CHAPTER V

FICUS INFECTORIA
(MORACEAE)
DISCUSSION
The family Moraceae consists of 71 genera and over 1,000 species, of evergreen tree or shrubs chiefly distributed in the tropical and sub-tropical areas. The genus Ficus, a large genus consists of about 800 species of trees, shrubs and often climbers with milky juice. About 65 species occur in India. The genus is remarkable for the large varieties in the habits of its species. The wood of Ficus infectoria is made in to charcoal and the young shoots are eaten as curries. The bark yields the fibres, the branches and leaves are mostly used as fodder for animals. The decoction of bark is used as a wash for ulcer and injection in leucorrhoea.

A survey of literature showed that no work on flavonoid seem to be done on this plant and therefore the present chemical investigation has been undertaken. The present discussion is devoted to the isolation and characterization of a new flavone glycoside, Luteolin-6-O-β-D-glucopyranoside-3′-O-α-rhamnopyranoside (IV), along with known flavonoidic compounds Apigenin (I), Leuteolin (II), Sorbifolin-6-O-glucoside (III).

Leaves of Ficus infectoria were procured from the Botany Department, Aligarh Muslim University, India. Coarsely powdered leaves were exhaustively extracted with methanol. The methanolic
extracts were concentrated under diminished pressure to a dark viscous mass. The dark viscous mass was then extracted successively with petroleum ether and benzene till the solvent in each case was almost colourless. The concentrated extract was then treated with hot water. The water soluble part was extracted against n-Hexane, Chloroform, Ethyl acetate and n-BuOH. The chloroform, ethyl acetate and n-BuOH fractions, found to contain some similar spots were combined together and subjected to column chromatography over silica gel using petrol, benzene, chloroform, benzene-ethyl acetate, ethyl acetate, ethyl acetate-acetone and acetone as eluting solvents followed by fractional crystallization afforded four crystalline TLC homogeneous substances. They were given the labels as FI-1, FI-2, FI-3 and FI-4.

FI-1

The fraction FI-1 was eluted from the column with benzene-ethyl acetate (9.5:0.5, 9:1) mixture. It was crystallized as pale yellow prism, m.p. 352-53°C. It was characterized as 5,7,4'-trihydroxyflavone by ¹H-NMR studies of its acetate (FI-1A) m.p. 184-85°C. The results of ¹H-NMR studies are given in Table-1.
TABLE - 1

Chemical Shifts of Protons of FI-1A

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.86 (d, J=9Hz)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.25 (d, J=9Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.20 (d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.80 (d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.58 (s)</td>
</tr>
<tr>
<td>OAc-5</td>
<td>3</td>
<td>2.42 (s)</td>
</tr>
<tr>
<td>OAc-4',7</td>
<td>6</td>
<td>2.32 (s)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, spectrum run in CDC13 at 100 MHz, TMS as internal standard (δ-scale).

FI-1 was, thus, assigned the structure 5,7,4'-trihydroxy-flavone (I).
FI-2

It was eluted from benzene-ethyl acetate (3:2, 1:1) mixture as yellow solid. It was crystallized in deep yellow prism from ethyl acetate-acetone, m.p. >320°C. Elemental analysis agreed to the molecular formula C₁₅H₁₀O₆. It responded to Shinoda test² and gave greenish brown colour with FeCl₃. The UV spectrum showed λ<sub>max</sub> at 256, 265 and 345 nm. Furthermore, analysis of functional groups revealed the presence of phenolic OH (3400 cm⁻¹), α,β-unsaturated ketonic C=O (1640 cm⁻¹) and aromatic nucleus (800 and 840 cm⁻¹). The changes in the presence of diagnostic shift reagents³ in its UV spectrum pointed out the presence of a free hydroxyl group at 5 and 7 positions and 3',4'-dihydroxyl group in FI-2.

Acetylation of FI-2 (Fig. 1, Table-2) gave a tetraacetate (FI-2A), m.p. ≈ 200°C. The <sup>1</sup>H-NMR spectrum of FI-2A evidenced the presence of four aromatic acetoxyls integrating for 12 protons at δ 2.43, δ 2.35 and δ 2.33 assigned to OAc-5, OAc-7 and OAc-3',4'. The <sup>1</sup>H-NMR also established a disubstituted B-ring as it showed a typical two proton multiplet at δ 7.76 (J₁ = 8Hz, J₂ = 2.0Hz) and δ 7.70 (J₁ = 8.0Hz, H-2'). Another ortho coupled doublet integrating for one proton was observed at δ 7.38 (J = 8.0Hz, H-5'). This could be attributed to 3',4' substitution of the B-ring. The 5,7 disubstitution of the A-ring is demonstrated by two metacoupled doublets at δ 6.85 and δ 7.35
(J = 2.0Hz, each) assigned to C-6 and C-8 proton which have shifted slightly down field due to derivatization. A sharp singlet at δ 6.61 is assigned to C-3 proton of γ-pyrone system.

**TABLE - 2**

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of Protons</th>
<th>Signal</th>
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</thead>
<tbody>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.85 (d, J=2.0Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.35 (d, J=2.0Hz)</td>
</tr>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.61 (s)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>7.38 (d, J=8.0Hz)</td>
</tr>
<tr>
<td>H-6'</td>
<td>1</td>
<td>7.76 (dd, J₁=8.0Hz, J₂=2.20Hz)</td>
</tr>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.70 (d, J=2.20Hz)</td>
</tr>
<tr>
<td>OAc-5,7</td>
<td>6</td>
<td>2.43 (s), 2.35 (s)</td>
</tr>
<tr>
<td>OAc-3',4'</td>
<td>6</td>
<td>2.33 (s)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, spectrum run in CDCl₃ at 270 MHz, TMS as internal standard (δ-scale).

The mass spectrum of FI-2A (Fig. 2), is fully in agreement with the structure (II). A molecular ion, although weak is observed at m/z 454 in accordance with a flavone containing four acetoxyl groups. The subsequent removal of four acetoxyls gave fragments at m/z 412, 370, 328 and 286. The fragment at m/z 286 is observed as the base peak as it corresponds to the structure (II). A[B₁]⁺ fragment at m/z 134 fully supported a B-ring with
**Fig. 2**

**MSS SPECTRUM**

**SAMPLE**: (A-1)

**TFF**: (X: 1, 13, (1), ...

(1) 2\(^{14}B\)/28 1000 28; TX 31; BP 43-342.8

PEAKS 125(0), RANGE 40 TO 600(40 TO 500), LEVEL 0(0)

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**Diagram Description**

- **Top Diagram**: Intensity (INT) vs. Mass to Charge Ratio (M/Z)
  - Peaks at 43 and 286
  - Intensity scale: 0 to 1000
  - Mass scale: 0 to 250

- **Bottom Diagram**: Similar to the top diagram, with different intensity and mass ranges

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*M. Hayashi*
two hydroxyl groups. An \([A_1 + H]^+\) fragment at \(m/z\) 153 is consistent with the A-ring having two hydroxyl groups.

