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# FLAVONOIDS FROM *BRICKELLIA CHLOROLEPIS* AND *B. DENTATA*

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**Key Word Index**—*Brickellia chlorolepis*; *B. dentata*; Compositae; Eupatorieae; 6-methoxyflavonol methyl ethers, glycosides and sulfate; 6-methoxyflavone methyl ethers; flavonol glycosides; flavonol methyl ether glycoside.

**Abstract**—Eleven flavonoids including three new glycosides were isolated from *Brickellia chlorolepis* and one new and nine known flavonoids were obtained from *B. dentata*. The new glycosides from *B. chlorolepis* are 6-methoxykaempferol 3-rhamnoglucoside, spinacetin 3-rhamnogalactoside and veronicafolin 3-rhamnoglucoside. The known compounds identified from *B. chlorolepis* are patuletin, casticin, artemetin, eupatolitin 3-galactoside, quercetin 3-rhamnogalactoside, rutin, isorhamnetin 3-galactoside and eupatin 3-$SO_3Ca_{1/2}$. *B. dentata* contains the new glycoside eupalitin 3-galactoside and nine known compounds: pectolinarigenin, salvigenin, eupafolin, cirsiliol, eupatorin, eupatolitin, eupatolitin 3-glucoside, eupatolitin 3-galactoside and eupatin.

## INTRODUCTION

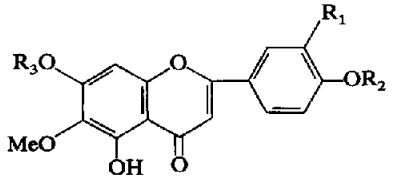
In a continuation of a phytochemical investigation of the genus *Brickellia* (Tribe Eupatorieae, Family Compositae) [1–3], we now report the isolation and characterization of eleven flavonoids from the leaves of *Brickellia chlorolepis* (Woot. and Standl) Shinners and ten from *B. dentata* (D. C.) Sch. Bip. Three of the flavonoids from *B. chlorolepis* are new glycosides: 6-methoxykaempferol 3-rhamnoglucoside, spinacetin 3-rhamnogalactoside, and veronicafolin 3-rhamnoglucoside. In addition, the new glycoside eupalitin 3-galactoside was obtained from *B. dentata*.

In contrast to the large amounts of several sulfated flavonoids found in *B. californica*, *B. laciniata* and *B. veronicaefolia* [1–3], only one sulfated flavonoid in small amounts was obtained from *B. chlorolepis* and none from *B. dentata*. *B. chlorolepis* is characterized by seven flavonol glycosides while *B. dentata* only produces 6-methoxyflavonoids, three of which are glycosides.

## RESULTS

Leaves of *B. chlorolepis* and *B. dentata* were both extracted by the same procedure; namely, an initial extraction with aqueous methanol and an extraction of the aqueous concentrate with a series of organic solvents: *n*-hexane, chloroform and ethyl acetate. The chloroform extract of *B. chlorolepis* yielded 6-methoxyquercetin 3,7,3′,4′-tetramethyl ether (**13**) [3, 4] (artemetin) and 6-methoxyquercetin 3,7,4′-trimethyl ether (**12**) [2–4] (casticin), while the EtOAc fraction afforded quercetin 3-rhamnogalactoside (**6**), rutin (**7**) [4], isorhamnetin 3-galactoside (**8**) [4], 6-methoxykaempferol 3-rhamnoglucoside (**9**), 6-methoxyquercetin 7-methyl ether (eupatolitin) 3-galactoside (**15**) [1–4], 6-methoxyquercetin 3′-methyl ether (spinacetin) 3-rhamnogalactoside (**17**), 6-methoxyquercetin 7,3′-dimethyl ether (veronicafolin) 3-rhamnoglucoside (**18**) and 6-methoxyquercetin (patuletin) (**11**) [2]. The remaining water layer contained 6-methoxyquercetin 7,4′-dimethyl ether (eupatin) 3-$SO_3Ca_{1/2}$ (**20**) [1–3].

