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6-METHOXYFLAVONOIDS FROM *BRICKELLIA LACINIATA* (COMPOSITAE)

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Key Word Index—*Brickellia laciniata*; Compositae; Eupatorieae; 6-methoxyflavonol methyl ethers; glycosides; sulfates; 6-methoxyflavone methyl ethers; flavone methyl ethers.

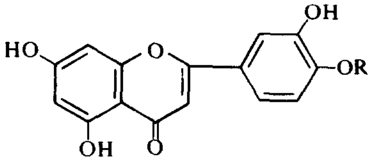
Abstract—One new and fourteen known flavonoids, including thirteen containing 6-methoxy groups, were isolated from *Brickellia laciniata*. The new flavonol is quercetagetin 6,4′-dimethyl ether. Among the known compounds identified were the 4′-methyl and 7,4′-dimethyl ethers of eupafolin and luteolin 4′-methyl ether, and the flavonols: patuletin, spinacetin, eupatolitin, eupatin, centaureidin, casticin, patuletin 3-glucoside and 3-galactoside, eupatolitin 3-galactoside, patuletin 3-SO_3K and eupatin 3-$SO_3Ca_{1/2}$.

INTRODUCTION

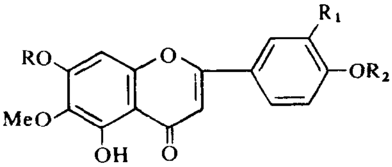
In continuation of the phytochemical investigations of the genus *Brickellia* [1], we now report the isolation and characterization of fifteen flavonoids from the leaves of *Brickellia laciniata* Gray. One of the flavonoids, quercetagetin 6,4′-dimethyl ether (laciniatin), is a new natural product.

RESULTS

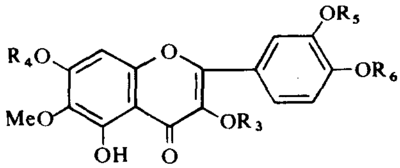
Leaves of *Brickellia laciniata* were extracted with aqueous methanol and the concentrated syrup partitioned between ethyl acetate and water. A heavy precipitate of sulfated flavonoids obtained from the water layer was analysed separately.



Diosmetin (1) R = Me



Desmethoxycentaureidin (2) R = H; R_1 = OH; R_2 = Me
Eupatorin (3) R, R_2 = Me; R_1 = OH



Patuletin (4) R_3, R_4, R_5, R_6 = H
Spinacetin (5) R_3, R_4, R_6 = H; R_5 = Me
Laciniatin (6) R_3, R_4, R_5 = H; R_6 = Me
Eupatolitin (7) R_3, R_5, R_6 = H; R_4 = Me
Eupatin (8) R_3, R_5 = H; R_4, R_6 = Me
Centaureidin (9) R_4, R_5 = H; R_3, R_6 = Me
Casticin (10) R_5 = H; R_3, R_4, R_6 = Me
Patuletin 3-glu (12) R_3 = glc; R_4, R_5, R_6 = H
Patuletin 3-gal (13) R_3 = gal; R_4, R_5, R_6 = H
Eupatolitin 3-gal (14) R_3 = gal; R_4 = Me; R_5, R_6 = H
Patuletin 3-SO_3K (15) R_3 = SO_3K; R_4, R_5, R_6 = H
Eupatin 3-$SO_3Ca_{1/2}$ (16) R_3 = $SO_3Ca_{1/2}$; R_4, R_6 = Me; R_5 = H

The aglycones found in the EtOAc layer were: luteolin 4′-methyl ether (**1**) [2, 3] (diosmetin); 6-methoxyluteolin 4′-methyl ether (**2**) [1, 2] (desmethoxycentaureidin); 6-methoxyluteolin 7,4′-dimethyl ether (**3**) [2] (eupatorin); quercetagetin 6,3′-dimethyl ether (**5**) [1, 2] (spinacetin); quercetagetin 6,4′-dimethyl ether (**6**) (laciniatin); quercetagetin 6,7,4′-trimethyl ether (**8**) [1, 2] (eupatin); quercetagetin 6,3,4′-trimethyl ether (**9**) [2] (centaureidin) and quercetagetin 6,3,7,4′-tetramethyl ether (**10**) [2] (casticin). The four glycosides isolated from the EtOAc layer were an incompletely identified derivative of eupalitin (6-methoxykaempferol 7-methyl ether) galactoside (**11**), patuletin (6-methoxyquercetin) 3-glucoside (**12**) and 3-galactoside (**13**) [1, 2] and eupatolitin (6-methoxyquercetin 7-methyl ether) 3-galactoside (**14**) [1, 2].