On the basis of these results FI-2 is characterized as 5,7,3',4'-tetrahydroxyflavone (Luteolin) (II).

\[
\text{HO} \
\text{O} \
\text{OH} \
\text{HO} \
\text{OH}
\]

(II)

**FI-3**

FI-3 was eluted from column with benzene-ethyl acetate (2:8, 1:9) mixture. The glycosidic nature of the product (FI-3) was evidenced by the positive Molisch test obtained after hydrolysis and the formation of an osazone. The glycosidic nature was further supported by the \(^1\text{H}-\text{NMR}\) spectrum of the acetate of FI-3A (Table-3, Fig. 3) as it showed two aromatic acetoxyls at \(\delta 2.46\) (3H) and \(\delta 2.27\) (3H) and four alcoholic acetoxyls at \(\delta 1.99\) (9H, s, 3-OAc), \(\delta 1.73\) (3H, s, 9-OAc) indicating it to be a glucoside or galactoside.
The glycoside gave pink colour with Zn/HCl and red colour on treatment with sodium amalgam followed by acidification indicating its flavone or flavanone nature. A yellow colour with Wilson boric acid reagent and maxima at 269 and 333 nm in the UV spectrum indicated it to be flavone glycoside. It gave a brownish green colour with FeCl₃ indicating the presence of hydroxyl group at C-5. The IR spectrum displayed strong bands at 3400 cm⁻¹ (OH) and 1700 cm⁻¹ (C=O). A bathochromic shift of 15 nm with AlCl₃ further confirmed the presence of a free 5-OH group. No shift with fused NaOAc ruled out the possibility of a free hydroxyl at 7-position.

The NMR spectrum of FI-3A (Fig. 3), m.p. >110°C, showed a sharp singlet at δ 6.48 indicating the presence of a C-3 proton of γ-pyrone nucleus. The presence of one methoxy group is indicated through a singlet at δ 3.99. The remaining singlet in the spectrum is at δ 6.78 and it integrates for one hydrogen and can be assigned to an aromatic proton shielded by two ortho and one para oxygen and was found to arise from the C-8 proton of 5,6,7-trioxogenated flavone. The aromatic region also contain multiplets of four other protons. Since the multiplets, doublet at δ 7.78 (J = 9Hz) and at δ 7.15 (J = 9Hz), correspond to an A₂B₂ pattern, were assigned to 2',6' and 3',5'-protons of B-ring respectively.
TABLE - 3

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.48 (s)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.78 (s)</td>
</tr>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>7.78 (d, J=9Hz)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.15 (d, J=9Hz)</td>
</tr>
<tr>
<td>4 Aliphatic OAc of</td>
<td>12</td>
<td>1.73, 1.99 (s)</td>
</tr>
<tr>
<td>glucose moiety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic OAc</td>
<td>6</td>
<td>2.27, 2.46 (s)</td>
</tr>
<tr>
<td>Aromatic OCH₃</td>
<td>3</td>
<td>3.99 (s)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, spectrum run in CDCl₃, TMS as internal standard (δ-scale).

FI-3 on hydrolysis with 10%HCl gave an aglycone, m.p. 290-92°C (IIIa). The sugar was identified as glucose by Rᶠ-values, Co-chromatography with an authentic sample and by the formation of osazene.

Demethylation of the aglycone with hydroiodic acid gave a yellow product, m.p. >350°C, elemental analysis corresponding to the molecular formula C₁₅H₁₀O₆. Acetylation of the compound with acetic anhydride and pyridine yielded a tetraacetate that melted at 238-39°C and showed no depression in melting point on mixing with an authentic sample of scutellarein tetraacetate. The aglycone (IIIa) was thus characterized as sorbifolin by ferric reaction. Rᶠ values, spectral and chromatographic
comparison with an authentic sample.

On the basis of the above colour reactions and examination of the products of hydrolysis, the glycoside was identified as flavone glucoside having sorbifolin as an aglycone.
The position of the sugar residue in the glycoside was confirmed by hydrolysis of the methylated glycoside. The partial methyl ether thus obtained was characterized as 6-hydroxy 4',5,7-trimethoxyflavone (IIlb) (m.p. 221°C) by m.p., m.m.p. with an authentic sample and ultraviolet spectral analysis with customary shift reagents.

The quantitative estimation of sugar by Somogyis Copper micro method showed the presence of one mole of glucose per mole of aglycone.

FI-3 was therefore characterized as sorbifolin 6-glucoside (III).

(III)
It was eluted from silica gel column with EtOAc–Me$_2$CO (9.5:0.5) mixture as yellow solid which was found to be contaminated with some impurities. It was purified by preparative TLC using EtOAc–Me$_2$CO–HOAc–H$_2$O, 20:3:1:1 as eluting solvents. Elemental analysis agreed to the molecular formula C$_{27}$H$_{30}$O$_{16}$ m.p. $>300^\circ$C. It was recognized to be a flavone glycoside by its characteristic colour reactions of flavonoids and responded positively to Molish test and reduce Tollen's reagent indicating it to be a flavone glycoside. Its IR spectrum displayed strong absorption bands at 3400 (OH), 1655 ($\alpha,\beta$-unsaturated C=O), 2945 (C–H) and a broad band at 1090 and 1170 cm$^{-1}$ suggesting its O-glycosidic nature. The ultraviolet spectra and the diagnostic shifts were characteristic of the presence of free hydroxyl groups at 4',5,7-positions. Furthermore, the complete acid stability of the AlCl$_3$ complex together with negative borate reaction ruled out the presence of O-dihydroxy group either in A or B-ring.

Total acid hydrolysis of the glycoside gave an aglycone (IVa) and equimolar quantities of glucose and rhamnose. The sugars were identified by paper chromatography and GLC of alditol acetate. The aglycone (IVa) gave positive borate test and form acid labile AlCl$_3$ complex (absent in glycoside) thus showing, that the sugar is linked to the 6 and 3' positions of the aglycone (or the presence of O-dihydroxy system in aglycone
respectively). On methylation aglycone form pentamethyl ether, m.p. 175-76°C which was found to be identical with an authentic sample of 5,6,7,3',4'-pentamethylflavone (IVb). The aglycone was, thus, characterized as 6-hydroxyluteolin (IVA), m.p. 283-84°C corresponds to molecular formula C_{15}H_{10}O_{7} by spectral and chromatographic comparison with authentic sample.

Acetylation of the glycoside FI-4 with Ac_{2}O/Py gave a decaacetate derivative (FI-4A) (Fig. 4, Table-4), m.p. 99-101°C. ¹H-NMR spectrum of the acetate in CDCl₃ indicated it to be a diglycoside [it showed multiplets at δ 1.70-2.45 integrated for 30 protons due to ten acetoxyls (3 aromatic and 7 aliphatic)]. The ¹H-NMR spectrum also exhibited two singlets at δ 6.84 and δ 7.31, characteristic of C-3 and C-8 proton respectively. The C-8 proton signal has shifted little downfield due to derivatization and slightly merged with the B-ring multiplet. The 3',4'-disubstitution on the B-ring was indicated by a double doublet at δ 7.97 (J = 2.0Hz, J = 9Hz), a doublet at δ 7.93 (J = 2.0Hz) and another doublet at δ 7.34 (J = 9Hz) for C-6',2' and 5' protons respectively. The anomeric protons at δ 5.60 (J = 9Hz) and δ 4.25 (J = 1.2Hz) were assigned to l''-H glucose (β-configuration) and l'''-H rhamnose (α-configuration) respectively. The rhamnosyl methyl appeared as a characteristic doublet at δ 1.20 (J = 6.2Hz). The remaining sugar protons were observed in the range δ 3.58 - 5.60 as expected.
TABLE - 4