The identities of patuletin, casticin, artemetin, eupatolitin 3-galactoside and eupatin 3-$SO_3Ca_{1/2}$ were established by direct comparison (TLC, UV, $^1$H NMR, MS) with authentic samples which were previously obtained from other *Brickellia* species [1–3]. The structure assignments for the known flavonols not previously isolated from *Brickellia* were determined by TLC, UV, NMR and MS and, when possible, co-chromatography (Tables 1 and 2). The structure




Pectolinarigenin (**1**) $R_1, R_3 = H$; $R_2 = Me$
Salvigenin (**2**) $R_1 = H$; $R_2, R_3 = Me$
Eupafolin (**3**) $R_1 = OH$; $R_2, R_3 = H$
Cirsiliol (**4**) $R_1 = OH$; $R_2 = H$; $R_3 = Me$
Eupatorin (**5**) $R_1 = OH$; $R_2, R_3 = Me$

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Table 1. Chromatographic data for flavonoids from *Brickellia chlorolepis* and *B. dentata**

| Compound | $R_f$ values TBA | HOAc | Colors at 350 nm† UV | UV/$NH_3$ | UV/NA |
|---|---|---|---|---|---|
| Salvigenin (**2**) | 0.91 | 0.10 | P | P | P |
| Quercetin 3-Gal-Rha (**6**) | 0.41 | 0.50 | P | Y | O |
| Rutin (**7**) | 0.49 | 0.64 | P | Y | O |
| Isorhamnetin 3-Gal (**8**) | 0.60 | 0.45 | P | Y | Y |
| 6-Methoxykaempferol 3-Glu-Rha (**9**) | 0.33 | 0.52 | P | Y | Y |
| Eupalitin 3-Gal (**10**) | 0.72 | 0.79 | P | Y | Y |
| Eupatolitin 3-Glu (**16**) | 0.58 | 0.50 | P | Y | O |
| Spinacetin 3-Gal-Rha (**17**) | 0.64 | 0.66 | P | Y | Y |
| Veronicafolin 3-Glu-Rha (**18**) | 0.44 | 0.60 | P | Y | Y |

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

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

Table 2. UV data for flavonoids from *Brickellia chlorolepis* and *B. dentata**

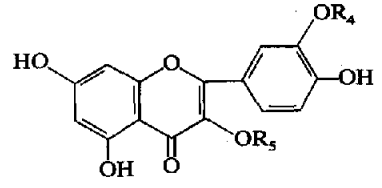
| Compound | MeOH ($\lambda_{max}$, nm) | NaOMe ($\lambda_{max}$, nm) | $AlCl_3$ ($\lambda_{max}$, nm) | $AlCl_3$/HCl ($\lambda_{max}$, nm) | NaOAc ($\lambda_{max}$, nm) | NaOAc/$H_3BO_3$ ($\lambda_{max}$, nm) |
|---|---|---|---|---|---|---|
| Salvigenin (**2**) | 329, 275 | 327, 276 | 352, 299, 290 sh, 260 sh | 351, 300, 290 sh, 260 sh | 331, 277 | 331, 277 |
| Quercetin 3-Gal-Rha (**6**) | 360, 332 sh, 262 sh, 254 | 410, 328 sh, 270 | 434, 304 sh, 270 | 400, 364, 264 | 387, 320 sh, 270 | 380, 260 |
| Rutin (**7**) | 362, 296 sh, 268 sh, 256 | 410, 330, 270 | 432, 304 sh, 274 | 400, 366 sh, 304, 266 | 400, 320 sh, 272 | 378, 262 |
| Isorhamnetin 3-Gal (**8**) | 352, 266 sh, 252 | 416, 330, 270 | 400, 366 sh, 298 sh, 262 | 400, 360 sh, 300 sh, 262 | 394, 274 | 356, 268 sh, 254 |
| 6-Methoxykaempferol 3-Glu-Rha (**9**) | 347, 275 | 412, 330, 280 | 380, 270 | 369, 290 sh, 265 | 347, 275 | 347, 273, 255 |
| Eupalitin 3-Gal (**10**) | 340, 271 | 398, 272 | 368, 305 sh, 279 | 360, 305 sh 280 | 408 sh, 344, 271 | 340, 271 |
| Eupatolitin 3-Glu (**16**) | 353, 262 sh, 257 | 410, 257 | 442, 305 sh, 257 | 380, 305 sh, 255 | 420 sh, 365, 257 | 380, 257 |
| Spinacetin 3-Gal-Rha (**17**) | 357, 272 sh, 257 | 422, 338, 273 | 390, 300 sh, 268 | 378, 300 sh, 266 | 390, 325, 273 | 357, 272, 257 |
| Veronicafolin 3-Glu-Rha (**18**) | 247, 275 sh, 258 | 418, 275 | 375,270 | 370, 267 | 430 sh, 347, 270 sh, 257 | 347, 270 sh, 257 |

