CHAPTER II

* ISOLATION AND STUDY OF A NOVEL ANTHRAQUINONE GLYCOSIDE [1,3, DIMETHOXY-6-METHYL ANTHRAQUINONE-8-0-β-D-GLUCOPYRANOSYL (1-→4)-O-α-L-RHAMNOPYRANOSIDE] FROM THE SEEDS OF IMPATIENS BALSAMINA LINN.

* This work has been accepted for publication in Asian Journal of Chemistry.
The plant *Impatiens balsamina* Linn.\(^1\)–\(^4\) (N.O. Balsaminaceae) is known Gulmendi in Hindi and is distributed throughout India and Ceylon upto 5,000 ft. and China and Malaya.

The plant is of upto 30 to 90 cm. height. Its stem is glabrous and leaves are alternate, upto 15 cm. long lanceolate, acuminate, deeply serrate and glabrous while flowers are irregular, often handsome, axillary and solitary.

The seeds of garden balsam are edible. They contain 27.0% of a viscous oil. The presence of β-amyrin, α-spinasterol and balsaminasterol has been reported in the unsaponifiable matter. The oil consists of following fatty acid, palmitic 4.68, stearic 5.16, arachidic 2.80, oleic 18.30, linoleic 9.17, linolenic 30.15 and parinaric 29.14%\(^5\).

Alcoholic extract of the flowers has been found to have adequate antibiotic activity against scleroting, fructicola and other pathogenic fungi and bacteria. It is reported to be useful for pains in the joints. When taken internally, it acts as an emetic, cathartic and diuretic. The flowers are cooling and
tonic useful when applied to burns and scalds. The active principle has been identified as 2-methoxy-1,4-naphthaquinone\textsuperscript{6}.

The deep red flower contain a monoglycosidic anthocyanin based on pelargonidin, whereas the roots and stems contains cyanidin monoglycoside\textsuperscript{7}.

In absence of adequate phytochemical investigations and inview of its important medicinal values it was thought worth while to investigate it phytochemically.

**ISOLATION OF THE GLYCOSIDE**

The seeds of *Impatiens balsamina* Linn (N.O. Balsaminaceae) was purchased from the local market of Sagar district and identified by the Botany Department of this University.

Air dried, powdered seeds of *Impatiens balsamina* were extracted with 95% ethanol. The total extract was concentrated under reduced pressure to thick viscous mass and resolved into water soluble and water insoluble parts. The concentrated water soluble part was successively extracted with petroleum ether, rectified spirit and ethylacetate. The petroleum ether soluble part on removal of the solvent yielded very small amount of gummy residue which could hardly afford for any
study. (The study of ethyl acetate soluble part is reported in chapter III of the thesis).

STUDY OF THE RECTIFIED SPIRIT SOLUBLE FRACTION

The rectified spirit soluble fraction was concentrated under reduced pressure to dark orange coloured mass and subjected to TLC examination, using ethylacetate : acetone : water (10 : 10 : 1) and iodine vapours as visualizing agent gave two spots. As such the concentrated rectified spirit extract was subjected to column chromatography and eluted with benzene : methanol in varying proportions.

Eluants from benzene : methanol (7:4) were of same $R_f$ value and so combined and on removal of the solvent yielded orange coloured needles (BS, 0.66%).

It responded to characteristic colour reactions \(^8-11\) for anthraquinone glycoside and positive Molisch's test.

STUDY OF THE ANTHRAQUINONE GLYCOSIDE (BS)

The anthraquinone glycoside (BS) was soluble in ethylacetate and crystallised with ethylacetate and light petroleum ether (1:1). It analysed for $\text{C}_{39}\text{H}_{34}\text{O}_{14}$; m.p. 240; and $M^+$ 606 (EIMS).
UV SPECTRUM OF THE ANTHRAQUINONE GLYCOSIDE (BS)

The wavelength of maximum absorption when recorded with shift reagent were at:

\[ \lambda_{\text{max}}^{\text{EtOH}} = 262, 292 \text{ and } 404 \text{ nm.} \]

IR SPECTRUM OF THE GLYCOSIDE

The prominent IR peaks of the glycoside (Fig. I) as observed and assignment of the functional groups in the molecule made with the help of available literature\textsuperscript{12,13} are recorded 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>3215</td>
<td>OH</td>
</tr>
<tr>
<td>2.</td>
<td>2932</td>
<td>C-H stretching vibration</td>
</tr>
<tr>
<td>3.</td>
<td>2896</td>
<td>-OCH(_3) group</td>
</tr>
<tr>
<td>4.</td>
<td>1760</td>
<td>Acetyl group</td>
</tr>
<tr>
<td>5.</td>
<td>1690, 1660</td>
<td>&gt;C = 0</td>
</tr>
<tr>
<td>6.</td>
<td>1678</td>
<td>C = C stretching vibration</td>
</tr>
<tr>
<td>7.</td>
<td>1628</td>
<td>-C-C aromatic stretching vibrations</td>
</tr>
<tr>
<td>8.</td>
<td>1600</td>
<td>Aromatic ring system</td>
</tr>
<tr>
<td>9.</td>
<td>1470</td>
<td>C-CH(_3) group</td>
</tr>
</tbody>
</table>
IR SPECTRUM OF THE GLYCOSIDE