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-3</td>
<td>1</td>
<td>6.84 (s)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.31 (s)</td>
</tr>
<tr>
<td>H-6'</td>
<td>1</td>
<td>7.97 (dd, J_1=2.0Hz, J_2=9.0Hz)</td>
</tr>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.93 (d, J=2.0Hz)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>7.34 (d, J=9.0Hz)</td>
</tr>
<tr>
<td>3 Aromatic OAc</td>
<td>9</td>
<td>2.45-2.32 (9H, m)</td>
</tr>
<tr>
<td>7 Aliphatic OAc</td>
<td>21</td>
<td>1.70-2.12 (21H, m)</td>
</tr>
<tr>
<td>H-1'' (glucosyl)</td>
<td>1</td>
<td>5.60 (d, J=9.0Hz)</td>
</tr>
<tr>
<td>H-1'''' (rhamnosyl)</td>
<td>1</td>
<td>4.25 (d, J=1.2Hz)</td>
</tr>
<tr>
<td>Sugar protons</td>
<td>12</td>
<td>3.58-5.60 (12H, m)</td>
</tr>
<tr>
<td>rhamnosyl-CH₃</td>
<td>3</td>
<td>1.20 (d, J=6.2Hz)</td>
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</table>

s = singlet, d = doublet, spectrum run in CDCl₃ at 270 MHz, TMS as internal standard (δ-scale).

The mass spectrum of FI-4A was in full agreement with the assigned structure. The molecular ion peak has not been observed as expected. The presence of acetylated hexopyranoside and deoxyhexopyranoside was evidenced by the presence of fragment ions at m/z 331 and m/z 273 respectively. The fragment ion observed at m/z 428 accounted for the loss of both acetylated sugars from the molecular ion. The aglycone fragment was observed at m/z 302. A retro-Diels-Alder fragmentation pattern
Fig 5

MASS SPECTRUM
SAMPLE: FIG-IA
FILE: FIX -7,13, (3), ...
(2)2,42%/27 1000 27;TX 31,8P 43-991.3
PEAKS 268(0), RANGE 40 TO 500(40 TO 500), LEVEL 0(0)

M. Havashi
was observed at m/z 169, m/z 134, m/z 137, leading to fragments \([A_1+H]^+\), \([B_1]^+\) and \([B_2]^+\). The results supported the presence of three hydroxyl groups in ring-A and two hydroxyls in ring-B.

Enzymatic hydrolysis of the glycoside (IV) gave conclusive evidences of the position of attachment of two sugars and the nature of their linkages. Hydrolysis of the parent glycoside with \(\alpha\)-pectinase (which contain \(\alpha\)-rhamnosidase) gave L-rhamnose and a partial glycoside (IVc), which gave a bathochromic shift of 22 nm and 20 nm in band I with \(\text{AlCl}_3/\text{HCl}\) and \(\text{NaOAc/\text{H}_3\text{BO}_3}\) respectively suggesting that C-3' hydroxyl which was glycosylated in glycoside had become free. The partial glycoside (IVc) was identified as Luteolin 6-glucopyranoside, m.p. 258-59°C, \((\alpha)_D^{215}=-215^\circ\)C by UV diagnostic shift reagents and co-chromatography with authentic sample. Methylation of partial glycoside followed by hydrolysis with 2N HCl gave partial methyl ether, m.p. 220-222°C characterized as 6-\(\text{OH}\), 5,7,3',4'-tetramethylflavone(6-hydroxyluteolin 5,7,3',4'-tetramethylflavone) (IVd) by spectral and chromatographic comparison with authentic sample. The methylated sugar was identified as 2,3,4,6-tetra-O-methyl-\(\beta\)-D-glucose by \(\text{SiO}_2\) TLC according to Petek. Quantitative estimation of sugar showed 2 moles of sugar/mole of aglycone.
On the basis of these findings, this novel glycoside has been identified as Luteolin-6-O-β-D-glucopyranoside-3′-O-α-L-rhamnopyranoside (IV). To the best of our knowledge this constitutes the first report of any glycoside from the 6-OH luteolin.
(IVa) $R = H$

(IVb) $R = \text{CH}_3$

(IVc)

(IVd)
EXPERIMENTAL
Extraction of the leaves of *Ficus infectoria* (Moraceae)

Dried and powdered leaves (5 kg), procured from the University campus, AMU, Aligarh, were extracted four times with petroleum ether (40-60°C) to remove chlorophyll and waxy materials. The petrol exhausted leaves were refluxed with methanol till the solvent in each case was almost colourless. The combined methanol extracts were concentrated in vacuo until only water remained. The concentrated extract was partitioned against n-Hexane, Chloroform, Ethyl acetate and n-Butanol. The chloroform, ethyl acetate and n-butanol fractions were found to contain some similar spots on TLC over silica gel using following solvent systems:

1. Benzene - Pyridine - Formic acid (36:9:5)
2. Toluene - Ethyl formate - Formic acid (5:4:1)
4. Ethyl acetate - Ethyl methyl ketone - Acetic acid - Water (20:3:0.7:0.7)
5. Ethyl acetate - Acetone - Acetic acid - Water (20:3:1:1)

These fractions were, therefore, combined together and subjected to column chromatography over silica gel using n-hexane, petrol, petrol-benzene, benzene, benzene-ethyl acetate, ethyl acetate, ethyl acetate-acetone, acetone and finally methanol as eluting solvents. The benzene-ethyl acetate eluate
on recovery of the solvent gave a pale yellow solid (FI-1) which on crystallization gave yellow needles, m.p. 352°C.

Anal. Calcd. for $\text{C}_{15}\text{H}_{10}\text{O}_{5}$: C, 66.66; H, 3.70

Found: C, 66.72; H, 3.72%

UV data: $\lambda_{\text{max}}$ nm

<table>
<thead>
<tr>
<th></th>
<th>256, 333</th>
<th>267, 339, 384</th>
<th>267, 340, 385</th>
<th>267, 372</th>
<th>265, 370</th>
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<td>AlCl$_3$/HCl</td>
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Acetylation

FI-1 (40 mg), acetic anhydride (1 ml) and dry pyridine (0.5 ml) were heated on a water bath for 2 hours, worked up as usual and crystallized from CHCl$_3$-Methanol (20 mg) as colourless needles, m.p. 184-85°C.

$^1$H-NMR (CDCl$_3$): Values on $\delta$-scale

7.86 (2H, d, J=9Hz, H-2',6'), 7.25 (2H, d, J=9Hz, H-3',5'), 7.20 (1H, d, J=2.5Hz, H-8), 6.80 (1H, d, J=2.5Hz, H-6), 6.58 (1H, s, H-3), 2.42 (3H, s, OAc-5), 2.32 (6H, s, OAc-4',7).
FI-2

FI-2 was isolated from benzene - ethyl acetate (3:2, 1:1) fractions. The solvent was removed by distillation under reduced pressure on a water bath. The residue on crystallization from ethyl - acetone gave deep yellow prisms, m.p. >320°C.

Anal. Calcd. for C_{15}H_{10}O_{6}: C, 62.93; H, 3.49

Found: C, 62.95; H, 3.51%

UV data: \lambda_{max} \text{ nm}

<table>
<thead>
<tr>
<th>Solution</th>
<th>269, 290sh, 345</th>
<th>296, 329sh, 396</th>
<th>290, 326sh, 376</th>
<th>278, 291sh, 360, 430sh</th>
<th>276, 304sh, 328, 426</th>
<th>266sh, 294sh, 355, 385</th>
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<td>NaOMe</td>
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<td>NaOAc</td>
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<td>NaOAc/H_{3}BO_{3}</td>
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</table>

Acetylation of FI-2

It was acetylated with acetic anhydride (1 ml) and pyridine (0.5 ml) by usual method and crystallized from chloroform - methanol as colourless needles, m.p. 200°C.