The water layer yielded 6-methoxyquercetin (**4**) [1, 2] (patuletin), 6-methoxyquercetin 7-methyl ether (**7**) [1, 2] (eupatolitin) and eupatolitin 3-galactoside (**14**). The yellow precipitate (2 g) which formed in the water layer yielded patuletin 3-SO_3K (**15**) and eupatin 3-$SO_3Ca_{1/2}$ (**16**) [1].

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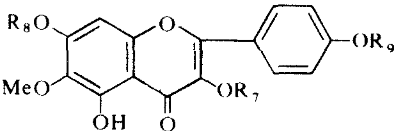

Eupalitin 3-gal der (**11**) R_7 = acyl(?)-gal; R_8 = Me; R_9 = H
Eupalitin R_8 = Me; R_7, R_9 = H

Quercetagetin 6,4′-dimethyl ether (**6**) (*laciniatin*)

The new 6-methoxyflavonoid was isolated as pale yellow crystals (from the EtOAc layer), mp 255°; λ_{max} (MeOH): 368, 272sh, 256 nm. The MS of **6** gave a molecular ion at *m/z* 346 ($C_{17}H_{14}O_8$) in accord with a flavone containing four hydroxyl and two methoxyl groups. The M − Me ion at *m/z* 331 (48 %) suggested a 6-methoxyl group and the A_1 − Me ion at 167 (11 %) and B_2 ion at *m/z* 151 (42 %) were in agreement with a flavonol containing one methoxyl group in both the A and B rings. The MS of the PDM derivative confirmed the presence of a 6-methoxyl group (M − Me as base peak at *m/z* 399) and that the A ring in the natural product also contained two hydroxyl groups (A_1 − Me at 201; 17 %) and that the B ring contained one hydroxyl and one methoxyl group (B_2 at *m/z* 168; 34 %).

The ^{1}H NMR spectrum of the trimethylsilyl ether of **6** in CCl_4 confirmed that it was a flavonol dimethyl ether: two methoxyl signals at δ 3.72 and 3.87; other signals established a 6-oxygenated quercetin derivative: a sharp singlet at δ 6.54 for H-8, a doublet (J = 9 Hz) at 6.84 for H-5′, a double doublet (J = 2.5 and 9.0 Hz) at 7.70 for H-6′ and a doublet (J = 2.5 Hz) at 7.60 for H-2′. That the H-6′ signal appeared downfield from the one for H-2′ is in accord with the presence of a C-4′ methoxyl group rather than one at the C-3′ position [3].

These spectral data indicated that the new flavonol is a 6-methoxyquercetin derivative containing a second methyl ether function at the 4′ position. That the compound contained 6- and 4′-methoxyl groups was further supported by the ^{1}H NMR spectrum of the trimethylsilyl ether of the compound in benzene-d_6: the upfield shifts of the methoxyl signals to 3.62 (+0.10 ppm) and 3.32 (+0.55 ppm) are in agreement with C-6 and C-4′ methoxyl groups, respectively [4]. The yellow fluorescence of **6** in UV light and the UV spectral shift with $AlCl_3$ in the presence of HCl (Δ + 62 nm relative to band I in MeOH) suggested a free C-3 hydroxyl group; 4′-substitution was indicated by a decrease in the intensity of band I with the addition of alkali. These data established that the new flavonol is quercetagetin 6,4′-dimethyl ether (**6**).

