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6-METHOXYFLAVONOLS FROM *BRICKELLIA VERONICAEFOLIA* (COMPOSITAE)

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Key Word Index—*Brickellia veronicaefolia;* Compositae; Eupatorieae; Alomiinae; 6-methoxyflavonol methyl ethers; glycosides; sulfates.

Abstract—Three new and eight known flavonols, all containing 6-methoxyl groups, were isolated from *Brickellia veronicaefolia.* The new compounds were eupatolitin 3-sulfate, 6-methoxyquercetin 7,3′-dimethyl ether (veronicafolin) 3-digalactoside and veronicafolin 3-sulfate. The known flavonoids were eupatolitin, quercetagetin 3,6,7-trimethyl ether, eupatin, casticin, artemetin, eupatolitin 3-galactoside, patuletin 3-sulfate and eupatin 3-sulfate.

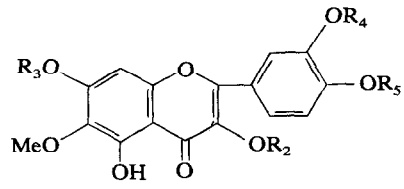
INTRODUCTION

In a continuation of our biochemical systematic investigation of the genus *Brickellia* [1, 2] we report the isolation and characterization of eleven flavonols from *Brickellia veronicaefolia* (HBK) Gray (Eupatorieae, Compositae). Three of the flavonoids, two of which contain sulfate groups, are new natural products. We previously described twenty-one flavonoids from *Brickellia californica* and *B. laciniata* including the known cytotoxic compounds eupatin and eupatorin [3, 4]. *B. veronicaefolia,* like the two previously investigated *Brickellia* species, contains a higher concentration of sulfated than non-sulfated flavonoid material.

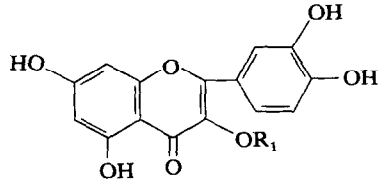
RESULTS

Leaves of *Brickellia veronicaefolia* were extracted with aqueous methanol and the syrup obtained after concentrating the extract was partitioned between water and a series of organic solvents: *n*-hexane, chloroform and ethyl acetate. The aglycones found in the *n*-hexane and chloroform extracts were 6-methoxyquercetin 7,4′-dimethyl ether (**4**) [1, 2, 5] (eupatin) and 6-methoxyquercetin 3,7,3′,4′-tetramethyl ether (**6**) [5] (artemetin). The ethyl acetate extract yielded additional eupatin and artemetin together with 6-methoxyquercetin 7-methyl ether (**2**) [1, 2, 5] eupatolitin), quercetagetin 3,6,7-trimethyl ether (**3**) [5], 6-methoxyquercetin 7-methyl ether (**2**) [1, 2, 5] (eupatolitin), quercetagetin 3,6,7-trimethyl ether (**3**) [5], methoxyquercetin 7,3′-dimethyl ether (veronicafolin) 3-digalactoside (**8**).

The yellow precipitate which formed in the water layer yielded eupatolitin 3-galactoside, 6-methoxyquercetin (patuletin) 3-SO_3^- (**9**) [1, 2], eupatin 3-SO_3^- (**12**) [1, 2], as well as two new sulfated flavonoids: eupatolitin 3-SO_3^- (**10**) and veronicafolin 3-SO_3^- (**11**). The water layer (supernatant) yielded additional eupatolitin 3-galactoside, patuletin 3-SO_3^- and eupatin 3-SO_3^- together with an incompletely characterized derivative of quercetin 3-glucoside (**1**) [5]. The identities of all known flavonoids were established by



