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FLAVONOL DERIVATIVES OF *ICHTHYOTHERE TERMINALIS*

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Key Word Index—*Ichthyothere*; Compositae; Heliantheae; Milleriinae; flavonols; *O*-methylation; glycosylation patterns; chemosystematics.

Abstract—The flavonoids of *Ichthyothere terminalis* are based upon quercetin, with minor amounts of kaempferol and dihydroquercetin. All glycosides are linked at position-3. Quercetin 3-glucoside, 3-galactoside, and 3-arabinoside comprise the monoglycoside fraction. The diglycoside fraction consists of quercetin 3-rutinoside, 3-rhamnosylgalactoside and 3-digalactoside. The single triglycoside present was shown to be quercetin 3-rhamnosylgalactosylgalactoside. A major constituent of the aglycone fraction was shown to be 3-*O*-methylquercetin. The flavonoid profile of *Ichthyothere terminalis* shows marked differences from those of the related genera *Clibadium* and *Desmanthodium*.

INTRODUCTION

Recent studies in our laboratory have dealt with flavonoid constituents of species of *Clibadium* [1] and *Desmanthodium* [2], both members of the Heliantheae and placed in the subtribe Milleriinae by Stuessy [3]. A third related genus, *Ichthyothere*, was also included in this subtribe [3]. The availability of two collections of *I. terminalis* (Spreng.) S. F. Blake afforded an opportunity to compare its flavonoid chemistry with that of the other two, putatively related, genera. This note records the results of that study.

RESULTS

The two collections of *I. terminalis* were chromatographically identical. Resolution of the combined material afforded four fractions: aglycones, mono-, di- and triglycosides. The aglycone fraction consisted of small amounts of kaempferol, quercetin and dihydroquercetin and a very large amount of 3-*O*-methylquercetin. Monoglycosides present were quercetin 3-glucoside and 3-galactoside in the approximate ratio of 1:3 along with a smaller amount of the 3-arabinoside. The diglycoside fraction consisted of quercetin 3-rutinoside and 3-rhamnosylgalactoside in a ratio of *ca* 1:3, along with quercetin 3-digalactoside. A pair of incompletely characterized quercetin 3-monoglycosides was also detected. These compounds, which appeared as a single spot, had R_f values *ca* half of those of quercetin 3-glucoside in both the aqueous and organic based systems routinely used in our laboratory. This behaviour is similar to that of the known quercetin 3-glucoside gallates [4] which suggested that ammonium hydroxide might release the parent glycoside. However, treatment with ammonium hydroxide for 20 min at room temperature had no effect upon the unknown and treatment for 90 min at room temperature brought about extensive degradation so that no recognizable flavonoid was found. Acid hydrolysis afforded quercetin, glucose and galactose (sugar ratio *ca* 1:3), and two blue fluorescent spots (366 nm). The limited amount of material available prevented further study.

DISCUSSION

Our earlier studies of the flavonoids of *Clibadium* [1] and *Desmanthodium* [2] showed a variety of flavonol derivatives in each genus. All members of the two genera studied accumulated kaempferol and quercetin mono- and diglycosides while one *Clibadium* species also produced an isorhamnetin glucoside. Seven of the eleven species of *Clibadium* and all three species of *Desmanthodium* examined had a series of compounds based upon quercetagetin (6-hydroxyquercetin). Derivatives of quercetagetin found in *Clibadium* were the 6-methyl ether (patuletin), the 3′-methyl ether, and the 6,3′-dimethyl ether. These all occurred as their 7-*O*-glucosides. *Desmanthodium* yielded quercetagetin 6- and 3′-methyl ethers as well as 3,6,3′-trimethylquercetagetin. 3,3′-Dimethylquercetin also occurred in all three species of *Desmanthodium*. As in *Clibadium* all the methylated flavonols found in *Desmanthodium* occurred as 7-glucosides. However, 7-galactosides of methylated flavonols were never seen in contrast to the regular occurrence of flavonol 3-glucoside and 3-galactoside mixtures.

Several features of the flavonoid complement of *I. terminalis* differed from *Clibadium* and *Desmanthodium*. Whereas *Clibadium* and *Desmanthodium* elaborated an array of *O*-methylated flavonols, *I. terminalis* showed only a single such compound, namely 3-*O*-methylquercetin. The capacity to make *O*-methylated flavonols is not a distinctive criterion,

Table 1. Distribution of biosynthetic capacities in analysed species of *Clibadium*, *Desmanthodium* and *Ichthyothere*

Biosynthetic capacity	Genus			
	Clibadium		*Desmanthodium*	*Ichthyothere*
	Group 1 (6 spp.)	Group 2 (5 spp.)	(3 spp.)	(1 sp.)
Kaempferol glycosides	+	+	+	−*
Quercetin glycosides	+	+	+	+
Quercetagetin derivatives	+	+	+	−
O-Methylation	−	+	+	+
3-*O*-Methylation	−	−	+	+
7-*O*-Glycosylation	+	+	+	−
Flavonol triglycosides	−	−	−	+
Dihydroflavonol	−	−	−	+

*A trace of the aglycone was found.

however, since several *Clibadium* species [1 and unpublished data] do not appear to possess such compounds. The position of *O*-methylation may be a better character since 3-*O*-methylation has not yet been reported in *Clibadium* whereas it does clearly occur in *Ichthyothere* and in all three species of *Desmanthodium* examined.

