FLAVONOIDS OF RHYNCHOCALYCACEAE (MYRTALES)

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ABSTRACT

Rhynchocalycaceae is a monotypic family represented by the rare *Rhynchocalyx lawsonioides* from South Africa. Although unquestionably myrtalean, it is isolated in the order with closest affinity to *Alzatea verticillata* in the monotypic Alzateaceae. Foliar flavonoids of *Rhynchocalyx* are reported for the first time. The flavonoid pattern comprises five quercetin 3-O-glycosides, present in approximately equal concentrations. These are quercetin 3-O-glucoside, 3-O-diglucoside, 3-O-rhamnoside, 3-O-xyloside, and 3-O-galactoside. Quercetin 3-O-glucoside and quercetin 3-O-diglucoside also occur in *Alzatea*. The pattern agrees with the typical profile of the Myrtales in which flavonols are common and flavones are rare. It differs in absence of myricetin, which is frequently found in the order. No more specific relationships are possible based on these flavonoid data due to the generalized nature and widespread occurrence of the compounds.

Rhynchocalycaceae is a newly recognized monotypic family based on the rare *Rhynchocalyx lawsonioides* Oliv. from Natal, South Africa. It has been regarded generally as a genus of the Lythraceae, related to *Lawsonia* in subtribe Lagerstromiinae (Oliver, 1895; Sprague & Metcalfe, 1937) although that position was rejected by the monographer of the family (Koehne, 1903). Recently, it was included in a remodeled Crypteroniaceae as sole member of the subfamily Alzateoideae, tribe Rhynchocalyceae (van Beusekom-Osinga & van Beusekom, 1975). Anatomical, embryological, and morphological data now support the relationship of *Rhynchocalyx* to the unigeneric Alzateaceae but reflect a degree of isolation that merits its recognition as a separate family (Johnson & Briggs, 1984; Graham, 1984).

Chemistry of *Rhynchocalyx* has not been previously reported. In this study, foliar flavonoids are isolated, identified, and compared to the general myrtalean flavonoid pattern.

MATERIALS AND METHODS

Dried leaf material of one population of *Rhynchocalyx lawsonioides* was examined (South Africa: Natal, *H. B. Nicholson s.n.* in 1982.) A voucher specimen is deposited at MO.

Techniques for chromatographic and spectral analyses of flavonoids follow those presented by Mabry et al. (1970). Briefly, the flavonoids were extracted overnight from the 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 various compounds present. The procedure presented by Mabry et al. 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.

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 1 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

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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 then visible as dark brown, red, or green bands. The aglycones also were run, along with authentic reference compounds, by circular thin-layer chromatography.

RESULTS

Five flavonol glycosides, all based on quercetin, were present in the population of *Rhynchocalyx* examined: (1) quercetin 3-O-xyloside, (2) quercetin 3-O-galactoside, (3) quercetin 3-O-rhamnoside, (4) quercetin 3-O-diglucoside, and (5) quercetin 3-O-glucoside. Each of the compounds was present in approximately equal concentrations. Compounds (2) and (5) were inseparable by the methods utilized but both sugars were noted in the hydrolysates. Rf values were consistent with the respective monoglycosides and not with that of a diglycoside.

CONCLUSIONS

The flavonoid pattern in *Rhynchocalyx* consists of a range of flavonol monoglycosides. The emphasis on flavonols in the genus is consistent with its classification in the Myrtales where common flavonols are the most frequent constituents (Bate-Smith, 1962; Gornall et al., 1979). Both quercetin 3-O-glucoside and quercetin 3-O-diglucoside are also found in *Alzatea* (Graham & Averett, 1984). Their wide dispersal in the angiosperms, however, precludes any taxonomic implication. Myricetin, typical of the order, is absent in both *Rhynchocalyx* and *Alzatea*. Flavonoid data support placement of *Rhynchocalyx* in the Myrtales but offer no further indication of phylogenetic relationship.

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