.10 gm),
on TLC examination, it gave single spot using EtOAc-MeOH-H₂O (12:5:3) and I₂ vapours as visualising agent, which was purified by column chromatography over si-gel column and eluted with EtOAc-Methanol in different proportions, the details of which are described below:

COLUMN CHROMATOGRAPHY

Length of the column - 150 cm.
Diameter of the column - 5.0 cm.
Weight of the si-gel - 150 gm.
Weight of crude extract - 2.10 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>1-5</td>
<td>EtOAc-Methanol (6:2)</td>
<td>Nil</td>
<td>Sticky mass</td>
</tr>
<tr>
<td>2.</td>
<td>6-11</td>
<td>EtOAc-Methanol (6:3)</td>
<td>Nil</td>
<td>No solid mass</td>
</tr>
<tr>
<td>3.</td>
<td>12-17</td>
<td>EtOAc-Methanol (6:4)</td>
<td>One</td>
<td>Compound (CS)</td>
</tr>
<tr>
<td>4.</td>
<td>18-23</td>
<td>EtOAc-Methanol (6:5)</td>
<td>Nil</td>
<td>No solid residue</td>
</tr>
</tbody>
</table>

Eluates collected from fractions EtOAc-Methanol (12-17) were found to have same Rf value and hence mixed together. Evaporation of the solvent yielded yellowish compound (CS) (1.40 gm) which was found
to be homogenous on TLC examination (EtOAc-MeOH-H₂O, 12:5:3).

STUDY OF THE FLAVANONE GLYCOSIDE CS

The compound (CS) crystallised from methanol, it analysed for molecular formula C₂₉H₃₆O₁₃, m.p. 299-300°C and [M]⁺ 592 EIMS.

It gave positive Molisch test for glycoside and also gave the following colour reactions:

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

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₂₉H₃₆O₁₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 58.76%</td>
<td>C 58.75</td>
</tr>
<tr>
<td>H 6.02%</td>
<td>H 6.04</td>
</tr>
</tbody>
</table>

Molecular weight - 592
(By EIMS)

ACETYLATION OF THE GLYCOSIDE CS

The acetylation of the glycoside was done by similar procedure as described on page no. 49 of the thesis.

The acetyl derivative crystallised from methanol as light yellow needles. It analysed for molecular formula C₄₁H₄₈O₁₉, m.p. 203°C, [M]⁺ 844 (EIMS).
ELEMENTAL ANALYSIS

Found
C 58.13%
H  5.65%

Calculated for $\text{C}_{41}\text{H}_{48}\text{O}_{19}$
C 58.29%
H  5.68%

Molecular weight - 844
(By EIMS).

ACID HYDROLYSIS OF THE GLYCOSIDE CS

The glycoside was hydrolysed in a similar way as described on page no. 49 of the thesis. Evaporation of the solvent yielded a yellow coloured compound (CS-1) (500 mg) m.p. 278°C, which was separated by filtration and aglycone and hydrolysate were examined separately.

STUDY OF THE AGLYCONE CS-1

The aglycone was crystallised from Methanol as yellow needles. It was found to be homogenous on TLC examination, using EtOAc-Methanol-water (10:6:4) and $\text{I}_2$ vapours as visualising agent. It analysed for $\text{C}_{17}\text{H}_{16}\text{O}_{4}$, m.p. 278-279°C and [M]$^+$ 284 (EIMS).

It showed the following colour reactions:

(i) Green colour with $\text{FeCl}_3$.
(ii) Red colour with Na-Hg/HCl.
ELEMENTAL ANALYSIS

Found

C  71.85%
H  5.62%

Molecular weight - 284
(By EIMS)

Calculated for C_{17}H_{16}O_{4}

C  71.83%
H  5.63%

ESTIMATION OF METHOXYL GROUP(S)

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

ALKALINE DEGRADATION OF THE AGLYCONE

About 250 mg. of the aglycone was treated with 50% ethanolic KOH, in the same way as described on page no. 79 of the thesis. The ethereal layer after washing with water, separated into two parts.

(i) The first part was treated with 50% NaHCO₃ solution (25 ml) and aqueous part after acidification yielded a compound (IIB) m.p. 199-200°C  199-200°C molecular formula C₉H₆O₂ and [M]+ 148 (EIMS). It was identified as cinnamic acid (confirmed by Co-PC and Co-TLC with authentic sample).

(ii) The second part of the ethereal layer was treated with 10% NaOH solution and the aqueous
part on acification gave another compound (IIA) m.p. 160°C, molecular formula C₈H₁₀O₃, [M]+ 154 (EIMS). It was identified as 2-methyl, 3-methoxy-0-resorcinol.
(Confirmed by mmp, Co-PC and Co-TLC) with authentic sample).

PARTIAL HYDROLYSIS OF THE GLYCOSIDE

The glycoside (250 mg) was treated with Kiliani mixture (HCl-CH₃COOH-H₂O) (20:30:50) in a 250 ml conical flask and left for seven days at room temperature. The contents were extracted with n-BuOH. Then n-BuOH soluble portion on PC examination gave two spots (Rf 0.56 and 0.35) in BAW (4:1:5) and aniline hydrogen phthalate as spraying agent which were separated by column chromatography over si-gel G, using CHCl₃-MeOH in various proportions.