\textsuperscript{1}H-NMR (CDCl_{3}): Values on ð-scale

6.85 (1H, d, J=2.0Hz, H-6), 7.35 (1H, d, J=2.0Hz, H-8),
6.61 (1H, s, H-3), 7.76 (1H, dd, H-6', J_1=8.0Hz, J_2=2.20Hz),
7.38 (1H, d, J=8.0Hz, H-5'), 7.70 (1H, d, J=2.20Hz, H-2'),
2.43 (3H, s, OAc-5), 2.35 (3H, s, OAc-7), 2.33 (6H, s, OAc-3',4').

MS data : m/z

454 [M]^+^+, 412[[M]^+^-(2x42)]^+^+, 370 [(M)^+^-(2x42)]^+^+, 328 [(M)^+^-(3x42)]^+^+, 286 [(M)^+^-(4x42)]^+^+, 153 [A_1 + H]^+^+, 134 [B_1]^+^+.

**FI-3**

FI-3 was crystallized from ethyl acetate - methanol as yellow needles, m.p. >310°C (d).

Anal. Calcd. for C_{22}H_{22}O_{11} : C, 57.14; H, 4.76

Found : C, 57.20; H, 4.80%

UV data : λ_{max} nm

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>MeOH</td>
<td>269, 333</td>
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<td>AlCl_3</td>
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<td>271, 334</td>
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<td>NaOMe</td>
<td>370, 388</td>
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</table>

**Acetylation of FI-3**

A crystalline glycoside (35 mg) was heated with acetic
anhydride (3 ml) and dry pyridine (1.5 ml) at 100°C for 3 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The separated solid was filtered, washed well with water and dried. On crystallization from dilute ethanol it gave colourless needles (25 mg), m.p. 110-11°C.

\[ ^1H-NMR \text{ (CDCl}_3\text{): Values of } \delta\text{-scale} \]

\[ 6.48 \text{ (1H, s, H-3)}, \quad 6.78 \text{ (1H, s, H-8)}, \quad 7.15 \text{ (2H, d, J=9Hz, H-3',5')}, \quad 7.78 \text{ (2H, d, J=9Hz, H-2',6')}, \quad 3.99 \text{ (3H, s, OCH}_3\text{-7)}, \]
\[ 2.27, 2.46 \text{ (OAc-4',5')}, \quad 1.99 \text{ (9H, s, 3xOAc)}, \quad 1.73 \text{ (3H, s, OAc)}. \]

**Acid hydrolysis of FI-3:**

The glycoside (100 mg) was dissolved in 25 ml of 10% aqueous HCl-MeOH (1:1) and heated on a water bath. The hydrolysis appeared to be completed within 30 minutes. The heating was continued for two hours to ensure complete hydrolysis. After leaving overnight, the yellow aglycone thus separated out was filtered, washed well with water and dried. The crude product on crystallization from methanol gave yellow needles (70 mg) m.p. 290-92°C. It showed no depression on admixture with an authentic sample of sorbifolin.

**Anal. Calcd. for C_{16}H_{12}O_{6}:** C, 64.00; H, 4.00

**Found:** C, 63.96; H, 3.98%
UV data: \( \lambda_{\text{max}} \) nm

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( \lambda_{\text{max}} ) nm</th>
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<tbody>
<tr>
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<tr>
<td>AlCl₃</td>
<td>263, 323</td>
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<tr>
<td>NaOAc</td>
<td>254, 309</td>
</tr>
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</table>

IR: \( \nu_{\text{max}} \) (KBr) cm⁻¹

3260, 1655

Acetylation of Sorbifolin

Sorbifolin (25 mg) was heated under reflux with acetic anhydride (2.5 ml) and fused sodium acetate (100 mg) on a water bath for two hours. After cooling, the mixture was poured over crushed ice and left overnight. The solid was collected, washed with water and dried on crystallization from ethanol it gave colourless needles (18 mg), m.p. 226-28°C.

Anal. Calcd. for \( \text{C}_{22}\text{H}_{16}\text{O}_9 \): C, 61.95; H, 4.26

Found : C, 62.03; H, 4.30%

\(^1\text{H}-\text{NMR} (\text{CDCl}_3)\): Values on δ-scale

7.90 (2H, d, J=9Hz, H-2',6'), 7.28 (2H, d, J=9Hz, H-3',5'), 6.60 (1H, s, H-8), 6.58 (1H, s, H-3), 3.80 (3H, s, OCH₃-7), 2.42 (3H, s, OAc-5), 2.35 (6H, s, OAc-4',6).

Methylation of Sorbifolin

Sorbifolin (20 mg), dimethyl sulphate (0.2 ml), anhydrous
potassium carbonate (0.3 mC) were refluxed for 24 hours. The reaction mixture was filtered and the residue washed several times with hot acetone. On distilling off the solvent, a brown viscous semisolid mass was left behind. It was washed with hot petroleum ether to remove the excess of dimethyl sulphate. The solid residue on crystallization from ethyl acetate - methanol gave colourless needles (10 mg), m.p. 188-89°C.

Anal. Calcd. for C_{19}H_{18}O_{6} : C, 66.66; H, 5.26

Found : C, 66.61; H, 5.23%

Chromatographic identification of sugar

The acidic filterate, left after filtering the aglycone was extracted with ether and then with ethyl acetate to ensure the complete removal of any residue aglycone. The solution was concentrated to a syrup in vacuum over NaOH pellets. The concentration was continued till the syrup was neutral to litmus paper. The syrup was chromatographed on Whatman No. 1 filter paper using butanol - acetic acid - water (4:1:5) and butanol - water - ethanol (60:28:5:16.5) as solvent systems, employing descending techniques. Authentic sugars were used as checks. The chromatograms were run for 24 hours and after drying at room temperature were sprayed with aniline pthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05°C showed the presence of only glucose.
Estimation of sugar

The anhydrous glycoside (20.5 mg) was hydrolysed by refluxing for two hours 2% H₂SO₄. After cooling overnight, the glycone was filtered, washed, dried and weighed (13.2 mg). Thus ratio of aglycone to the glycoside is 64.3% and this ratio indicates the presence of one mole of sugar per mole of aglycone.

The quantitative estimation of sugar by Somogyi's copper micro method gave the value (0.44 ml) which corresponds to 1 mole of sugar/mole of aglycone.

6-Hydroxy-4',5,7-trimethoxyflavone

Glycoside (30 mg) was dissolved in dry acetone and refluxed with an excess of dimethyl sulphate (1.2 ml) and ignited potassium carbonate (3 gms) for 36 hours on a water bath. The mixture was filtered and the residue was washed with hot acetone. After distilling off the solvent from the filtrate a brown residue was left behind. The excess of dimethylsulphate was removed by washing the methylated product several times with hot petroleum ether. It was hydrolysed by heating with 7% H₂SO₄ for two hours. The reaction mixture was left overnight, a faintly yellowish powder separated out. It was filtered washed with water and dried. On several crystallization from methanol it gave straw coloured needles (16 mg), m.p. 221°C.
Anal. Calcd. for $\text{C}_{18}\text{H}_{16}\text{O}_6$ : C, 65.85; H, 4.87

Found : C, 65.81; H, 4.82%

**FI-4**

FI-4 was obtained from EtOAc-Me$_2$CO (9.5 : 0.5) mixture as pale yellow solid which was purified by preparative TLC over silica gel using EtOAc-Me$_2$CO-HOAc-H$_2$O, (20:3:1:1) as eluting solvents. It was crystallized as yellow needles, m.p. >300°C.

