APPENDICES
Milano, 14th May 1991

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Dear Sir,

This is to inform you that we should publish your paper
"APIGENIN-4'-O-B-D XYLOFURANOSYL (1-24)-O-B-D-GLUCOPYRANOSIDE FROM IMPATIENTS BALSAMINA LINN" as "Phytochemical communications" and we ask you, should you agree, to re-write it as per the enclosed outline.

Therefore, we are returning you, herewith attached, your above paper looking forward to your news.

Yours faithfully,

[Signature]
Dr/A. Bonati

enc.
A NOVEL FLAVONOIDAL GLYCOSIDE FROM IMPATIENS BALSAMINA LINN.

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Natural Products Laboratory  
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The plant Impatiens balsamina Linn\(^1-4\) (N.O. Balsaminaceae) in known Gulmendi in Hindi and is distributed throughout in India. The seeds of Impatiens balsamina Linn was purchased from the local market of Sagar district, November 1988 and identified by Chairman, Botany Department of this University and a herbarium specimen No. XI has been deposited at herbarium room no. 36 of Chemistry Department.

Alcoholic extract of the flowers has been found to have adequate antibiotic activity against sclerotina, 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 tonic useful when applied to burns and scalds. The active principle has been identified as 2-methoxy-1, 4-naphthaquinone\(^5\).

The details of previously isolated constituents from Impatiens balsamina Linn are summarised below;
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The present paper deals with the isolation and identification of a novel flavonoidal glycoside; Apigenin-4'-O-β-D-xylofuranosyl (1→4) O-β-D-glucopyranoside from the seeds of Impatiens balsamina.

The ethylacetate part of air dried and powdered seeds of Impatiens balsamina on its column chromatography on Si-gel and elution with ethylacetate/methanol gave a yellow coloured substance, 0.086% (l), which was found to be homogeneous on TLC (ethylacetate : methanol : water, 14:8:2, Rf = 0.62). It analysed for molecular formula, C_{26}H_{28}O_{14}, m.p. 240°, M^+ 564. It gave positive Molisch test for glycoside and positive colour reactions for flavonoidal glycoside^{12,13}.
On hydrolysis with 7% ethanolic H₂SO₄ (1) gave an aglycone (2), m.p. 348°C, C₁₅H₁₀O₅, M⁺ 270 and sugar moieties. The sugars were identified as D-glucose and D-xylose (by Co-Pc and Co-TLC), which were in equimolar ratio (1:1)¹⁴.

Preparation of its triacetyl derivative, m.p. 189°C, C₂₁H₁₆O₈, M⁺ 396 indicated the presence of three free OH groups in the (2). Formation of 1:3:5 - trihydroxy benzene on alkaline degradation confirmed the presence of OH groups at C₅ and C₇ in the (2). A bathochromic shift of 26 nm in band I with AlCl₃ (relative to MeOH) and 7 nm in band II with NaOAc (relative to MeOH) further confirmed the presence of OH groups at C₅ and C₇ respectively¹⁵.

Formation of p-hydroxy benzoic acid on alkaline degradation confirmed the presence of OH group at C-4' in the (2), which was further confirmed by a bathochromic shift of 32 nm in presence of NaOAc/H₃BO₃ relative to band I in MeOH confirmed the presence of free OH group at C-4,¹⁶ and this bathochromic shift was not observed when examined in the UV spectrum of glycoside, suggesting the involvement of C₄'-OH in glycosylation with the disaccharide moiety&identifying the aglycone as apigenin.

(1) On graded hydrolysis by Kiliani mixture¹⁷ liberated D-xylose first followed by D-glucose suggesting that D-xylose was the terminal sugar and D-glucose was linked to the aglycone (2).
(1) On enzymatic hydrolysis\textsuperscript{18} with emulsin yielded D-xylose and D-glucose indicating that the D-xylose was attached to D-glucose through \( \beta \)-linkage and further confirmed that D-glucose was attached to the aglycone by \( \beta \)-linkage. Pyranose form of sugars were confirmed by periodate oxidation\textsuperscript{19} and acid hydrolysis of permethylated glycoside yielded 2,3,5-tri-O-methyl xylose and 2,3,4,6-tetra-O-methyl glucose thereby confirming that xylose was attached to glucose by (1\( \rightarrow \)4) linkage and glucose with aglycone (2) by \( \text{C}_{1} \). Thus (1) was identified as; Apigenin-4'-O-\( \beta \)-D-xylofuranosyl (1\( \rightarrow \)4)-O-\( \beta \)-D-glucopyranoside.