EXPERIMENTAL

Plant material. Leaves and vouchers of *B. laciniata* were collected 14 miles north of Alpine, Jeff Davis County, Texas (a voucher specimen, A. M. Powell and J. F. Weedin 3175, is deposited in the Herbarium, Sul Ross University, Alpine, Texas). The plant material was air-dried prior to extraction.

General techniques. Column chromatography employed polyamide, Polyclar AT (GAF Corp), Si gel 60, 70–230 mesh, (Merck), Sephadex LH-20 and Sephadex G-10 (Pharmacia); PC and electrophoresis were carried out on Whatman 3MM paper. Precoated cellulose plates (Merck); polyamide, Polygram, Polyamid-6 (Macherey-Nagel and Co.) and Si gel 60 GF-254 (Merck) were used for TLC. PLC was carried out with Si gel G (type 60) plates without fluorescent indicator (Merck). The solvents used were: BPMM (C_6H_6–petrol (65–110°)–MeCOEt–MeOH, 60:26:7:7); BMM (C_6H_6–MeCOEt–MeOH, 4:3:3); CAA ($CHCl_3$–Me_2CO–HCOOH, 9:2:1); BPA (C_6H_6–Py–HCOOH, 36:9:5); TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and *n*-BAW, upper layer (*n*-BuOH–HOAc–H_2O, 4:1:5). The flavonoids were visualized either by UV light + NH_3 or by spraying with NA (Naturstoffreagenz A Carl Roth, Germany) in MeOH. Hydrolyses were carried out with 0.1 N TFA on a steam cone for 30 min for the sulfated flavonoids and 50 min for the flavonoid glycosides. Hydrolysis of sulfated flavonoids was carried out with Sulfatase from limpets, type V, 1000 units (Sigma 8629) at 37° for 3 hr. All the hydrolysates were analysed for sugars and sulfates (with $BaCl_2$). Mps were uncorr. The analysis of Ca and K was carried out on a Perkin–Elmer 306 spectrophotometer. All the flavonoids were purified prior to spectral analyses by standard procedures [2, 3] over Sephadex LH-20: using MeOH for flavonoid aglycones or 80 % aq. MeOH for the glycosides and sulfates.

Chromatographic, UV, MS and NMR data are presented in Tables 1–4 for those compounds for which spectral data have not been previously reported [1].

Extraction, purification and identification of flavonoids from Brickellia laciniata. Ground leaf material of *Brickellia laciniata* (468 g) was extracted × 6 with 6 l. 80 % and 6 l. 50 % aq. MeOH (until the extract was colorless). The combined extracts were evapd until only H_2O remained and extrd with 8 l. EtOAc. The

Table 1. Chromatographic data (R_fs × 100 and colors) for flavonoids from *Brickellia laciniata**

	Cellulose				Polyamide		Silica gel		Colors†		
	HOAc										
Compound	15 %	40 %	TBA	*n*-BAW	BMM	BPMM	CAA	BPA	UV (350)	UV/NH_3 (350)	UV/NA (254)
Diosmetin (**1**)	3	32	71	87	50	4	50	45	p	p	ol-y
Eupatorin (**3**)	9	56	85	86	84	55	58	50	p	p	w ol-y
Laciniatin (**6**)	6	31	50	77	61	12	51	48	y	y	y
Centaureidin (**9**)	24	63	79	90	93	27		75	p	p	p
Casticin (**10**)	17	69	91	92	86	65	60	55	p	p	w ol-y
Eupalitin 3-acyl(?)-gal (**11**)	71	80	80	81	93	3	13	21	p	g	p
Eupalitin	11	41	81	85					y	y	y

* TLC data on cellulose microcrystalline Merck; polyamide MN (polygram) and silica gel GF 254 Merck; for solvent key, see Experimental.

†Color key: p = purple; y = yellow; ol = olive; g = green; w = weak. NA refers to Naturstoffreagenz A in MeOH.

