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METHYLATED FLAVONOIDS FROM *ARTEMISIA MONOSPERMA*

By

S.I. ISMAIL*, A.M. RIZK**, F.M. HAMMOUDA* and N.M. HASSAN*
*Pharmaceutical Sciences Department, National Research Centre, Dokki, Cairo, Egypt
**Chemistry Department, Faculty of Science, University of Qatar, Doha, Qatar

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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 (Khafagy *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

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 ($CHCl_3$/MeOH). The NMR (DMSO-d_6 at 60 Hz) in δ-scale using TMS as internal standard showed proton signals at 4.0 ppm, (9 H), 3 (-OCH_3); 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); $C_{18}H_{16}O_7$, 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; $C_{16}H_{12}O$, 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/H_2O) (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-d_6) showed signals at 3.8 ppm, d (6 H), (2, -OCH_3); 3.93 ppm, s (3 H), (-OCH_3); 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); $C_{18}H_{16}O_8$, 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-d_6) showed proton signals at 3.81 ppm, d (6 H), (2 -OCH_3); 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), $C_{17}H_{14}O_8$, 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.

Table 1

UV Spectra of the isolated flavonoids

Additions to methanol	λ max (nm)							
	Flavonoid I		Flavonoid II		Flavonoid III		Flavonoid IV	
	Band I	Band II	Band I	Band II	Band I	Band II	Band I	Band II
None	345	276	290 345 sh	242 251 s 268	371	264 310 sh	314 369 sh	263 288 sh
NaOMe	228 404 sh	272	303 390 sh	269	330 423 sh	270	318 409	273
$AlCl_3$	272	262 289 sh	269 361 sh 390	266 276 s	327 405 sh	275 295 sh	326 403	272 294 sh
$AlCl_3$/HCl	366	259 289 sh	294 355 sh 385	263 276 sh	325 402 sh	275 294 sh	324 401	270 292
NaOAc	408	284	385	280 323 sh	412	272	392	272 294 sh
NaOAC/H_3BO	347	275	349	252 278 sh	375	270	378	271

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 triaacetate derivative. The 2′,3,5′-trimethoxy-4′,5,7-trihydroxyflavone (flavonoid III) was proved by UV, NMR and MS. The NMR

showed the characteristic pattern of 2′,3,4′,5,5′,7-hexa-substituted flavone. Three of these substituents are present as -OCH_3 groups as assigned by proton signals at 3.8 ppm (OCH_3-2′, OCH_3 -5′) and 3.93 (OCH_3-3). 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), (OCH_3-2′, OCH_3-5′). The MS showed M^+ at 346 corresponding to a dimethlyether 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.

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الفلافونيـــدات الميثيليـــة من نبـــات العـادر

شمس الدين امبابي إسماعيل - عبد الفتـــاح محمد رزق
فايـــزة محمـــد حمـــوده و ناهـــد محمد حسـن

أسفرت دراسة الفلافونيدات لنبات العادر عن فصل وتعريف أربعة مركبات هي : كيرستين ٣,٣,١٤,١ - تراي ميثيل إثير ، دايزموتين (ليتيولين ٤١ - ميثيل أثير) ، ٢,٣,٥,١ - تراي ميثوكس - ٤,٥,٧,١ - تراي هيدروكس فلافون ، ٢١ و ٥١ - داي ميثوكس - ٣,٤,٥,٧,١ - تتراهيدروكس فلافون .