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FLAVONOL DERIVATIVES OF *DESMANTHODIUM* (COMPOSITAE)

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Key Word Index—*Desmanthodium*; Compositae; Heliantheae; Milleriinae; flavonols; *O*-methylation; chemosystematics.

Abstract—The flavonoids of three species of *Desmanthodium* are based upon kaempferol, quercetin and quercetagetin. Sugar substitutions comprise glucosides, galactosides, rhamnosides, rutinosides and diglucosides. Four different *O*-methylated compounds occur in field populations of the genus, but they are found in all species and are therefore not useful for sectional or subgeneric delimitations. The flavonoid profile of *Desmanthodium* is very similar to that of *Clibadium*, which parallels their close morphological affinity.

INTRODUCTION

As a continuation of our biosystematic study of the subtribe Milleriinae of the Heliantheae, we undertook an examination of the flavonoid chemistry of three species of *Desmanthodium* Benth: *D. fruticosum* Greenm., *D. hondurense* Molina and *D. perfoliatum* Benth. The genus consists of ten species and is known from Mexico and Central America [1]. A recent communication [2] described our study of the flavonoids of the closely related genus *Clibadium*. To the best of our knowledge, no other work on the flavonoids of *Desmanthodium* has been described.

RESULTS

The flavonoids of the *Desmanthodium* species examined in this study were all based upon kaempferol, quercetin and quercetagetin. Kaempferol and quercetin occurred as their 3-*O*-glucosides and 3-*O*-galactosides while quercetin also occurred as the 3-*O*-rhamnoside in *D. perfoliatum*. The diglycoside fraction consisted of the 3-*O*-rutinosides, 3-*O*-rhamnosylgalactosides and 3-*O*-diglucosides of both kaempferol and quercetin. These diglycosides were observed in all specimens taken from the field; they were apparently absent from greenhouse material.

A set of *O*-methylated compounds based upon quercetin and quercetagetin was also found. 3-*O*-Methylquercetagetin 7-*O*-glucoside and 3,3′-di-*O*-methylquercetin 7-*O*-glucoside were found in all species, although only a trace of the monomethyl ether was seen in the greenhouse-grown sample of *D. perfoliatum*. Quercetagetin 7-*O*-glucoside was observed as a major component of greenhouse *D. perfoliatum* and as a trace constituent of *D. hondurense*. 3′-*O*-Methylquercetagetin 7-*O*-glucoside was observed only in the cultivated *D. perfoliatum*. 6-*O*-Methylquercetagetin (patuletin) 7-*O*-glucoside was observed in all samples although there was some doubt about its presence in *D. perfoliatum* collected from nature. 3,6,3′-Tri-*O*-methylquercetagetin 7-*O*-glucoside was seen in all specimens except cultivated *D. perfoliatum*.

In the case of *D. perfoliatum* two samples of plant material were examined, one collected in the field and the other grown from seed obtained from the same collection. These differed in their flavonoid patterns as described above. Four collections of *D. hondurense* and six collections of *D. fruticosum* showed essentially identical 2-dimensional thin layer chromatograms, at least with regard to their flavonoid patterns.

DISCUSSION

Although only three of the ten species of *Desmanthodium* have been examined for flavonoids, these taxa are diverse morphologically and represent a reasonable sampling of the genus. Six and four populations of *D. fruticosum* and *D. hondurense*, but only one of *D. perfoliatum*, have been analyzed, respectively.

The results, summarized in Table 1, indicate that the occurrence of flavonoids within each species of *Desmanthodium* is uniform. This contrasts with the situation in the related genus, *Clibadium*, in which much infraspecific chemical variation is known [2, 3]. The only variation of this type in *Desmanthodium* is between field- and greenhouse-grown material of *D. perfoliatum*. Seeds from the field population were collected and one plant survived in the greenhouse (not yet having flowered), from which leaves were taken for flavonoid analysis. It is difficult to interpret presence or absence of compounds in the greenhouse material because the genetic composition of this individual is presumably different from that of plants collected in the field (the breeding system is not known). The nature of the growth conditions in the greenhouse, including light quality and intensity, could influence the production of flavonoids [4]. As a result the concentrations of several flavonoids and their glycosides may have dropped below detectable levels, whereas those of quercetagetin 7-*O*-glucoside and quercetagetin-3′-methyl ether 7-*O*-glucoside have increased.

