METHYLATED FLAVONOIDs FROM 
ARTEMISIA MONOSPERMA

By

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ABSTRACT
Investigation of the flavonoid constituents of Artemisia monosperma resulted in
the isolation and identification of four methylated flavonoids: quercetin 3,3',4'-
trimethylether, diosmetin (luteolin 4'-methylether), 2',3,5'-trimethoxy-4',5,7-
trihydroxyflavone and 2',5'-dimethoxy-3,4',5,7-tetrahydroxyflavone.

INTRODUCTION
Artemisia species have been reported to contain several flavonoids, most of which
are methylated ones (Rodriguez et al., 1972; Segal et al., 1973; Hurabielle et al.,
1982; Bouzid et al., 1982; Belenovskaya et al., 1982; Li and Mabry, 1982). Previous
investigation of the flavonoid constituents of Artemisia monosperma revealed the
identification of several flavonoid glycosides and methylated flavonoids (Hafagy
et al., 1979; Saleh et al., 1985, 1987). In the present work other four methylated
flavonoids were isolated.

EXPERIMENTAL
Plant material: Artemisia monosperma Del. (Compositae) was collected from the
Western desert of Egypt (Cairo-Alexandria Road).

Extraction and Fractionation of the Flavonoids
About 3.5 kg of the air dried plant (leaves and flowers) were perculated with
ethanol. The alcoholic extract was concentrated in vacuo to 1.5 l, diluted with
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water and filtered. The filtrate was extracted with hexane (discarded), followed by chloroform (200 gm). About 100 gm of the residue were subjected to column chromatographic fractionation using silica gel. Elution was affected with chloroform ethyl acetate (50:50), collecting 200 ml fractions.

TLC: a- Silica gel G, developed with chloroform-methanol-formamide (80:19:1), or benzene-pyridine-formic acid (36:9:5); b- Polyamide, developed with n-butanol-acetic acid-water (6:2:1).

Flavonoid I (Quercetin 3,3',4'-trimethylether)
Fractions 5-36, gave yellow needles, m.p. 209-210°C (CHCl₃/MeOH). The NMR (DMSO-d₆ at 60 Hz) in δ-scale using TMS as internal standard showed proton signals at 4.0 ppm, (9 H), 3 (-OCH₃); 6.25 ppm, d (1 H), H-6; 6.42 ppm, d (1 H), H-8; 7.0 ppm, s (1 H), H-5; 7.63 ppm, m (2 H), H-2, H-6. MS showed M⁺ at m/e 244 (100); C₁₈H₁₆O₇, calculated: C, 62.50, H, 4.65; found: C, 62.48, H, 4.57%.

Flavonoid II (Diosmetin, luteolin 4'-methylether)
Fractions 39-41 gave yellow needles, m.p. 260-261°C (MeOH) (undepressed). The NMR spectrum agreed with that of diosmetin. MS showed M⁺ at m/e 300; C₁₆H₁₂O, calculated: C, 64.00, H, 4.00; found, C, 63.72, H, 4.15%. The flavonoid acetate (acetic anhydride/pyridine at 25°C for 2 days) melted at 196-199°C (MeOH/H₂O) (undepressed with authentic diosmetin triacetate).

Flavonoid III (2',3,5'-trimethoxy-4',5,7-trihydroxyflavone)
Fractions 42-46 afforded yellow needles, m.p. 271-272°C (MeOH). The NMR (DMSO-d₆) showed signals at 3.8 ppm, d (6 H), (2 -OCH₃); 3.93 ppm, s (3 H), (-OCH₃); 6.60 ppm, s (1 H), H-3; 6.97 ppm, s (1 H), H-6; 7.13 ppm, s (1 H), H-8; 7.48 ppm, (1 H), H-6. MS showed M⁺ at m/e 360 (55); C₁₈H₁₆O₇, calculated: C, 60.00, H, 4.44; found, C, 59.75, H, 4.25%.

Flavonoid IV (2',5'-Dimethoxy-3,4',5,7-tetrahydroxyflavone)
Fractions 47-52 afforded yellow needles, m.p. 291-292°C (MeOH). The NMR spectrum (DMSO-d₆) showed proton signals at 3.81 ppm, d (6 H), (2 -OCH₃); 6.62 ppm, s (1 H), H-6; 6.67 ppm, s (1 H), H-8; 7.07 ppm, s (1 H), H-3; 7.43 ppm, s (1 H), H-6. MS showed M⁺ at m/e 346 (100), C₁₇H₁₄O₈, calculated: C, 58.95, H, 4.04; found: C, 58.52, H, 3.93%.

