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A PHYTOCHEMICAL STUDY OF SELECTED PODOSTEMACEAE: SYSTEMATIC IMPLICATIONS

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ABSTRACT

Podostemum ceratophyllum contains γ-mangostin and its 6-glucoside. The aglycone is also present in *Marathrum, Oserya,* and *Vanroyenella,* but is absent from *Tristicha.* Anthocyanins were identified from all genera, but no additional flavonoids were detected. Condensed and hydrolyzable tannins, iridoids, cyanogenic glycosides, and alkaloids were lacking in all Podostemaceae examined. Phytochemical constituents do not clarify the uncertain systematic affinity of Podostemaceae, but emphasize the generally accepted taxonomic isolation of this family.

Key words: *Marathrum, Oserya, Podostemum, Tristicha, Vanroyenella,* Podostemaceae, xanthone glucoside, γ-mangostin, comparative phytochemistry, aquatic plants.

INTRODUCTION

The Podostemaceae are the largest family of aquatic flowering plants (48 genera, 268 species) (Cook 1990; Novelo and Philbrick 1993). The family's distribution is largely pantropical with about 40 species occurring in Africa, 20 in Australasia, and the remainder mostly in tropical America. *Podostemum ceratophyllum* Michx. is one of the few temperate members of the family and the only taxon that occurs in the United States (Philbrick and Crow 1983). Members of this strictly aquatic family occur attached to rocks in clear running water of streams and rivers, especially waterfalls and rapids. They exist most of the time submerged, but produce aerial flowers and fruits when the water levels drop (Philbrick 1984). Podostemaceae are highly modified vegetatively and diverse in form and organization, but relatively simple anatomically (Rutishauser and Huber 1991).

The phylogenetic placement of Podostemaceae is problematic, in part because the family is taxonomically very isolated (Cronquist 1981). The placement of Podostemaceae in their own order (Podostemales) by most authors (Takhtajan 1969; Cronquist 1981; Thorne 1992) or as the sole member of a superorder (Podostemiflorae, Dahlgren 1980) attests to their unclear affinities and isolated systematic position. Taxonomic affinities of Podostemaceae are uncertain, but suggested Rosalean candidates include Saxifragaceae and Crassulaceae (Tahktajan 1969; Cronquist 1981; Thorne 1992) or perhaps Hydrostachyaceae (Cronquist 1981).

A goal of the present study was to phytochemically characterize representative members of Podostemaceae, whose chemistry is virtually unknown and to de-

termine if comparative phytochemistry could clarify systematic relationships either within the family or with other angiosperm families.

MATERIALS AND METHODS

Plant materials were collected from the field by the authors (*Marathrum, Oserya, Tristicha, Vanroyenella*) or generously provided by colleagues (*Podostemum ceratophyllum*). Voucher specimens are deposited at RSA-POM. Samples of air-dried and powdered whole plants were extracted overnight with 80% MeOH. Extracts were filtered and concentrated under vacuum, then subjected to 2D-PC on Whatmann 3MM paper using TBA and 15% HOAc as solvents (Mabry, Markham, and Thomas 1970). Phenolic compounds detected on chromatograms under UV illumination were purified by repeated 1D-PC using TBA.

Anthocyanin pigments were purified by 1D-PC using BAW as solvent and were identified based on chromatographic properties in four solvents (Harborne 1967).

Purified xanthones were characterized chromatographically and UV-spectroscopically using standard methods (Mabry et al. 1970). Silica gel TLC of xanthone aglycones was according to the methods of Saleh (1974). Enzymatic hydrolysis of the xanthone glycoside followed standard procedures for flavonoids (Mabry et al. 1970).

Additional classes of phytochemicals were examined using the following methods: ellagitannins (Bate-Smith 1972), iridoids (Weiffering 1966), cyanogenic glycosides and proanthocyanins (Gibbs 1974), and alkaloids (Hultin and Torssell 1965).

RESULTS

Examination of methanolic extracts of whole plants by paper chromatography revealed the presence of phenolic compounds. Compounds were detected whose chromatographic mobilities suggested flavonoid aglycones and glycosides, but whose fluorescence color on paper (red or orange under UV illumination turning to fluorescent yellow in the presence of ammonia) was more characteristic of xanthones. Flavonoids (except anthocyanins) were not detectable in any samples examined.

