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FLAVONOIDS FROM *BRICKELLIA GLUTINOSA*[1]

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In a continuation of a phytochemical investigation of the genus *Brickellia* (Tribe Eupatorieae, Subtribe Alomiinae, Family Compositae) (1-6), we now report the isolation and characterization of eleven flavonoids from *Brickellia glutinosa* A. Gray *e.g.*, 5,7,3′-trihydroxy-3, 6, 4′-trimethoxy-7-O-β-D-glucosylflavone (centaurein) (7),5,7,4′-trihydroxy-3, 6, 3′- trimethoxyflavone- 4′-sulfate (jaceidin-4′-sulfate) (8),5,7,3′, 4′-tetrahydroxy-3, 6-dimethoxy-7-*O*-β-D-glucosylflavone (quercetagetin-3,6-dimethylether-7-*O*-β-D-glucoside) (9), 5, 7, 3′,4′-tetrahydroxy-3-methoxyflavone (quercetin-3-methylether), 3,5,7, 3′,4′-pentahydroxyflavone (quercetin), 5, 3′, 4′-trihydroxy-3, 6, 7-trimethoxyflavone (quercetagetin-3,6,7-trimethylether) (10), 5,3′,4′-trihydroxy-3,7-dimethoxyflavone (quercetin-3,7-dimethylether) (11), 3,5,3′,4′-tetrahydroxy-6, 7-dimethoxyflavone (eupatolitin) (10), 5, 3′-dihydroxy-6, 7, 2′, 4′, 5′-pentamethoxyflavone (brickellin) (10), 5,7-dihydroxy-3, 6, 3′, 4′-ttetramethoxyflavone (quercetagetin-3, 6, 3′, 4′-tetramethylether) (10), 5,4′-dihydroxy-3,6,7-trimethoxyflavone (penduletin) (12), and the aldose reductase (AR) inhibitory activity of five of these flavonoids.

Many flavonoids have been established as effective inhibitors of AR, the enzyme that converts D-glucose and D-galactose to sorbitol. This inhibitory activity is of importance as this property imparts potential therapeutic value to flavonoids in the treatment of diabetic and galactosemic cataract (13).

The previous chemotaxonomic work on *Brickellia* indicated that this genus produced flavonoids which, based on established structure-activity relationships, would be potent inhibitors of AR (14). Additionally, the flavonoid pattern of these *Brickellia* species and related members of the Alomiinae is proving to be useful in assessing the generic alignments in the tribe (15).

RESULTS AND DISCUSSION

Centaurein and quercetagetin-3,6-dimethyl-ether-7-*O*-β-D-glucoside were isolated from the aqueous extract of *B. glutinosa* including a new compound jaceidin-4′-sulfate. Quercetin-3-methylether, quercetin, and quercetagetin-3,6,7-trimethylether were isolated from the EtOAc extract and quercetin-3,7-dimethylether, eupatolitin, brickellin, quercetagetin-3, 6, 3′, 4′-tetramethylether, and penduletin were obtained from the $CHCl_3$ extract.

The presence of the sulfate moiety in jaceidin-4′-sulfate was demonstrated by migration of the compound to the anode during thin-layer electrophoresis, precipitation of $BaSO_4$ after addition of $BaCl_2$ to the dilute HCl hydrolysate of jaceidin-4′-sulfate, negative ion fast atom bombardment ms, and enzymatic hydrolysis with sulfatase. The hydrolysis

[1]This work was performed in partial fulfillment of the requirements for the degree of Doctor of Philosophy for Robert S. Goodwin.

product of jaceidin-4′-sulfate was identified as jaceidin from uv and pmr spectra and by co-chromatography with an authentic reference standard (8).

The linkage of the sulfate moiety to the 4′ position was established by comparison of the uv spectra before and after hydrolysis of jaceidin-4′-sulfate. In accordance with flavonoids with a substituted 4′-OH, the absorbance of Band I of jaceidin-4′-sulfate was less intense in the NaOMe spectrum compared to the MeOH or MeOH/HCl spectrum. After hydrolysis, the absorbance of Band I in NaOMe was greater than that of Band I in MeOH. This indicated that the 4′-OH was unsubstituted after hydrolysis and that the sulfate was bound to the 4′ position of jaceidin-4′-sulfate (16).

The phytochemistry of *B. glutinosa* indicates that it is clearly a member of the section Bacchariđeae of *Brickellia*, all of which are distinguished by the presence of highly methoxylated, 6-methoxy flavonoid aglycones, glycosides, and sulfates (1-6).