The distribution of flavonoids among the three species of *Desmanthodium* is essentially the same (Table 1). A trace of quercetagetin 7-*O*-glucoside occurs in *D. hondurense* and not the others, but this is also found in the greenhouse

Table 1. The major flavonoids of three species of *Desmanthodium*

Flavonol	Presence/absence in*			
	D. fruticosum	*D. hondurense*	*D. perfoliatum* Field	*D. perfoliatum* Greenhouse
Kaempferol glycosides				
3-*O*-Galactoside	+	+	+	n.d.
3-*O*-Glucoside	+	+	+	+
3-*O*-Gal-rhamnoside	+	+	+	n.d.
3-*O*-Rutinoside	+	+	+	–
3-*O*-Diglucoside	+	+	+	–
Quercetin glycosides				
3-*O*-Galactoside	+	+	+	n.d.
3-*O*-Glucoside	+	+	+	+
3-*O*-Rhamnoside	–	–	+	–
3-*O*-Rutinoside	+	+	+	tr
3-*O*-Diglucoside	+	+	+	tr
3-*O*-Rhamnoside	+	+	+	n.d.
Methylated derivatives				
Patuletin-3-*O*-glycoside	+	+	?	+
3-Methylquercetagetin 7-*O*-glucoside	+	+	+	tr
3,3′-Dimethylquercetin 7-*O*-glucoside	+	+	+	+
3,3′6-Trimethylquercetagetin 7-*O*-glucoside	+	+	+	–
Quercetagetin 7-*O*-glycoside	–	tr	–	+
Quercetagetin 3′-methyl ether 7-*O*-glycoside	–	–	–	+

*n.d. = not determined, tr = trace. Numbers of populations analysed were 6, 4, 1 and 1, respectively.

material of *D. perfoliatum* (not from the field-collected material, however). Also, *D. perfoliatum* (field) has traces of patuletin 3-*O*-glucoside in contrast to the other two species, but greenhouse material of the same taxon showed detectable quantities.

The close morphological relationship of *Desmanthodium* to *Clibadium* [1] is paralleled by the similarity of flavonoids. In both genera, kaempferol and quercetin occur with several methylated derivatives. Both contain quercetagetin and accumulate derivatives methylated at the 3′- and 6-positions. *Desmanthodium* is different in accumulating mono-methyl derivatives at position 3, quercetin dimethyl ethers and trimethyl compounds. By contrast, *Clibadium* accumulates dimethyl derivatives of quercetagetin and *Desmanthodium* does not. However, these compounds would be expected to occur in the latter genus, probably at low (and perhaps undetectable) concentrations because they are probably biosynthetic intermediates.

Morphologically, *Clibadium* is closest to *Ichthyothere*, a genus of about 15 species primarily from northeastern South America, but for which no flavonoid data are presently available. (The two genera are quite similar, however, in their polyacetylenes [2, 5].) *Desmanthodium* relates to *Clibadium* somewhat more distantly than the latter does to *Ichthyothere*. The meager chromosomal data support this interpretation. *Clibadium* is $n = 16$ and 24 (probably $x = 8$) [6–12], and *Ichthyothere* is $n = ca\ 33$ (probably *ca* 32, also $x = 8$) [13], but *Desmanthodium* is different with $n = 18$ [14, 15], with probably $x = 9$. Recently Robinson described two new subtribes of the Heliantheae [16]: Clibadiinae and Desmanthodiinae. Although details of these new subtribes have not yet been provided, such as the genera included other than the obvious types, it seems unlikely that *Clibadium* and *Desmanthodium* are so distantly related that they deserve to be separated at the subtribal level. Certainly the flavonoid data provide no support for such a view.

EXPERIMENTAL

Source of plants (All *Stuessy* and *Gardner* are at OS; asterisks indicate collections from which the initial analysis for flavonoids was done.) *Desmanthodium fruticosum* Greenm.: Mexico: Guerrero, *4206*, *4215*; Jalisco, *4135*, *4147*; Michoacán, *4173*; Nayarit, *4115*.* *D. hondurense* Molina: Honduras: Comayagua, *4408*,* *4414*; Ocotopeque, *4390*, *4401*. *D. perfoliatum* Benth.: Mexico: Chiapas, *4306**.