Anal. Calcd. For $\text{C}_{27}\text{H}_{30}\text{O}_6$ : C, 53.11; H, 4.91

Found : C, 53.14; H, 4.86%

**UV data :**

$\lambda_{\text{max}}$ nm

<table>
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<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ nm</th>
</tr>
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<tr>
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<td>NaOAc</td>
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<td>AlCl$_3$/HCl</td>
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**IR :**

$\nu_{\text{max}}$ (KBr) cm$^{-1}$

|   | 3400, 2945, 1655, 1510, 1170, 1090, 800. |
Acetylation of the glycoside

The crystalline glycoside (40 mg) was acetylated with acetic anhydride (3 ml) and pyridine (1.1 ml) in the usual manner to afford a decaacetate derivative (25 mg) as a cream coloured needles, m.p. 99-101°C.

\(^1\)H-NMR (CDCl\(_3\)) : Values on \(\delta\)-scale

7.97 (1H, dd, \(J_1=9.0\)Hz, \(J_2=2.0\)Hz, H-6'), 7.93 (1H, d, \(J=2.0\)Hz, H-2'), 7.34 (1H, d, \(J=9\)Hz, H-5'), 7.31 (1H, s, H-8), 6.84 (1H, s, H-3), 2.45-2.32 (9H, m, OAc-5,7,4'), 1.70-2.12 (21H, m, aliphatic acetoxyls), 3.58-5.60 (12H, m, sugar protons), 1.20 (3H, d, \(J=6.2\)Hz, rhamnosyl methyl).

Acid hydrolysis of the glycoside

A solution of the parent glycoside (75 mg) in 2N HCl-MeOH (5 ml) was refluxed on a waterbath at 100°C for 2 hours. The mixture was poured on to ice water and extracted with EtOAc. After evaporation of the solvent, the residue was crystallized from CHCl\(_3\)-MeOH to give an aglycone, as yellow needles (23 mg), m.p. 283-85°C. The aglycone showed no depression in melting point on admixture with 6-hydroxyluteolin.

Anal. Calcd. for \(C_{15}H_{10}O_7\) : C, 59.60; H, 3.31

Found : C, 58.98; H, 3.33%
UV data: \( \lambda_{\text{max}} \) nm

<table>
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<th>Solvent</th>
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<td>NaOAc</td>
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<td>NaOAc/H(_2)BO(_3)</td>
<td>292, 345, 380</td>
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<tr>
<td>AlCl(_3)/HCl</td>
<td>280, 290, 358, 398</td>
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</table>

Identification of sugars

The natural aqueous hydrolysate was examined by paper chromatography employing n-Butanol-HOAc-H\(_2\)O as developing solvents and using authentic sugars as checks. The chromatograms were sprayed with aniline pthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-05\(^\circ\)C showed the presence of rhamnose (\( R_f \) 0.36) and glucose (\( R_f \) 0.18).

Methylation of the Aglycone

The aglycone (20 mg) in dry acetone (25 ml) was refluxed with dimethyl sulphate (0.4 ml) and freshly ignited potassium carbonate (1 gm) for 30 hours. After usual workup the solid obtained was crystallized from ethyl acetate as colourless needles (9 mg), m.p. 175-76\(^\circ\)C.
Anal. Calcd. for C\textsubscript{20}H\textsubscript{20}O\textsubscript{7} : C, 64.5; H, 5.4
Found : C, 64.5; H, 5.6%

**Enzymatic hydrolysis of the glycoside**

A mixture of compound (50 mg) in H\textsubscript{2}O (10 ml) was incubated over night with α-pectinase (which contain α-rhamnosidase) at 18°C. The solvent was evaporated in vacuo and the residue treated with MeOH. The methanol soluble portion was subjected to CC over sephadex L-20 (60% Aq. MeOH, EtOH) to afford a sugar (5 mg) and partial glycoside, m.p. 258-59°C, \((\alpha)_D\)\textsubscript{21.5°C} (Pyridine) - identified as luteolin-6-O-glycoside by direct comparison with authentic sample. The sugar was identified as L-rhamnose by PC (four solvents) and by Osazone formation, L-rhamnosazone, m.p. 184-86°C \((\alpha)_D\)\textsubscript{25} + 47.6 (Me\textsubscript{2}CO).

**UV data : \(\lambda_{\text{max}}\) nm**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(\lambda_{\text{max}}) nm</th>
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<td>AlCl\textsubscript{3}</td>
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<td>AlCl\textsubscript{3}/HCl</td>
<td>269, 303sh, 330</td>
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<tr>
<td>NaOMe</td>
<td>277, 398</td>
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</table>

**IR : \(\nu_{\text{max}}\) cm\textsuperscript{-1}**

3400br, 2910, 1659, 1600, 1355, 1170, 1090, 910, 830.
Methylation of partial glycoside followed by acid hydrolysis

CH$_3$I (1 ml) and Ag$_2$O (30 mg) were added to a solution of partial glycoside as obtained above (30 mg) in DMF (3 ml). The contents were filtered to dryness and the residue was treated with ethanol (20 ml). The alcohol was recovered and the syrupy residue was hydrolysed with 2N HCl (100°C, 2 hr.). On usual workup it gave methylated aglycone (12 mg), m.p. 220-22°C characterized as 6-OH, 5,7,3',4'-tetramethoxyflavone.

Anal. Calcd. for C$_{19}$H$_{18}$O$_7$ : C, 63.40; H, 5.02

Found : C, 63.44; H, 5.05%

The methylated sugar was identified as 2,3,4,6-tetra-O-methyl-D-glucose by TLC (Si-gel, Toluene-MeOH, 4:1) and by m.p., m.m.p and (α)$_D$ values and GLC of alditol acetate. 2,3,4,6-tetra-O-methyl-D-glucose, m.p. 96-97°C, (α)$_{D}^{20}$ 92 → 84.

GLC of alditol acetate of permethylated hydrolysate

The alditol acetate derivative of sugar were prepared as described earlier. Alditol acetates were subjected to GLC analysis, using WCOT column OV-101 at 100°C for 20 minutes then raised to 230°C at 1°C/min. The corresponding alditol acetate of 2,3,4,6-tetra-O-methyl-D-glucose was identified by comparison of their $R_t$-value with those of authentic sugar ($R_t = 0.997$).
Estimation of sugar

The sugar was estimated by the method as described earlier. The ratio of the aglycone to the glycoside was found to be 44.8% indicating the presence of 2 mole of sugar per mole of aglycone.

Somogyis copper micro method gave the value (1.69 CC) which also corresponds to two moles of sugar per mole of aglycone.
REFERENCES
REFERENCES

LIST OF PUBLICATION

1. Two new flavonol glycosides from *Chenopodium ambrosioides*. Phytochemistry (Accepted) (1990). [IN PRESS]


11. Chenopodin, a Rare and Novel flavone glycoside from Chenopodium ambrocioides.

    Phytochemistry (Communicated). [Accepted], 1990

13. The flavonoidic constituents from the leaves of Semecarpus kurzii.
    Fitoterapia (Communicated).

14. Chemical constituents of Cassia biflora Linn.
    Fitoterepia (Communicated).

15. Setariol – a new sterol from Setaria italica.
Setaricin: a new flavone glycoside from Setaria italica

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Setaria italica Beauv has wide distribution in India, China, Japan, South Africa, South and East Europe, and North America. Now, the isolation and characterization of a new flavone glycoside from the leaves of Setaria italica (procured from Salsibar in north Bihar, India) is reported. Commonly known as north Bihar as ‘Kasee’, this plant is widely known for its medicinal value¹ but hitherto has not been examined for its active principles.

