CHAPTER II

CHENOPODIUM AMBROSIOIDES
(CHENOPODIACEAE)
DISCUSSION
FLAVONOIDS FROM THE FRUITS OF *CHENOPODIUM AMBROSIOIDES*

(*CHENOPODIACEAE*)

The family *chenopodiaceae* consists of 102 genera and about 1400 species of evergreen trees, shrubs and weeds, distributed mostly in the xerophytic and halophytic areas of central Asia, salt plains of Australia and North America etc. The genus *Chenopodium* consists of about 120 species, 45 of which are distributed in India. *Chenopodium ambrosioides* is reported to possess many medicinal properties. It is used as an anthelmintic and is particularly effective in expulsion of hook worms. Its root contains saponin.

The medicinal value of the plant and the absence of any work on the flavonoidic glycosides attracted our attention. The present work deals with the isolation and characterization of three new flavonol glycosides, Kaempferol-3-α-rhamnopyranoside-4'-β-xylopyranoside (IV), Kaempferol-3-α-rhamnopyranoside-7-β-xylopyranoside (V) and 4'-desmethylabrectorin-7-α-rhamnopyranopyranoside-3'-β-xylopyranoside (VI) along with Kaempferol (I), Isorhamnetin (II) and Quercetin (III) from the fruits of *Chenopodium ambrosioides*.

Fresh fruits of *Chenopodium ambrosioides* were procured from the Aligarh Muslim University, Campus, India were defatted by extraction with hot petrol (40-60°) and the residue were
extracted twice with methanol (10:1). The methanol extract were combined together and concentrated under reduced pressure to give brown gummy mass which was dissolved in hot water. After cooling, water was extracted with EtOAc and n-BuOH. The n-BuOH fraction after TLC examination were found identical with EtOAc fraction except for one additional spot in n-BuOH fraction. The ethyl acetate fraction showed the presence of five spots on TLC over silica gel which were separated by preparative TLC (silica gel EtOAc-Me₂CO-AcOH-H₂O, 30:3:1:1) and labelled as CA-1, CA-2, CA-3, CA-4 and CA-5. The other one component CA-6 was obtained from n-BuOH fraction by preparative TLC over silica gel (EtOAc-Me₂CO-HOAc-H₂O, 30:3:1:1).

**CA-1**

The band CA-1 was eluted with methanol recovery of the solvent left a yellow solid. It was crystallized from benzene-methanol as pale yellow needles, m.p. 278-80°C. It was characterized as Kaempferol (I) by comparison with an authentic sample⁷ (Rᶠ value, m.p., m.m.p., co-chromatography). It was further confirmed by UV and ¹H-NMR studies of its acetate, CA-1A, m.p. 180-82°C(Table 1 and 2).
### TABLE - 1

<table>
<thead>
<tr>
<th>Reagent</th>
<th>CA-1</th>
<th>Kaempferol</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>254, 266, 367</td>
<td>253sh, 266, 294sh, 322sh, 367</td>
</tr>
<tr>
<td>(\lambda_{\text{max}}) nm</td>
<td>260sh, 268, 425</td>
<td>260sh, 268, 303sh, 350, 428</td>
</tr>
<tr>
<td>AlCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>258sh, 267, 423</td>
<td>256sh, 269, 303sh, 348, 428</td>
</tr>
<tr>
<td>AlCl&lt;sub&gt;3&lt;/sub&gt;/HCl</td>
<td>276, 306, 388</td>
<td>274, 303, 387</td>
</tr>
<tr>
<td>NaOAc/H&lt;sub&gt;3&lt;/sub&gt;BO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>268, 322sh, 374</td>
<td>267, 297sh, 320sh, 372</td>
</tr>
</tbody>
</table>

### TABLE - 2

<table>
<thead>
<tr>
<th>Assignments</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2',6'</td>
<td>2</td>
<td>8.25 (d, J = 9Hz)</td>
</tr>
<tr>
<td>H-3',5'</td>
<td>2</td>
<td>7.19 (d, J = 9Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>6.85 (d, J = 2.5Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td>1</td>
<td>6.62 (d, J = 2.5Hz)</td>
</tr>
<tr>
<td>OAc-5,7,3,4'</td>
<td>12</td>
<td>2.45-2.34</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, spectrum run in CDCl<sub>3</sub> at 100 MHz, values on \(\delta\)-scale, TMS as internal standard.

CA-1 was, therefore, assigned the structure 3,5,7,4'-tetrahydroflavone (I).
The band CA-2 on elution with methanol gave deep yellow colour solid. It was crystallized from benzene - methanol as dark yellow sharp needles, m.p. 290-92°C. CA-2 was found to be isorhamnetin by direct comparison with authentic sample (m.p., m.m.p., R_f value, co-chromatography). It was further confirmed by its UV and _1H_NMR studies (Table 3a, 3b).

**TABLE - 3a**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>CA-2</th>
<th>Isorhamnetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>270, 301sh, 320, 375</td>
<td>253, 267sh, 306sh, 326sh, 370</td>
</tr>
<tr>
<td>NaOMe</td>
<td>284, 329, 432(Dec)</td>
<td>240sh, 271, 328, 435(Dec)</td>
</tr>
<tr>
<td>AlCl_3</td>
<td>264, 304sh, 368sh, 428</td>
<td>264, 304sh, 361sh, 431</td>
</tr>
<tr>
<td>AlCl_3/HCl</td>
<td>271, 302sh, 357, 420</td>
<td>242, 272, 302sh, 357, 428</td>
</tr>
<tr>
<td>NaOAc</td>
<td>274, 325, 389(Dec)</td>
<td>260sh, 274, 320, 393(Dec)</td>
</tr>
<tr>
<td>NaOAc/H_3BO_3</td>
<td>270sh, 328sh, 380</td>
<td>255, 270sh, 306sh, 326sh, 377</td>
</tr>
</tbody>
</table>
On the basis of these findings CA-2 was, therefore, assigned the structure as 3'-methoxy-3,5,7,4'-tetrahydroxy-flavone (II).