The same xanthone aglycone (based on fluorescence color and chromatographic properties) was observed from *Podostemum ceratophyllum, Marathrum haenkeanum* Engler, *M. elegans* van Royen, and *M.* sp. (Novelo and Philbrick, unpublished), *Oserya coulteriana* Tul., and *Vanroyenella plumosa* Novelo & Philbrick, but was undetectable in *Tristicha trifaria* (Bory ex. Willd.) Sprengel. In addition, a xanthonelike compound whose chromatographic properties suggested a glycoside was detected in *P. ceratophyllum.* This putative aglycone (compound 1) and glycoside (compound 2) were purified from *P. ceratophyllum* and chemically characterized. The presence of the xanthone glycoside cannot be unequivocally excluded from other genera examined since very limited material (less than 2 g) from these taxa was available for extraction and analysis.

UV-spectroscopic properties of compounds 1 and 2 are typical of xanthones in general (Hostettmann and Hostettmann 1989) and 1,3,6,7-tetraoxygenated xanthones in particular (Imperato 1991).

The xanthone aglycone (compound 1) has been provisionally identified as

γ-mangostin [1,3,6,7-tetrahydroxy-2,8-di(3,3-dimethylallyl) xanthone]. This identification is based upon agreement between TLC (silica gel) mobility values of compound 1 and γ-mangostin in 10 solvents (#2, 3, 4, 5, 10, 15, 22, 28, 31, and 35 from Saleh 1974). In addition, methanolic UV-spectroscopic features of compound 1 agree with those for γ-mangostin presented by Jefferson, Quillinan, Scheinmann, and Sim (1970). Compound 1 also exhibits spectroscopic shifts in diagnostic reagents similar to those for 1,3,6,7-tetrahydroxy-8-(3,3-dimethylallyl) xanthone (= 2-deprenyl-γ-mangostin) presented by Nielsen and Arends (1979).

Acid hydrolysis of compound 2 (glycoside) yielded compound 1, as shown by cochromatography on paper in four solvents (TBA, BAW, water, 15% HOAc) indicating that compound 2 is an O-glycoside of compound 1. Enzymatic (β-glucosidase) hydrolysis of compound 2 yielded compound 1, as shown by TLC (silica gel) cochromatography in 10 solvents from Saleh 1974 (listed above) indicating that the sugar moiety is glucose.

The xanthone glycoside (compound 2) has been provisionally identified as γ-mangostin-6-O-glucoside based on comparison of UV-spectroscopic features of compound 2 with those of magniferin-6-O-glucoside and mangiferin-7-O-glucoside (Goetz and Jacot-Guillarmod 1977). This xanthone glycoside is previously unreported.

Two anthocyanins were the only flavonoids detectable among Podostemaceae samples examined. These compounds were identified as cyanidin 3-glucoside (from *P. ceratophyllum*) and pelargonidin 3-rhamnoside 5-glucoside (from *Marathrum* sp. [Novelo and Philbrick, unpublished data]) based on chromatographic mobilities of the glycoside in four solvents (BAW, 1% HCl, 15% HOAc, Bu-HCl) and the aglycone in BAW and Forrestals (Harborne 1967).

A partially purified (due to limited samples) anthocyanin from *Tristicha trifaria* cochromatographed in the four above-mentioned solvents with cyanidin 3-glucoside from *P. ceratophyllum* and a standard purified from *Camellia japonica.* Partially purified anthocyanins from *Oserya coulteriana* and *Vanroyenella plumosa* cochromatographed as above with pelargonidin 3-rhamnoside 5-glucoside from *Marathrum* sp.

There was no chromatographic evidence for the occurrence of gallic or ellagic acid in either hydrolyzed or nonhydrolyzed sample extracts for any species of Podostemaceae. Ellagitannins were undetectable in all Podostemaceae samples using Bate-Smith's (1972) chromogenic test. Similarly, plant samples upon hydrolysis (2N HCl) yielded only anthocyanidin amounts attributable to naturally occurring, endogenous anthocyanins and not due to the presence of proanthocyanidins. Thus, no condensed or hydrolyzable tannins were detectable among Podostemaceae examined.

Tests for iridoids, cyanogenic glycosides, and alkaloids were uniformly negative among all examined Podostemaceae.

DISCUSSION

Xanthone Chemistry

The number of known naturally occurring xanthone compounds has increased dramatically in the last decade due largely to advances in structure determination using spectroscopic techniques (Hostettmann and Wagner 1977; Sultanbawa 1980;

Bennett and Lee 1989). Currently over 200 xanthone aglycone structures are known, whose diversity derives from the variety of substitution patterns of hydroxyl-, methoxyl-, and isoprenyl-groups. Xanthones usually occur naturally as aglycones, but glycosides (both C- and O-) are known, as discussed below.