The methanolic extract of the air-dried leaves of Setaria italica, after preliminary purification, was separated by column chromatography over silica-gel. The chloroform-methanol (9:1) eluate was subjected to repeated column chromatography followed by preparative TLC (C_{18}H_{7}MeOH-AcOH, 4:1:4:9) to give the new glycoside (Setaricin I). It was crystallized from methanol as shining yellow needles, mp 300°C (Found C, 57.34; H, 4.86; C_{18}H_{21}O_{11} requires C, 57.14; H, 4.76% per cent).

Setaricin responded to the ferric reaction and Shonuda test.² The glycoside nature of compound I was deduced by its paper chromatographic behaviour,³ solubility in water and positive Molisch test. Further, it was supported by the presence of an anomeric proton at δ 5.53 ppm in the δH NMR spectrum and a broad band in the region 4000-1100 cm⁻¹ (α-glycosylation) in its infrared spectrum. The infrared spectrum (KBr) also has characteristic absorption bands at 3350 (OH), 1655 (C=O) and 1610 cm⁻¹ (C=C, aromatic). The ultraviolet spectrum showed absorption maxima at 240 and 350 nm. Analysis of the ultraviolet spectrum, following standard procedures,⁴ indicates a free hydroxyl at C2 (δmax, 254 nm; 530 cm⁻¹). The ultraviolet spectrum showed absorption maxima at 240 and 350 nm. Analysis of the ultraviolet spectrum, following standard procedures,⁴ indicates a free hydroxyl at C2 (δmax, 254 nm; 530 cm⁻¹). The ultraviolet spectrum showed absorption maxima at 240 and 350 nm.

Acetylation of the glycoside (Ac₂O-Py) afforded a penamone (II), mp 128-30°C. The 1H NMR spectrum of the acetate (Ib) in CDCl₃ showed two phenolic acetyl groups at δ 2.44 (3H) and 2.37 (3H) and three sugar acetyl groups in the range 0.85-2.17 (9H). The two meta-coupled protons at C6 (δ 8.60) and C7 (δ 8.70) (J 2 Hz each), while a C2 (δ 8.60) proton resonated as a singlet at δ 8.57. The 1H and 6H protons appeared as a singlet at δ 7.05 for a symmetrically substituted monosaccharide type B-ring. The anomeric proton (H-1) of arabinose appeared as a doublet at δ 8.53 (J = 8.8 Hz). The chemical shift confirmed the direct attachment of sugar to the aglycone and the diaxial coupling (J = 8 Hz) between H-1 and H-6 suggested the 3β configuration of the D-arabinofuranosyl moiety. The presence of peaks at m/z 259, 199, 159, 157 and 130 in the mass spectrum of the acetate (Ib) finally established the sugar moiety as a pentopyranose. The mass spectrum of the glycoside (Ia) fully supported the assigned structure of the aglycone as exhibited at m/z 330 (M⁺-gly) as the base peak. This was further supported by EDA fragmentation² representing a series of peaks at m/z 153 [A⁺-H, 98], 152 [A⁺], 149 [B⁺-H], 148 [B⁺], 137 [B⁺-H₂] and B (m/z: 131 [B⁺-H], 178 [B⁺]) which are indicative of the presence of two hydroxyl groups in ring A and two methoxyl and one hydroxyl group in ring B of the aglycone (II).

Kahn methylation (Me₂DMS/Ag₂O) of compound (Ia) followed by acetylation afforded a compound (II) which, by paper chromatographic comparison with an authentic sample,¹ (Found C 61.98; H, 4.33% Calc for C_{18}H_{21}O_{11} as C 61.14; H 4.24% per cent).

Contributions should have novelty and be original in substance. Manuscripts must be submitted in accordance with the instructions to authors, which were published in the 16 January issue. Authors are requested to note that manuscripts which do not accord with these instructions are currently being returned without consideration. In the case of overseas contributors, return is by special rate in order to expedite publication of accepted manuscripts; proofs are not returned to authors outside the UK.

References

Received 8 May 1989.
Two flavonol glycosides from Chenopodium ambrosiodes

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(Received 15 March 1998)

Key Words: Chenopodium ambrosiodes — Flavonol glycosides — Kelsoflavone — 3-O-b-D-glucopyranosyl-2-0-b-D-glucopyranosyl-9-flavone — Quercetin.

Abstract — Two new flavonol glycosides, kelsoflavone 3-O-b-D-glucopyranosyl-2-0-b-D-glucopyranosyl-9-flavone, were isolated from the leaves of Chenopodium ambrosiodes. Their structures were established using spectroscopic and chemical evidence.

INTRODUCTION

The genus Chenopodium consists of 200 species, 45 of which are distributed in India. Chenopodium ambrosiodes L. is reported to possess many medicinal properties [1]. The plant is used as an anthelmintic and is frequently used in the treatment of leucorhoea. Previous work on the secondary chemistry of the genus is scarce [2-6]. We now report two new flavonol glycosides (1 and 2) along with kaempferol, neohesperitin, and quercetin that have been isolated from the fruits of C. ambrosiodes.

RESULTS AND DISCUSSION

The ethyl acetate soluble portion of the defatted methanolic extract of the leaves was subjected to column chromatography on TLC (silica gel, F254, COH2O-BuOH-H2O, 3:2:1:3). The mixture was resolved into several components on the basis of color reactions and reversed phase high performance liquid chromatography. Component 1 was analysed for C14H16O4. Its IR spectrum showed sharp absorption bands at 3350 and 1655 cm⁻¹ corresponding to alcoholic and carboxylic groups respectively. A white crystalline compound (mp 235-239) with a free hydroxyl group at the 3-position and a methoxyl group at the 7-position was isolated as its potassium salt. Its IR spectral data are consistent with a kaempferol residue. Component 2 was subjected to column chromatography on TLC (silica gel, F254, COH2O-BuOH-H2O, 3:2:1:3). The mixture was resolved into several components on the basis of spectral data and reversed phase high performance liquid chromatography. Component 2 was analysed for C14H16O4. Its IR spectrum showed sharp absorption bands at 3350 and 1655 cm⁻¹ corresponding to alcoholic and carboxylic groups respectively. A white crystalline compound (mp 235-239) with a free hydroxyl group at the 3-position and a methoxyl group at the 7-position was isolated as its potassium salt. Its IR spectral data are consistent with a kaempferol residue.

Enhanced by Daidal Media.

Acknowledgments: The authors thank Prof. A A Zaheer, Department of Chemistry, Aligarh Muslim University, Aligarh and Prof. A M Alam, the President of the Council of Scientific and Industrial Research (CSIR) New Delhi for a new research fellowship.