Extraction and isolation procedures. Plant material was extracted repeatedly with boiling 80% MeOH. Combined extracts were evapd to dryness *in vacuo*, the residue re-extracted with hot water and filtered using a diatomaceous filter aid. Extraction of the aq. fraction with water-saturated *n*-BuOH afforded the polyphenolic fraction. After removal of *n*-BuOH under red. pres., the residue was taken up in MeOH for storage. Subsequent fractionations were accomplished using column chromatography on Sephadex LH-20 followed by partition separations, all as described by Wilkins and Bohm [17]. Final purifications were accomplished by prep. TLC [17].

Identification of compounds. The mono- and diglycosides of kaempferol and quercetin were identified by routine methods including UV analysis [18], partial and total hydrolyses and comparison with known compounds. In the diglycosides, the comparison standards were the corresponding compounds obtained from *Clibadium* [2]. Quercetagetin 7-*O*-glucoside, 6-*O*-methylquercetagetin (patuletin) 7-*O*-glucoside, and 3′-*O*-

methylquercetagetin 7-*O*-glucoside were identified by comparison with compounds isolated from *Clibadium* species [2].

The structure of 3-*O*-methylquercetagetin 7-*O*-glucoside was arrived at in the following way. UV characteristics suggested it to be a flavonol. Acid hydrolysis was very slow under conditions which cleave 3-*O*-glycosides readily; the sugar released was glucose. That it was a flavonol 7-*O*-glucoside was confirmed by the absence of an acetate-induced shift of band II. Acetate did cause a significant bathochromic shift of band I and addition of borate indicated the presence of 3′,4′-dihydroxylation. This agreed with the yellow-orange colour reaction observed with diphenylboric acid ethanolamine complex which is characteristic of the quercetin-type B-ring. Addition of $AlCl_3$ yielded a band I shift of 87 nm; addition of HCl resulted in a band I bathochromic shift of 19 nm relative to the MeOH spectrum. This behaviour indicates the presence of 6-oxygenation plus a substituted 3-hydroxyl function (either sugar or methyl) [18]. Both the glycoside and the aglycone appeared dark under UV light which requires that the 3-position be substituted. Since there is a sugar at the 7-position, the methyl group must be at position 3. TLC against a series of methylated standards suggested that the unknown aglycone bears a single *O*-methyl function. Thus, the compound must be 3-*O*-methylquercetagetin 7-*O*-glucoside.

The structural determination of 3,3′-di-*O*-methylquercetin 7-*O*-glucoside proceeded as follows. The compound exhibited UV characteristics of a flavonol and appeared as a dark absorbing spot on chromatograms. Acid hydrolysis, which yielded glucose, was very slow indicating glycosylation at the 7-position. The aglycone also appeared dark, suggesting that the 3-position was still substituted. UV spectral properties suggested the presence of 3′,4′-oxygenation but the absence of both a borate shift and the typical quercetin yellow-orange colour with the diphenylborate spray argued for substitution at one of these positions. Acetate did not alter the band II position of the glycoside but did produce a band I shift suggesting an unsubstituted 4′-hydroxyl. $AlCl_3$ gave a 56 nm bathochromic shift of band I which was stable with acid; therefore, 6-oxygenation could be eliminated. TLC against methylated standards suggested the presence of two *O*-methyl functions. The compound is thus 3,3′-di-*O*-methylquercetin 7-*O*-glucoside.

Finally, the trimethylated flavonoid was determined by the following means. This compound also exhibited the UV and hydrolytic characteristics of a flavonol 7-*O*-glucoside having 3′,4′-oxygenation and oxygenation at position 6. The compound was dark under UV before and after hydrolysis indicating the 3-*O*-methyl group. The ^1H NMR spectrum of the glucoside showed the quercetagetin pattern (H-8, H-2′, H-5′ and H-6′) plus three *O*-methyl groups. UV spectral data agreed with those published [18] for jacein, 3,6,3′-tri-*O*-methylquercetagetin 7-*O*-glucoside.

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