Table 2. UV data (λ_{max}, nm) for flavonoids from *Brickellia laciniata*

	MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+H_3BO_3
Diosmetin (**1**)	342, (290), 266, 248, (240)	380, (304), 268, (230)	384, 360, 294, (274), 260	380, 352, (290), 274, 258	368, 320, 274	342, 266
Eupatorin (**3**)	338, 272, (250), 240	376, 326, 266, (240)	370, (290), 284, 260	364, 282, 258	336, 268	338, 272, (250)
Laciniatin (**6**)	368, (272), 256	410, 272	428, (368), 264	430, (368), 262	386, (275), 264	368, (272), 256
Centaureidin (**9**)	348, (266), 254	386, (304), 270	377, (304), 264	366, (304), 264	374, (298), 270	350, (268), 254
Casticin (**10**)	346, (270), 256	384, 338, (290), 268	378, (302), (278), 266	366, (278), 264	346, (268), 256	346, (266), 254
Eupalitin 3-acyl(?)-gal (**11**)	340, 268	398, 272	368, (300), 270	360, (308), 265	(396), 350, 268	342, 266
Eupalitin	355, (260), 256	455, (375), 272	410, (375), 264	410, (370), 260	(415), 356, (270), 264	358, (280) 265

H_2O layer yielded a yellow ppt. (2 g) which was analysed separately.

EtOAc *extract*. The EtOAc extract (20 g) was chromatographed over 3 polyamide columns (7.5 × 100 cm, 500 g each) and eluted with (1) $CHCl_3$–MeOH–MeCOEt-2,4-pentanedione (20:10:5:1) and (2) $CHCl_3$–MeOH–MeCOEt–2,4-pentanedione (10:10:5:1). The polarity of the solvent was increased until the column was finally eluted with MeOH. The yields noted below represent the total amount of each cpd from the original 20 g of extract. Eupatorin (**3**), mp 185°, 20 mg and casticin (**10**), mp 208–210°, 10 mg, eluted together and were further separated on a Si gel column with (1) $CHCl_3$–MeOH (3:1) and then with increasing polarity of solvent until the column was eluted finally with $CHCl_3$–MeOH (1:1). Diosmetin (15 mg) which eluted next was further purified over a second polyamide column using C_6H_6–petrol–MeCOEt–MeOH (20:26:3.5:3.5). Desmethoxycentaureidin (**2**; 15 mg), centaureidin (**9**; 15 mg), and an eupalitin 3-galactosyl derivative (**11**; 4 mg) eluted together and were further separated on a polyamide column with C_6H_6–MeCOEt–MeOH (4:3:3). That **11** is a eupalitin 3-*O*-galactosyl derivative is indicated by spectral and hydrolytic data: the MS of the underivatized cpd gave an aglycone ion at *m/z* 330 in accord with a flavone containing 3 hydroxyl and 2 methoxyl groups; acid hydrolysis gave galactose, the purple color in UV light changing to yellow after hydrolysis indicates that the sugar is attached to the 3-hydroxyl. Moreover, the UV data indicated 6- and 7-methoxyl functions and free 5 and 4′-hydroxyl groups (Table 2). However the R_f values in TBA and HOAc (Table 1) suggest that the galactosyl moiety is acylated. There was insufficient material to complete the analysis; therefore, **11** is tentatively designated as a eupalitin 3-acylgalactoside.

Eupatin (**8**; 50 mg) eluted next followed by laciniatin (**6**; 25 mg), which was further purified over a small polyamide column using C_6H_6–petrol–MeCOEt–MeOH (60:26:7:14). Spinacetin (**5**; 8 mg) and eupatolitin 3-gal (**14**; 30 mg) eluted separately in the next fractions with MeOH–MeCOEt (10:5). Patuletin 3-glucoside (**12**) and 3-galactoside (**13**) were isolated from the last fractions as a mixture (15 mg) and could not be separated by PC, TLC or column chromatography. UV spectra clearly indicated a patuletin 3-glycoside and after hydrolysis of the mixture, the aglycone was identified as patuletin and the sugars as glucose and galactose. MS of the permethyl ethers and mobility of the natural products on TLC established the presence of two monoglycosides rather than a diglycoside.

