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FLAVONOIDS FROM *ARTEMISIA FRIGIDA*

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Key Word Index—*Artemisia frigida*; Compositae; 6-methoxyflavone methyl ethers; flavones; flavone methyl ethers; 6-methoxyflavonol methyl ether.

Abstract—Twelve flavonoids including a new flavone were isolated from *Artemisia frigida*. The structure of the new highly oxygenated flavone was determined by spectroscopic methods as 5,7,3′,4′-tetrahydroxy-6,5′-dimethoxyflavone. The known compounds are quercetagetin 3,6,3′,4′-tetramethyl ether, eupatilin, jaceosidin, hispidulin, eupafolin, luteolin 3′,4′-dimethyl ether, tricin, chrysoeriol, apigenin, luteolin and luteolin 7-glucoside.

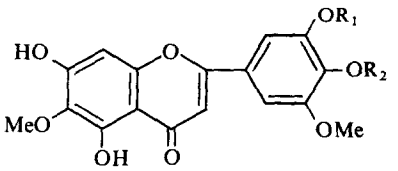
INTRODUCTION

We previously reported [1] the isolation of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**1**) and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone (**2**) from *Artemisia frigida* Willd. (Compositae). The present paper describes the isolation of twelve additional flavonoids, including a new highly oxygenated flavone from the same species.

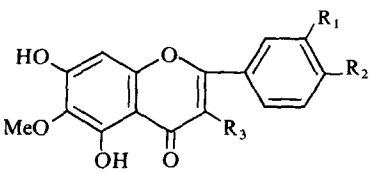
RESULTS AND DISCUSSION

Chromatographic separation of the chloroform and ethyl acetate extracts of a concentrated aqueous methanol extract of *Artemisia frigida* afforded in addition to **1**, **2** and the new flavone **3**, eleven known compounds: quercetagetin 3,6,3′,4′-tetramethyl ether (**4**), eupatilin (**5**), jaceosidin (**6**), hispidulin (**7**), eupafolin (**8**), luteolin 3′,4′-dimethyl ether, tricin, chrysoeriol, apigenin, luteolin and luteolin 7-glucoside [4]. Since we have previously reported **1** and **2** [1], here we present only the detailed data for the characterization of **3**.

The MS of **3** exhibited a molecular ion peak at *m/z* 346 (100%) for $C_{17}H_{14}O_8$ in accord with a flavone containing four hydroxyl and two methoxyl groups (Table 2). **3** appeared as a purple fluorescent spot on a paper chromatogram in UV light, changing to yellow with ammonia indicating the presence of free 5- and 4′-hydroxyl groups. **3** also gave an orange-yellow colour with NA indicating an *ortho*-dihydroxyl group in the B-ring. UV maxima in methanol at 352 and 273 nm and the shifts obtained with diagnostic reagents (Table 3) suggested the presence of a methoxyl group at C-6 (Δ + 20 nm for Band I in $AlCl_3$/HCl compared to Band I in MeOH) and hydroxyl groups at positions 5, 7, 3′ and 4′ [2]. The ^{1}H NMR spectrum of the TMSi ether of **3** (in CCl_4) exhibited two singlets at δ 3.71 and 3.87 for two methoxyl groups. In addition, singlets at δ 6.34 and 6.53 could be assigned to H-3 and H-8, respectively, and a two-proton singlet appeared at δ 6.97, which is characteristic for H-2′ and H-6′ in a 3′,4′,5′-trisubstituted B-ring (Table 4). Since the UV and NMR data established the



1 R_1 = Me; R_2 = H
2 R_1 = H; R_2 = Me
3 R_1 = R_2 = H



4 R_1 = R_2 = R_3 = OMe
5 R_1 = R_2 = OMe; R_3 = H
6 R_1 = OMe; R_2 = OH; R_3 = H
7 R_1 = R_3 = H; R_2 = OH
8 R_1 = R_2 = OH; R_3 = H

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Table 1. Chromatographic data* for flavonoids from *Artemisia frigida*