**Experimental**

Air dried powdered seeds (3.0 Kg) were extracted with 95% ethanol. The alcoholic extract was concentrated under reduced pressure 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 ethylacetate soluble part was concentrated under reduced pressure to a yellow coloured mass (7.4 gm). It showed a single spot (\( \text{Rf} = 0.52 \)) when examined by thin layer chromatography on si-gel G plates using ethylacetate:methanol (98:1) and sulphuric acid as visualizing agent. It was purified over Si-gel column and eluated ethylacetate:methanol:water (14:8:2, \( \text{Rf} = 0.62 \)) gave yellow coloured compound, 2.6 gm (l), m.p. 240\({}^\circ\text{C}\), molecular
formula, C\textsubscript{26}H\textsubscript{28}O\textsubscript{14}, M\textsuperscript{+} 564 (found C = 55.30%, H = 4.95%, calculated for C = 55.32%, H = 4.97%). It gave red colour with Mg and HCl and positive Molisch test. UV \(\lambda_{\text{max}}\) \text{MeOH} 268, 313, \(\lambda_{\text{max}}\) \text{MeOH+NaOAc} 276, 297, 362, \(\lambda_{\text{max}}\) \text{MeOH+AlCl\textsubscript{3}/HCl} 278, 300, 368 nm; IR \(\nu_{\text{max}}\) \text{KBr} 3348, 2904, 1682, 1612, 1562, 1512, 1282, 1150 and 1730 cm\textsuperscript{-1}.

Compound (1): m.p. 240\textdegree}C, C\textsubscript{26}H\textsubscript{28}O\textsubscript{14}, the acetyl derivative of (1) was prepared by acetic anhydride and pyridine, m.p. 118\textdegree}C, C\textsubscript{42}H\textsubscript{44}O\textsubscript{22}, M\textsuperscript{+} 900, \textsuperscript{1}H\text{NMR} (CDCl\textsubscript{3}, 60 MH\textsubscript{2}\text{SO}) 7.26 (2H, d, J=9 Hz, H-2', H-6'), 6.75 (2H, d, J=9 Hz, H-3', H-5'), 6.23 (1H, s, H-3), 6.49 (1H, d, J=2.5 Hz, H-6), 6.37 (1H, d, J=2.0 Hz, H-8), 2.48 (3H, s, 5-OAc), 2.40 (3H, s, 7-OAc), 5.47 (1H, d, J = 7 Hz, 1''-anomeric proton), 2.03 (3H, s, 2''-OAc), 2.05 (3H, s, 3''-OAc), 3.94 (3H, s, 6''-OAc), 5.66 (1H, d, J = 7 Hz, 1'''-anomeric proton), 2.06 (3H, s, 2'''-OAc), 2.07 (3H, s, 3'''-OAc), 2.09 (3H, s, 5'''-OAc), 3.62-4.32 (11H, m, protons of sugar residue.

Compound (2): m.p. 348\textdegree}C, C\textsubscript{15}H\textsubscript{10}O\textsubscript{5}, triacetyl derivative, m.p. 189\textdegree}C, C\textsubscript{21}H\textsubscript{16}O\textsubscript{8}, M\textsuperscript{+} 396, \textsuperscript{1}H\text{NMR} (CDCl\textsubscript{3}, 60 MH\textsubscript{2}\text{SO}) 7.24 (2H, d, J = 9 Hz, H-2', H-6'), 6.77 (2H, d, J = 9 Hz, H-3', H-5'), 6.23 (1H, s, H-3), 6.48 (1H, d, J = 2.5 Hz, H-6), 6.36 (1H, d, J = 2.0 Hz, H-8), 2.33 (3H, s, 4'-OAc), 2.47 (3H, s, 5'-OAc), 2.39 (3H, s, 7-OAc).
\[ R = \text{Sugars; D-xylose} \]
\[ \text{D-glucose} \]

(1)

Acknowledgements:

Authorress is greatful to Chairman UGC, New Delhi for awarding a minor research project and thanks are due to Director, CDRI, Lucknow for spectral analysis.
References:


Dr. R.N. Yadav
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Sagar-470003 (M.P.)

Dear Dr. Yadav,

We are pleased to inform you that your research paper entitled, "A novel rutinoguine glycoside from impatiens Balsamina linn scott" (Index No. 511/31) by R.N. Yadav and Jasvinder Jain has been accepted for publication in Asian Journal of Chemistry.

Yours Sincerely

R.K. Agarwal

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Note: Subscription must be sent either by M.O. or by D.D. drawn on any bank at Delhi or Ghaziabad in favour of ASIAN JOURNAL OF CHEMISTRY, SAHIBABAD.
A NOVEL ANTHRAQUINONE GLYCOSIDE FROM IMPATIENS BALSAMINA L. SEEDS

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Department of Chemistry,
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ABSTRACT

A novel anthraquinone glycoside 1,3,4-dimethoxy-6, methyl anthraquinone-8-0-β-D-glucopyranosyl(1→4)-O-α-L-rhamnopyranoside.