Those compounds isolated in sufficient quantity were assayed for aldose reductase inhibitory activity according to the method of Varma *et al.* (14). Table 1 compares the inhibitory activity of these flavonoids to two compounds with established potency, quercitrin (14) and 1,3-dioxo-1H-benz[de]isoquinoline (alrestatin) (17). All compounds tested show substantial inhibition of AR and, with the exception of centaurein and penduletin, the levels of inhibitory activity are similar. The data confirm the structure-activity relationships established by Varma *et al.* (14). The low activity of penduletin can be explained by the absence of a catechol group on the side phenyl, and the decreased activity of centaurein by glycosylation at the 7 position.

EXPERIMENTAL

PLANT MATERIAL.—Samples of *B. glutinosa* were collected in August 1981, approximately fifteen miles southwest of Cuatrocienegas in Coahuilla, Mexico. A voucher specimen (Norris #70) is deposited at the Lundell Herbarium, University of Texas at Austin.

GENERAL EXPERIMENTAL PROCEDURES.—Chemical structures of compounds were elucidated using pmr, uv, and mass spectral and chromatographic techniques in conjunction with comparisons of appropriate reference compounds when available. Sugars were identified from their gas-liquid chromatograms after hydrolysis and trimethylsilylation (18). Tlc on polyamide (19), silica gel, and cellulose plates was also utilized.

Uv spectra were recorded on a Gilford 2600 ultraviolet-visible spectrophotometer. Pmr spectra of the trimethylsilyl ethers were recorded on a Varian T-60A spectrometer in CCl_4 with TMS as internal standard. Electron impact mass spectra were obtained on an Extranuclear EL 1000 mass spectrometer. Negative ion mass spectra of jaceidin-4′-sulfate were obtained with an AEI/Kratos MS-50 mass spectrometer, using fast atom bombardment. Gc was performed on a Varian Aerograph Series 2100 instrument equipped with a flame ionization detector using 3% SE-30 on Chromosorb W. Silica gel 60 and cellulose tlc plates were obtained from EM Laboratories Inc., Darmstadt, Germany. Sulfatase and β-D-

TABLE 1. Inhibition of Aldose Reductase by Flavonoids from *Brickellia glutinosa*

Compound	Inhibition Percentage[a]			
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M
Centaurein	72	47	0	
Quercetin 3-methyl ether	100	95	78	28
Quercetagetin 3,6,7-trimethyl ether	100	88	63	26
Quercetin 3,7-dimethyl ether	100	97	71	16
Penduletin	97	64	21	0
Quercitrin[b]	100	91	82	57
1,3-Dioxo-1H-benz[de]isoquinoline[b]	100	92	44	17

[a]The numbers indicate percentage of inhibition of the aldose reductase activity as compared to controls when the reaction was carried out in the absence of inhibitors.

[b]Not isolated from *Brickellia glutinosa*.

glucosidase were supplied by Sigma Chemical Co., St. Louis, MO.

EXTRACTION AND ISOLATION.—Air-dried stems, leaves, and flowers (1265 g) were defatted by extracting four times with one-liter portions of petroleum ether. After drying, the marc was exhaustively extracted with seven 700-ml portions of absolute MeOH. The petroleum ether was discarded and the MeOH extract was taken to dryness under reduced pressure. The residue from the MeOH extract (173 g) was suspended in 500 ml of H_2O and partitioned five times with 250 ml of $CHCl_3$. Following this shake-out, the aqueous phase was partitioned with five 200-ml volumes of EtOAc. The $CHCl_3$ and EtOAc extracts were then taken to dryness.

The aqueous phase was reduced to half its volume and refrigerated. A yellow precipitate (1.2 g) formed, which was separated from the aqueous supernatant. The precipitate from the aqueous phase was chromatographed over two 6×100 cm Polyclar AT[2] columns in absolute MeOH. Three fractions were collected; the compound in the first fraction (1500 ml) was identified as centaurein (289 mg). The second fraction was chromatographed over a 3×40 cm Polyclar AT column in 50% MeOH and yielded jaceidin-4′-sulfate (4 mg) in 50 ml of eluate. The third fraction was combined with a fraction from the aqueous supernatant.

The aqueous supernatant was adjusted to pH 2 and passed over an acidified 6×36 cm Amberlite XAD-2[3] column (20). Impurities were eluted with 1200 ml of H_2O at pH 2. After washing with 1500 ml of H_2O to neutrality, the flavonoid-containing fraction was eluted with 1200 ml each of 50% and 100% MeOH. The methanolic eluates were taken to dryness under reduced pressure. The residues were combined and chromatographed over a 6×100 cm Polyclar AT column in absolute methanol. A band was eluted and combined with the third fraction from the aqueous precipitate.