* All spectra were recorded using standard procedures [5] on a Beckman DB Spectrophotometer; sh: shoulder.

assignments for the new compounds are discussed separately.

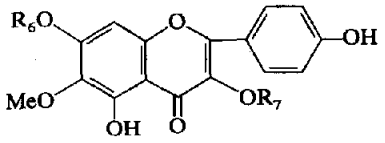
Spectral (UV, MS) and hydrolytic analyses established the new glycosides to be 6-methoxykaempferol 3-rhamnoglucoside (**9**), 6-methoxyquercetin 7,3'-dimethyl ether (veronicafolin) 3-rhamnoglucoside (**18**) and 6-methoxyquercetin 3'-methyl ether (spinacetin) 3-rhamnogalactoside (**17**). The linkages in the disaccharide moieties in both **9** and **18** were assigned on the basis of lack of hydrolysis with $\beta$-glucosidase.

In addition to the data mentioned above, the $^1$H NMR spectrum of **17** (as TMSi derivative in $CCl_4$) showed a doublet at $\delta$ 0.9 for rhamnose Me, sugar protons (10 H) at 3.30–3.65, two methoxyl signals at 3.74 and 3.88, rhamnose $H_1$ at 4.35, galactose at 5.65 (in accord with a 3-*O*-rhamnogalactosyl moiety). The other signals appeared at $\delta$ 6.5 for H-8, a doublet ($J$ = 9 Hz) at 6.8 for H-5', a double doublet ($J$ = 2.5 and 9 Hz) at 7.5 for H-6' and a doublet ($J$ = 2 Hz) at 7.75 for H-2'. The two methoxyl resonances did not exhibit any significant shift in benzene-$d_6$ in accord with C-3' (3.64; $\Delta$ +0.10 ppm) and C-6' (3.88; $\Delta$ 0.0) methoxyl groups.



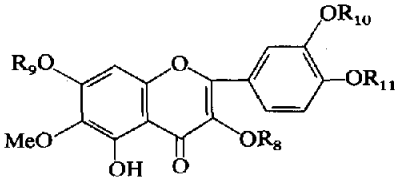

Quercetin 3-Gal-Rha (**6**) $R_4$ = H; $R_5$ = Gal-Rha
Rutin (**7**) $R_4$ = H; $R_5$ = Glu-Rha
Isorhamnetin 3-Gal (**8**) $R_4$ = Me; $R_5$ = Gal

The flavonoids obtained from the hexane–chloroform extract of *B. dentata* were 6-methoxyapigenin 4'-methyl ether (**1**) [1, 4] (pectolinarigenin), 6-methoxyapigenin 7,4'-dimethyl ether (**2**) [4] (salvigenin), 6-methoxyluteolin (**3**) [1, 4] (eupafolin), 6-methoxyluteolin 7,4'-dimethyl ether (**5**) [2, 4] (eupatorin) and 6-methoxyquercetin, 7-methyl



6-Methoxykaempferol 3-Glu-Rha (**9**) $R_6 = H$; $R_7 = Glu\text{-}Rha$
Eupalitin 3-Gal (**10**) $R_6 = Me$; $R_7 = Gal$.