INTRODUCTION

Medicinal use attributed to members of the natural order Balsaminaceae are well known and so it attracted our attention on Impatiens balsamina Linn\textsuperscript{1,2} (N.O. Balsaminaceae) is known Gulmendi in Hindi and is distributed throughout in India. Alcoholic extract of the flowers is reputed for curing 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.

Earlier workers\textsuperscript{3,4} have already reported the presence of 2-hydroxy-1,4-naphthoquinone, 2-methoxy-1,4-naphthoquinone from the seeds and leaves of the plant. The present paper deals with the isolation and identification of a novel anthraquinone, 1,3,4-dimethoxy-6, methyl anthraquinone-8-0-β-D-glucopyranosyl (1→4)-O-α-L-rhamnopyranoside.
RESULTS AND DISCUSSION

The water soluble part of the rectified spirit extract of the 95% concentrated ethanolic extract of dried and powdered seeds gave crude glycoside (0.66%), which was found to be homogeneous on TLC (Benzene) and on column chromatography yielded orange needles from (ethylacetate : light petroleum ether 1:1), m.p. 238-40°C, molecular formula C_{29}H_{34}O_{14}^{[1]}, M^+ = 606 (R_f = 0.64). It gave positive Molisch test and responded to all the colour tests^5-8 characteristic of an anthraquinone glycoside.

On acid hydrolysis with 7% ethanolic H_{2}SO_{4}^{[1]} gave an aglycone [2] (0.42%) m.p. 312°, C_{17}H_{14}O_{5} and sugar moities. The sugars were identified as rhamnose and glucose (by co-pc and CO-TLC). The anthraquinone skeleton was further supported by the isolation of 2-methyl anthracene (m.p. 205-206°) from Zn-dust distillation of [2]. \textsuperscript{1}H NMR spectrum of monoacetyl derivative of [2] showed signals at δ = 7.18 (d, J = 2.4 Hz, C_{2}-1H), δ = 7.38 (d, J = 2.5 Hz, C_{4}-1H), δ = 7.92 (d, J = 2.5 Hz, C_{5}-1H), δ = 6.98 (d, J = 2.0 Hz, C_{7}-1H), δ = 3.98 (s, 3H, C_{1}, -OCH_{3}), δ = 3.86 (s, 3H, C_{3}-OCH_{3}), δ = 2.53 (s, 3H, C_{6}-CH_{3}), δ = 2.56 (s, 3H, C_{8}-OAc); UV in D_{2}O \lambda_{max} (E75): 265, 282 and 414 nm; EIMS M^+ 298, m/z = 270, 242, 214, 213, 136, 106; IR ν_{max} 3300, 2947, 2892, 1632, 1680, 1666, 1656, 1598, 1478 cm\textsuperscript{-1}.

The IR peak at ν_{max} 3220 cm\textsuperscript{-1} indicated the presence of free OH group. Preparation of a mono-acetyl derivative
C_{19}H_{16}O_{6}, m.p. 202^\circ, M^+ 340, suggested one acetylable OH group in the [2]. The [2] gave a red complex with zirconium nitrate solution, soluble in HCl showing the presence of a hydroxyl group at position C-8^9.

The ethanolic solution of the [2] formed a complex with ethanolic copper sulphate showing the presence of a hydroxyl function in the \(\alpha\)-position of the \(>\text{C}=O\) group.\(^{10}\)

The presence of only \(\alpha\)-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 [2].

A peak in the IR spectrum of [2] at 2892 cm\(^{-1}\) indicated the presence of \(-\text{OCH}_3\) group(s) in it. Methoxy group estimation (21.12%) was done by Zeisel\(^{11}\) method which confirmed the presence of two methoxyl groups in [2].

The [2] gave red colour on treatment with concentrated H\(_2\)SO\(_4\) showing the presence of at least one methoxyl group in any \(\alpha\)-position\(^{12}\). Thus the two methoxyl groups present in the [2], one is of B-position and the other is at \(\alpha\)-position.

The \(^1\)H NMR of acetylated derivative of the [2] showed singlet at \(\delta = 3.98\) and \(\delta = 3.86\) integrating for 3 protons and confirmed the presence of two methoxyl groups at position 1 and 3 in the ring (A).