The combined fractions from the aqueous precipitate and supernatant were chromatographed over three 6×100 cm Polyclar AT columns in 100% MeOH and yielded quercetagetin-3,6-dimethyl ether-7-*O*-β-D-glucoside (32 mg) in 200 ml of eluate.

The residue from the EtOAc extract (8.5 g) was chromatographed over a 6×100 cm Polypenco[4] column in 90% MeOH and three fractions were collected. The second fraction (1500 ml) and third fraction (3500 ml) contained quercetin-3-methyl ether (230 mg) and quercetin (650 mg), respectively. The first fraction was chromatographed over a 6×100 cm Polyclar AT column in 100% MeOH. Two bands were collected; the first provided additional centaurein (100 mg) from 500 ml of eluate, and the second yielded quercetagetin-3,6,7-trimethyl ether (47 mg) from 250 ml.

The residue from the $CHCl_3$ extract (146 g) was suspended in a mixture of 250 ml of petroleum ether and 250 ml of MeOH and partitioned with 250 ml of H_2O. Centrifugation permitted more efficient separation of the phases, and the H_2O-MeOH phase was taken to dryness under reduced pressure. This residue (61 g) was chromatographed over a 6×100 cm Polyclar AT column using absolute MeOH. Six fractions were collected, and of these, fractions 3,5 and 6 provided additional quantities of quercetagetin-3,6,7-trimethylether, quercetin-3-methylether and quercetin, respectively. Fraction 2 (750 ml) yielded quercetin-3,7-dimethyl ether (124 mg). Fraction 4 was chromatographed over a 3×40 cm Polyclar AT column using 90% MeOH. The eluate (100 ml) contained eupatolitin (17 mg). Fraction 1 was chromatographed sequentially over 6×100 cm Polyclar AT and Polypenco columns in 75% MeOH, followed by a 6×40 cm silica gel column in $CHCl_3$-MeOH (95:5 and 50:50). Two bands were collected, A and B. Band B was chromatographed over a 6× 100 cm silica gel column using $CHCl_3$-MeOH (95:5 and 50:50). Two bands were collected, C and D. Bands A and C were combined and chromatographed over a 3×40 cm silica gel column in CHCl-MeOH (95:5) followed by chromatography over a 6×100 cm Polypenco column using 75% MeOH. The eluate (125 ml) yielded brickellin (19 mg). Band D was chromatographed sequentially over a 6×100 cm silica gel column with $CHCl_3$-MeOH (95:5) and a 6×100 cm Polyclar AT column using 100% $CHCl_3$ followed by $CHCl_3$-MeOH (75:25). Two fractions were collected; one contained quercetagetin-3,6,3′,4′-tetramethyl ether (142 mg) in 900 ml of absolute $CHCl_3$, the other yielded penduletin (536 mg) in 3000 ml of $CHCl_3$-MeOH (75:25).

JACEIDIN-4′-SULFATE:—Uv (on paper: purple with and without NH_3), λ max (MeOH) 257, 280, 343 (MeOH/HCl) 257, 272sh, 348 (NaOMe) 276, 336, 399 ↓ ($AlCl_3$) 272, 280sh, 299sh, 377, 404sh ($AlCl_3$/HCl) 270, 281, 299sh, 369, 404sh (NaOAc) 272, 335, 376 (NaOAc/H_3BO_3) 258, 272sh, 352 nm; negative ion fast atom bombardment ms *m/z* (relative intensity) 439 [$(M-H)^-$, 9], 359 [$(M-HSO_3)$ ′-4].

JACEIDIN[5].—Uv (on paper: purple with NH_3,

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[2]Polyclar AT was supplied by GAF Corporation, New York.

[3]Amberlite XAD-2 was supplied by Mallinckrodt, St. Louis, MO.

[4]Polypenco was obtained from Polyumer Corporation, Reading, PA.

[5]Obtained by acid hydrolysis of jaceidin-4′-sulfate.

yellow-green without NH_3), λ max (MeOH) 256, 271, 351 (NaOMe) 272, 308, 392 ↓ ($AlCl_3$) 268, 281sh, 302sh, 378, 405sh ($AlCl_3$/HCl) 267, 281, 299sh, 370, 408sh (NaOAc) 272, 380, (NaOAc/H_3BO_3) 259, 271, 350 nm; pmr 7.60 (m, 2H, H-2′, 6′), 6.87 (d, J=8 Hz, H-5′), 6.50 (s, H-8), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3).

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