Flavonoid	($R_f \times 100$) in Cellulose 15% HOAc	Cellulose 40% HOAc	Cellulose TBA	Cellulose BAW	Polyamide BMM	Polyamide BPMM	Silica gel CAA	Silica gel BPA	UV (366)†	Color in UV/NH_3 (366)†	UV/NA (254)†
5,7,4′-Trihydroxy-6,3′,5′-trimethoxyflavone (**1**)	6	34	64	77	89	35	76	52	p	y	p-br
5,7,3′-Trihydroxy-6,4′,5′-trimethoxyflavone (**2**)	15	56	86	91	88	42	79	59	p	p	p
5,7,3′,4′-Tetrahydroxy-6,5′-dimethoxyflavone (**3**)	3	25	53	71	71	8	50	21	p	y	or -y
Quercetagetin 3,6,3′,4′-tetramethyl ether (**4**)	13	63	85	92	94	85	87	78	p	p	p
Eupatilin (**5**)	6	46	81	90	92	77	87	73	p	p	p
Jaceosidin (**6**)	9	41	76	84	84	32	76	56	p	y	p
Hispidulin (**7**)	9	45	82	90	75	17	72	54	p	y	ol-y
Eupafolin (**8**)	5	31	61	80	60	7	49	22	p	y	or -y
Luteolin 3′,4′-dimethyl ether	4	34	78	87	79	47	85	68	p	p	br

* TLC using cellulose, polyamide MN Polygram and Si gel GF 254 plates; solvent systems are described in the Experimental.

† UV, long wavelength 366 nm; short wavelength 254 nm. p = purple, y = yellow, ol = olive, or = orange, br = brown.

Table 2. MS data for flavonoid aglycones from *Artemisia frigida**

Flavonoid	M+	[M−H]	$[M-Me]^+$	$[M-18]^+$	$[M-HCO]^+$	$[M-COMe]^+$	$[A_1-Me]^+$	$[A_1-MeCO]^+$	$[A_1-MeCO-CO]^+$	$[B_1]^+$	$[B_2]^+$	$[A_1+H]^+$
5,7,3′,4′-Tetrahydroxy-6,5′-dimethoxyflavone (**3**)	346	345	331	328	317	303	167	139	111	164	167	
	(100)	(19)	(72)	(62)	(9)	(44)	(16)	(15)	(14)	(8)	(16)	
Quercetagetin 3,6,3′,4′-tetramethyl ether (**4**)	374	373	359	356	345	331	167	139	111	162	165	
	(100)	(31)	(69)	(27)	(3)	(50)	(3)	(3)	(2)	(2)	(11)	
Eupatilin (**5**)	344	343	329	326	315	301	167	139	111	162	165	
	(100)	(8)	(70)	(59)	(10)	(52)	(14)	(22)	(5)	(6)	(6)	
Jaceosidin (**6**)	330	329	315	312	301	287	167	139	111	148	151	
	(100)	(8)	(63)	(53)	(8)	(47)	(19)	(24)	(3)	(8)	(8)	
Hispidulin (**7**)	300	299	285	282	271	257	167	139	111	118	121	
	(100)	(14)	(68)	(61)	(10)	(71)	(17)	(16)	(1)	(7)	(5)	
Eupafolin (**8**)	316	315	301	298	287	273	167	139	111	134	137	
	(100)	(14)	(76)	(72)	(13)	(81)	(17)	(23)	(2)	(13)	(6)	
Luteolin 3′,4′-dimethyl ether	314	313	299	296	285	271				162	165	153
	(100)	(24)	(90)	(8)	(23)	(40)				(2)	(3)	(45)

* MS were recorded at 70 eV, source temp. 200° and probe temp. from 50 to 425°. Values are given in m/z and in parentheses the % abundance relative to the base peak. The A_1, B_1 and B_2 terminology for the fragments is given in [4].