The m-di-substituted pattern in the ring A and C is supported by the \(^1\)H NMR spectrum of acetylated derivative of the [2] showed signals at \(\delta = 7.18\) (d, \(J = 2.4\) Hz, C\(_2\)-1H), \(\delta = 7.38\) (d, \(J = 2.5\) Hz, C\(_4\)-1H), \(\delta = 7.92\) (d, \(J = 2.5\) Hz, C\(_5\)-1H) \(\delta = 6.98\) (d, \(J = 2.0\) Hz, C\(_7\)-1H).
In the IR spectrum peak at $\nu_{\text{max}}^\text{KBr} 1478 \text{ cm}^{-1}$ indicated the presence of methyl group in the [2]. Estimation of methyl group by the semi-micro apparatus as mentioned by Belcher and Godbert$^{13}$ (4.9992%) confirmed the presence of one methyl group in the [2] and [2] was identified as 1,3,6-dimethoxy-8-hydroxy-6-methyl anthraquinone.

The [2] gave a red complex with zirconium nitrate solution, soluble in HCl, indicating the presence of -OH group at position C-8 in the [2] but its absence in the [1], clearly indicating the involvement of 8-OH in glycosylation with the diaccharide moiety. The [1] on graded hydrolysis by killani mixture liberated first glucose followed by rhamnose indicating that glucose was the terminal unit and rhamnose was attached to aglycone. Periodate oxidation$^{14}$ of the [1] indicated the presence of a disaccharide having both the units in pyranose form and the linkage between the sugars was on enzymatic hydrolysis with emulsin gave glucose and unhydrolysed agly-rhamnose, which on further hydrolysis with enzyme tokadiastase yielded aglycone[2] and rhamnose proving $\beta$-linkage between glucose and rhamnose and $\alpha$-linkage between rhamnose and aglycone[2].

Acid hydrolysis of permethylated glycoside resulted 2,3,4,6-tetra-O-methyl glucose and 2:3 di-O-methyl L-rhamnose thereby confirming that glucose was attached to rhamnose by (1$\rightarrow$4) linkage and rhamnose with aglycone [2] by C$_1$. Thus [1]
was identified as; 1,3-dimethoxy-6, methyl anthraquinone-8-
O-β-D-glucopyranosyl (1→4)-O-α-L-rhamnopyranoside and was
further supported by $^1$HNMR spectrum of its hexa-acetyl
derivative showed singlet at $\delta = 7.16$ (d, $J = 2.4$ Hz, $C_2$-1H),
$\delta = 7.35$ (d, $J = 2.5$ Hz, $C_4$-1H), $\delta = 7.94$ (d, $J = 2.5$ Hz,
$C_5$-1H), $\delta = 6.96$ (d, $J = 2.0$ Hz, $C_7$-1H), $\delta = 3.96$ (s, 3H,
$C_1$-OCH$_3$), $\delta = 3.84$ (s, 3H, $C_3$-OCH$_3$), $\delta = 2.51$ (s, 3H, $C_6$-CH$_3$),
$\delta = 4.38$ (d, $J = 7.5$ Hz, 1-anomeric proton-$C_1$), $\delta = 4.26$ (d,
$J = 2$ Hz, 1-anomeric proton-$C_1$), $\delta = 2.08$ (s, 3H, $C_2$-OAc),
$\delta = 3.00$ (s, 3H, $C_3$-OAc), $\delta = 0.74$ (s, 3H, $C_6$-CH$_3$), $\delta = 3.02$
(s, 3H, $C_2$-OAc), $\delta = 2.98$ (s, 3H, $C_3$-OAc), $\delta = 2.04$ (s, 3H,
$C_6$-OAc), $\delta = 3.90$ (s, 3H, $C_6$-OAc), $\delta = 4.51$-4.82 (m, 4-protons
of rhamnosyl unit), $\delta = 5.44$ (m, 6-protons of glucose unit).

**EXPERIMENTAL**

Dried and powdered seeds were extracted exhaustively
with 95% ethanol. The extract was concentrated under reduced
pressure and the concentrated extract poured into water. The
concentrated water soluble part was successively extracted
with rectified spirit and concentrated under reduced pressure.
The concentrated extract poured in 400 ml of water and the
coloured precipitate (5.8g) thus obtained was subjected to
column chromatography.

**ISOLATION AND IDENTIFICATION**

The coloured precipitate showed a single spot on tlc
Si, gel G plates using Benzene. The fraction was purified
over a Si gel column (60-120 mesh) and eluted with ethylacetate.
light petroleum (1:1) providing compound [1] as orange
coloured needles (0.66%), m.p. 238-240° (Found: C = 57.38%,
H = 5.58%, C_{29}H_{34}O_{14} calcd. for: C = 57.42% H = 5.61%);
UV \text{EtOH} \quad \lambda_{\text{max}} \quad 262, 292 and 404 nm; EIMS M^+ 606, m/z = 443,
427, 298, 270, 242, 214, 213, 136, 106; IR \quad \nu_{\text{max}} \quad 3215,
2932, 2896, 1760, 1690, 1660, 1678, 1628, 1600, 1470 cm^{-1}.