(II)
CA-3

The band CA-3 was also eluted with methanol. On several crystallization from methanol it gave yellow crystallized microscopic needles, m.p. $312^\circ$C. The structure was confirmed by UV and $^1H$-NMR studies of parent compound and its acetate, m.p., $194-95^\circ$C. The result of UV spectra of CA-3 and Quercetin are recorded in Table-4.

**TABLE - 4**

UV data of CA-3 and Quercetin

<table>
<thead>
<tr>
<th>Reagent</th>
<th>CA-3</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>256, 270sh, 301sh, 372</td>
<td>255, 269sh, 301sh, 370</td>
</tr>
<tr>
<td>NaOMe</td>
<td>247sh, 321(Dec)</td>
<td>247sh, 321(Dec)</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>274, 304sh, 334, 458</td>
<td>272, 304sh, 333, 458</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>264, 358, 427</td>
<td>265, 301sh, 359, 428</td>
</tr>
<tr>
<td>NaOAc</td>
<td>257sh, 274, 329, 390</td>
<td>257, 274, 390(Dec)</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>264, 303sh, 389</td>
<td>261, 301sh, 388</td>
</tr>
</tbody>
</table>

The UV spectral data of CA-3 were found identical with quercetin. The results of $^1H$-NMR spectrum are recorded in Table-5.
TABLE - 5

Chemical Shifts of protons of CA-3

<table>
<thead>
<tr>
<th>Assignment</th>
<th>No. of protons</th>
<th>Signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2'</td>
<td>1</td>
<td>7.73 (d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-6'</td>
<td>1</td>
<td>7.65 (q, J₁=2.5Hz, J₂=8.5Hz)</td>
</tr>
<tr>
<td>H-5'</td>
<td>1</td>
<td>7.28 (d, J=8.5Hz)</td>
</tr>
<tr>
<td>H-8</td>
<td>1</td>
<td>7.24 (d, J=2.5Hz)</td>
</tr>
<tr>
<td>H-6</td>
<td></td>
<td>6.80 (d, J=2.5Hz)</td>
</tr>
<tr>
<td>5xOAc</td>
<td>15</td>
<td>2.42 (s), 2.32 (m)</td>
</tr>
</tbody>
</table>

s = singlet, d = doublet, q = quartet, m = multiplet, spectrum run in CDCl₃ at 60 MHz, TMS being an internal standard (δ-scale).

¹H-NMR spectrum of the acetate taken in CDCl₃ showed signals due to five phenolic acetyl groups at δ 2.32 - 2.42. The A-ring proton signal showed doublets at δ 6.80 and 7.24 (J = 2.5Hz) assigned to 6 and 8 positions respectively. The B-ring protons showed an ABX pattern, two doublets at δ 7.73 (J = 2.5Hz) for H-2' and δ 7.28 (J = 8.5Hz) for H-5' and a quartet at 7.65 (J = 2.5Hz and 8.5Hz) for H-6'.

Considering the above facts, CA-3 was assigned the structure as 5,7,3,3',4'-pentahydroxyflavone (III).
The glycosidic nature of the product (CA-4) was evidenced by the positive Molisch test obtained after hydrolysis and by the formation of an Osazone. The glycosidic nature was further confirmed by the $^1$H-NMR spectrum of the acetate of CA-4 (Table-6) as it showed two aromatic acetoxyls at $\delta$ 2.45 (3H) and $\delta$ 2.34 (3H) and six aliphatic acetoxyls at $\delta$ 1.98-2.20 (18H, m, 6xOAc) indicating it to be a diglycoside.

The glycoside gave pink colour with Zn/HCl and red colour on treatment with sodium amalgam followed by acidification$^{10}$ indicating its flavanone or flavone nature. A yellow colour with Wilson boric acid reagent$^{11,12}$ and maxima at 242sh, 269, 315sh, 345 in the UV spectrum indicated it to be a flavonol glycoside. It gave a brownish green colour with FeCl$_3$ indicating
the presence of hydroxy group at C-5. The IR spectrum showed
strong absorption bands at 3420 (OH), 1655 (C=O), 2950 (C–H),
1620 (C=C, aromatic) and a broad band at 1100–1000 cm⁻¹ indi­
cating its glycosidic nature. A bathochromic shift of +40 nm
in band I with AlCl₃ further confirmed the presence of a free
5-OH group. The presence of free 7-OH group is confirmed by a
bathochromic shift of +11 nm in band II with fused NaOAc.

Total acid hydrolysis of the glycoside with 2N HCl yielded
equimol. mixture of L-rhamnose, D-xylose (PC and GLC) and an
aglycone, m.p. 280–81°C characterised as Kaempferol (IVa) by
spectral and chromatographic comparison with authentic sample¹³.