ether (**14**) [2–4] (eupatolitin). The ethyl acetate extract and the water layer yielded 6-methoxyluteolin 7-methyl ether (**4**) [1, 4] (cirsiliol), eupatolitin (**14**), eupatolitin 3-galactoside (**15**), eupatolitin 3-glucoside (**16**), eupatin (**19**) and the new flavonol 6-methoxykaempferol 7-methyl ether (eupalitin) 3-galactoside (**10**) (Tables 1 and 2). The identities of all the known flavonoids were established by direct comparison (TCL, UV, $^1$H NMR and MS) with authentic samples which except for salvigenin, eupatolitin 3-glucoside and eupalitin 3-galactoside were previously obtained from other *Brickellia* species [1–3]. The latter compound showed a purple UV fluorescence on PC (yellow after hydrolysis), changing to yellow both with ammonia and when the TLC plates were sprayed with Naturstoffreagenz A in MeOH. The UV and MS data and $R_f$ values established the compound to be a flavonol 3-glycoside (Tables 1 and 2). Hydrolysis with 0.1 N TFA yielded galactose as the only sugar and eupalitin (TLC, UV and MS comparison with a standard sample). The $^1$H NMR spectrum of **10** (TMSi derivative in $CCl_4$) exhibited signals for two rotamers: 6 sugar protons between $\delta$ 3.4 and 3.6, two methoxy signals at 3.90 and 3.73 and two galactose $H_1$ doublets ($J = 7$ Hz) at 5.7 and 5.9. Other signals established a 6-oxygenated kaempferol derivative: a sharp singlet at 6.5 for H-8, two doublets ($J = 9$ Hz) near 6.88 for H-3′ and H-5′ and two doublets ($J = 9$ Hz) near 7.96 for H-2′ and H-6′. As expected, when the compound was examined in $C_6D_6$ the C-7 methoxyl signal exhibited a large shift of 0.51 ppm, while the C-6 methoxyl signal shifted only 0.02 ppm. The spectral and hydrolytic data established that the new glycoside is 6-methoxykaempferol 7-methyl ether 3-galactoside (**10**).



Patuletin (**11**) $R_8, R_9, R_{10}, R_{11} = H$
Casticin (**12**) $R_{10} = H$; $R_8, R_9, R_{11} = Me$
Artemetin (**13**) $R_8, R_9, R_{10}, R_{11} = Me$
Eupatolitin (**14**) $R_8, R_{10}, R_{11} = H$; $R_9 = Me$
Eupatolitin 3-Gal (**15**) $R_{10}, R_{11} = H$; $R_9 = Me$; $R_8 = Gal$
Eupatolitin 3-Glu (**16**) $R_{10}, R_{11} = H$; $R_9 = Me$; $R_8 = Glu$
Spinacetin 3-Gal-Rha (**17**) $R_9, R_{11} = H$; $R_{10} = Me$; $R_8 = Gal\text{-}Rha$
Veronicafolin 3-Glu-Rha (**18**) $R_{11} = H$; $R_9, R_{10} = Me$; $R_8 = Glu\text{-}Rha$
Eupatin (**19**) $R_8, R_{10} = H$; $R_9, R_{11} = Me$
Eupatin 3-$SO_3$ $Ca_{1/2}$ (**20**) $R_{10} = H$; $R_9, R_{11} = Me$; $R_8 = SO_3Ca_{1/2}$

## EXPERIMENTAL

*Plant material.* Leaves and vouchers of *B. chlorolepis* were collected in October 1977 in Sul Ross Hill, Alpine, Brewster County, Texas (voucher specimen J. F. Weedin 930 is deposited in the Herbarium, Sul Ross University, Alpine, Texas). Leaves and vouchers of *B. dentata* were collected in August 1977, in McKinney Falls State Park, Travis County, Texas (voucher specimen B. N. Timmermann 877 is deposited in Lundell Herbarium, The University of Texas at Austin). The plant material of both species was air-dried prior to extraction.