Table 3. MS data for flavonoid aglycones from *Brickellia laciniata**

Compound	M^+	[M − H]	[M − Me]	[M − 18]	[M − HCO]	[M − COMe]	[A_1 − Me]	[A_1 − COMe]	[A_1 − COMe − CO]	[B_1]	[B_2]
PDM-Diosmetin (**1**)	351 (100)	350 (55)	—	333 (13)	322 (9)	—	168† (7)	140‡ (25)	112 (16)	165 (31)	168 (6)
Eupatorin (**3**)	344 (100)	343 (40)	329 (95)	326 (14)	315 (35)	301 (38)	181 (32)	153 (51)	125 (17)	148 (12)	151 (39)
Laciniatin (**6**)	346 (100)	345 (28)	331 (48)	328 (78)	317 (40)	303 (95)	167 (11)	139 (11)	111 (6)	148 (7)	151 (42)
PDM-Laciniatin (**6**)	414 (98)	413 (61)	399 (100)	396 (39)	385 (6)	311 (5)	201 (17)	173 (28)	145 (12)	165 (10)	168 (34)
Casticin (**10**)	374 (100)	373 (60)	359 (67)	356 (26)	345 (19)	331 (30)	181 (24)	153 (29)	125 (9)	148 (8)	151 (36)
Eupalitin	330 (85)	329 (20)	315 (30)	312 (54)	301 (25)	287 (100)	181 (7)	153 (32)	125 (16)	118 (5)	121 (56)

* 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 ref. [1].

† $A_1 - CD_3$.

‡ $A_1 - CO - CD_3$.

Table 4. ^1H NMR spectra of *Brickellia laciniata* flavonoids*

							OMe†							
							CCl_4				C_6H_6			
Compound	H-2′	H-6′	H-5′	H-8	H-6	H-3	3	4′	6	7	3	4′	6	7
Diosmetin (**1**)‡	7.4, *d* (2.5)	7.5, *dd* (9.0)	7.10, *d* (9.0)	6.46	6.22	6.55		3.97						
Eupatorin (**3**)	7.26, *d* (2.5)	7.40, *dd* (2.5; 9.0)	6.88, *d* (9.0)	6.56		6.30		3.72	3.96	3.88		3.22 Δ = +0.50	3.74 Δ = +0.22	3.26 Δ = +0.62
Laciniatin (**6**)	7.60, *d* (2.5)	7.70, *dd* (2.5; 9.0)	6.84, *d* (9.0)	6.54				3.87	3.72			3.32 Δ = +0.55	3.62 Δ = +0.10	
Casticin (**10**)	7.56, *d* (2.5)	7.60, *dd* (2.5; 9.0)	6.88, *d* (9.0)	6.48			3.88	3.72	3.94	3.84	3.78 Δ = +0.10	3.14 Δ = +0.58	3.94 Δ = 0	3.26 Δ = +0.58

* Spectra were recorded in CCl_4 and C_6D_6 (only OMe signals are recorded for this solvent). Values are given in ppm (δ scale) relative to TMS as an internal standard. Numbers in parentheses denote coupling constants in Hz. Signals are singlets unless otherwise stated: *d* = doublet, *dd* = double doublet.

† Some OMe signal assignments may need to be interchanged especially in CCl_4.

‡ Recorded in MeOH-d_4, underivatized.

Water layer. The H_2O layer (12 g) was chromatographed on a Sephadex G-10 column using H_2O, MeOH–H_2O (1:1), and MeOH as eluents. This fraction yielded eupatolitin 3-galactoside (100 mg), patuletin (6 mg) and eupatolitin (5 mg).

Water precipitate. The H_2O ppt. (2 g) which was separated by centrifugation from the H_2O layer, was chromatographed over 4 polyclar columns (7.5 × 100 cm; 500 g each) packed in H_2O. The cpds were eluted with H_2O and increasing amounts of MeOH. Patuletin 3-SO_3K (**15**; 20 mg) and eupatin 3-$SO_3Ca_{1/2}$ (**16**; 1.2 g) were isolated separately. Four minor flavonol 3-sulfates were also detected but were not further identified.

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