Acid hydrolysis of the glycoside:

400 mg of compound was refluxed with 7% alc H_2SO_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 [2] as a precipitate which was
separated. The aqueous layer was worked up separately for the
identification of sugars and on paper chromatographic examination
(BAW 4:1:5) showed the presence of D-glucose and L-rhamnose.

Zn-Dust distillation of the aglycone:

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

Acetylation of the Aglycone:

50 mg of the compound was dissolved in 2 ml of pyridine
and treated with 1 ml of acetic anhydride. The mixture was
heated for 10 minutes over the water bath, left overnight, precipitated with ice water, washed and crystallised acetyl derivative obtained m.p. 202°, C_{19}H_{16}O_{6}, M^+ 340.

Graded hydrolysis of compd. [1]:

Compd. [1] was mixed with killani mixture and left for two days partial hydrolysis was monitored by pc. After appearance of glucose the unhydrolysed agly-rhamnose was separated m.p. 200°, analysed for C_{23}H_{24}O_{9}.

Periodate oxidation:

Compd. [1] was dissolved in methanol and kept with sodium metaperiodate for 2 days, liberated formic acid and consumed periodate was estimated by Jone's method.

Permethylation and Acid hydrolysis:

Compd. [1] was treated with CH_{3}I and Ag_{2}O in dimethyl formamide at room temp. After two days the reaction mixture was filtered and the residue was washed with chloroform. The filtrate was conc. and hydrolysed by 7.5% alc. H_{2}SO_{4}. After usual work up methylated sugars were identified on PC as: 2,3,4,\textit{\textalpha{}}-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-glucose.

Enzymatic hydrolysis:

Compd. [1] dissolved in ethyl alcohol and an aqueous solution of emulsin was mixed and left at room temp. for 48 hours. The examination of hydrolysate showed presence of glucose. The unhydrolysed agly-rhamnose was separated and again treated with aqueous solu. of tokadiastase for 8 hrs resulting into aglycone [2] and rhamnose.
ACKNOWLEDGEMENTS

The authors wish to thank Central Drug Research Institute, Lucknow for spectral analysis.

REFERENCES


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


*LUTEOLIN-5-O-α-L-RHAMNOPYRANOSYL (1→4)-O-β-D-GLUCOPYRANOSIDE, A NOVEL FLAVONOID GLYCOSIDE FROM IMPATIENS SCABRIDA D.C.*

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A novel flavonoid glycoside, Luteolin-5-O-α-L-rhamnopyranosyl (1→4)-O-β-D-glucopyranoside, has been isolated from Impatiens Scabrida D.C. and characterised by physical and chemical methods.

**EXPERIMENTAL AND DISCUSSION**

*Impatiens Scabrida* D.C. belongs to the natural order Balsaminaceae and is distributed throughout India. It is an annual herb with sessile and narrow leaves. The Ayurvedic system of medicines describes the oil of the plant to be used as a semidrying oil. The present communication deals the isolation and structural elucidation of a novel flavonoid glycoside.

Powdered plant (2.5 kg) of I. Scabrida D.C. was extracted with 95% ethanol and concentrated under reduced pressure and the concentrated extract was poured into excess of distilled water. The water soluble part was concentrated to a dry viscous mass and extracted with ethyl acetate and methanol successively. The ethylacetate fraction gave the colour reactions