Xanthone aglycones have been reported from eight angiosperm families (Hostettmann and Hostettmann 1989), but occur commonly in only two families (Clusiaceae and Gentianaceae). Xanthone O-glycosides have been reported from three families (Gentianaceae, Polygalaceae, and Clusiaceae), but occur commonly only among Gentianaceae. Xanthone C-glycosides in general and the compound mangiferin in particular represent a special case. Mangiferin has been reported from 19 angiosperm families and several ferns (Richardson 1983). Mangiferin, however, is thought to be biogenetically distinct from (and therefore nonhomologous with) other polyhydroxyanthones (Bennett and Lee 1989).

Little was known regarding the phytochemistry of Podostemaceae until the occurrence in this family of biphenyls and xanthones was reported recently (Burkhardt, Schild, Becker, and Gruber 1992). The occurrence of xanthones in Podostemaceae and the present report of a xanthone O-glycoside are noteworthy because of the limited distribution of these classes of compounds.

Two xanthone oxidation patterns have been detected among Podostemaceae taxa examined to date: 1) 1,3,5-trihydroxylation in *Mourera fluviatilis* (Burkhardt et al. 1992) and 2) 1,3,6,7-tetrahydroxylation in *Marathrum, Oserya, Podostemum,* and *Vanroyenella* (present report). Methoxylation of the xanthone nucleus has not been observed in Podostemaceae although methoxylated biphenyls were reported from *Mourera* (Burkhardt et al. 1992). In other families (Clusiaceae [Bennett and Lee 1989] and Gentianaceae [Schaufelberger and Hostettmann 1988]) xanthone distribution data have provided useful taxonomic insights at the generic and tribal levels. Since only six of the 48 genera recognized among Podostemaceae have been examined phytochemically, variation in hydroxylation pattern detected among examined taxa suggests that a survey of additional genera may provide valuable insights into suprageneric relationships within the family.

The 1,3,6,7-tetrahydroxyxanthone pattern observed among Podostemaceae has a sporatic distribution among angiosperms, most commonly found as the C-glucosylxanthone, mangiferin. The distribution of this xanthone tetraoxygenation pattern, excepting mangiferin, is limited to three families (Clusiaceae, eight genera; Moraceae, three genera; and one species of the fern genus *Cystopteris* [Athyriaceae]).

Isoprenyl substitution of xanthones is common and widely distributed among Clusiaceae (but is unknown from Gentianaceae) and also occurs in Moraceae (Sultanabawa 1980; Bennett and Lee 1989). The present report, in addition to the previously reported occurrence of monoprenylated (6-desoxyjacareubin) and diprenylated (trapeziafoliaxanthone) trihydroxyxanthones from *Mourera* (Burkhardt et al. 1992), suggest that isoprenyl substitution of xanthones is a phytochemical characteristic of the Podostemaceae. Isoprenyl substitution of 1,3,6,7-tetraoxygenated xanthones as found in Podostemaceae is restricted to reports from the Clusiaceae, as is the single report of an incompletely characterized 1,3,6,7-tetrahydroxyxanthone O-glucoside (nonprenylated) from *Garcinia mangostana* (Holloway and Scheinmann 1975).

O-glycosylation of xanthones has been reported from only three families (Gen-

tianaceae, Polygalaceae, Clusiaceae) (Hostettmann and Hostettmann 1989). Xanthone O-glucosides are common in Gentianaceae, but quite rare in Polygalaceae and Clusiaceae (two compounds). Whereas isoprenyl substitution of xanthones is common in Clusiaceae and unknown in Gentianaceae, O-glucosylation is rare in Clusiaceae and common in Gentianaceae. *Podostemum ceratophyllum,* uniquely to date, combines these two chemical features by producing an isoprenylated O-glucosylated xanthone. The present report represents the first known occurrence of a prenylated xanthone O-glucoside.

Although the xanthone chemistry of Podostemaceae might initially suggest a relationship with Clusiaceae or Gentianaceae, such a conclusion is not supported by other sources of systematic data. Further, xanthone chemistry of Podostemaceae exhibits structural features characteristic of both Clusiaceae (prenylation) and Gentianaceae (O-glycosylation), but combines them in a manner unique to Podostemaceae. Xanthone production in Podostemaceae appears to be of an independent, nonhomologous origin to that in other families and emphasizes the generally accepted view of the taxonomic isolation of Podostemaceae.