FIG 113
PRESENCE OF THE OH GROUP(S)

The IR spectrum of the glycoside showed peak at $\nu_{\text{KBr max}}^{\text{max}}$ 3215 cm$^{-1}$ suggesting the OH group(s) in it. The compound underwent acetylation ($\text{Ac}_2\text{O}/\text{pyridine}$) yielding an acetylated product, m.p. 166°, molecular formula, $\text{C}_{41}\text{H}_{46}\text{O}_{20}$ and $M^+$ 858 (EIMS).

In the IR spectrum of the acetate, appearance of peak at 1760 cm$^{-1}$ and disappearance of hydroxyl peak at 3215 cm$^{-1}$ indicated that all the hydroxyl group had undergone acetylation.

Estimation of the acetyl groups (29.82%) by Wiesenerberger method$^{14}$ as described by Belcher and Godbert$^{15}$ suggested the presence of six hydroxyl groups in it.

PRESENCE OF METHYL GROUP(S)

In the IR spectrum peak at $\nu_{\text{KBr max}}^{\text{max}}$ 1470 cm$^{-1}$ indicated the presence of methyl group(s) in the glycoside. Estimation of methyl groups by the semimicro method as mentioned by Belcher and Godbert$^{15}$ confirmed the presence of one methyl group in it.

PRESENCE OF METHOXYL GROUP(S)

In the IR spectrum peak at $\nu_{\text{KBr max}}^{\text{max}}$ 2896 cm$^{-1}$
indicated the presence of methoxyl group(s) in the glycoside. Estimation of methoxyl groups (10.2120%) in it by Zeisel's method\textsuperscript{16} confirmed the presence of two methoxyl group in it.

The structure of the glycoside (BS) was elucidated by its acid hydrolysis.

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

The glycoside on its acid hydrolysis (7% alc. H\textsubscript{2}SO\textsubscript{4}) yielded an aglycone (BS\textsubscript{1}) and sugar moiety (ies) which were separated by filtration and examined separately.

**STUDY OF THE AGLYCON (BS\textsubscript{1})**

The aglycone was found to be homogeneous by TLC (Benzene : Methanol, R\textsubscript{f} = 0.58). The aglycone crystallised from methanol as light orange coloured crystalline substance and had m.p. 312°. It analysed for molecular formula, C\textsubscript{17}H\textsubscript{14}O\textsubscript{5}, M\textsuperscript{+} 298 (EIMS). The anthraquinonoidal nature of the aglycone was confirmed on the basis of colour reactions\textsuperscript{17}.

The anthraquinone skeleton was further supported by the isolation of 2-methyl anthracene, m.p. 206° from its Zn - dust distillation.
UV SPECTRUM OF THE AGLYCONES (BS₁)

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

\[ \lambda_{\text{max}} \text{EtOH} = 265, 282 \text{ and } 414 \text{ nm.} \]

IR SPECTRUM OF THE AGLYCONES (BS₁)

The significant IR peaks for the aglycone (Fig. II) as observed and the structural units inferred with the help of available literature\(^{18,19}\) are tabulated 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>3220</td>
<td>OH grouping</td>
</tr>
<tr>
<td>2.</td>
<td>2947</td>
<td>C-H stretching</td>
</tr>
<tr>
<td>3.</td>
<td>2892</td>
<td>-OCH(_3) group</td>
</tr>
<tr>
<td>4.</td>
<td>1756</td>
<td>Acetyl group</td>
</tr>
<tr>
<td>5.</td>
<td>1680</td>
<td>C=C stretching vibrations</td>
</tr>
<tr>
<td>6.</td>
<td>1666, 1656</td>
<td>&gt;C=O</td>
</tr>
<tr>
<td>7.</td>
<td>1632</td>
<td>C-C aromatic stretching vibrations</td>
</tr>
<tr>
<td>8.</td>
<td>1594</td>
<td>Aromatic ring system</td>
</tr>
<tr>
<td>9.</td>
<td>1478</td>
<td>C-CH(_3) group</td>
</tr>
</tbody>
</table>
IR SPECTRUM OF THE AGLYCONE
PRESENCE OF HYDROXYL GROUP(S)

The IR peak at $\nu_{\text{max}}^\text{KBr} 3220 \text{ cm}^{-1}$ indicated the presence of free OH group (s) in the aglycone, on its acetylation ($\text{Ac}_2\text{O} + \text{pyridine}$) it formed an acetate, m.p. 202$^\circ$, molecular formula, $C_{19}H_{16}O_6$ and $M^+ 340$ (EIMS). Estimation of the acetyl group (12.40%) by Wiesenberger method$^{14}$ as described by the Belcher and Godbert$^{15}$ suggested the presence of one OH group in it.