Table 3. UV data for flavonoids from *Artemisia frigida**

Flavonoid	MeOH (λ_{max}, nm)		NaOMe (λ_{max}, nm)		$AlCl_3$ (λ_{max}, nm)		$AlCl_3$/HCl (λ_{max}, nm)		NaOAc (λ_{max}, nm)		NaOAc/H_3BO_3 (λ_{max}, nm)	
5,7,3′,4′-Tetrahydroxy-6,5′-dimethoxy-flavone (**3**)	352,	273	410, 276sh,	336, 256	432, 304sh, 272	356sh, 280,	372, 280	300sh,	406, 263	328sh,	436sh, 264sh	373,
Quercetagetin 3,6,3′,4′-tetramethyl ether (**4**)	346, 254	272,	374, 294,	310, 273	371, 279,	297sh, 262	402sh, 281,	365, 261	375, 274	310,	348, 254sh	271,
Eupatilin (**5**)	340, 238	272,	371, 275	310,	366, 258	282,	361, 256	288,	369, 273	309,	341,	272
Jaceosidin (**6**)	345,	273	408, 276sh,	336, 256	374, 281,	296sh, 259	366, 259	286,	401, 274	324sh,	348,	273
Hispidulin (**7**)	335,	274	394, 276	326,	363, 282sh,	302, 264sh	356, 284sh,	300, 262sh	389, 306,	329, 274	340,	272
Eupafolin (**8**)	346,	269	401, 264	336,	424, 304sh,	340sh, 273	365, 278,	296sh, 258	399, 268	335sh,	428sh, 262	372,
Luteolin 3′,4′-dimethyl ether	341, 250sh,	269, 238	370, 276	312,	382sh, 293sh, 260	357, 277,	381sh, 292sh, 257	352, 277,	367, 276	313,	343,	268

* All UV spectra were recorded using standard procedures [2].

Table 4. ^{1}H NMR data for TMSi ethers of flavonoids from *Artemisia frigida*.*

TMSi ethers of flavonoids	H-3	H-6	H-8	H-2′	H-3′	H-5′	H-6′	—OMe† CCl_4 3	CCl_4 6	CCl_4 3′	CCl_4 4′	CCl_4 5′	C_6D_6 3	C_6D_6 6	C_6D_6 3′	C_6D_6 4′	C_6D_6 5′
3	6.34 *s*		6.53 *s*	6.97 *s*			6.97 *s*		3.71 *s*			3.87 *s*		3.62 *s* Δ = +0.09			3.30 *s* Δ = +0.57
4			6.48 *s*	7.53 *d* (2.5)		6.82 *d* (9.0)	7.53 *dd* (2.5, 9.0)	3.79 *s*	3.70 *s*	3.86 *s*	3.86 *s*		3.86 *s* Δ = −0.06	3.65 *s* Δ = +0.05	3.53 *s* Δ = +0.33	3.42 *s* Δ = +0.44	
5	6.27 *s*		6.50 *s*	7.30 *m*		6.77 *d* (9.0)	7.30 *m*		3.70 *s*	3.84 *s*	3.86 *s*			3.63 *s* Δ = +0.07	3.38 *s* Δ = +0.46	3.35 *s* Δ = +0.51	
6	6.32 *s*		6.54 *s*	7.34 *m*		6.85 *d* (9.0)	7.34 *m*		3.73 *s*	3.91 *s*				3.64 *s* Δ = +0.09	3.29 *s* Δ = +0.62		
7	6.36 *s*		6.55 *s*	7.73 *d* (9.0)	6.85 *d* (9.0)	6.85 *d* (9.0)	7.73 *d* (9.0)		3.75 *s*					3.66 *s* Δ = +0.09			
8	6.27 *s*		6.50 *s*	7.35 *m*		6.85 *d* (9.0)	7.35 *m*		3.72 *s*					3.65 *s* Δ = +0.07			

* Spectra were recorded in CCl_4 and C_6D_6. Values are given in ppm (δ-scale) relative to TMS as an internal standard. Numbers in parentheses denote coupling constants in Hz. Signals are indicated as follows: *s* = singlet; *d* = doublet; *dd* = double doublet; *m* = multiplet.