References

The paper deals with the isolation and characterisation of tricin - 7-O-α-arabinoside and named as sotaricin from leaves extract of Setaria italica. The plant is used as sedative to the gravid uterus and widely known for its medicinal value. This novel glycoside is being reported for the first time. This also constitutes the first report of any flavone glycoside from this plant and only second report of tricin glycoside from the family Gramineae. It is interesting to note that the configuration of arabinose is β (instead of α ) which is of rare occurrence.
Isolation and Characterization of a C-Galactopyranosylflavone from Semen Carpospermum amurense

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Section of Pharmaceutical Chemistry, Aligarh Muslim University, Aligarh - 202002, India

Abstract

A new C-galactopyranosylflavone, 3-β-D-galactopyranosyl-5'-O-glucoside of 7-methyl-2'-3'-6'-triindoorazarin-8'-oxygenylflavone, was isolated from the roots of Semen Carpospermum amurense. The structure was determined on the basis of UV, mass, and NMR spectral data. The flavone was found to be a potent inhibitor of human renin in vitro.

Introduction

Studies on the isolation and characterization of flavonoids from natural sources have been of great interest in recent years. The unique chemical and biological properties of flavonoids make them attractive for various applications in medicine, agriculture, and food industry. In this study, we report the isolation and structural elucidation of a new C-galactopyranosylflavone from Semen Carpospermum amurense, a traditional medicinal plant used in various folk remedies.

Experimental

The plant material was dried and extracted with methanol. The crude extract was subjected to silica gel column chromatography, followed by preparative thin-layer chromatography (PTLC) using ethyl acetate:hexane (9:1) as the eluent. The pure compound was obtained as a white amorphous solid.

Results and Discussion

The isolated compound was identified as 3-β-D-galactopyranosyl-5'-O-glucoside of 7-methyl-2'-3'-6'-triindoorazarin-8'-oxygenylflavone (Fig. 1). The UV spectrum showed absorption maxima at 310, 345, and 420 nm, characteristic of flavone derivatives. The NMR spectral data, including 1H NMR and 13C NMR, confirmed the structure of the isolated compound. The IR spectrum showed characteristic absorption bands for C=O and OH groups.

Conclusion

The results of this study demonstrate the potential of Semen Carpospermum amurense as a source of novel flavonoids with intriguing structural features. Further studies are necessary to evaluate the biological activities of this compound.

References


Acknowledgments

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research (CIHR). The authors are grateful to the reviewers for their valuable comments.

Received 18th July 2018, Paper 0(10)04(10)

Scheme

Scheme showing the structure of the isolated flavone. The flavone was shown to have absorption maxima at 310, 345, and 420 nm, characteristic of flavone derivatives.

References


Acknowledgments

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research (CIHR). The authors are grateful to the reviewers for their valuable comments.

Received 18th July 2018, Paper 0(10)04(10)

Scheme

Scheme showing the structure of the isolated flavone. The flavone was shown to have absorption maxima at 310, 345, and 420 nm, characteristic of flavone derivatives.
Mesuein: a novel flavanone glycoside from Mesua ferrea

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The genus Mesua is distributed chiefly in tropical Asia. Mesua ferrea commonly known as 'Nagkesar' is widely known for its medicinal value. Raju et al. have reported the presence of two new flavanones from the stems of Mesua ferrea. Now, the isolation and characterisation are reported from the leaves of the same plant of mesuein, the first flavanone glycoside with a C-methyl substituent in the B-ring and a sugar linkage unusual to flavanones. Mesuein (Ia), a new flavanone glycoside, isolated from C₂₆H₄₀O₉, m.p > 250°C, has been isolated from the aconitine-soluble portion of the methanolic extract by column chromatography and preparative t.l.c. over silica gel. The BOA-O-Me-C-O (7-3) eluate was subjected to repeated column chromatography followed by preparative t.l.c. (BIOAc-R-MeC-O-HO-A-MeC-O, 9:3:13) to give the new flavanone, mesuein (Ia). It was crystallised from ethanol as pale yellow needles, m.p. > 250°C (Found C, 54.6; H, 5.0; C₂₆H₄₀O₉ requires C, 55.0; H, 5.5 per cent). It responded positively to the Shimada test, Molisch test and the ultraviolet spectrum showed absorption maxima at 275 nm (Band II). Analysis of functional groups revealed the presence of phenolic OH (3450 cm⁻¹), w-unsaturated ketonic C=O (1680 cm⁻¹) and a complex aromatic substitution pattern (1500, 1385, 1205, 1146, 800 cm⁻¹) over a strong band at 2900 cm⁻¹. The colour reaction, infrared and ultraviolet spectral evidence with the diagnostic shift reagents are consistent with those of a flavanone skeleton bearing hydroxyl groups at the 4',5,7-positions.

Total acid hydrolysis with 0.2N hydrochloric acid gave rhamnose and galactose (p.c. and g.l.c.) in equimolar quantity and an aglycone (II) which rapidly decomposed in the presence of sodium hydroxide (as shown by its ultraviolet spectrum: thus as a characteristic feature of 3',4'-dihydroxyflavonol) thus showing that the sugar is linked to the 3'-position of the aglycone. The presence of four hydroxyl (tetracenter) and one C-methyl group (H n.m.r. signal at δ 2.4 corresponding to 3H of C(3M)) was in agreement with the structure of the aglycone being 5-C-methyl 3',4',5,7-tetrahydroxyflavone (5-C-methyl crocetinoid) corresponding to the molecular formula C₂₆H₃₄O₉. Found C, 63.0; H, 4.9 C₂₆H₃₄O₉ requires C, 63.5; H, 4.6 per cent. On acetylation (Ac₂O-Py) the glycoside formed a crystalline nonaceteate (Ib) m.p. 85-6°C. The H n.m.r. spectrum of compound (Ib) indicated it to be a flavanone-glycoside it showed multiplets at δ 2.0-2.4 integrating for 30 protons due to one C-methyl group and nine acetoxy (3 aromatic and 6 aliphatic). A multiplet centred at δ 3.20 as assigned to the C-2 methyl and a double doublet at δ 5.20 (J 7.5 and 10 Hz) assigned to the C-2 proton. The 5,7-disubstitution was demonstrated by the presence of two more coupled doublets at δ 6.82 and 7.28 assigned to the O(5) and O(8) protons which have shifted considerably downfield owing to derivatisation as shown from the aglycone (II) n.m.r. spectrum. A doublet at δ 7.75 is assigned to the '6-proton of the B-ring. The mass spectrum of compound (Ib) fully supported the structure of compound (Ia) as it exhibited M⁺ at m/z 428 (M+O) in accordance with the compound tetracyclic structure containing three acetoxy and C-methyl substituent. The subsequent removal of three acetoxy groups gave fragments at m/z 366, 344 and 302. The fragment at 302 was observed as the base peak as it corresponds to the aglycone (II). Other major fragments were observed at m/z 287, 283, 271, 236, 245, 153, 137, 111.

Kuhn methylation (Me_2DCl/AgO) of compound (Ia) followed by acid hydrolysis (0.3N HCl, 4h, under reflux) gave 2,3,4-tri-O-methyl-4'-hamnosone, 2,3,4,6-tetra-O-methyl-D-galactose and the diastereides to be galactosyl (1->4)hamnoside and an aglycone characterised as 5-C-methyl 4',5,7-tri-O-ethyl-crocinot from spectral studies. The sugar moieties were found to be attached to the 3' position by the formation of this partial methyl ether as well as by comparison of the ultraviolet spectrum of the aglycone and glycoside in the presence of sodium hydroxide. Further evidence for the vicinal dihydroxy system in the aglycone was obtained by conversion into the dihydroxy methylketone derivative with diphenyl dichloromethane. However, the glycoside failed to form this derivative thus confirming the sugar linkage at the 3'-position. Ellman hydrolys of the glycoside gave only galactose in the aqueous hydrolysate showing it to be terminal and β-linked to rhamnose.

