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TWO METHYLATED FLAVONES FROM *ARTEMISIA FRIGIDA*

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Key Word Index—*Artemisia frigida*; Compositae; Anthemideae; new flavones; 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone; 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone.

Abstract—Two new highly oxygenated flavones were isolated from *Artemisia frigida*. Their structures were determined by spectroscopic methods as 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone.

INTRODUCTION

Leaves of *Artemisia frigida* Willd. (Compositae) have similar mats of pubescence and glandular trichomes as do those of *A. ludoviciana* var. *ludoviciana* Nutt.; however, the former are not eaten by the grasshopper *Hypochlora alba* while the leaves of the latter provide the primary food source for this insect. In connection with an investigation of the chemical and physical bases of the feeding deterrents of *A. frigida* to phytophagous insects, a joint project with Professors H. Knutson and T. Hopkins, Kansas State University, two new flavone aglycones were isolated from this species. This paper reports their isolation and structure determination as 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**1**) and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone (**2**).

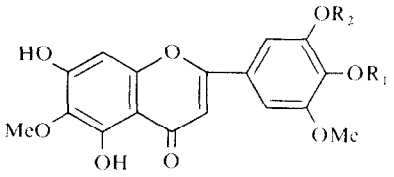
RESULTS AND DISCUSSION

Ground air-dried leaves and stems of *A. frigida* were extracted with aqueous methanol and the aqueous layer obtained after removal of the methanol was partitioned between hexane, chloroform and ethyl acetate. Chromatographic separation of the chloroform extract afforded **1** and **2**. The MS of **1** (mp 187–189°) exhibited a molecular ion at *m/e* 360 (100%) for $C_{18}H_{16}O_8$ in accord with a flavone containing three hydroxyl and three methoxyl groups (Table 1). Flavone **1** appeared as a purple fluorescent spot on a paper chromatogram under UV light and changed to yellow-green with ammonia, indicating the presence of free 5- and 4′-hydroxyl groups. When a cellulose TLC plate was sprayed with Naturstoffreagenz A (NA), the spot turned yellow indicating a 4′-hydroxyl but no *ortho*-dihydroxyl group in the B-ring. Compound **1** exhibited UV maxima in methanol at 348 ($\varepsilon = 33{,}300$) and 273 ($\varepsilon = 17{,}100$)/nm and the shifts obtained with diagnostic reagents (Table 2) suggested the presence of a methoxyl group at C-6 ($\Delta + 25$ nm for Band I in $AlCl_3$/HCl compared to Band I in MeOH) and hydroxyl groups at positions 5, 7 and 4′ [1]. The ^1H NMR spectrum of the TMSi ether of **1** (in CCl_4) exhibited a signal at δ 3.88 for two methoxyl groups and another singlet at 3.71 for a third methoxyl group. In addition, singlets were observed at δ 6.30 and 6.53 for H-3 and H-8, respectively, and a two-proton singlet appeared

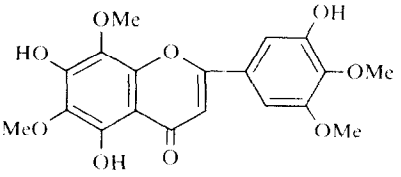
Table 1. MS data for flavones **1** and **2** from *Artemisia frigida**

Fragments	Compounds			
	Flavone **1**	PDM-Flavone **1**	Flavone **2**	PDM-Flavone **2**
$M^{+\cdot}$	360 (100)	411 (70)	360 (100)	411 (22)
$[M-H]^+$	359 (18)	410 (28)	359 (10)	410 (3)
$[M-Me]^+$	345 (75)	396 (100)	345 (68)	396 (100)
$[M-18]^{+\cdot}$	342 (64)	393 (22)	342 (57)	393 (4)
$[M-HCO]^+$	331 (8)	382 (21)	331 (7)	382 (2)
$[M-COMe]^+$	317 (51)	368 (36)	317 (39)	368 (1)
$[A_1-Me]^+$	167 (19)	201 (16)	167 (12)	201 (4)
$[A_1-MeCO]^+$	139 (22)	173 (28)	139 (16)	173 (9)
$[A_1-MeCO-CO]^+$	111 (10)	145 (13)	111 (4)	145 (3)
$B_1^{+\cdot}$	178 (8)	195 (10)	178 (3)	195 (1)
B_2^+	181 (5)	198 (10)	181 (3)	198 (2)

* MS were recorded at 70 eV, source temp. 200° and probe temp. from 50° to 425°. Values are given in *m/e* and the percentage abundance relative to the base peak is given in parentheses. The A_1, B_1 and B_2 terminology for the fragments is given in [6].



1 $R_1 = H$, $R_2 = Me$
2 $R_1 = Me$, $R_2 = H$



3 Scaposin

at 6.98 for H-2′ and H-6′. Since the UV and NMR data established the oxygenation pattern for **1** as well as the presence of hydroxyl groups at the 5, 7 and 4′ positions, the three methoxyl groups could be assigned to the other available sites, namely C-6, C-3′ and C-5′. The benzene-induced shifts of the methoxyl resonances for the TMSi ether of **1** supported these assignments; 6-OMe: δ 3.71 to 3.70, $\Delta = +0.01$ ppm, and 3′- and 5′-OMe's: δ 3.88 to 3.33, $\Delta = +0.55$ ppm, and 3.88 to 3.36, $\Delta = +0.52$ ppm [2].

