FLAVONOIDS OF ALZATEACEAE (MYRTALES)

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ABSTRACT

Three leaf flavonoids are reported from the Alzateaceae, a monotypic family of the New World tropics. Two are flavonol 3-O-glycosides: quercetin 3-O-glucoside and quercetin 3-O-diglucoside. The third is tentatively identified as 5,4′dihydroxy flavone. The presence of these flavonols is consistent with the position of *Alzatea* in the Myrtales. The profile differs from the common pattern of the order in absence of myricetin and is further distinguished by absence of C-glycoflavones and the presence of a flavone, supporting the segregation of *Alzatea* as a distinct family within the Myrtales. More specific relationships with taxa in the order cannot be suggested on this biochemical evidence because of the widespread occurrence of flavonols in the Myrtales.

Alzateaceae is a monotypic family of the New World tropics, long known from Peru and Bolivia, and more recently discovered in the low montane rain forests of Panama and Costa Rica. The single species, *Alzatea verticillata* Ruiz & Pavón was first described from Peru in 1798. Proposed affinities have since included nine families in five orders (Graham, 1984). In recent years the genus has been considered either a member of the Lythraceae (Lourteig, 1965) or the Crypteroniaceae in the order Myrtales (van Beusekom-Osinga & van Beusekom, 1975). It has been most closely associated with the African genus *Rhynchocalyx,* also of uncertain affinities. Classification and relationships of these infrequently collected genera have been restricted to comparison of macromorphological characters.

On the basis of newly accumulated evidence, much of it presented in this volume, the myrtalean position of *Alzatea* is confirmed. Anatomical studies demonstrate the presence of the internal phloem and vestured pitting definitive of Myrtales (van Vliet, 1975). Embryologically, six of seven ordinal characteristics are present (Tobe & Raven, 1984). Presence of ellagic acid in the leaves (Graham, 1984) is consistent with the Myrtales, an order especially characterized by ellagitannins (Bate-Smith, 1962). Within the order, *Alzatea* takes an isolated position. It retains some seemingly ancestral features, such as trilacunar nodes and generalized pollen features, but these are associated with a number of unique apomorphic attributes. Phylogenetically, *Alzatea* is separated from its nearest relative, *Rhynchocalyx,* by a substantial suite of specialized characters supporting recognition of the monotypic Alzateaceae.

Chemical characteristics, whose usefulness in suggesting phylogenies and taxonomic classification is widely accepted (Stuessy & Crawford, 1983), are not well known for *Alzatea.* Ellagic acid and flavonoid mono- and diglycosides, including 3-OH-flavonols, are reported but have not been specifically identified (Graham, 1984). In this study the foliar flavonoids are isolated and identified, and comparison made to the generalized myrtalean flavonoid profile.

MATERIALS AND METHODS

Dried leaf material of two populations of *Alzatea* was examined for flavonoids. Voucher specimens are deposited at MO (Costa Rica: Cartago, *Gómez 18725, 18728*; Panama: Chiriquí, *Knapp & Vodicka 5532*).

Techniques for chromatographic and spectral analyses of the flavonoids follow those presented by Mabry et al. (1970). Briefly, the flavonoids were extracted overnight from leaves with 85% methanol. The resulting extract was applied to Whatman 3MM chromatographic paper both directly and after concentration on a rotary evaporator. Solvent systems of t-butanol, glacial acetic acid, and water (3 : 1 : 1 v/v) and 15% glacial acetic acid in water were used to develop two-dimensional chromatograms. The chromatograms were observed over ultraviolet light and in the presence of ammonia vapor to detect color characteristics of the compounds present. The procedure presented by Mabry et al. (1970) for the isolation and spectral analyses of the compounds was followed with the exception that fused sodium acetate was used for determining the spectral curve for that reagent.

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ANN. MISSOURI BOT. GARD. 71: 855–857. 1984.

TABLE 1. Absorption maxima for compound three ($max^{n.m.}$).

MEOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	H_3BO_3
277, 327	273, 388	280, 305, 346	282, 306, 345	275, 328	328

Acid and enzyme hydrolyses were carried out routinely for glycosidic characterization and to obtain the aglycone for positive identification. Acid hydrolyses were carried out in 5% HCl at 70°C for about one hour. Normally this treatment is sufficient to remove O-glycosides from the flavonoid skeleton. Enzyme hydrolyses were accomplished at 27°C in water. These techniques as well as other pertinent data concerning the characterization of phenolic glycosides are discussed by Harborne (1965).

β-D-Glucosidase was regularly employed because this enzyme is reliable for detecting the presence of glucose. The flavonoid glycosides on which enzyme hydrolysis was not effective were hydrolyzed in acid as outlined above. The resulting sugar was then taken up in water and spotted on cellulose thin-layer plates along with standard sugars for comparison. Circular thin-layer chromatograms were developed in ethyl acetate, pyridine, and water (6 : 3 : 2 v/v) as described by Exner et al. (1977). After drying, the TLC plates were sprayed with a 0.1 M solution of p-anisidine and pthalic acid in 96% ethanol and placed in an oven at 130°C for ten minutes. The sugars were visible as dark brown, red, or green bands. The aglycones also were run, along with authentic reference compounds, by circular thin-layer chromatography.

RESULTS

Three flavonoids were present in the two populations of *Alzatea* examined. Two of the compounds were flavonol 3-O-glycosides: quercetin 3-O-glucoside and quercetin 3-O-diglucoside. The third was tentatively identified as a 5,4′dihydroxy flavone. The latter compound is similar to apigenin in Rf values (0.86, TBA and 0.06, HOAc) and in color (purple in UV). Spectral data for the two compounds, however, differ significantly (Table 1). The data indicate fewer hydroxyls than apigenin and the absence of a 7 hydroxyl. Thus, while the identification of the third compound is less than certain, there are few other possibilities compatible with these data.

DISCUSSION

The emphasis on flavonols in *Alzatea* is consistent with its taxonomic placement in the Myrtales where the flavonoid profile of the order consists of common flavonols and their O-methyl derivatives (Bate-Smith, 1962). The same flavonol glycosides are also found in the related genus *Rhynchocalyx* (Averett & Graham, 1984) and are nearly ubiquitous in the woody angiosperms (Gottlieb, 1975). *Alzatea* is distinctive in the absence of myricetin, which is otherwise common in the order, and in the absence of C-glycoflavones reported from Lythraceae, Combretaceae, Onagraceae, and Myrtaceae (Gornall et al., 1979). The presence of a flavone is unusual for Myrtales, where flavones are very rare (Gornall et al., 1979). In lack of myricetin and presence of a flavone, *Alzatea* flavonoids are more similar to those of Rosales than Myrtales. The two orders are believed to share a common early ancestor (Dahlgren & Thorne, 1984) and the *Alzatea* pattern could reflect that putative relationship. Further evaluation of the presence of the flavone and the limited flavonoid profile exhibited are not feasible since Alzateaceae combines a mixture of primitive and advanced characters and the flavonoids may equally represent an early evolutionary or later reduced condition. The flavonoid data are consistent with other systematic information; viz. *Alzatea* has many common features of the Myrtales, but also sufficient differences to justify recognition as its own family in the order.

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