The detail study of the column chromatography is described below:

COLUMN CHROMATOGRAPHY

Length of the column - 100 cm.
Diameter of the column - 3.0 cm.
Weight of the si-gel - 30 gm.
Weight of the n-Butanol soluble portion - 300 mg
<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>CHCl₃:MeOH (4:1)</td>
<td>One</td>
<td>CSP-1</td>
</tr>
<tr>
<td>2.</td>
<td>6-10</td>
<td>CHCl₃:MeOH (4:2)</td>
<td>Two</td>
<td>CSB-1 and CSP-2</td>
</tr>
<tr>
<td>3.</td>
<td>11-15</td>
<td>CHCl₃:MeOH (4:3)</td>
<td>One</td>
<td>CSP-2</td>
</tr>
</tbody>
</table>

**STUDY OF FRACTIONS (1-5)**

The fractions (1-5) were of the same Rf value and so they were mixed together. On removal of the solvent, it gave compound (CSP-1). It crystallised from methanol and analysed for molecular formula, C₂₃H₂₅O₉, m.p. 285°C [M]+ 446 (EIMS).

**ELEMENTAL ANALYSIS OF CSP-1**

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₂₃H₂₅O₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 62.0%</td>
<td>C 62.02%</td>
</tr>
<tr>
<td>H 5.62%</td>
<td>H 5.61%</td>
</tr>
</tbody>
</table>

Molecular weight - 445 (By EIMS)
PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE CSP-1

The proaglycone (CSP-1) 75 mg was dissolved in DMF (8 ml) in a 100 ml conical flask and then Me I (10 ml) and Ag₂O (120 mg) were added to it and the mixture was refluxed for 24 hours at room temperature. The reaction mixture was filtered and the contents was washed with DMF. The filtrate was concentrated under reduced presure to gave a syrupy mass and hydrolysed with 7% H₂SO₄ to give the aglycone and methylated sugars.

After the removal of the aglycone, the aqueous layer was neutralised with BaCO₃ and BaSO₄ filtered off. The filtrate was concentrated and studied by Pc on Whatman filter paper no.1 using BAW (4:1:5) as solvent system and aniline hydrogen phthalate as visualising agent. The methylated sugar was identified as 2,3,4,6-tetra-O-methyl-D-glucose.

STUDY OF FRACTIONS (11-15)

The fractions (11-15) were found to have the same Rf value and therefore they were combined together. On evaporation of the solvent gave compound (CSP-2), which crystallised from methanol, and analysed for C₂₉H₃₆O₁₃, m.p. 301-302°C and [M]⁺ 592 (EIMS).
ELEMENTAL ANALYSIS OF CSP-2

Found
C 58.77%
H 6.02%
Molecular weight - 592
(By EIMS)

Calculated for C_{29}H_{36}O_{13}
C 58.75%
H 6.04%

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE CSP-2

The permethylation and hydrolysis of the proaglycone (CSP-2) was done in the similar way to that for proaglycone (CSP-1). It suggested the presence of aglycone (by mmp and Co-PC and Co-TLC) and methylated sugars (by Co-PC and mmp Co-TLC with authentic sample).

IDENTIFICATION OF SUGARS AFTER HYDROLYSIS

The hydrolysate was neutralised with BaCO₃ and BaSO₄ filtered off. The filtrate was concentrated under reduced pressure and studied by PC using solvent system (i) EtOAc-H₂O-pyridine (12:5:4) (ii) n-butanol OHAc-Water (4:1:5) and aniline hydrogen pthalate as spraying agent. The results are tabulated in VII.
### Table VII

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent system</th>
<th>Rf value</th>
<th>Sugar identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reported</td>
<td>Found</td>
</tr>
<tr>
<td>1.</td>
<td>EtOAc-H$_2$O-Pyridine</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>(12:5:4)</td>
<td>0.49</td>
<td>0.48</td>
</tr>
<tr>
<td>2.</td>
<td>N-butanol-OHAc-Water</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>(4:1:5)</td>
<td>0.37</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**Periodate Oxidation of the Glycoside**

The glycoside (100 mg) dissolved in methanol and treated with sodium meta periodate (25 ml) in a (100 ml) conical flask. The reaction mixture was left for two days at room temperature. Simultaneously a blank was run in the similar way. The quantity of sodium meta periodate consumed and formic acid liberated was estimated by Jone’s method.

**Enzymatic Hydrolysis of the Glycoside**

The glycoside dissolved in ethanol (25 ml) and treated with enzyme tokadiastase (35 ml) in a 100 ml conical flask and the content was allowed to stay at room temperature for two days and filtered. The aglycone and hydrolysate were studied separately.

The hydrolysate was concentrated and studied by PC on Whatman No.1 filter paper and BAW (4:1:5)
solvent system. The sugar was identified as L-rhamnose (Rf 0.38). The aglycone was identified as proaglycone (CSP-1), m.p. 285°C (by Co-TLC and mmp).

The proaglycone, (20 mg) was dissolved in ethanol and mixed with equal volume of almond emulsin solution in a 100 ml conical flask. The reaction mixture was allowed to left at room temperature for 24 hours and then filtered. The hydrolysate on examination by PC, was found to contain D-glucose (Rf 0.17) and the aglycone was identified as 5-hydroxy, 6-methyl-7-methoxy flavanone.
REFERENCES