POSITION OF THE OH GROUP AT C-8

(i) The aglycone gave a red complex with zirconium nitrate solution, soluble in HCl, showing the presence of a hydroxyl group at position C-8$^{20}$.

(ii) The ethanolic solution of the aglycone formed a complex with ethanolic copper sulphate showing the presence of a hydroxyl function in the $\alpha$-position, of the $>\text{C}=\text{O}$ group$^{21}$.

(iii) The presence of only hydroxyl group is further supported by the peaks at 1632 cm$^{-1}$ and 1680 cm$^{-1}$ in the IR spectrum and $\lambda_{\text{max}}$ at 414 nm in the UV spectrum of the aglycone$^{22}$.

PRESENCE OF METHYL GROUP(S)

In the IR spectrum peak at $\nu_{\text{max}}^\text{KBr} 1478 \text{ cm}^{-1}$ indicated the presence of methyl group(s) in the aglycone.
Estimation of methyl group by the semimicro method as mentioned by Belcher and Godbert\(^{15}\) (4.9992\%) confirmed the presence of one methyl group in it. The \(^1\)HNMR spectrum of acetylated derivative of the aglycone showed singlet at \(\delta = 2.53\) integrating for 3 protons and confirmed the presence of one methyl group at position 6 in the anthraquinone\(^{26,27}\).

**PRESENCE OF THE METHOXYL GROUP(S)**

Another peak at \(\sqrt{KBr} 2892 \text{ cm}^{-1}\) in the IR spectrum of the aglycone indicated the presence of \(-\text{OCH}_3\) group(s) in it. Methoxy group estimation (21.12\%) was done by Zeisel's\(^{16}\) method which confirmed the presence of two methoxyl groups in it.

**POSITION OF THE METHOXYL GROUP(S)**

The compound gave red colour on treatment with concentrated sulphuric acid showing the presence of at least one methoxyl group in \(\alpha\)-position in the anthraquinone\(^{23}\). Signal in the \(^1\)HNMR at \(\delta = 7.38 (2H,d,J= 2.5 \text{ Hz}, C_2\text{-H},C_4\text{-H})\) indicating metaprotons thereby confirming the presence of another methoxyl group at \(\beta\)-position in the anthraquinone\(^{24}\).

Thus out of the two methoxyl group present in the aglycone, one is of \(\beta\)-position and the other is at \(\alpha\)-position.
The $^1$HNMR spectrum of acetylated derivative of the aglycone showed singlet at $\delta = 3.98$ and $\delta = 3.86$ integrating for 3 protons and confirmed the presence of two methoxy groups at position 1 and 3 in the ring$^{25}$.

Signals in $^1$HNMR of acetylated derivative of the aglycone at $\delta = 7.38(2H,d,J = 2.5\text{ H} , C_2-H, C_4 - H)$ and $\delta = 7.92(2H,d,J = 2.4\text{ H} , C_5-H, C_7 - H)$, these values supported m-di-substituted pattern in the ring A and C$^{24}$.

Oxidation of the Aglycone (BS$_1$)

The aglycone on oxidation with KMnO$_4$ gave 1:3-dimethoxy phthalic acid, m.p. 244$^\circ$, molecular formula, C$_8$H$_{12}$O$_6$, M$^+$ 204, as one of the product which placed two methoxyl groups in position 1 and 3.

Thus the aglycone was assigned the structure (I) as 1,3-dimethoxy-8-hydroxy-6-methylantraquinone.

![Structure (I)](image)

The above proposed structure (I) of the aglycone was further supported by its $^1$HNMR and mass spectral studies.
$^1$H NMR SPECTRUM OF MONOACETYL DERIVATIVE OF AGLYCONE (BS$_2$)

The chemical shifts obtained in the $^1$H NMR spectrum (Fig. III) of acetylated derivative and structural units inferred with the help of available literature$^{26,27}$ are recorded in table III and further supported its identity as a 1,3-dimethoxy-8-hydroxy-6 methyl anthroquinone.