The structure of **1** was confirmed by MS: a peak of 75% relative intensity was observed at *m/e* 345 ($M^+ - Me$), a result which is characteristic for 6-methoxy-flavones [3–4]; other fragments from both **1** and its PDM derivative established the presence of two methoxyls and one hydroxyl in the B-ring and two hydroxyls and one methoxyl in the A-ring (Table 1). These spectral findings established **1** to be 5,7,4′-trihydroxy-6,3′,5′-trimethoxy-flavone.

The MS of aglycone **2** (mp 243–245°) also gave a molecular ion at *m/e* 360 suggesting that it differed from **1** only in the location of the three hydroxyl and three methoxyl groups. Like compound **1**, **2** also appeared as a purple fluorescent spot on a paper chromatogram under UV light; however, in contrast to **1** this color was unchanged when treated with ammonia vapor or when the cellulose TLC plates were sprayed with NA. These findings indicated that **2** contained a 4′-methoxyl substituent. While Band II in the UV spectrum of **2** was similar to the Band II observed for **1** (274 nm with $\varepsilon = 12\,200$ for **2**), **2** differed from **1** by exhibiting a Band I previously observed for scaposin (**3**) [5], a flavone which contains a 3′-hydroxy-4′,5′-dimethoxy B-ring (Band I at 332 nm and $\varepsilon = 15\,200$ for **2** versus 330 nm and $\varepsilon = 15\,900$ for **3**). UV shifts with diagnostic reagents (Table 2) suggested that **2** contained hydroxyl groups at positions 5 and 7 and a methoxyl at C-6. Thus, these findings supported the structure assignment as shown in **2**. This structure was confirmed by ^{1}H NMR and MS. The ^{1}H NMR spectrum of the TMSi ether of **2** (in CCl_4) exhibited one-proton singlets at δ 6.28 and 6.53 for H-3 and H-8, respectively, and a two-proton singlet at 6.94 for H-2′ and

Table 2. UV data for flavones **1** and **2** from *Artemisia frigida**

Reagent	Flavone **1**		Flavone **2**	
	λ_{max} nm	RA†	λ_{max} nm	RA
MeOH	348	1.0	332	1.0
	273	0.5	274	0.9
NaOMe	419	1.0	372	1.0
	340	0.3	306	0.6
	276sh	0.3	270	1.2
	258	0.6		
$AlCl_3$	386	1.0	358	1.0
	304sh	0.3	298sh	0.6
	282	0.5	284	0.7
	256	0.4		
$AlCl_3$–HCl	373	1.0	353	1.0
	304sh	0.4	289	0.8
	282	0.5		
	253sh	0.5		
NaOAc	414	1.0	368	1.0
	326	0.4	300sh	0.8
	276sh	0.7	273	1.5
	260	0.8		
NaOAc–H_3BO_3	352	1.0	334	1.0
	272	0.8	274	0.9

* The UV spectra were recorded using standard procedures [1].

† The relative absorptivities (RA) are presented for the λ_{max}s obtained for each compound, using the longest wavelength peak as 1.0.

H-6′. A methoxyl signal at δ 3.72 could be assigned to the 6-OMe ($\Delta_{\delta CCl_4-\delta C_6D_6} = 0.01$ ppm) while signals at δ 3.80 ($\Delta = +0.19$) and 3.90 ($\Delta = +0.58$) were assigned to the 4′ and 5′ methoxyl groups, respectively [2]. A small upfield shift for a C-4′ methoxyl group flanked by two vincinal oxygenated substituents has been previously observed (Sakakibara, M. and Mabry, T. J., unpublished results). In addition to the molecular ion at *m/e* 360 (100%), a strong peak at *m/e* 345 ($M^+ - Me$, 68%) supported the presence of a 6-OMe group. Other MS data for **2** and its PDM derivative supported the structural assignment (Table 1). The spectral data established the structure of **2** to be 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone.

EXPERIMENTAL

Mps are uncorr. Column chromatography employed Polyclar AT (GAF Corp.) and Sephadex LH-20 (Pharmacia). **1** and **2** were purified over Sephadex LH-20 using MeOH for elution.

Extraction and isolation. Ground, dried leaves and stems (1.58 kg) of *Artemisia frigida* Willd. (collected by Dr. Greg Mulkem, near Fargo, N. D. Aug. 18, 1979; voucher specimen G. M.–R. K. No. 1 is deposited in the Lundell Herbarium, The Univ. of Texas at Austin) were extracted with 85% aq. MeOH (10 l. × 3) and 50% aq. MeOH (10 l. × 2). The extracts were combined and evapd. under red. pres. until only H_2O remained. The ppt. obtained on standing the concentrate in the cold for 2 days was filtered off and the aq. layer partitioned with *n*-hexane (1.5 l. × 4), $CHCl_3$ (1.2 l. × 8) and EtOAc (1.2 l. × 15).

The $CHCl_3$ extract (25 g) was chromatographed over a polyclar column (8 × 50 cm) packed in C_6H_6–MeOH (95:5) and eluted with C_6H_6–MeOH in increasingly polar ratios (19:1, 9:1, 17:3, 3:1, 7:3, 3:2 and 1:1). Fractions were collected by monitoring in UV light; **1** and **2** were obtained from bands 5 and 6, respectively. After their purification over Sephadex LH-20, both compounds were recryst. from MeOH to give fine yellow crystals. **1** had R_f values (Whatman No. 1 paper) of 0.58 (TBA) and 0.06 (15% HOAc) while **2** had 0.78 (TBA) and 0.13 (15% HOAc).

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