On the basis of these results, this novel flavanone glycoside has been identified as 5'-C-methyl crocetin 3'-O-B-D-galactopyranosyl (1->4)-α-L-rhamnoside. As far as the authors are aware, this is the first flavanone glycoside having a C-methyl substituent in the B-ring. Moreover, such a sugar combination doesn't appear to have been reported in the flavone series though it has been reported in flavonoid. Prof. Dr. M.S. Ahmad, Chairman, Department of Chemistry is thanked for the provision of facilities and Professor Dr. W. Wagner, Institut fur Pharmazeutische Biologie, Munchen, West Germany is thanked for 'H n.m.r. and mass spectral measurements.

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References
Communications to the Editor

Contributions should have novelty and must be brief. Manuscripts must be submitted in accordance with the instructions to authors, which are published in the 6 July issue (p458). Authors are requested to note that manuscripts which do not accord with these instructions are currently being returned without consideration; in the case of overseas contributions return is by airmail. In order to expedite publication of accepted manuscripts, proofs are not circulated to authors outside the UK.

Semecarpetin: a new flavone glycoside from Semecarpus kurzii

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The genus *Semecarpus* (Anacardiaceae) is well known for elaborating flavonoid compounds. There are about 40 species under this genus and *Semecarpus Kurzii* Linn is rather a rare species for which no flavone glycoside is on record. Current investigation has revealed the presence of two new flavone glycosides. One is now reported and the other will be described elsewhere after further work on its characterisation.

Semecarpetin (I), C_{19}H_{22}O_{13}, m p > 275°C, has been isolated from the ethanolic extract of the leaves of *Semecarpus kurzii* (procured from Andamans) by column chromatography with silica gel (EtOAc/Me2CO, 9:1) and crystallised from methanol as pale yellow needles. It was recognised to be a flavone from its positive Shinoda test and ultraviolet spectrum. Its infrared spectrum displayed strong absorption bands at 3450 (OH) and 1650 cm⁻¹ (C=O). The high molecular weight of the compound, its paper chromatographic behaviour, solubility in water, and positive Molisch test suggested it to be a glycoside. The ultraviolet spectrum showed absorption maxima at 290, 334 nm and changes in the presence of classical reagents pointed to the presence of free hydroxyl groups at the 5- and 7-positions and a solitary methoxyl group at the 4'-position.

Total acid hydrolysis of the glycoside (2M HCl; 2h at 100°C) gave an aglycone and equimolar quantities of rhamnose and glucose (PC, g.l.c. of aldihit acetate). The aglycone was characterised as 4'-methoxyscutellarein, m p 268-70°C, by spectral and chromatographic comparison with an authentic sample (Found C, 63.95; H, 3.96. C_{14}H_{12}O_{5} requires C, 64.00; H, 4.00 per cent).

On acetylation (Ac_2O-Py) the glycoside formed an octaacetate as a white amorphous powder, m p 166-8°C. The 'H n.m.r. spectrum (270 MHz, CDCI₃) of the acetate gave signals centred at δ 8.07 (2H, d, J 9 Hz, H-2',6'), 7.40 (2H, d, J 9 Hz, H-3',5'), 6.87 (1H, s, H-8), 6.65 (1H, s, H-3), 3.79 (3H, s, OCH₃), 2.35, 2.48 (3H each, s, OAc-7,5), 2.02-2.09 (18H, m, 6xOAc), 3.40-5.44 (sugars), 0.88 (3H, d, J 6 Hz, rhamnosyl-CH₃). The position of the H-1 proton of the rhamnosyl portion δ 4.42 (d) and glucosyl δ 5.14 (d) along with the integration of the region δ 4.42-5.44 and δ 3.40-4.40 (ratio 8:4) fully supported the 6-O-rutinosyl group.

Thus, the spectral evidence indicated that the sugar molecules were attached as a disaccharide to the 6-position of the aglycone and this was confirmed by Kuhn methylation (MeI/DMF/Ag_2O) of the glycoside (I) followed by acid hydrolysis (0.3N HCl, 4h under reflux) to give a partial methyl ether characterised spectrally as 6-hydroxy-4',5,7-trimethoxyflavone (Found C, 65.80; H, 5.10. C_{15}H_{19}O_{5} requires C, 65.90; H, 4.90 per cent). The methylated sugars were identified as 2,3,4-tri-O-methyl L-rhamnose and 2,3,4-tri-O-methyl D-glucose by SiO₂-t.l.c. and paper chromatography according to Petek.

On the basis of these results, the glycoside was identified as 4'-methoxyscutellarein 6-O-rutinoside and named Semecarpetin. As far as the authors are aware this novel flavone glycoside is being reported for the first time.

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References

4 Vendanthan, T.N.C., Subramanyam, S.S., & Harborne, J.B., Phytochemistry, 1979, 18, 294
In the present studies we have carried out systematic chemical investigation of four important medicinal plants and isolated and elucidated the structures of a number of compounds, including 11 new constituents. These products may be helpful to other researchers who are mainly concerned with biological activity of herbal constituents.

The theoretical part of the thesis includes a critical review of the chemistry of flavonoidic compounds and highlights the recent advances in the analytical techniques applied to their isolation and structure elucidation.

The different compounds isolated from these plants belonging to different families are as follows:

1. Flavonoids from the fruits of *Chenopodium ambrosioides* (Chenopodiaceae)
   
   I  Kaempferol  
   II Isorhamnetin  
   III Quercetin  
   IV Kaempferol-3-α-rhamnopyranoside-4′-β-xylopyranoside  
   V Kaempferol-3-α-rhamnopyranoside-7-β-xylopyranoside  
   VI 4′-Desmethylnabrectorin-7-α-rhamnopyranoside-3′-β-xylopyranoside.
IV, V compounds are new compounds – paper accepted in Phytochemistry (1990).

VI compound is also a new compound – paper communicated in J.C.R. (1990) and presented in XVth International Conference on Group phenol held at Strasbourg, France on 9-11th July (1990).

2. Flavonoids from the leaves of *Semecarpus kurzii* (Anacardiaceae).

I  Gallic acid
II  Apigenin
III Naringenin
IV  Ethyl Gallate
V  Eriodictyol


The paper is presented in XVth International Conference held on Group phenols in Strasbourg, France, on 9-11th July (1990).

X Quercetin-3-O-rhamnoside

XI Apigenin 7-0-neohesperidoside

3. Flavonoids from the leaves of *Ficus infectoria* (Moraceae)

I Apigenin

II Luteolin

III Sorbifolin 6-0-glucoside


4. The chemical constituents from *Setaria italica* (Garminae)

I β-Sitosterol

II β-Amyrin

III Taraxerone

IV Myricetin


The paper is also presented in 3rd International Conference on Flavonoid in biology and Medicine on 14th Nov. 1989, in Singapore.
VI  4,14,24-Destrimethyl 7(8), 20(21)-diene-cyclo euphorbande - 3α, 14α, 26-triol - setariol.

VII  8,3'-Dimethoxy-5,4'-dihydroxyflavone-7-0-β-D-gluicoside - a new flavone glycoside - paper communicated in Phytochemistry (1990).