**TABLE - III**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Value</th>
<th>Pattern</th>
<th>J value ($H_2$)</th>
<th>No. of protons</th>
<th>Structure (assignment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.38</td>
<td>d</td>
<td>2.5</td>
<td>2</td>
<td>H-2, H-4</td>
</tr>
<tr>
<td>2.</td>
<td>7.92</td>
<td>d</td>
<td>2.4</td>
<td>2</td>
<td>H-5, H-7</td>
</tr>
<tr>
<td>3.</td>
<td>3.98</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>1 - OCH$_3$</td>
</tr>
<tr>
<td>4.</td>
<td>3.86</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>3 - OCH$_3$</td>
</tr>
<tr>
<td>5.</td>
<td>2.53</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6 - CH$_3$</td>
</tr>
<tr>
<td>6.</td>
<td>2.56</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>8 - OAC</td>
</tr>
</tbody>
</table>

**MASS SPECTRUM OF THE AGLYCONE**

EI mass spectrum of aglycone showed the fragment ion peaks at, $M^+$ 298, m/z = 270, 242, 214, 213, 136, 106.

The various species assigned to the fragments are
$^1$H NMR SPECTRUM OF THE ACETYLATED AGLYCONE
SCHEME - I
described in the scheme I and further confirmed its identity as; 1,3 dimethoxy-8-hydroxy-6-methyl anthraquinone.

**STUDY OF THE SUGAR MOIETY (IES)**

The aqueous hydrolysate obtained by the hydrolysis of the glycoside was neutralised with BaCO₃ and BaSO₄ filtered off. The filtrate was concentrated to an orange syrupy mass. It was examined by co-paper chromatography and found to contain L-rhamnose and D-glucose.

**QUANTITATIVE ESTIMATION OF THE SUGAR**

The quantity of sugars in the glycoside was estimated by the procedure of Mishra and Rao²⁸ which revealed that the sugars were present in glycoside in equimolar ratio (1:1).

**PERIODATE OXIDATION OF THE GLYCOside**

The sodium meta periodate oxidation²⁹ of the glycoside consumed 3 molecules of periodate with the liberation of 1.04 moles of formic acid indicated the presence of one molecule of rhamnose and one molecule of glucose attached to the molecule of aglycone and simultaneously confirmed that both the sugars were in the pyranose form.
POSITION OF ATTACHMENT OF THE SUGAR

There were the two possibilities of attachment of both the sugars to the aglycone.

1. Either both the sugars were attached on different C-atoms of aglycone.

2. Or both, glucose and rhamnose were attached on the same C-atom as disaccharide.

Out of these possibilities the linking of the sugars as disaccharide was confirmed by the fact that during the periodate oxidation the glycoside consumed 3 moles of sodium metaperiodate with the liberation of 1.04 moles of formic acid. These values were in accordance with the disaccharide nature of the sugar and not monosaccharide.

Therefore, the sugars must be attached with the same carbon atom of the aglycone since there is only one free -OH group in the aglycone at C-8 position of ring C, hence the same -OH must be involved in the glycosylation with sugar of disaccharide nature. This has also been confirmed by UV spectral study\textsuperscript{30,31}.

Keeping all the facts together a tentative structure to the glycoside was assigned as (II) which was further supported by its \textsuperscript{1}HNMR and mass spectral studies.
SEQUENCE OF THE SUGAR RESIDUE(S)

The glycoside on graded hydrolysis with Kiliani mixture\textsuperscript{32} liberated D-glucose first followed by L-rhamnose suggesting that D-glucose was the terminal sugar and L-rhamnose was linked to the aglycone.

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

STUDY OF THE PROAGLYCONE (BSA-1)

The proaglycone analysed for the molecular formula, $C_{23}H_{24}O_{9}$, m.p. 200\textdegree, $M^+$ 444. On hydrolysis with 7\% H$_2$SO$_4$ it furnished the aglycone 1,3 dimethoxy-8-hydroxy-6 methyl anthraquinone (m.m.p. and Co-TLC) and L-rhamnose.

The proaglycone BSA-1 was hydrolysable by enzyme tokadiastase\textsuperscript{33} solution and afforded L-rhamnose and aglycone thereby suggesting the presence of $\alpha$-linkage between the aglycone and L-rhamnose.
PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (BSA-1)

The proaglycone (BSA-1) on permethylation by Khun's procedure followed by hydrolysis yielded aglycone and 2,3,4-tri-O-methyl rhamnose (Co-Pc) which indicated that C-1 of L-rhamnose was involved in glycosylation and also confirmed the pyranose form of the rhamnose.

Thus the proaglycone (BSA-1) was assigned the structure (III) as: 1,3-dimethoxy-6 methyl-8-O-\(\alpha\)-L-rhamnose.