* This paper has been communicated for publication in Journal of Institution of Chemists.
indicating the presence of flavonoidal glycoside \(^2,^3[1]\), (0.096\%), mp. 227°C, molecular formula \(C_{27}H_{30}O_{15}\), molecular weight \(M^+ 594\), (found C = 54.52\%, H=5.02\%, calculated for C=54.54\%, H=5.05\%). Its acetyl derivative had mp. 166\°C. Its UV spectrum showed characteristic peaks at \(\lambda_{\text{MeOH}}^{\text{max}}\) 343, 275, 254 nm; NaOMe 405, 268, 233(sh); AlCl\(_3\), 421, 354(sh), 300(sh), 274, 242(sh), AlCl\(_3\)/HCl, 376, 357, 298, 258 nm; NaOAC, 400, 300, 256nm; NaOAC/H\(_3\)BO\(_3\), 393, 261nm; and IR \(\nu_{\text{KBr}}^{\text{max}}\) cm\(^{-1}\) at 3334, 2892, 1677, 1593, 1280, 764. \(^1\)HNMR of the glycoside \(\delta = 0.76\) (m, 3H, C\(_6\)'-CH\(_3\)); \(\delta = 1.98\) (S, 3H, C\(_3\)'-OAc); \(\delta = 2.04\) (S, 3H, C\(_4\)'-OAc); \(\delta = 2.09\) (S, 3H, C\(_2\)'-OAc); \(\delta = 2.35\) (S, 3H, C\(_4\)-OAc); \(\delta = 2.41\) (S, 3H, C\(_3\)'-OAc); \(\delta = 2.44\) (S, 3H, C\(_2\)-OAc); \(\delta = 3.00\) (S, 3H, C\(_2\)'-OAC); \(\delta = 3.02\) (S, 3H, C\(_3\)'-OAC); \(\delta = 3.92\) (S, 3H, C\(_6\)'-OAC); \(\delta = 4.40\) (d, J=7.6 Hz, 1H, C\(_1\)'-anemic proton); \(\delta = 5.46\) (d, J=2, Hz, 1H, C\(_1\)' anemic sugar proton); \(\delta = 6.52\) (d, J=2.4 Hz, C\(_6\)-1H); \(\delta = 6.58\) (d, J=2.0 Hz, C\(_8\)-1H); \(\delta = 6.62\) (S, 1H, C\(_3\)-1HO); \(\delta = 7.03\) (d, J=2.5 Hz, C\(_5\)'-1H); \(\delta = 7.42\) (d, J=2.5 Hz, C\(_2\)'-1H); \(\delta = 7.66\) (dd, J=2.5, 9.0 Hz, C\(_6\)'-1H) and different peaks in mass spectrum at \(M^+ 594\), and m/e 286, 258, 257, 169, 168, 140, 118.

On hydrolysis with 7\% methanolic H\(_2\)SO\(_4\)[1] gave an aglycone[2] mp.330°, molecular formula \(C_{15}H_{10}O_{6}\), \(M^+ 286\), (found C=62.94\%, H=3.4\%, calculated, C=62.93\%, H=3.4\%) which crystallised from methanol into yellow crystals.
Its acetyl derivative had mp. 138 °C its UV spectrum showed characteristic peaks at \( \lambda_{\text{max}} \) 347, 293, 265, 235 nm; \( \lambda_{\text{max}} \) 403, 328, 267 nm; \( \text{AlCl}_3 \), 428, 327, 297, 290 nm; \( \text{AlCl}_3/\text{HCl} \), 386, 351, 296, 273, 268 nm; \( \text{NaOAc} \), 386, 325, 265nm; \( \text{NaOAc/H}_3\text{BO}_3 \), 435, 373, 259 nm; and IR \( \nu_{\text{KBr}} \) cm\(^{-1}\) 3346, 2882, 1678, 1600, 1294, 762.

\( ^1\text{HNMR} \) of the tetra acetyl derivative of aglycone \( \delta = 2.35 \) (S, 3H, C\(_4\)OAC); \( \delta = 2.41 \) (S, 3H, C\(_3\)OAC); \( \delta = 2.44 \) (S, 3H, C\(_7\)OAC); \( \delta = 2.46 \) (S, 3H, C\(_5\)OAC); \( \delta = 6.52 \) (d, J = 2.4 Hz, C\(_6\)-1H); \( \delta = 6.58 \) (d, J = 2.0 Hz, C\(_8\)-1H); \( \delta = 6.62 \) (S, 1H, C\(_3\)-1H); \( \delta = 7.03 \) (d, J = 2.5 Hz, C\(_5\)-1H); \( \delta = 7.42 \) (d, J = 2.5 Hz, C\(_2\)-1H); \( \delta = 7.66 \) (dd, J = 2.5, 9.0 Hz, C\(_6\)-1H) and different peak in mass spectrum at M\(^+\) 286 and m/e 258, 257, 153, 152, 134, 132, 124.

A peak at 3346 cm\(^{-1}\) in the I.R. spectrum of the[2] showed the presence of -OH groups in it. The number of OH groups were estimated by the acetylation with AC\(_2\)O/Pyridine yielded tetra acetyl derivative, thereby confirming the presence of four hydroxy groups in it.

Formation of 4:5 dihydroxybenzoic acid (proteocatecheuic acid) on alkaline degradation of the[2] confirmed the presence of OH groups at C-3' and C-4' in the[2]. The presence of hydroxyl group at C-4' was further confirmed by positive shinoda test.\(^4\)
A bathochromic shift of 24 nm of band I in presence of NaOAC/H$_3$BO$_3$ relative to band I in MeOH confirmed the presence of -OH groups at C-3' and C-4'.

Formation of Phloroglucinol on alkaline degradation of the [2] showed the presence of two-OH groups at C-5 and C-7 respectively.