The ¹H-NMR spectrum of CA-4 (acetate) (Fig. 1), m.p. 125–
270°C showed two meta coupled doublets at δ 6.79 and 7.09
(J = 2.2Hz) were attributed to C-6 and C-8 protons respectively.
Two ortho coupled doublets at δ 7.90 (J = 8.5Hz) and δ 7.28
(J = 8.5Hz) which corresponded to A₂B₂ pattern were assigned to
C-2',6' and C-3',5' protons of B-ring respectively. The anomeric
protons at δ 5.19 (J = 9.5Hz) and δ 5.63 (J = 1.2Hz) were assi­
gned to H-1''' xylose (β-configuration) and H-1'' rhamnose
(α-configuration) respectively. The rhamnosyl methyl appeared
as a doublet at δ 1.23 (J = 6.1Hz). The remaining sugar protons
were observed in the range δ 3.77 - 5.63.
The mass spectrum of the acetylated glycoside (Fig. 2) was in agreement with the assigned structure of the glycoside. The mass spectrum showed the presence of acetylated pentopyranoside, m/z 259 and acetylated hexopyranoside, m/z 273. The fragment ions observed at m/z 642 and m/z 628 accounted for the loss of acetylated pentopyranoside and hexopyranoside respectively from the molecular ion. The loss of both acetylated sugar moieties gave fragment at m/z 370. The aglycone fragment
Fig 2

CAF-2
SAMPLE NO.: 185 SCAN NO.: 40*44 TIME(MIN): 3.1

[Graph showing mass spectrometry data]
Was observed at m/z 286. A retro-Diels-Alder fragmentation pattern was observed at m/z 153 and m/z 121 leading to fragments \([A_1+H]^+\) and \(B_2^+\). The result supported the presence of two hydroxyl groups in ring-A and one hydroxyl group in ring-B. Other prominent sugar fragments appeared at m/z 213, 171, 153, 111, 97, 96.

Enzymatic hydrolysis of the parent glycoside (IV) gave conclusive evidences of the position of attachment of two sugars and the nature of their linkages. Hydrolysis of (IV) with β-xylosidase gave D-xylose and a partial glycoside (IVb) which gave a bathochromic shift of +62 nm with NaOMe in band I without a decrease in intensity (absent in glycoside). Thus showing that C-4' hydroxyl which was glycosylated in (IV) had become free. The partial glycoside (IVb) was identified as Kaempferol-3-rhamnoside, m.p. 170-72°C by UV diagnostic shift reagents and co-chromatography with authentic sample. Methylation of partial glycoside (IVb) followed by hydrolysis with 2N HCl gave a partial methyl ether (IVc), m.p. 135-36°C characterized as 3-OH,5,7,4'-Trihydroxyflavone (Kaempferol 5,7,4'-trimethyl ether) by spectral and chromatographic comparison with authentic sample. The methylated sugars were identified as 2,3,4-tri-O-methyl-L-rhamnose by SiO₂ TLC according to Petek. This finally established that L-rhamnose was α-linked at C-3 while D-xylose was β-linked at 4'-position. Quantitative estimation of sugar by Somogyis copper micro method indicated the
presence of 2 moles of sugar/mole of aglycone.

On the basis of these data, compound CA-4 was identified as Kaempferol-3-α-rhamnopyranoside-4'-β-xylopyranoside (IV) which is a new natural product.

(IV)

IV $R_1, R_2, R_3 = H, R_4 = $Xyl; $R_5 = $Rhm.

IVa $R_1, R_2, R_3, R_4, R_5 = H$

IVb $R_1, R_2, R_3, R_4 = H, R_5 = $Rhm.

IVc $R_1, R_2, R_4 = $CH$_3$, $R_3, R_5 = H$
CA-5 was obtained as pale yellow granules, m.p. 300°C, analysed for C_{26}H_{28}O_{14} gave all colour reactions given by CA-4. The striking similarity of IR, ^{1}H-NMR and EI mass spectrum between 4 and 5 suggested that they have same chromophore. The UV spectrum showed \lambda_{\text{max}} at 238sh, 260, 360. A bathochromic shift of +48 nm with AlCl_3 in band I and +58 nm with NaOMe in band I pointed out the presence of free hydroxyl group at C-5 and C-4' position on a 3,7-disubstituted flavonol glycoside.

Total acid hydrolysis with 2N HCl gave equimol. mixture of L-rhamnose, D-xylose and Kaempferol.

The glycoside formed a crystalline octaacetate, m.p. 130-32°C. The ^{1}H-NMR spectrum of the acetate (Fig. 3) evidenced the expected signals in the aromatic region. Two metacoupled doublets at \delta 6.78 and \delta 7.08 (J = 2.2Hz) were attributed to C-6 and C-8 protons respectively. Two ortho coupled doublets at \delta 7.88 (J = 9Hz) and \delta 7.28 (J = 9Hz) which corresponded to \text{A}_2\text{B}_2 pattern were assigned to C-2',6' and C-3',5' protons of the B-ring. The anomic protons at \delta 5.19 (d, J = 8.3Hz) and \delta 5.63 (d, J = 1.0Hz) were assigned to H-1 xylose (\beta-configuration) and H-1 rhamnose (\alpha-configuration) respectively. The rhamnosyl methyl appeared as a doublet at \delta 1.23 (d, J = 6.1Hz). The remaining sugar protons were observed in the range \delta 3.78-5.63.
The aliphatic acetoxyls appeared as multiplet integrating for eighteen protons in the range of $\delta$ 1.98-2.20. Two aromatic acetoxyls appeared at $\delta$ 2.4 and $\delta$ 2.34 assigned to OAc-5 and OAc-4', respectively.

The mass spectrum of the glycoside (Fig. 4) was in agreement with the assigned structure of the glycoside. The mass spectrum showed the presence of same fragment ions as given by CA-4(Scheme).