(III)

STUDY OF THE PROAGLYCONE (BSA-2)

The proaglycone (BSA-2) analysed for \(C_{29}H_{34}O_{14}\), m.p. 238-240 °C, \(M^+\) 606. On acid hydrolysis with 7% alc. \(H_2SO_4\) it yielded 1,3-dimethoxy-8-hydroxy-6 methyl anthraquinone (m.m.p. and Co-TLC) and L-rhamnose and D-glucose (Co-Pc and Co-TLC).
PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (BSA-2)

The proaglycone (BSA-2) was permethylated and subsequently hydrolysed when it yielded the aglycone and 2,3,4,6-tetra-O-methyl glucose and 2,3-di-O-methyl rhamnose showing the presence of L-rhamnose and D-glucose in pyranose form and also that C-4 OH group of the L-rhamnose was linked with C-1 OH of D-glucose.

ENZYMATIC HYDROLYSIS

The glycoside when hydrolysed by enzyme emulsin yielded proaglycone (BSA-1) and D-glucose (Co-Pc) thereby indicating β-linkage between BSA-1 and D-glucose.

The proaglycone (BSA-1) on its hydrolysis with tokadiastase solution yielded 1,3, dimethoxy-8-hydroxy-6 methyl anthraquinone (m.m.p., Co-TLC) and L-rhamnose, confirmed α linkage between 1,3, dimethoxy-8-hydroxy-6 methyl anthroquinone and L-rhamnose.

The above facts when put together concluded that 8-OH of aglycone was linked with C-1 of the L-rhamnose via α-linkage and C-4 of the L-rhamnose was attached to the C-1 of D-glucose via β-linkage.

Thus the structure to the glycoside was assigned (IV) as; 1,3, dimethoxy-6-methyl anthraquinone-8-O-β-D-glucopyranosyl (1→4)-O-α-L-rhamnopyranoside.
\(^1\)HNMR spectrum of hexa acetyl derivative of the glycoside further supported the above structure (IV). The important peaks obtained in the \(^1\)HNMR spectrum (Fig. IV) of the hexa acetyl derivative of the glycoside and the structural units inferred with the help of literature\(^{36}\) are recorded in 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>7.35</td>
<td>d</td>
<td>2.5</td>
<td>2</td>
<td>H-2, H-4</td>
</tr>
<tr>
<td>2.</td>
<td>7.94</td>
<td>d</td>
<td>2.4</td>
<td>2</td>
<td>H-5, H-7</td>
</tr>
<tr>
<td>3.</td>
<td>3.96</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>1-OCH₃</td>
</tr>
<tr>
<td>4.</td>
<td>3.84</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>3-OCH₃</td>
</tr>
<tr>
<td>5.</td>
<td>2.51</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6-CH₃</td>
</tr>
<tr>
<td>6.</td>
<td>4.38</td>
<td>d</td>
<td>7.5</td>
<td>1</td>
<td>1' anomeric proton</td>
</tr>
<tr>
<td>7.</td>
<td>4.26</td>
<td>d</td>
<td>2</td>
<td>1</td>
<td>1'' anomeric proton</td>
</tr>
<tr>
<td>8.</td>
<td>2.08</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>2'' OAC</td>
</tr>
<tr>
<td>9.</td>
<td>3.00</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>3''-OAC</td>
</tr>
<tr>
<td>10.</td>
<td>0.74</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6'' -CH₃</td>
</tr>
<tr>
<td>11.</td>
<td>3.02</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>2.04</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>4'''-OAC</td>
</tr>
<tr>
<td>14.</td>
<td>3.90</td>
<td>s</td>
<td>-</td>
<td>3</td>
<td>6'''-OAC</td>
</tr>
<tr>
<td>15.</td>
<td>4.61-4.82</td>
<td>m</td>
<td>-</td>
<td>4</td>
<td>4-proton of rhamnosyl unit</td>
</tr>
<tr>
<td>16.</td>
<td>5.54-5.56</td>
<td>m</td>
<td>-</td>
<td>6</td>
<td>6-protons of glucose unit</td>
</tr>
</tbody>
</table>
MASS SPECTRUM OF THE GLYCOSIDE

EI mass spectrum of glycoside showed the fragment ion peaks at: \( M^+ \) 606, \( m/z = 443, 427, 298, 270, 242, 214, 213, 136, 106 \).