A bathochromic shift of 55 nm in band I with AlCl$_3$ (relative to MeOH) and 18 nm in band II with NaOAC (relative to MeOH) further confirmed the presence of -OH groups at C$_5$ and C$_7$ respectively.$^{5,6}$

Formation of above degradation products established the identity of the[2] as 5,7,3',4'-tetrahydroxy flavone.

The aqueous hydrolysate was found to be a mixture of two sugars D-glucose and L-rhamnose. (CO-Pc and CO-TLC). The sodium metaperiodate Oxidation$^7$ of the glycoside consumed 3.02 molecule of periodate and liberated 1.14 molecule of formic acid indicating the presence of disaccharide and also that both the sugars were in the pyranose$^{8}$ form.

[1] on permethylation$^9$ followed by hydrolysis yielded 2,3,4,6-α-L-rhamnose and 2,3,4-tri-O-methyl rhamnose (by CO-Pc and CO-TLC) indicating that the linkage between the sugars was through (1→4).
Enzymatic hydrolysis$^{10}$ of [1] indicated $\beta$-linkage between the sugars and the aglycone.

Thus [1] was identified as Luteolin-5-O-$\alpha$-L-rhamnopyranosyl (1$\rightarrow$4)-O-\(\beta\)-D-glucopyranoside.

REFERENCES

Dear Dr. R. Yadav,

We are pleased to inform you that the research paper entitled, "Coscoptin-7-(β-D-glucopyranoside), A novel flavonoid glycoside from Ipomoea scambrida D.C." (Index No. 464/51) by Basant Jain and Basanti Jain has been accepted for publication in Asian Journal of Chemistry. The paper has been included for Volume 4 (1992) of the journal.

Yours Sincerely,

[Signature]

Dr. R. K. AGARWAL

Note: Subscription must be sent either by M. O. or by D. D. drawn on any bank at Delhi or Ghaziabad in favour of ASIAN JOURNAL OF CHEMISTRY, SAHIBABAD.
Gossypetin-7-C-β-D-glucopyranoside, a novel flavonoid glycoside from Impatiens scabra L. C.

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ABSTRACT

Phytochemical examination of Impatiens scabra resulted in the isolation and identification of a novel flavonoid glycoside, Gossypetin-7-0-β-D-glucopyranoside.

INTRODUCTION

Impatiens scabra L. C. (N. C. Balsaminaceae) is distributed throughout India. It is an annual herb with sessile and narrow leaves. The Ayurvedic system of medicine describes the oil of the plant to be used as a semidrying oil. The present paper deals with the isolation and structural elucidation of a novel flavonoid glycoside.

EXPERIMENTAL

The methanolic extract of the water soluble part of the 95% concentrated ethanolic extract of dried and powdered plant when worked up by C.C. gave crude glycoside (0.084 %). Methanolic extract on TLC examination showed two spots which was separated by column chromatography on Si-gel C. One of the compd. eluted with ethylacetate: methanol:water gave a brown amorphous compound m.p. 208°C, molecular formula C_{21}H_{20}O_{13}, m/z 480 (M^+). It gave positive Molisch test and responded to all the colour tests of flavonoidal glycosides [1], the study of which is in progress.
On acid hydrolysis with 7% ethanolic H₂SO₄ gave an aglycone and the sugar moiety, which was identified as D-glucose (by Co-Fc and Co-TLC).

The aglycone C₁₅H₁₀O₈, m.p. 312°, m/e = 290, 208, 169, 168, 140, 143, 132, Crystallised in yellow needle, UV \( \lambda_{max} \text{NaOH} \) 256, 270, 375 nm, \( \lambda_{max} \text{AlCl₃} \) 257, 272, 446 nm, \( \lambda_{max} \text{NaOAc+H₃BO₃} \) 266, 395 nm, IR \( \nu_{max} \text{KBr} \) 3388, 2916, 1680, 1600, 1280, 1114, 810 cm⁻¹, ¹H NMR spectrum of hexa-acetyl derivative of the aglycone showed signals at \( \delta = 6.48 (d, J=2, C₆-1H) \),

\[ \delta = 7.53 (d, J = 2.5, C₂, -1H), \delta = 6.60 (d, J = 8.5, C₅, -1H), \delta = 7.63 (d, d, J = 2.5, 9, C₆, -1H), \delta = 2.34 (s, 3H, C₃, -OAc), \delta = 2.36 (s, 3H, C₄, -OAc), \delta = 2.48 (s, 3H, C₃-OAc), \delta = 2.45 (s, 3H, C₅-OAc), \delta = 2.41 (s, 3H, C₇-OAc), \delta = 2.39 (s, 3H, C₈-OAc). \]

A peak at 3388 cm⁻¹ in the IR spectrum of the [2] showed the presence of OH groups in it. The number of OH groups were estimated by the acetylation with AC₂O/pyridine yielded hexa-acetyl derivative, thereby confirming the presence of six hydroxyl groups in it.