Enzymatic hydrolysis of the glucoside CA-5 gave conclusive evidence of the position of attachment of both sugars and their linkages in the glycoside. Enzymatic hydrolysis of the parent glycoside (V) with $\beta$-xylosidase gave the same products as given by CA-4. However, a bathochromic shift of 12 nm in band II with NaOAc (absent in glycoside indicated that D-xylose was attached at C-7 position in CA-5). Methylation and subsequent acid hydrolysis gave similar products as identified in CA-4. On the basis of these findings, compound CA-5 was identified as Kaempferol-3-$\alpha$-rhamnopyranoside-7-$\beta$-xylopyranoside (V).
Fig 4

CAF-3
SAMPLE NO.: 186 SCAN NO.: 29134 TIME (MIN): 2.2

[Graph showing mass spectrometry data with peaks labeled at various masses, such as 50, 111, 127, 139, 153, 171, etc., with intensity values on the y-axis and mass values on the x-axis.]
CA-6

CA-6 was obtained by preparative TLC of n-BuOH fraction over silica gel (EtOAc-Me₂CO-AcOH-H₂O, 30:3:1:1) as yellow solid. It was crystallized from methanol as yellow needles, m.p. 247-48°C. Elemental analysis agreed to the molecular formula C₂₇H₃₀O₁₄. The glycosidic nature of the product (CA-6) was evidenced by the positive Molisch test obtained after hydrolysis and by spectrum of its acetate. It gave a green colour with FeCl₃ and pink colour with Zn/HCl. The ultra-violet spectrum showed λmax at 252 and 342 nm. A bathochromic shift of +59 nm with NaOMe in band I without a decrease in intensity indicated the presence of a free 4'-hydroxyl group. Absence of any shift with +NaOAc and NaOAc/H₃BO₃ ruled out the presence of O-dihydroxyl grouping.

The infrared spectrum displayed a carbonyl group at 1655 cm⁻¹ phenolic OH at 3440 cm⁻¹ and a complex aromatic substitution pattern at 1500, 1355, 1210, 1150, 800 cm⁻¹ besides a strong band at 2950 cm⁻¹. The IR and UV spectral studies with diagnostic shift reagents¹⁷ suggested that CA-6 is a 7,3'-substituted flavone glycoside bearing one hydroxy and one methoxy group at 4' and 6 position respectively.

Total acid hydrolysis of CA-6 with 2N HCl yielded equimol. mixture of L-rhamnose and D-xylose (PC and GLC) and an aglycone (VIb) which gave a bathochromic shift of 8 nm with NaOAc and
21 nm with NaOAc/H₃BO₃ (absent in glycoside) thus showing that the sugar is linked to the 7 and 3'-position of the aglycone. Kuhn methylation of aglycone gave tetramethyl ether, m.p. 221-22°C which was found identical with an authentic sample of abrectorin tetramethyl ether. The aglycone was thus characterized as 4'-Demethyl abrectorin, m.p. 232-34°C corresponding to the molecular formula C₁₆H₁₂O₆ by chemical and spectral studies.

Acetylation of the glycoside with Ac₂O/Py gave a hepta-acetate derivative (VIa), m.p. 115-13°C. In the ¹H-NMR spectrum of the acetate (Fig. 5), the flavone nucleus was evidenced from the characteristic one proton singlet (C-3H) of the unsaturated C-ring at δ 6.54. Two more singlets at δ 8.09 and δ 6.69 were assigned to C-5 and C-8 proton while a three proton singlet at δ 3.95 was attributed to the solitary methoxy group at C-6 position. Furthermore, a three proton multiplet at δ 7.12 and δ 7.02 showed the presence of C-2', C-5' and C-6' protons of the B-ring. As a result of glycosylation, the proton signal of C-2' shifted downfield in comparison with those of C-5' and C-6', thus indicating that one of the position of glycosylation was the C-3' hydroxy group. The anomeric protons at δ 5.36 (J = 1.5Hz) and δ 5.19 (J = 8.2Hz) were assigned to H-1'' rhamnose (α-configuration) and H-1'''-xylose (β-configuration), respectively. The rhamnosyl methyl appeared as a characteristic
multiplet at $\delta$ 0.88. The other sugar protons were observed in the range $\delta$ 3.74-5.58. The solitary aromatic MeCO appeared as a singlet at $\delta$ 2.32 while six aliphatic MeCO appeared as multiplet at $\delta$ 2.02 – 2.24.

The EI mass spectrum of CA-6 acetate (VIa) (Fig. 6, Scheme 2) was in full agreement with the assigned structure. The molecular ion peak as expected was not observed. The presence of acetylated pentopyranoside and deoxyhexopyranoside was evidenced by the fragment ions at m/z 259 and m/z 273 respectively. The loss of these two ions alternatively from the molecular ion gave fragments at m/z 614 and m/z 600. The fragment ion observed at m/z 342 accounted for the loss of both the acetylated sugars from the molecular ion. A retro-Diels-Alder fragmentation pattern representing ring A [m/z 167 ($A_1$+H)$^+$] and ring B [m/z 134 ($B_1$)$^+$] are indication of the presence of one hydroxy and one methoxy groups in ring A and two hydroxy groups in ring B of the aglycone.

The position of the sugar residue in the glycoside was confirmed by permethylation of the glycoside followed by hydrolysis with $\beta$-xylosidase gave 2,3,4-tri-O-methyl-L-rhamnose identified by SiO$_2$ TLC according to Petek$^{15}$ and a partial glycoside which gave a bathochromic shift of 24 nm with NaOAc/H$_3$BO$_3$ suggesting that C-3' hydroxy which was glycosylated in VI had become free. The partial glycoside on subsequent hydrolysis
Fig 6

CAF-4

![Graphical representation of a mass spectrum with labeled peaks at various masses.](image-url)
Scheme-2
with 2N HCl gave 2,3,4-tri-O-methyl-L-rhamnose and an aglycone (VIc), m.p. 229-30°C characterized as abrectorin by chromatographic and spectral comparison with authentic sample\textsuperscript{18}. This finally established that D-xylose was $\beta$-linked at C-3' while L-rhamnose was $\alpha$-linked at C-7 position. Quantitative estimation of sugar\textsuperscript{16} showed two moles of sugar per mole of aglycone.