The various species assigned to the fragments are described in the scheme II and further confirmed its identity as 1,3, dimethoxy-6 methyl-8-\( \beta \)-D-glucopyranosyl (1\( \rightarrow \)4)-O-\( \alpha \)-L-rhamnopyranoside.
Scheme II
EXPERIMENTAL

The seeds of *Impatiens balsamina* Linn. (Fam. Balsaminaceae) was purchased from the local market of Sagar district. Air dried and powdered seeds (3.0 Kg) of *Impatiens balsamina* were extracted with 95% ethanol in 5 lit. R.B. flask, fitted with condenser and the extraction continued for a month. The total extract was concentrated under reduced pressure to a thick viscous mass (208 g) and resolved into water soluble and water insoluble parts. The concentrated water soluble part was successively extracted with petroleum ether, rectified spirit and ethylacetate. The *pétroleum ether soluble* part on removal of the solvent yielded very small amount of gummy residue which could hardly afford for any study. (The study of ethylacetate soluble part is reported in chapter III).

STUDY OF THE RECTIFIED SPIRIT SOLUBLE PART

The rectified spirit soluble part was concentrated under reduced pressure to dark orange coloured mass (5.8 gm.) and subjected to TLC examination using ethylacetate : acetone : water (10 : 1 : 1) and iodine vapours as visualizing agent, gave two spots. As such the concentrated rectified spirit extract was subjected to column chromatography and eluated with benzene : methanol in varying proportions.
COLUMN CHROMATOGRAPHY

Length of the column 50 cm.
Diameter of the column 3 cm.
Weight of the si-gel 150 g.
Weight of the crude extract 4.2 g.

**TABLE - V**

<table>
<thead>
<tr>
<th>No.</th>
<th>01 - 11</th>
<th>Benzene : Methanol (7:2)</th>
<th>No solid substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-do-</td>
<td>(7:4)</td>
<td>yielded the compound (BS)</td>
</tr>
<tr>
<td>2.</td>
<td>-do-</td>
<td>(7:6)</td>
<td>No crystalline material</td>
</tr>
</tbody>
</table>

**STUDY OF FRACTIONS (12-30)**

On removal of the solvent from fractions collected (12-30) and crystallisation from ethylacetate; light petroleum ether (1:1) an orange coloured substance (BS 1.99 gm) was obtained which was found to be homogeneous on Tlc (Rf 0.62, Benzene : Methanol, 7:4).

**STUDY OF THE GLYCOSIDE (BS)**

The orange coloured compound was soluble in ethylacetate and methanol. It analysed for C$_{29}$H$_{34}$O$_{14}$, m.p. 240°, M$^+$ 606.
It gave Molisch's test and colour reaction characteristic of an anthraquinone glycoside:

(i) Concentrated sulphuric acid gave yellow orange red to reddish brown colour with the alcoholic solution of the compound.

**ELEMENTAL ANALYSIS**

\[
\begin{align*}
\text{Found} & & \text{Calculated for C}_{29}\text{H}_{34}\text{O}_{14} \\
C &= 57.38\% & C &= 57.42\% \\
H &= 5.58\% & H &= 5.61\% \\
\text{Molecular weight} &= 606 & \text{Molecular weight} &= 606 \\
\text{(By mass spectroscopy)} & & \text{(By mass spectroscopy)}
\end{align*}
\]

**ACETYLATION OF THE GLYCOSIDE (BS)**

The compound (100 mg), acetic anhydride 7 ml and 2 ml of pyridine were taken in 50 ml RB fask fitted with air condenser. The reaction mixture was refluxed on a water bath for 4 hrs. and poured into ice cold water (75 ml) resulting into a precipitate which was taken into a 150 ml separating funnel and shaken with solvent ether (50 ml). The ethereal layer was washed with water and NaHCO\textsubscript{3} solution. It was dried over anhydrous sodium sulphate. Removal of solvent ether and crystallisation of the residue from methyl alcohol yielded colourless needles (65 mg), which analysed for C\textsubscript{41}H\textsubscript{46}O\textsubscript{20}, m.p. 168° and M\textsuperscript{+} 858.
ELEMENTAL ANALYSIS

Found
C = 57.291%
H = 5.31%
Percentage of acetyl groups = 29.82%
Molecular weight = 858
(By mass spectroscopy)

Calculated for \( \text{C}_{41}\text{H}_{46}\text{O}_{20} \)
C = 57.3426%
H = 5.3613%
Percentage of acetyl groups = 29.37%
Molecular weight = 858
(By mass spectroscopy)

ACID HYDROLYSIS OF THE GLYCOSIDE (BS)

The glycoside (400 mg) was refluxed with 7% alc. \( \text{H}_2\text{SO}_4 \) (50 ml) in a 150 ml R.B. flask on a water bath for eight hours. Then 50 ml of water was added to the reaction mixture and alcohol was removed by its distillation under reduced pressure, when it yielded an aglycone as a precipitate which was separated. The aqueous layer was worked up separately for the identification of sugars and on paper chromatographic examination showed the presence of D-glucose and L-rhamnose.