Formation of 4:5 dihydroxy-benzoic acid (protocatechuic acid) on alkaline degradation of the [2] confirmed the presence of OH groups at C-3' and C-4' in the [2].
A bathochromic shift of 20 nm in the bond I and hypsochromic shift of 34 nm upon addition of AlCl₃ and H₃BC₃ in the UV spectrum of [2] further confirmed the presence of OH groups at C₃ and C-4', respectively⁴.

Formation of chloroglucinol on alkaline degradation of the [2] showed the presence of two -OH groups at C-5 and C-7 respectively.

Spectral shift⁵ with AlCl₃ (bathochromic shift 26 nm) and NAOAC (bathochromic shift 20 nm) indicated the presence of -OH groups at C-5 and C-7 respectively.

Yellow fluorescence of the [2] in UV light⁶ and the spectral shift with AlCl₃ in presence of HCl relative to band (I) in MeOH indicating a free 3'-OH group in the aglycone.

A characteristic colour reaction with Zn/Hg and Zircenium oxychloride in citric acid⁷ further suggested the presence of -OH group at C-3.

The [2] gave a red complex with Zircenium nitrate solution soluble in HCl showing the presence of a hydroxyl group at position C₆, which was further confirmed by a signal at δ = 2.39 in the 1H NMR spectrum of acetylated aglycone.


The aqueous hydrolysate on chromatographic study revealed the presence of only D-glucose (by GC-PC and GC-TC). The sodium metaperiodate oxidation⁸ of the [1] consumed 0.01 mole of periodate.
and liberated 1.07 mole of formic acid indicating the presence of a monosaccharide and also that it was in the pyranose form.

[1] on permethylation\textsuperscript{10} followed by hydrolysis yielded 2:3:4:6-tetra-O-methyl-L-glucose (by CO-PC and CO-TLC) confirmed that the sugar was attached via its C\textsubscript{1}-OH to the C\textsubscript{7}-OH of the aglycone.

[1] on hydrolysis with emulsion\textsuperscript{11} indicated \(\beta\)-linkage between the sugar and the aglycone thereby confirming that the glycoside was Cossyrtarin-7-C-\(\beta\)-D-glucopyranoside which was further supported by its spectral analysis. UV\(\lambda_{\text{max}}^{\text{H}_{2}\text{O}}\): 244, 272, 363 nm, \(\lambda_{\text{max}}^{\text{AcCl}}\): 295, 364 nm, \(\lambda_{\text{max}}^{\text{H}_{2}\text{O}}\): 224, 270, 362 nm, IR\(\nu_{\text{max}}^{\text{IR}}\): 3400, 2910, 1675, 1598, 1112, 1275, 815 cm\textsuperscript{-1}, \(^1\text{H}{^1}\text{HNR}\) \(\delta = 6.46\) (\(\delta, \text{J}=2, \text{C}_{6}-1\text{H}\)).

\(\delta = 7.54\) (d, \(\text{J}=2.5\), C\textsubscript{2}-1H), \(\delta = 6.86\) (d, \(\text{J}=8.5\), C\textsubscript{5}-1H), \(\delta = 7.61\) (d, \(\text{J}=2.5\), 9, C\textsubscript{6}-1H), \(\delta = 2.31\) (s, 3H, C\textsubscript{3}-6AC), \(\delta = 2.35\) (S, 3H, C\textsubscript{4}-6AC), \(\delta = 2.47\) (S, 3H, C\textsubscript{3}-CAC), \(\delta = 2.44\) (S, 3H, C\textsubscript{5}-CAC), \(\delta = 2.40\) (S, 3H, C\textsubscript{6}-6AC), \(\delta = 5.7\) (d, \(\text{J}=7\), C\textsubscript{1}-anomeric proton), \(\delta = 5.46\) (m, 6-protons of sugar residue), \(\delta = 2.06\) (S, 3H, C\textsubscript{2\text{\textsuperscript{n}}}-CAC), \(\delta = 2.10\) (S, 3H, C\textsubscript{3\text{\textsuperscript{n}}}-CAC), \(\delta = 2.02\) (S, 3H, C\textsubscript{4\text{\textsuperscript{n}}}-CAC), \(\delta = 3.92\) (S, 3H, C\textsubscript{6\text{\textsuperscript{n}}}-CAC), M\textsuperscript{+} 480, m/e=318, 290, 288, 169, 168, 140, 134, 132.

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