On the basis of these findings, this novel flavone glycoside has been identified as 4'-Demethyl abrectorin-7-$\alpha$-rhamnopyranoside-3'-$\beta$-xylopyranoside (VI). This constitutes the first report of any glycoside from this aglycone. Furthermore, xylose and rhamnose appears to be commonly occurring sugars in this species and thereby serving as an important taxonomic marker.

\begin{equation}
\text{(VI)} \quad R = H
\end{equation}

\begin{equation}
\text{(VIa)} \quad R = \text{Ac}
\end{equation}
EXPERIMENTAL
Extraction of the fruits of Chenopodium ambrosioides
(Chenopodiaceae)

Fresh fruits (3 kg) were exhaustively extracted with petrol (40-60\(^\circ\)) and the residue was extracted with methanol. The combined methanolic extract was concentrated under reduced pressure to give a brown gummy mass which was dissolved in boiling water. After cooling, water was successively extracted with Et\(_2\)O, EtOAc and n-BuOH. The EtOAc fraction on TLC (Si gel, EtOAc-Me\(_2\)CO-HOAc-H\(_2\)O, 30:3:1:1) showed five spots. The mixture was resolved into individual components by repeated column chromatography followed by preparative TLC using the same solvent system. These compounds were labelled as CA-1, CA-2, CA-3, CA-4, CA-5, CA-6.

CA-1

It was crystallized from methanol-benzene as yellow needles, m.p. 278-80\(^\circ\)C, was characterized as Kaempferol by UV and \(^1\)H-NMR studies of parent compound and its acetate.

Anal. Calcd. for C\(_{15}\)H\(_{10}\)O\(_6\) : C, 62.93; H, 3.49

Found : C, 62.80; H, 3.44\%.
UV spectral data (λ<sub>max</sub> nm)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>254, 266, 376</td>
</tr>
<tr>
<td>AlCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>260sh, 268, 452</td>
</tr>
<tr>
<td>AlCl&lt;sub&gt;3&lt;/sub&gt;/HCl</td>
<td>258sh, 267, 423</td>
</tr>
<tr>
<td>NaOAc</td>
<td>276, 306, 388</td>
</tr>
<tr>
<td>NaOAc/H&lt;sub&gt;3&lt;/sub&gt;BO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>268, 322sh, 374</td>
</tr>
</tbody>
</table>

Acetylation of CA-1

A mixture of CA-1 (50 mg), pyridine (1 ml) and acetic anhydride (2 ml) was heated on a water bath for 2 hrs. The reaction mixture was poured over crushed ice. The solid was washed well and dried. On crystallized from CHCl<sub>3</sub>-MeOH, gave colourless needles of Kaempferol tetraacetate (25 mg) (CA-1A) m.p. 180-82°C.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): Values on δ-scale

8.25 (2H, d, J = 9Hz, H-2',6'), 7.19 (2H, d, J = 9Hz, H-3',5'), 6.85 (1H, d, J = 2.5Hz, H-8), 6.62 (1H, d, J = 2.5Hz, H-8), 2.45-2.34 (12H, s, 4xOAc).

CA-2

It was crystallized from benzene - methanol as sharp yellow needles, m.p. 290-92°C. It was characterized as isorhamnetin by direct comparison of its UV and <sup>1</sup>H-NMR spectral data with isorhamnetin.
Anal. Calcd. for C_{16}H_{12}O_{7} : C, 67.59; H, 3.79

Found : C, 67.60; H, 3.80%

UV spectral data with shift reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Absorption Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>270, 301sh, 320, 375</td>
</tr>
<tr>
<td>NaOMe</td>
<td>284, 329, 432(Dec)</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>264, 304sh, 368sh, 428</td>
</tr>
<tr>
<td>AlCl₃/HCl</td>
<td>271, 302sh, 357, 420</td>
</tr>
<tr>
<td>NaOAc</td>
<td>274, 325, 389(Dec)</td>
</tr>
<tr>
<td>NaOAc/H₃BO₃</td>
<td>270sh, 328sh, 380</td>
</tr>
</tbody>
</table>

¹H-NMR (CDCl₃) : Values on δ-scale

7.80 (1H, d, J = 2.5Hz, H-2'), 7.64 (1H, d, J = 9Hz, H-6'), 6.92 (1H, d, J = 9Hz, H-5'), 6.68 (d, J = 2.5Hz, H-8'), 6.23 (1H, d, J = 2.5Hz, H-6), 3.60 (3H, s, 3'-OCH₃).

CA-3

It was eluted from band CA-3 as yellow solid. It was crystallized as yellow crystalline microscopic needles, m.p. 312°C.

Anal. Calcd for C_{15}H_{10}O_{7} : C, 59.62; H, 3.31

Found : C, 59.70; H, 3.33%.
UV data : \( \lambda_{\text{max}} \) nm

<table>
<thead>
<tr>
<th></th>
<th>MeOH</th>
<th>NaOMe</th>
<th>AlCl(_3)</th>
<th>AlCl(_3)/HCl</th>
<th>NaOAc</th>
<th>NaOAc/H(_3)B(_3)O(_3)</th>
</tr>
</thead>
</table>

**Acetylation of CA-3**

The aglycone (25 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand for 24 hours at room temperature. After usual workup it was crystallized from ethylacetate as cream coloured needles (10 mg), m.p. 194-95°C.