*Extraction, purification and identification of flavonoids from* B. chlorolepis *and* B. dentata. General chromatographic and electrophoretic techniques which were employed here were described in detail in reports on other *Brickellia* species [1–3]. Ground leaf material (131 g) of *B. chlorolepis* was extracted ×5 with 7 l. 80% and 5 l. 50% aq. MeOH until the extract was colorless. The extracts were then combined and evapd under red. pres. until only $H_2O$ remained. The aq. layer was extracted successively with 800 ml *n*-hexane, 2 l. $CHCl_3$, and 2 l. EtOAc. PC indicated that the flavonoids in the hexane and $CHCl_3$ extracts were similar and therefore these extracts were combined. Similar results with PC were obtained with the EtOAc extract and the $H_2O$ layer and therefore these were also combined. In the case of *B. chlorolepis*, the combined hexane (4.8 g) and $CHCl_3$ (28 g) extracts produced a yellow ppt. (700 mg) which was filtered off and chromatographed over a Polyclar column (7×90 cm, 600 g) packed in MeOH–$H_2O$ (4:1). The column was eluted first with MeOH and then with EtOAc. Artemetin (400 mg) eluted first as a single compound, followed by a small amount of an unidentified flavone. The material from the supernatant (200 mg) was chromatographed on a cellulose column (9× 30 cm, 90 g) with 15% HOAc. The flavonoids isolated from this column were additional artemetin (100 mg), 2 mg of an unidentified flavone which was also present in the ppt., casticin (10 mg) and veronicafolin 3-rhamnoglucoside (50 mg). The material from the EtOAc extract and the supernatant from the $H_2O$ layer were combined (40 g) and chromatographed on a Polyclar column (8×60 cm, 500 g) packed in the elution solvent. The column was eluted first with $CHCl_3$–MeOH–MeCOEt–2,4-pentanedione (20:10:5:1) followed by $CHCl_3$–MeOH–MeCOEt–2,4-pentanedione (10:10:5:1). The polarity of the solvent was increased until the column was finally eluted with MeOH. Patuletin (6 mg) and eupatolitin 3-galactoside (30 mg) eluted first as single compounds and these were further purified by Sephadex LH-20 columns using MeOH and MeOH–$H_2O$ (4:1), respectively. Quercetin 3-*O*-rhamnogalactoside (5 mg), rutin (6 mg) and isorhamnetin 3-galactoside (6 mg) eluted together and were further separated on a Polyclar column (5×80 cm, 200 g) first with $C_6H_6$–MeCOEt–MeOH (4:3:3) followed by $C_6H_6$–MeCOEt–MeOH (4:3:6:) and finally MeOH. 6-Methoxykaempferol 3-rhamnoglucoside eluted next and spinacetin 3-rhamnogalactoside (30 mg) was isolated as the last compound when the column was eluted with MeOH. The ppt. (15 mg) that formed in the $H_2O$ layer was filtered off and recrystallized from hot MeOH–$H_2O$. The material was first chromatographed over a Polyclar column (3×20 cm, 50 g) with $H_2O$ and then over Sephadex LH-20 with MeOH–$H_2O$ (4:1). Only one flavonoid, eupatin 3-$SO_3$ $Ca_{1/2}$ (8 mg), was obtained.

The extraction and chromatographic techniques used for the isolation of the flavonoids from 123 g of dry leaf material of *B. dentata* were similar to those already described for *B.*

*chlorolepis*. The combined hexane (3.5 g) and $CHCl_3$ (10 g) extracts yielded pectolinarigenin (8 mg), salvigenin (20 mg), eupafolin (15 mg), eupatorin (30 mg) and eupatolitin (10 mg). The material from the EtOAc extract (2.1 g) and $H_2O$ layer (2.5 g) were combined; by standard chromatography cirsiliol (20 mg), eupalitin 3-galactoside (40 mg), eupatolitin (10 mg), eupatolitin 3-galactoside (30 mg), eupatolitin 3-glucoside (15 mg) and eupatin (20 mg) were obtained.

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