Eupatolitin (**2**) R_2,R_4,R_5 = H; R_3 = Me
Quercetagetin 3,6,7 tri-OMe (**3**) R_4,R_5 = H; R_2,R_3 = Me
Eupatin (**4**) R_2,R_4 = H; R_3,R_5 = Me
Casticin (**5**) R_4 = H; R_2,R_3,R_5 = Me
Artemetin (**6**) R_2R_3,R_4,R_5 = Me
Eupatolitin 3-gal (**7**) R_2 = GAL; R_3 = Me; R_4,R_5 = H
Veronicafolin 3-gal-gal (**8**) R_2 = gal-gal; R_5 = H; R_3,R_4 = Me
Patuletin 3-SO_3^- (**9**) $R_2 = SO_3^-$; R_3,R_4,R_5 = H
Eupatolitin 3-SO_3^- (**10**) $R_2 = SO_3^-$; R_4,R_5 = H; R_3 = Me
Veronicafolin 3-SO_3^- (**11**) $R_2 = SO_3^-$; R_5 = H; R_3,R_4 = Me
Eupatin 3-SO_3^- (**12**) $R_2 = SO_3^-$; R_4 = H; R_3,R_5 = Me



Quercetin 3-glc der. (**1**) R_1 = acyl(?)-glc

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Table 1. Chromatographic data for flavonoids from *Brickellia veronicaefolia**

Compound	R_f values TBA	HOAc	Colors at 350 nm† UV	UV/NH_3	UV/NA
Quercetin 3-acyl(?)-glc (**1**)	0.65	0.45	P	Y	O
Quercetagetin 3,6,7-tri-OMe (**3**)	0.82	0.18	P	YB	O
Artemetin (**6**)	0.82	0.16	P	P	P
Veronicafolin 3-gal-gal (**8**)	0.69	0.65	P	YB	Y
Veronicafolin 3-SO_3^- (**11**)	0.32	0.56	P	Y	Y
Veronicafolin‡	0.75	0.08	Y	Y	Y

* 2D chromatograms on Whatman 3MM paper were developed first in TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and then in 15% HOAc.

† P = purple; Y = yellow; B = brown; O = orange. NA refers to Naturstoffreagenz A in MeOH.

‡ Obtained only as the aglycone of **8** and **11**.

direct comparison (TLC, UV, NMR, MS) with authentic samples, which except for artemetin and quercetagetin 3,6,7-trimethyl ether were previously obtained from other *Brickellia* species [1, 2]. The structure assignments for the new compounds are discussed separately.

6-*Methoxyquercetin* 7,3′-*dimethyl ether* (*veronicafolin*) 3-*digalactoside* (**8**) *and veronicafolin* 3-SO_3^- (**11**)

Compounds **8** and **11** are new flavonoids since the aglycone (named here veronicafolin) has not been previously isolated albeit synthesized [6]. The glycoside of veronicafolin was isolated from the EtOAc extract whereas the sulfate was obtained from the water layer.

6-Methoxyquercetin 7,3′-dimethyl ether 3-digalactoside (**8**) showed a purple fluorescence on paper in UV light (yellow after hydrolysis) changing to yellow-brown with ammonia and to yellow when the TLC plates were sprayed with NA. These color changes are in accord for a flavonol 3-*O*-glycoside with free 5 and 4′-hydroxyl groups. The UV spectral data (Table 2) also indicated that **8** was a 3-*O*-substituted flavonoid and further suggested the presence of a 6-methoxyl group (band I bathochromic shift of 22 nm in $AlCl_3$/HCl compared to band I in MeOH). A shift in band I of 72 nm with an increase in intensity with added NaOMe confirmed the presence of a 4′-hydroxyl group. The absence of band III in the NaOMe spectrum and the absence of a NaOAc shift indicated a 7-*O*-substituent. The MS of the PDM derivative of **8** gave an aglycone ion at *m/e* 394 (95%) in accord with **8** containing (before derivatization) three methoxyl and two hydroxyl groups. (An ion at *m/e* 394 corresponds to 3-hydroxy-6,7,3′-trimethoxy-5,4′-dideuteriomethoxyflavone.) The M-Me ion from the PDM derivative of **8** at *m/e* 379 (100%) further supported the presence of a 6-methoxyl group; moreover the A_1 ion at *m/e* 213 (10%), A_1-Me at *m/e* 198 (6%) and a B_2 ion at 168 (18%) together were in agreement with **8** containing one hydroxyl and two methoxyl groups in the A-ring and one hydroxyl and one methoxyl group in the B-ring.