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

7.73 (1H, d, J = 2.5Hz, H-2'), 7.65 (1H, d, J = 2.5Hz and J = 8.5Hz, H-6'), 7.28 (1H, d, J = 8.5Hz), 7.24 (1H, d, J = 2.5Hz, H-8), 6.80 (1H, d, J = 2.5Hz, H-6), 2.42-2.32 (15H, m, 5xOAc).

**CA-4**

CA-4 was obtained as pale yellow granules on crystallization from chloroform - methanol, m.p. 262-63°C.
Anal. Calcd. for C_{26}H_{28}O_{14}: C, 56.11; H, 5.03

Found: C, 56.15; H, 5.08%.

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

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( \lambda_{\text{max}} ) nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>242 sh, 269, 315 sh, 345</td>
</tr>
<tr>
<td>AlCl(_3)</td>
<td>250 sh, 275, 305 sh, 340, 398</td>
</tr>
<tr>
<td>AlCl(_3)/HCl</td>
<td>277, 299, 340, 395</td>
</tr>
<tr>
<td>NaOAc</td>
<td>280, 354, 395</td>
</tr>
<tr>
<td>NaOAc/H(_3)BO(_3)</td>
<td>271, 318 sh, 352</td>
</tr>
<tr>
<td>NaOMe</td>
<td>255 sh, 296, 355, 405</td>
</tr>
</tbody>
</table>

Acetylation of CA-4

The crystalline glycoside (45 mg) was acetylated with dry Ac\(_2\)O/Py (1:1) at room temperature for 48 hr. It was cooled to room temperature and poured to crushed ice. The separated solid was filtered, washed with distilled water and dried. It was crystallized from chloroform - methanol as cream needles, m.p. 125-27°C.

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

6.79 (1H, d, \( J = 2.2 \text{Hz} \), H-6), 7.09 (1H, d, \( J = 2.2 \text{Hz} \), H-8), 7.90 (2H, d, \( J = 8.5 \text{Hz} \), H-2',6'), 7.28 (2H, d, \( J = 8.5 \text{Hz} \), H-3',5'), 5.19 (1H, d, \( J = 9.5 \text{Hz} \), H-1'''xylose), 5.63 (1H, d, \( J = 1.2 \text{Hz} \), H-1''rham.), 1.23 (3H, d, \( J = 6.1 \text{Hz} \), rham-CH\(_3\)), 3.77-5.63 (10H, m, gly-H), 2.45 (3H, s, OAc-5), 2.34 (3H, s, OAc-7), 1.98-2.20 (18H, m, aliphatic MeCO).
**Mass : (m/z)**

642 \([\text{M-acetylated pentose} + \text{H}^+]^+)\, 628 \([\text{M-acetylated hexose} + \text{H}^+]^+)\, 370 \([\text{M-acetylated pentose} - \text{acetylated hexose} + 2\text{H}]^+)\, 286 \([\text{M-614}]^+)\, 273 \([\text{(rham)Ac}_3]^+)\, 259 \([\text{(xyl)Ac}_3]^+)\, 153 \([\text{A}_1+\text{H}]^+)\, 121 \([\text{B}_2]^+)\.

**Hydrolysis of CA-4**

The glycoside CA-4 was hydrolysed with 2N HCl - MeOH (5 ml) (100°C, refluxed for 2 hr.). Water was added and the mixture was extracted with ethylacetate. The aqueous hydrolysate was neutralized with Ag$_2$CO$_3$, ppt filtered off and the filterate evaporated in vacuo giving a residue.

**Identification of aglycone**

The aglycone in EtOAc fraction was crystallized from CHCl$_3$-MeOH as yellow needles, m.p. 280-81°C and identified as Kaempferol by spectral and chromatographic comparison with authentic sample.

Anal. Calcd. for C$_{15}$H$_{10}$O$_6$ : C, 62.93; H, 3.49

**Found** : C, 62.80; H, 3.44%.

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

<table>
<thead>
<tr>
<th>MeOH</th>
<th>249sh, 265, 295sh, 320sh, 370</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl$_3$</td>
<td>259sh, 266, 300sh, 364, 421</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>259sh, 265, 301sh, 364, 422</td>
</tr>
</tbody>
</table>
Identification of sugars

The neutral hydrolysate was concentrated and chromatographed on Whatman No. 1 filter paper using n-BuOH - acetic acid - water (4:1:5) and EtOAc-Pyridine-H_2O (2:1:2) as solvent systems, employing the 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 Rhamnose (0.37, 0.28) and Xylose (0.30, 0.22).

GLC of TMSi ethers of sugars:

The TMSi ethers of sugar was obtained by taking 15 mg of sugar in drying pyridine (0.5 ml) and hexamethyldisilazane (0.2 ml) in a 10 ml round bottom flask. To this solution 0.2 ml of trimethylchlorosilane was added and flask was stoppered and allowed to stand at room temperature for 45 minutes. The solution was then dried and taken in heptane. The heptane soluble TMSi ether derivatives of sugar were then subjected to GLC (2% OV-1, column temp. 150-250°C, 10 min. dect. temp. 300°C, N_2, 50 ml/min) along with silyl derivatives of standard sugars (R_t 3.9, 3.8 min for rhamnose, 3.9, 4.5 min for xylose). The observed R_t - values were in agreement with those of an authentic sample of rhamnose and xylose.
Enzymatic hydrolysis of CA-4

A mixture of compound CA-4 (100 mg) and β-xylosidase (10 mg) was incubated in \((\text{NH}_4)_2\text{SO}_4\)-NaOAc buffer (pH 5.0) at 250°C for 30 hr and then after addition of water, it was extracted with n-BuOH. The BuOH extract was chromatographed on silica gel column to give a partial glycoside, m.p. 170-72°C, identified as kaempferol-3-O-rhamnoside. From the H$_2$O layer, D-xylose was identified by PC (four solvents).