STUDY OF THE AGLYCONE (BS₁)

The aglycone crystallised from methanol as light orange coloured crystalline substance. It was found to be homogeneous by Tlc (Benzene : Methanol \( R_f = 0.58 \)). It analysed for molecular formula, \( \text{C}_{17}\text{H}_{14}\text{O}_5 \), m.p. 312°, \( M^+ 298 \) (EIMS). It was soluble in alkalies with orange colour changing to deep orange red.
ELEMENTAL ANALYSIS

Found
C = 68.40%
H = 4.95%
Molecular weight = 298
(By mass spectroscopy)

Calculated for C$_{17}$H$_{14}$O$_{5}$
C = 68.45%
H = 4.98%
Molecular weight = 298
(By mass spectroscopy)

Zn DUST DISTILLATION OF THE AGLYCOME (BS$_{1}$)

The compound (40 mg) and Zn dust (2 gm) was taken in a pyrex glass tube sealed and heated upto dull red, yellow coloured compound, m.p. 205-206° was obtained at the upper end of the tube and was identified as 2-methyl anthracene.

ACETYULATION OF THE AGLYCOME (BS$_{1}$)

The acetylation of the aglycone was done in a similar way as described on page No. 42 of the thesis.

The acetyl derivative was obtained as colourless crystals, analysed for C$_{19}$H$_{16}$O$_{6}$, m.p. 202° and M$^+$ 340 (EIMS).

ELEMENTAL ANALYSIS

Found
C = 66.088%
H = 4.69%
Percentage of acetyl group = 12.40%
Molecular weight = 340
(By mass spectroscopy)

Calculated for C$_{19}$H$_{16}$O$_{6}$
C = 67.058%
H = 4.7058%
Percentage of acetyl group = 12.64%
Molecular weight = 340
(By mass spectroscopy)
OXIDATION OF THE AGLYCONES (BS₁)

The compound (50 mg) was suspended in acidic KMnO₄ solution (70 ml) in a 250 ml good joint conical flask and refluxed for 10 hours. The reaction mixture was separated from the precipitated MnSO₄ and the water removed by slow evaporation, when prism shaped crystals m.p. 156° were obtained. The compound was identified as 1:3 dimethoxy phthalic acid (by m.m.p. and Co-Tlc) molecular formula, C₈H₁₀O₆, m.p. 244°, M⁺ 204 (EIMS).

PARTIAL HYDROLYSIS OF THE GLYCOSIDE (BS)

The glycoside (200 mg) was taken with Kiliani mixture (75 ml, HCl : Acetic acid : Water, 15 : 35 : 50) in a 150 ml conical flask and left for 2 days at room temperature. The contents of the flask were extracted with ethanol and ethanol soluble part on examination by P.C. showed two spots (Rf 0.38 and 0.52 in BAW).

The ethanol soluble part was concentrated and chromatographed over silica gel (60 - 120 mesh) column. The column was eluted with CCl₄ : C₂H₅OH in various proportions. The details of the column chromatography is as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of ethanol soluble part</td>
<td>200 mg</td>
</tr>
<tr>
<td>Length of the column</td>
<td>50 cm</td>
</tr>
<tr>
<td>Diameter of the column</td>
<td>3.0 cm</td>
</tr>
<tr>
<td>Weight of the Si-gel</td>
<td>40 g</td>
</tr>
</tbody>
</table>
TABLE - VI

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fractions</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:Ethanol (1:1)</td>
<td>One</td>
<td>BSA-1</td>
</tr>
<tr>
<td>2.</td>
<td>9 - 14</td>
<td>CCl₄:Ethanol (1:2)</td>
<td>Two</td>
<td>Mixture of two</td>
</tr>
<tr>
<td>3.</td>
<td>15 - 20</td>
<td>CCl₄:Ethanol (1:3)</td>
<td>One</td>
<td>BSA-2</td>
</tr>
<tr>
<td>4.</td>
<td>21 - 25</td>
<td>Ethanol</td>
<td>Nil</td>
<td>-</td>
</tr>
</tbody>
</table>

The fraction (1-8) were of same Rf value and so mixed together. The removal of solvent from combined fraction yielded a compound BSA-1 which crystallised from ethanol, m.p. 202° and molecular formula, C₂₃H₂₄O₉, M⁺ 444.