The UV spectra of the four flavonoids are shown in Table 1.
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Table 1

UV Spectra of the isolated flavonoids

<table>
<thead>
<tr>
<th>Additions to methanol</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavonoid I</td>
</tr>
<tr>
<td></td>
<td>Band I</td>
</tr>
<tr>
<td>None</td>
<td>345</td>
</tr>
<tr>
<td>NaOMe</td>
<td>228</td>
</tr>
<tr>
<td>404 sh</td>
<td>262</td>
</tr>
<tr>
<td>AlCl(_3)</td>
<td>272</td>
</tr>
<tr>
<td>AlCl(_3)/HCl</td>
<td>366</td>
</tr>
<tr>
<td>NaOAc</td>
<td>408</td>
</tr>
<tr>
<td>NaOAc/H(_2)BO</td>
<td>347</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

TLC of the chloroform fraction revealed the presence of eight components, four of which have been isolated applying column chromatographic technique using silica gel G. The NMR spectrum of flavonoid I showed characteristic pattern of 3,3',4',5,7-pentasubstituted flavone, three of them are methoxyl groups assigned by proton signals at 4.0 ppm. Moreover, the presence of a low field signal at 12.35 ppm corresponds to hydrogen-bonded OH group at C-5. The MS showed fragments at 153 corresponding to the ring A and m/e at 167 corresponding to the ring B which proved that two of the three methoxyl groups are attached to ring B (C-3', C-4'). Fragments at m/e 329 (M\(^+\) - CH\(_3\)) and 301 (M\(^+\) - CH\(_3\) + CO) were also found. The UV spectra showed the presence of free OH groups at C-7 denoted by the bathochromic shift of band II with NaOAc and at C-5 (bathochromic shift of band I with AlCl\(_3\) and AlCl\(_3\)/HCl). This confirmed that the three -OCH\(_3\) groups must be located at C-3, C-3' and C-4'. These findings showed that the flavonoid I is quercetin 3,3',4'-trimethylether.

The identification of flavonoid II as diosmetin was proved by UV, TLC, NMR, MS and the preparation of its triacetate derivative. The 2',3,5'-trimethoxy-4',5,7-trihydroxyflavone (flavonoid III) was proved by UV, NMR and MS. The NMR
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displayed the characteristic pattern of 2',3,4',5,5',7-hexa-substituted flavone. Three of these substituents are present as -OCH₃ groups as assigned by proton signals at 3.8 ppm (OCH₃-2', OCH₃-5') and 3.93 (OCH₃-3). The MS spectrum showed a molecular ion M⁺ at m/e 360 corresponding to the molecular formula of trimethylether of a hexahydroxyflavone. Moreover, the fragmentation pattern showed m/e at 153 corresponding to ring A fragment and at 167 corresponding to the ring B fragment with three methoxyl groups located in this ring. The UV spectra revealed the presence of free -OH groups at C-5 and C-7.

The NMR of the flavonoid IV showed the same substitution pattern as flavonoid III but with only two of them are methoxyl groups (proton signal at 3.81 ppm), (OCH₃-2', OCH₃-5'). The MS showed M⁺ at 346 corresponding to a dimethylether of a hexahydroxy flavone. The fragmentation pathway undergoes the retro-Diels-Alder reaction with hydrogen transfer to ring A characteristic for highly substituted flavonols, which increases the stability of the molecular ion peak. This was confirmed by the presence of the molecular ion peak at the base peak. Moreover, the fragmentation pattern revealed that the two methoxyl groups must be located at ring B. The UV spectra revealed the presence of free -OH groups at C-3, C-5, C-7 and C-4' (Table 1). All these data showed that the flavonoid IV is probably 2',5'-dimethoxy-3,4',5,7-tetrahydroxyflavone.

REFERENCES


الفلافونيدات الميثيلية من نباتات العادر

شمس الدين امبابي إسماعيل ـ عبد الفتاح محمد زرق
فايزة محمد حموده ـ ماهند محمد حسن

تُتناول دراسة الفلافونيدات لنباتات العادر عن فصل وتعريف أربعة مركبات هي:
كيرستين 2,3,4,7-4,0,2-تراي ميثيل إثير، دايزومتين (ليثولين 4,0-ميثيل أثير)،
7,8-تراي ميثوكس – 4,1-تراي هيدروكس فلافون، 2,6-دای
ميثوكس – 7,0,1,4-7,0,1,4-تترابهيدروكس فلافون.