The MS of the PDM aglycone (veronicafolin) exhibited an aglycone ion at *m/e* 411 (65%) and M-Me *m/e* 396 (100%) as expected for 6-methoxyquercetin 7,3′-dimethyl ether (see Tables 1 and 2 for the chromatographic and UV spectral data for this aglycone). Together, these findings indicate that **8** is a 3-

Table 2. UV data for flavonoids from *Brickellia veronicaefolia**

	MeOH	+NaOMe	+$AlCl_3$	λ_{max}, nm in +$AlCl_3$/HCl	+NaOAc	+NaOAc–H_3BO_3	+HCl†
Quercetin 3-acyl (?)-glc (**1**)	352, 260	410, 270	430, 272	398, (360), 268	394, 272	374, 260	
Quercetagetin 3,6,7-tri-OMe (**3**)	354, (268), 257	410, 272	440, 276	374, 263	404, 263	374, 262	
Artemetin (**6**)	342, (272), 253	336, (274), 252	378, 264	359, (276) 258	338, (294), 256	344, (270), 253	
Veronicafolin 3-gal-gal (**8**)	348, (270), 252	420, 274	390, 264	370, 264	420, 258	354, 254	
Veronicafolin 3-SO_3^- (**11**)	330, 284	388, 274	378, (276), 264	368, (278), 264	370, 272	347, (268), 252	366, 254
Veronicafolin‡	360, 258	dec	390, 260	380, 260	dec	364, 258	

* All UV spectra were recorded using standard procedures [5, 7].

† The UV spectra of sulfated flavonoids with added reagents are best compared with the spectrum of the compound in MeOH + HCl not MeOH.

‡ Obtained only as the aglycone of **8** and **11**.

O-glycoside of 6-methoxyquercetin 7,3'-dimethyl ether. Since only galactose was detected in the hydrolysate of **8** and since the R_f values in TBA and HOAc (Table 1) favored two sugar moieties, **8** corresponds to 6-methoxyquercetin 7,3'-dimethyl ether 3-digalactoside.

Compound **11** gave veronicafolin and sulfate on mild hydrolysis (with 0.1 N TFA or sulfatase) and since it shows a purple fluorescence on paper in UV light, changing to yellow after hydrolysis, it should be the 3-SO_3^- of veronicafolin. In agreement with these results, the electrophoretic mobility on paper indicated one sulfate group. Therefore **11** is 6-methoxyquercetin 7,3'-dimethyl ether 3-SO_3^-.

6-*Methoxyquercetin* 7-*methyl ether* (*eupatolitin*) 3-SO_3^- (**10**)

Since compound **10** gave eupatolitin and sulfate on hydrolysis with both 0.1 N TFA and sulfatase and was purple when viewed in UV light changing to yellow after hydrolysis, it should be eupatolitin 3-SO_3^-. The orange color with NA established a 3',4'-dihydroxyl system, and the electrophoretic mobility on paper is in accord with one sulfate group. Therefore **10** is identified as eupatolitin 3-SO_3^-.

EXPERIMENTAL

Plant material. Leaves and vouchers of *Brickellia veronicaefolia* were collected 100 km S.W. of Monterrey, Nuevo Leon, Mexico (voucher specimen B. L. Turner, s.n., is deposited in the Lundell Herbarium, University of Texas at Austin). The plant material was air-dried prior to extraction.

Chromatographic and UV spectral data are presented in Tables 1 and 2 for compounds for which spectral data have not been previously reported [1, 2]. MS were recorded on a Du Pont 21-491 mass spectrometer. ^{1}H NMR spectra were recorded on a Varian HA 100 spectrometer.