ELEMENTAL ANALYSIS OF PROAGLYCONE BSA-1

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for C₂₃H₂₄O₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>C = 62.15%</td>
<td>C = 62.16%</td>
</tr>
<tr>
<td>H = 5.38%</td>
<td>H = 5.40%</td>
</tr>
<tr>
<td>Molecular weight = 444</td>
<td>Molecular weight = 444</td>
</tr>
<tr>
<td>(By mass spectroscopy)</td>
<td>(By mass spectroscopy)</td>
</tr>
</tbody>
</table>

PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (BSA-1)

The proaglycone BSA-1 (45 mg) was treated with methyl iodide (4.5 ml) and Ag₂O (120 mg) in dimethyl
formamide (6.0 ml) in a 50 ml conical flask and left for two days at room temperature. The contents were filtered and residue was washed with dimethyl formamide. The filtrate was concentrated under reduced pressure to a solid mass, which when hydrolysed with 7% $\text{H}_2\text{SO}_4$, gave the aglycone and methylated sugar.

After separation of the aglycone the aqueous part was neutralised with $\text{BaCO}_3$ and $\text{BaSO}_4$ filtered off. On concentration the filtrate was examined on paper chromatography on Whatman filter paper No. 1 using ethylacetate : water : pyridine (3 : 3 : 1) solvent system and aniline hydrogen phthalate as spraying agents. The sugar was identified as 2,3,4-tri-O-methyl rhamnose.

STUDY OF FRACTIONS 15–20

The fractions were of same $R_f$ value and so were mixed together and on evaporation of the solvent yielded a compound which crystallised from ethanol, molecular formula, $C_{29}H_{34}O_{14}$, m.p. 240°-1° and $M^+$ 606.

ELEMENTAL ANALYSIS OF THE PROAGLYCONE (BSA–2)

<table>
<thead>
<tr>
<th>Found</th>
<th>Calculated for $C_{29}H_{34}O_{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C = 57.38%$</td>
<td>$C = 57.42%$</td>
</tr>
<tr>
<td>$H = 5.58%$</td>
<td>$H = 5.61%$</td>
</tr>
<tr>
<td>Molecular weight = 606</td>
<td>Molecular weight = 606</td>
</tr>
<tr>
<td>(By mass spectroscopy)</td>
<td>(By mass spectroscopy)</td>
</tr>
</tbody>
</table>
PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE (BSA-2)

The permethylation and hydrolysis of the proaglycone BSA-2 was carried out in the same way as described for BSA-1. It showed the presence of 2,3,4,6-tetra-O-methyl glucose (by Co-Pc and Co-TLC).

IDENTIFICATION OF SUGARS AFTER HYDROLYSIS

The aqueous hydrolysate (50 ml) obtained after hydrolysis of the glycoside was neutralised with BaCO₃ and BaSO₄ filtered off. The filtrate was concentrated to yellow syrupy mass under reduced pressure and examined by paper chromatography on Whatman filter paper No. 1 using aniline hydrogen phthalate as detecting agent. The results are recorded in table - VII.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent system</th>
<th>R_f 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>Butanol:Acetic Acid:Water 94:1:5)</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>2.</td>
<td>Ethylacetate:Water:Pyridine (2:2:1)</td>
<td>0.23</td>
<td>0.25</td>
</tr>
</tbody>
</table>
<pre><code>                                  |           | 0.37      | 0.35             | L-Rhamnose      |
                                  |           | 0.49      | 0.50             | L-Rhamnose      |
</code></pre>

PERIODATE OXIDATION OF THE GLYCOSIDE (BS)

20 mg of the glycoside was dissolved in ethanol (15 ml) in a conical flask and treated with sodium meta
periodate (10 ml of 0.01 N). The reaction mixture was allowed to stand for the two days at the room temperature. A blank was also run with the same procedure. The quantity of sodium periodate consumed and formic acid liberated were estimated by Jones's method.

ENZYMATIC HYDROLYSIS OF THE GLYCOSIDE (BS)

The glycoside (30 mg) was dissolved in ethanol (20 ml) and mixed with almond emulsion solution (15 ml) in a conical flask fitted with stopper. The reaction mixture was left for 72 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 Whatman No. 1 filter paper and BAW (4 : 1 : 5) solvent system. The sugar was identified as D-glucose.

The aglycone was identified as proaglycone BSA-1, m.p. 202° (by Co-T., m.m.p.).

The proaglycone BSA-1 (18 mg) was dissolved in ethanol (10 ml) and mixed with equal vol. of solution of tokadiastase (15 mg) in a conical flask.

The contents were left for 8 hrs. and filtered. The aglycone was identified as 1,3, dimethoxy-8-hydroxy-
6-methyl anthraquinone, m.p. 212° (by m.m.p. and Co-TLC).
The hydrolysate when examined on PC, was found to contain
L-rhamnose.
REFERENCES


10. Zeisel's, Monatsh, 6, 989 (1885).