Anthocyanin Chemistry

Anthocyanins are common foliar and floral pigments in plants. Two anthocyanins were identified from examined Podostemaceae. Cyanidin 3-glucoside is present in *Podostemum* and *Tristicha* and pelargonidin 3-rhamnoside 5-glucoside is present in *Marathrum, Vanroyenella,* and *Oserya.* Cyanidin glucosides occur widely in leaves and flowers and pelargonidin glycosides often occur in flowers, but the occurrence of a pelargonidin glycoside in leaves is quite rare (Harborne 1967) and is noteworthy in Podostemaceae.

A particularly notable feature of the phytochemistry of Podostemaceae is the absence of additional detectable flavonoid compounds which are nearly universal plant constituents. Anthocyanin production is one of the end products of flavonoid biosynthesis and anthocyanin occurrence in Podostemaceae indicates that flavonoid biosynthesis capacity has not been lost in this family, but rather that no biogenic intermediates are accumulated. Whereas quantitative and qualitative reductions of foliar flavonoids in aquatic plants has been reported (Les and Sheridan 1990), the loss of flavonoids is not a general feature of the aquatic habitat in which Podostemaceae occur. Other aquatic plants sequester typical suites of phenolic compounds characteristic of their taxonomic grouping (Scogin 1992*a, b*). In spite of their physicochemical similarities, xanthones do not replace flavonoids in terrestrial plant groups in which they cooccur (Harborne and Mabry 1982). However, the possibility remains that xanthones may have functionally replaced flavonoids in Podostemaceae in the aquatic environment.

Intergeneric Relationships

No proposals regarding intergeneric relationships among Podostemaceae have been presented in the systematic literature. The current limited survey base (both taxonomically and phytochemically) precludes definitive conclusions regarding relationships among genera of Podostemaceae based on chemical constituents. However, several patterns should be noted which suggest that further investigations might yield useful data.

Table 1. Phytochemical profiles of Podostemaceae and putatively related families.

	Podostemaceae	Saxifragaceae*	Crassulaceae*
Ellagic acid	−	+	−
Pelargonidin	+	−	+
Flavonols	−	+	+
Flavones	−	+	−
C-glycosyl flavones	−	−	+
Proanthocyanidins	−	+	+
Iridoids	−	−	−
Cyanogenic glycosides	−	+	+
Xanthones	+	−	−
Alkaloids	−	+	+
Biphenyls	+	−	−

* Data from Gibbs (1974), −: compounds not detected, +: compounds present in at least some species.

Similarities in anthocyanin chemistry indicate a phenetic similarity within the *Marathrum-Oserya-Vanroyenella* and *Podostemum-Tristicha* generic groupings. In addition, the genus *Tristicha,* which has frequently been treated as the separate family Tristicaceae, stands phytochemically apart from other examined genera by virtue of its lack of detectable xanthones. *Podostemum* stands apart at present from the other xanthone-producing genera as the only genus with detectable xanthone O-glycosides. This may be an erroneous distinction because only *Podostemum ceratophyllum* was available as a large (ca. 100 g) bulk sample for analysis. Very limited samples of other genera may have resulted in failure to detect xanthone glycoside presence. The xanthones of *Mourera* (Burkhardt et al. 1992) exhibit an oxygenation substitution pattern different from that noted in the present study, suggesting that xanthone oxygenation patterns within the family may exhibit characteristic taxonomic or phytogeographic distributions. Further study will be required to explore these possibilities.

Family Relationships

Uncertainty regarding the systematic affinities of the Podostemaceae have been longstanding and affinities remain unclear. The only clear consensus among systematists is that the family is very isolated (Dahlgren 1980; Cronquist 1981). The extreme representation of that isolation has been the suggestion of Cusset and Cusset (1988) that members of the Podostemaceae are best treated as a third class of angiosperms, the Podostemopsida. These taxa have also been treated as a superorder, the Podostemiflorae (Dahlgren 1980). The most frequent current treatment is as an order, the Podostemales, within a superorder Rosanae (Thorne 1992) or Rosidae (Cronquist 1981) with likely relationships with Crassulaceae or Saxifragaceae. A relationship between Podostemaceae and Hydrostachyaceae has occasionally been proposed based largely on their shared river-rapids habitat, but there is no phytochemical support for this suggestion (Scogin 1992*a*).

A phytochemical comparison among Podostemaceae, Crassulaceae, and Saxifragaceae is presented in Table 1. No compelling phytochemical similarity is present between Podostemaceae and either of these putative relatives. Phytochemical data at present serves only to reinforce the general agreement regarding

the systematically isolated position of Podostemaceae and currently affords no insight into its systematic affiliations.

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