Methylation of Partial Glycoside

CH$_3$I (1 ml) and Ag$_2$O (30 mg) were added to a solution of partial glycoside (30 mg) in DMF (3 ml). The mixture was stirred in dark at room temperature for 48 hr. The contents were filtered and the residue washed with little DMF. The filtrate was evaporated to dryness and the residue washed with little DMF. The filtrate was evaporated to dryness and the residue was treated with ethanol (25 ml). The alcohol was recovered and the syrupy residue was hydrolysed with 2N HCl. On usual workup it gave 3-OH, 5,7,4'-trimethoxyflavone (14 mg) m.p. 135-36°C. Calcd. for C$_{18}$H$_{16}$O$_6$: C, 65.85; H, 4.87; Found: C, 65.96; H, 4.98%.

Quantitative estimation of sugars

The anhydrous glycoside (25 mg) was hydrolysed by refluxing with 0.2N HCl. After cooling over night, the aglycone was
was dried and weighed (11.5 mg), the ratio of aglycone to glycoside is 44.2% indicating the presence of two moles of sugar/mole of aglycone.

CA-5

CA-5 was eluted from fifth band with methanol as yellow granules, m.p. 300°C.

Anal. Calcd. for $C_{25}H_{20}O_{14}$: C, 56.11; H, 5.03

Found: C, 56.30; H, 5.09%

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

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>238sh, 260, 310sh, 360</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>244sh, 274, 306, 359sh, 408</td>
</tr>
<tr>
<td>AlCl₃/HCl</td>
<td>265, 290sh, 356, 403</td>
</tr>
<tr>
<td>NaOMe</td>
<td>245, 272, 304sh, 362, 418</td>
</tr>
<tr>
<td>NaOAc</td>
<td>265, 319sh, 362, 396</td>
</tr>
<tr>
<td>NaOAc/H₃BO₃</td>
<td>264, 316sh, 262</td>
</tr>
</tbody>
</table>

IR $\nu_{\text{max}}$ KBr cm⁻¹

3440 (OH), 1650 (C=O), 2950 (C–H), 1620 (C=C), 1120-1000 (C–O).

Acid hydrolysis

The acid hydrolysis of CA-5 with 2N HCl (at 100°C, 2 hr)
gave same products as given by CA-4. The sugars were identified as L-rhamnose and D-xylose by co-chromatography and GLC (TMSi ether). The aglycone gave yellow needles, m.p. 280-81°C characterized as Kaempferol by spectral and chromatographic comparison with authentic sample.

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

Found : C, 62.80; H, 3.44%.

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

<table>
<thead>
<tr>
<th>Solution</th>
<th>$\lambda_{\text{max}}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>249sh, 265, 320sh, 370</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>259sh, 266, 300sh, 364, 421</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>256sh, 265, 301sh, 364, 422</td>
</tr>
<tr>
<td>NaOMe</td>
<td>260sh, 282, 315, 430</td>
</tr>
<tr>
<td>NaOAc</td>
<td>277, 300, 398</td>
</tr>
</tbody>
</table>

Acetylation of CA-5

CA-5 (25 mg) was acetylated with acetic anhydride and pyridine and worked up as described earlier. It was crystallized from CHCl$_3$-MeOH as colourless needles (15 mg), m.p. 130-32°C.

$^1$H-NMR : Values on $\delta$-scale

7.88 (2H, d, $J = 9$Hz, H-2',6'), 7.28 (2H, d, $J = 9$Hz, H-3',
5'), 7.08 (1H, d, J = 2.2Hz, H-8), 6.78 (1H, d, J = 2.2Hz, H-6),
5.19 (1H, d, J = 8.3Hz, H-1'' xylose), 5.63 (1H, d, J = 11Hz,
H-1'' rham), 1.23 (3H, d, J = 6.1Hz, rham-CH3), 1.23 (3H, d, J = 6.1Hz, rham-CH3), 3.78-5.63 (10H, m, sugar proton), 2.40 (3H, s, OAc-5), 2.34 (3H, s, OAc-4'),
1.98-2.20 (18H, m, aliphatic MeCO).

MS data ; m/z

642 [M-acetylated pentose +H+]•, 628 [M-acetylated hexose +H]+•, 600 [M-acetylated pentose -Ac + H+]••, 558 [M-acetylated pentose +H+ - 2xAc']••, 586 [M-acetylated hexose +H+ - Ac']••, 370
[M-acetylated pentose - acetylated hexose + 2H+]••, 286

Enzymatic hydrolysis

Hydrolysis of CA-5 with β-xylosidase was carried out under the same conditions as CA-4 to obtain the same product.

CA-6

The CA-6 was obtained from n-BuOH fraction by preparative TLC over silica gel using EtOAc-Me2CO-HOAc-H2O, 30:3:1:1 as developing solvent. CA-6 was obtained as yellow solid, crystallized from CHCl3-MeOH as yellow needles, m.p. 247-49°C.
Anal. Calcd. for $C_{27}H_{30}O_{14}$: C, 56.2; H, 5.1

Found: C, 56.3; H, 5.2%

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

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>252, 265sh, 342</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>251sh, 267, 360</td>
</tr>
<tr>
<td>AlCl$_3$/HCl</td>
<td>250sh, 267, 344, 362</td>
</tr>
<tr>
<td>NaOAc</td>
<td>254, 268sh, 398</td>
</tr>
<tr>
<td>NaOAc/H$_3$BO$_3$</td>
<td>255, 270sh, 343</td>
</tr>
<tr>
<td>NaOMe</td>
<td>261, 274, 335sh, 401</td>
</tr>
</tbody>
</table>

IR $\nu_{\text{KBr}}$ cm$^{-1}$

2950, 1655, 1500, 1210, 1355, 1150, 800.