† Some OMe signal assignments may need to be interchanged.

oxygenation pattern of **3**, the presence of hydroxyl groups at the 5, 7, 3′ and 4′ positions and one methoxyl group at C-6, the second methoxyl must be assigned to the only available position, namely C-5′. The benzene-induced shifts of the methoxyl resonances for the TMSi ether of **3** supported these assignments: 6-OMe, δ 3.71 to 3.62, $\Delta + 0.09$ ppm; and 5′-OMe, δ 3.87 to 3.30, $\Delta = +0.57$ ppm [3]. The MS of **3** exhibited a strong fragment peak at *m/z* 331 (72%) typical for 6-methoxyflavones. Other fragments from **3** established the presence of two hydroxyls and one methoxyl in both the A- and B-rings. These spectral data established the structure of **3** as 5,7,3′,4′-tetrahydroxy-6,5′-dimethoxyflavone.

Eupatilin (**5**) showed similar UV spectra and had the same R_f value (cellulose TLC developed with 40% HOAc) as eupatorin (5,3′-dihydroxy-6,7,4′-trimethoxyflavone) but comparison of the ^{1}H NMR spectra of **5** (as the TMSi ether) measured in CCl_4 and C_6D_6 (Table 4) did not unequivocally locate the methoxyl groups. However, the ^{1}H NMR spectrum of the acetate of **5** (in $CDCl_3$) gave the proton signal of H-8 at δ 7.26 indicating a downfield shift of 0.76 ppm, suggesting that the C-8 proton was affected by acetyl groups at both C-5 and C-7 (*meta*-acetate gives about 0.15 ppm shift while a *para*-acetate gives *ca* 0.5 ppm shift) [5–8]. Furthermore, MS of **5** gave the B_1 and B_2 fragments at *m/z* 162 and 165, respectively.

Quercetagetin 3,6,3′,4′-tetramethyl ether (**4**), jaceosidin (**6**), hispidulin (**7**), eupafolin (**8**), tricin and chrysoeriol were identified by UV, ^{1}H NMR, MS and, except for **4** and **8**, co-chromatography with authentic samples (Tables 1–4). Luteolin, apigenin and luteolin 3′,4′-dimethyl ether were identified by UV, MS and co-chromatography with authentic samples. The identity of luteolin 7-glucoside was determined by UV, NMR, co-chromatography and acid hydrolysis.

EXPERIMENTAL

Plant material. The aerial parts of *A. frigida* were collected by Dr. Greg Mulkem, near Fargo, N. D. 18 August 1979. Voucher specimen G.M.–R.K. No. 1 is deposited in the Lundell Herbarium, The University of Texas at Austin.

General techniques. Mps are uncorr. Column chromatography employed Polyclar AT (GAF) and Sephadex LH-20 (Pharmacia). Precoated cellulose plates (E. Merck), polyamide, Polygram, Polyamide-6 (Macherey-Nagel) and Si gel 60 GF-254 (E. Merck) were used for TLC. The solvent systems were: TBA (*t*-BuOH–HOAc–H_2O, 3:1:1); BAW, (*n*-BuOH–HOAc–H_2O, 4:1:5 upper layer); BMM (C_6H_6–MeCOEt–MeOH, 4:3:3); BPMM [C_6H_6–petrol (65–110°)–MeCOEt–MeOH, 60:26:7:7]; CAA ($CHCl_3$–Me_2CO–HCO_2H, 9:2:1) and BPA (C_6H_6–pyridine–HCO_2H, 36:9:5). All flavonoids were purified over Sephadex LH-20 using MeOH before spectral analyses by standard procedures [2, 4]. Flavonoids were visualized either by UV light + NH_3 or by spraying with NA (Naturstoffreagenz-A) in MeOH.