Extraction, purification and identification of flavonoids from Brickellia veronicaefolia. General chromatographic and electrophoretic techniques are as previously described for similar studies on other *Brickellia* species [1, 2]. Ground leaf material (80 g) of *B. veronicaefolia* was extracted ×5 with 6 l. 80% and 6 l. 50% aq. MeOH. The combined extracts were concd under red. pres. at 25° until only H_2O remained. The aq. layer was extracted with 2 l. of *n*-hexane followed by 3 l. $CHCl_3$ and finally with 3 l. EtOAc. The remaining aq. layer was concd *in vacuo* to a small vol. (200 ml) and stored at 2° to allow sulfated flavonoids to ppt. PC indicated that the flavonoids in the hexane and $CHCl_3$ extracts were similar and therefore these extracts were combined.

(*A*) *Hexane–*$CHCl_3$ *extract.* The material from the combined hexane–$CHCl_3$ extract (13 g) was divided into two equal portions and the portions were chromatographed separately over Si gel columns (7.5 × 100 cm, 700 g each) packed in the elution solvent. Each column was eluted first with $CHCl_3$–MeOH, 9:1. The polarity of the solvent was increased (9:2; 6:4) until the column was finally eluted with MeOH. The yields noted below represent the total amount of each cpd obtained from the original 13 g of extract. Artemetin (**6**), 300 mg, eluted together with the chlorophylls and a small amount of an unidentified flavone; the flavonoid material was separated from the chlorophylls on a cellulose column with 15% HOAc. The first 5 fractions contained artemetin mixed with a small amount of the unknown flavone while subsequent fractions contained only artemetin. The latter material was recrystallized from hot MeOH to give pure artemetin (by TLC, PC). Further elution of the Si gel column gave 50 mg of eupatin (**4**).

(*B*) EtOAc *extract.* The material from the EtOAc extract (20 g) was chromatographed over a polyclar column (7.5 × 100 cm, 500 g) packed in the elution solvent. The column was first eluted with $CHCl_3$–MeOH–MeCOEt–2,4-pentanedione (20:10:5:1) and then with $CHCl_3$–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 flavonoids isolated sequentially from this column were artemetin (**6**), 20 mg; casticin (**5**), 20 mg; eupatin (**4**), 10 mg; eupatolitin (**2**), 20 mg; eupatolitin 3-galactoside (**7**), 25 mg; quercetagetin 3,6,7-trimethyl ether (**3**), 15 mg; and veronicafolin 3-digalactoside (**8**), 10 mg.

(*C*) *Water layer.* 1. *Precipitate:* A yellow ppt. (600 mg) in the H_2O layer was filtered off and recrystallized from MeOH–H_2O and chromatographed over a polyclar column (7.5 × 100 cm, 400 g) with MeOH–H_2O. The flavonoids isolated from this column were eupatolitin 3-galactoside (**7**), 15 mg; patuletin 3-SO_3^- (**9**), 60 mg; eupatin 3-SO_3^- (**12**), 300 mg and two new sulfated flavonoids, eupatolitin 3-SO_3^- (**10**), 50 mg and veronicafolin 3-SO_3^- (**11**), 30 mg.

2. *Supernatant:* The material from the supernatant (200 mg) was chromatographed on a Sephadex G-10 column [2] (6 × 70 cm, 50 g) using H_2O; MeOH–H_2O (1:1) and MeOH as eluents. The non-flavonoid material eluted with H_2O, and the flavonoids were isolated when MeOH was employed as eluent: eupatolitin 3-galactoside (**7**), 30 mg; eupatin 3-SO_3^- (**12**), 100 mg; patuletin 3-SO_3^- (**9**), 10 mg and a quercetin 3-glucoside derivative (**1**), 10 mg. The latter cpd gave quercetin and glucose on hydrolysis and the color in UV light (purple before hydrolysis, yellow afterwards) indicated that the sugar moiety is at C-3. Moreover, the UV data indicated that the 5,7,3' and 4' positions were free. However, the R_f values of **1** in TBA and HOAc (Table 1) indicated that the cpd is not a simple quercetin 3-glucoside; it is likely that the glucosyl moiety is acylated and, accordingly, the cpd is designated as quercetin 3-acyl (?)-glucoside.

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