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FLAVONOIDS OF *ASANTHUS*, A SEGREGATE GENUS OF *BRICKELLIA*

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Asanthus R.M. King and H. Robinson (Family Compositae, Tribe Eupatorieae, subtribe Alomiinae), a group of three Chihuahuan desert species defined by King and Robinson on the basis of stylar and other micromorphological features (1), has been chemically examined in conjunction with our systematic investigation of the genus *Brickellia* Ell. (2) and its relatives. B.L. Robinson originally included *Asanthus* in *Brickellia* while King and Robinson, in their sweeping reorganization of the Eupatorieae, segregated *Asanthus* from *Brickellia*. Recently, two of the species in *Asanthus*, *A. thrysiflora* Gray and *A. solidaginifolia* Gray, have been treated as one taxon (3).

Our previous chemical studies have shown flavones, flavonols, flavonol glycosides and sulfates, all with 6-methoxylation, to be characteristic of the main evolutionary line in *Brickellia* (4-10), and these chemical characters, when used in conjunction with morphology and cytology, have aided in assessing the affinities of taxa within the group. For example, chemical data were important in supporting the new genera *Flyriella* R.M. King and H. Robinson (11) and *Brickelliastrum* R.M. King and H. Robinson (12), previously associated with *Brickellia*, where the absence of 6-methoxylation in flavonoids (13, 14) coupled with other taxonomic evidence supported their separate generic status.

In our present study, we found that *Asanthus* not only produces 6-methoxylated flavones like those of *Brickellia* but also produces non 6-methoxylated flavonol mono and diglycosides, similar to those from *Flyriella* and *Brickelliastrum*. Eleven flavonoids were isolated from *A. solidaginifolia*, including 6-methoxyapigenin and its 4′ methyl ether; 6-methoxyluteolin and its 4′-methyl ether; kaempferol and its 3-*O*-β-D-glucoside; quercetin, its 3-*O*-β-D-galactoside, 3-*O*-β-D-rhamnogalactoside, and 3-methyl ether 7-*O*-β-D-glucoside; and isorhamnetin 3-*O*-β-D-glucoside. *A. thrysiflora* afforded five compounds which were also present in *A. solidaginifolia*, namely, 6-methoxyapigenin, 6-methoxyluteolin, quercetin and its 3-*O*-β-D-galactoside and 3-*O*-β-D-rhamnogalactoside.

These chemical results support earlier conclusions that *A. solidaginifolia* is not only closely related to *A. thrysiflora* but adds weight to their treatment as a single species, in agreement with McVaugh (3). Moreover, the data suggest that *Asanthus* is chemically distinct from the core group of *Brickellia* and may occupy an intermediate position between it and other members of the Alomiinae.

EXPERIMENTAL

PLANT MATERIAL.—*A. solidaginifolia* (Sundberg and Lavin #2743) was collected in the State of Chihuahua, 19.2 miles W. of Hwy 45 and 1.0 mile E. of Cumbres de Majalca, Mexico in September 1984. Material of *A. thrysiflora* (Norris #48) was collected in January 1982, in Mexico, State of Aguascalientes, about 18 miles W. of Ciudad Aguascalientes on Hwy. 70 to Cavillo. Voucher materials are deposited in The Plant Resources Center at The University of Texas at Austin, Austin, Texas.

EXTRACTION AND ISOLATION OF FLAVONOIDS.—Dried leaves and stems (900 g) of *A. solidaginifolia* were extracted with 85% and 50% aqueous MeOH. The combined extracts were concentrated to an aqueous layer under reduced pressure, and the concentrate was partitioned against CH_2Cl_2 and EtOAc. The concentrate from the CH_2Cl_2 (15 g) and the EtOAc (28 g) extracts were chromatographed over Polyclar AT (GAF Corp.) columns packed initially in toluene and gradually altered in 10% increments to 100% MeOH and finally concluded with Me_2CO-MeOH (1:1). Fractions, which were collected by monitoring the column with uv light, were further separated by paper chromatography using 15% HOAc on Whatmann 3MM paper. The EtOAc fraction yielded kaempferol, quercetin, transilin 7-*O*-glucoside, and isorhamnetin 3-*O*-glucoside while pectolinarigenin, 6-methoxyluteolin 4′-methyl ether, and quercetin 3-*O*-β-D-galactoside were isolated from the CH_2Cl_2 extract. Dinatin, 6-methoxyluteolin, kaempferol 3-*O*-β-D-glucoside, and quercetin 3-*O*-β-D-rhamnogalactoside were detected in both the CH_2Cl_2 and EtOAc fractions.

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Leaf material of *A. thrysiflora* (159.1 g), washed with CH_2Cl_2, yielded 24 g of nonpolar extract. When this material was passed over a Polyclar AT column eluted with CH_2Cl_2-MeOH (1:1) with increasing polarity toward MeOH and MeOH-H_2O (1:1), it yielded a mixture of flavones which were separated on a second Polyclar column utilizing toluene-MeOH (8:2) in a gradient to MeOH. The latter column yielded the two major nonpolar flavonoids, the 6-methyl ethers of apigenin and luteolin. The more polar compounds (a diglycoside, a monoglycoside and their aglycone) which occur inside the leaves, were obtained as a mixture after extraction with 80% and 50% aqueous MeOH of the ground CH_2Cl_2-washed leaf material. The mixture was separated on a cellulose column using 40% HOAc; and the fractions obtained, which were subsequently purified on Polyclar in TBA (*t*-BuOH-HOAc-H_2O, 3:1:1), afforded quercetin, its 3-*O*-β-D-galactoside and 3-*O*-β-D-rhamnogalactoside. All compounds were purified over Sephadex LH-20 in 80% or 100% MeOH prior to analysis by uv, ^{1}H nmr (as trimethylsilyl ethers), ms, color reactions on paper under uv light, and comparisons with authentic samples. Hydrolysis of the glycosides (0.1 N TFA, 2 h) yielded the expected aglycones and sugar residues.

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FLAVONOID COMPOUNDS FROM *BALLOTA HIRSUTA*

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From the aerial parts of *Ballota hirsuta* Benth. (Labiatae) fourteen flavonoid compounds, six glycosides, and eight aglycones have been isolated and identified. Previously, only 5-hydroxy-7,4′-dimethoxyflavone had been reported from the genus (*Ballota pseudodictamnus*) (1). The aglycones salvigenin (5-hydroxy-6,7,4′-trimethoxyflavone), kumatakenin (5,4′-dihydroxy-3,7-dimethoxyflavone), genkwanin (5,4′-dihydroxy-7-methoxyflavone), ladanein (5,6-dihydroxy-7,4′-dimethoxyflavone), nuchensin (5,6,3′-trihydroxy-7,4′-dimethoxyflavone), isokaempferide (5,7,4′-trihydroxy-3-methoxyflavone), apigenin and luteolin, the flavonoid *O*-glycosides apigenin-7-(*p*-coumaroyl)-glucoside, apigenin-7-glucoside, luteolin-7-glucoside, quercetin-3-glucoside and luteolin-7-rutinoside, and the flavone-C-glucoside vicenin-2 (apigenin-6,8-di-*C*-glucoside) have been isolated and characterized by uv (2-5) and eims (6-8) and by chromatographic comparisons with authentic compounds.

Within the Labiatae, kumatakenin and isokaempferide have been found previously only in *Salvia glutinosa* (9), nuchensin in *Teucrium nuchense* (10), and quercetin-3-glucoside in *Clechoma hederacea* (11).