Isolation of flavonoids. Ground, dried leaves and stems (1.58 kg) were extracted with 85% aq. MeOH (10 l. × 3) and 50% aq. MeOH (10 l. × 2). The combined extracts were evapd under red. pres. until only H_2O remained. The ppt. obtained on standing in the cold for 2 days was removed 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 conc $CHCl_3$ extract (25 g) was chromatographed over a polyclar column using a mixture of C_6H_6–MeOH with an increasing ratio of MeOH. Thirteen fractions were collected by monitoring the elution in UV light. The conc EtOAc extract (21 g) was chromatographed over a polyclar column (500 g) packed in MeOH–MeCOEt–Me_2CO–H_2O (3:3:1:13) and eluted with the same solvent system. Several unidentified *C*-glycosylflavonoids were isolated. After 19 fractions were collected (each 500 ml), five well-separated bands appeared on the Polyclar column. The absorbent was mechanically separated into 5 parts giving bands 14–18.

Identification of the isolated flavonoids. Quercetagetin 3,6,3′,4′-tetramethyl ether (**4**), from band 1, was recryst. from MeOH, yield 12 mg, as yellow needles, mp 157–158° (lit. [9] 152–153° or 158–160°). It was identical by UV, MS and ^{1}H NMR with **4** [9] (Tables 1–4).

Eupatilin (**5**), from band 2, was recryst. from MeOH, yield 596 mg, as yellow rhombohedral plates, mp 240–242° (lit. [10, 11] 240–241.5° or 234–236°). ^{1}H NMR of the acetate of **5**: δ 2.37 (*s*, 3 H, C_7–OAc), 2.48 (*s*, 3 H, C_5-OAc), 3.86 (*s*, 3 H, C_6-OMe), 3.93 (*s*, 6 H, $C_{3',4'}$-OMe), 6.55 (*s*, 1 H, C_3-H), 6.94 (*d*, 1 H, $C_{5'}$-H, $J = 9.0$ Hz), 7.26 (*s*, 1 H, C_8-H), 7.26–7.51 (*m*, 2 H, $C_{2',6'}$-H). For UV, ^{1}H NMR and MS see Tables 2–4.

5,7,3′,4′-Tetrahydroxy-6,5′-dimethoxyflavone (**3**), from band 12, was recryst. from MeOH, yield 15 mg, as yellow needles. For UV, MS, ^{1}H NMR and R_f values see Tables 1–4.

Jaceosidin (**6**), from band 7, was recryst. from MeOH, yield 149 mg, as pale yellow needles, mp 220–222° (lit. [12] 219–221°).

Hispidulin (**7**), from band 9 ($CHCl_3$ extract) and band 17 (EtOAc extract), was recryst. from MeOH, yield 299 mg, as pale yellow needles, mp 289–291° (lit. [10] 288–289°, [13] 289–290°, [14] 291–292°).

Eupafolin (**8**), from band 13 ($CHCl_3$ extract) and band 15 (EtOAc extract), was recryst. from 80% MeOH, yield 22 mg, as pale yellow needles, mp 260–262° (lit. [15] 262–264° and [16] 258–262°). It was identical by UV, MS and ^{1}H NMR with **8** [10].

Luteolin 3′,4′-dimethyl ether was obtained from band 4 as a mixture with two other flavonoids. The mixture was separated over a Sephadex LH-20 column eluting with MeOH. Twelve fractions were collected. Fraction 11 appeared as one flavonoid which recryst. MeOH as pale yellow needles. From the UV, MS and color data, it was found to be luteolin 3′,4′-dimethyl ether [2]. Fractions 9 and 10 were combined and separated on Whatman 3MM paper to give luteolin 3′,4′-dimethyl ether along with another compound. The latter exhibited the following UV spectral data: λ_{max} nm: 333, 273 (MeOH); 387, 302 sh, 275 (NaOMe); 358, 300, 284, 262 sh ($AlCl_3$); 353, 298, 284, 260 sh ($AlCl_3$/HCl), 388, 272 (NaOAc) and 337, 272 (NaOAc/H_3BO_3). On the basis of these data, it is tentatively assigned a 5,4′-dihydroxy-6,7-dimethoxyflavone structure.

Tricin from band 8 (yield 26 mg), chrysoeriol from band 10 (yield 18 mg), apigenin from band 11 (yield 6 mg), luteolin from band 14, EtOAc extract (yield 6 mg), and luteolin 7-glucoside from band 18, EtOAc extract (yield 26 mg), were identified by standard procedures.

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