**Acetylation of Glycoside**

The crystalline glycoside (20 mg) was acetylated with Ac$_2$O/Py (1:1, 3 ml) in the usual manner to afford a hepta-acetate derivative (14 mg) as cream coloured needles, m.p. 115-16°C.

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

7.02, 7.12 (3H, m), 8.09 (1H, s), 6.69 (1H, s), 6.54 (1H, 3.95 (3H, s), 5.19 (1H, d, $J = 8.2$Hz), 5.39 (1H, J = 1.5Hz),
2.32 (3H, s), 2.02-2.24 (18H, m), 3.74-5.58 (11H, m), 0.88 (3H, m).

**Acid hydrolysis of the Glycoside**

A solution of glycoside CA-6 (50 mg) in 2N HCl-MeOH (1:1, 5 ml) was refluxed on a water bath for 2 hr. The mixture was poured into ice-water and extracted with EtOAc. After evaporation of solvent, the residue was crystallized from CHCl₃-MeOH to give an aglycone as yellow needles (22 mg), m.p. 232-34°C.

Anal. Calcd. for C₁₆H₁₂O₆ : C, 64.0; H, 4.0

Found : C, 64.2; H, 4.0%

**UV data:**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(\lambda_{\text{max}}) nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>251, 265sh, 344</td>
</tr>
<tr>
<td>NaOAc</td>
<td>259, 270sh, 390</td>
</tr>
<tr>
<td>NaOAc/H₃BO₃</td>
<td>255, 365</td>
</tr>
</tbody>
</table>

**IR data:**

3400, 2950, 1650

**Methylation of the aglycone**

The aglycone (200 mg) was methylated with MeI (1 ml) and
HCONMe$_2$ (3 ml) in the presence of Ag$_2$O (30 mg). The mixture was stirred in dark at room temperature for 48 hours. The contents was evaporated to dryness and the residue was treated with ethanol (25 ml). The alcohol was recovered and the mass on crystallization gave pale yellow needles, m.p. 221-22°C.

Anal. Calcd. for C$_{19}$H$_{18}$O$_6$: C, 66.6; H, 5.3

,Found : C, 66.5; H, 5.5%.

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

7.52 (1H, s), 6.87 (1H, s), 6.60 (1H, s), 3.99 (12H, s), 7.45 (2H, m), 7.02 (1H, d).

Identification of sugars

The aqueous hydrolysate after removing the last traces of aglycone was concentrated to a syrup in vacuum over KOH pellets. The sugars were identified by paper chromatography in two different solvent systems namely, n-BuOH-AcOH-Water (4:1:5, upper layer) and ethyl acetate - acetic acid - water (3:1:3) using authentic sugars as checks. The $R_f$ - values of sugars were identical with those of rhamnose (0.37, 0.34) and xylose (0.28, 0.26).

GLC of TMS1 ether derivative

The TMS1 ether derivative of sugars were prepared as
described earlier. GLC of TMSi ether confirmed them to be rhamnose and xylose.

**Enzymatic hydrolysis of permethylated glycoside**

Permethylated glycoside (100 mg) and β-xylosidase (10 mg) was incubated in (NH₄)₂SO₄-NaOAc buffer (pH 5.0) at 25°C for 30 hr. and then after addition water, it was extracted to give a partial glycoside, m.p. 215°C. Hydrolysis of partial glycoside with 2N HCl gave an aglycone, m.p. 229-30°C characterized as abrectorin by chromatographic and spectral comparison with authentic sample.

**Anal. Calcd. for C₁₇H₂₄O₆ :** C, 64.9; H, 4.4
**Found :** C, 65.5; H, 4.6%

The methylated sugars were identified as 2,3,4-tri-O-methyl-D-xylose, m.p. 91-92°C (α)D + 18° (in water) and 2,3,4-tri-O-methyl-L-rhamnose syrup + 26° (in water). It gave a crystalline phenylhydrazide, m.p. 177°C.

**Quantitative estimation of sugar**

The anhydrous glycoside (35 mg) was hydrolysed by refluxing for two hours with 2% H₂SO₄. After cooling over night, the aglycone was filtered, washed, dried and weighed (15.6 mg). Thus ratio of the aglycone to the glycoside is 15.6:35 and this
ratio indicated the presence of two moles of sugar per mole of aglycone.

Somogyis copper micro method gave the value (1.67 cc) which also corresponds to two moles of sugar per mole of aglycone.
REFERENCES
REFERENCES


5. D.J. Crawford and T.J. Mabry, Biochem. Syst. Ecol., 6,
   189 (1978).

   Quim, 27, 175 (1982).

7. W. Rahman, Kh. Ishratullah, H. Wagner, O. Seligmann,
   V.M. Chari and B.G. Osterdahl, Phytochemistry, 17, 1064
   (1978).

8. A.G. Valesi, E. Rodriguez, G. Vander Velde and T.J. Mabry,
   Phytochemistry, 11, 2821 (1972).

9. V.M. Chari, R.J. Grayer-Barkmeijer, J.B. Harborne and


11. T.J. Mabry, K.R. Markham and M.B. Thomas, 'The Systematic