7-*O*-Glycosylation was found in all *Clibadium* and *Desmanthodium* species where methylated flavonoids were present. However, 7-*O*-glycosylation was not observed in *I. terminalis*. It is, therefore, interesting to speculate on the nature of the substrate requirements for 7-*O*-glucosylation. The enzyme could require a relatively less polar substrate, such as the *O*-methylated compounds found in *Clibadium* and *Desmanthodium*. Although two exceptions exist, quercetin 7-glucoside in *C. sessile* [1] and patuletin 3-glucoside in the *Desmanthodium* species [2], *O*-methylation is usually associated with 7-glycosylation. *I. terminalis* produced a comparatively large amount of 3-*O*-methylquercetin which was seen only as the aglycone. Because glycosylation at position-3 is precluded by the methyl group, it is possible that 7-*O*-glycosylation cannot occur in this species.

The occurrence of only a trace of kaempferol, with a lack of any glycosylated forms, and the finding of dihydroquercetin also serve to distinguish *I. terminalis* from the other two genera. Finally, *I. terminalis* accumulates quercetin 3-rhamnosylgalactosylgalactoside to the extent that it is a major component of the flavonoid profile. Triglycosides were not encountered in any of the species of *Clibadium* and *Desmanthodium* examined. The biosynthetic capacities of the three genera are summarized in Table 1.

In a recent paper on the terpenoid chemistry of four species of *Ichthyothere*, Bohlmann *et al.* [5] stated that the overall chemistries of *Clibadium* and *Ichthyothere* are distinct except for the presence of the polyacetylenic alcohol ichthyothereol and its acetate in both *C. sylvestre* [6] and *I. terminalis* [7]. Our current findings on the flavonoids of *I. terminalis* support the idea that significant chemical differences exist between the two genera. Differences in chromosome numbers between the two genera have also been found [2 and references cited therein]. A survey of further *Ichthyothere* species is clearly needed.

EXPERIMENTAL

Source of plants. Brazil: Amazonas, Manaus, 28 Nov. 1981, Nelson 866 (NY); Pará, Mun. Tucuruí, 25 km S. of Represa Tucuruí on the road (BR 422) to Breu Branco, 16 Mar. 1980, Plowman, Rosa and Rosario 9625 (OS).

Extraction and isolation procedures. The procedures used for isolation of flavonoids were those used in the earlier studies [1, 2]. Solvent systems and chromatographic methods were described by Wilkins and Bohm [8]. The UV spectroscopic methods used were those described by Mabry *et al.* [9].

Identification of compounds. The mono-, di- and triglycosides of quercetin were determined by standard means including comparative TLC with standards, partial and total hydrolysis with trifluoroacetic acid, and colour reactions with ethanolamine diphenylborinate. Ammonium hydroxide hydrolyses were performed by adding one drop of conc. NH_4OH from a Pasteur pipette to a soln of the unknown in 1 ml H_2O. At the end of the hydrolysis period the reaction mixture was evaporated to dryness *in vacuo* and the residue taken up in a few drops of MeOH for chromatography.

The structure of 3-*O*-methylquercetin was confirmed by ^1H NMR. The compound was dark under UV, gave no sugar upon acid hydrolysis, exhibited UV spectral behaviour characteristic of a 3-substituted quercetin derivative and had chromatographic behaviour suggesting the presence of at least one methyl group. The structure of dihydroquercetin was established through its conversion to quercetin by heating with a soln of sodium metabisulfite. Its UV spectrum originally suggested a reduced heterocyclic flavonoid which was supported by a strong positive test with Mg–HCl.

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THE GLYCOALKALOIDS OF *SOLANUM DEMISSUM*

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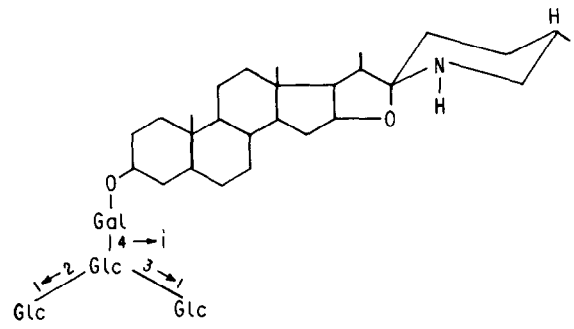
Key Word Index—*Solanum demissum*; Solanaceae; glycoalkaloids; commersonine; neotomatine.

Abstract—Two glycoalkaloids previously unreported in *Solanum demissum* have been isolated and identified as commersonine and neotomatine.

Solanum demissum has been reported to contain the glycoalkaloids demissine and tomatine. We now wish to report the isolation and characterization of two glycoalkaloids previously unreported from *S. demissum* in accession No P.I. 205514.

The glycoalkaloid fraction of *S. demissum* foliage was isolated by basic precipitation using standard techniques. TLC of this fraction indicated the presence of four compounds, two of which were readily identified as demissine and tomatine on the basis of TLC and GC/MS characterization of the aglycones. The aglycones of the glycoalkaloids were identified by GC/MS following hydrolysis in hydrochloric acid in methanol; the structure of the carbohydrate moieties were determined by permethylation analysis. One of these unknown compounds was identified as commersonine, a glycoalkaloid we recently isolated from *S. commersonii* [2]. The remaining glycoalkaloid which we have named neotomatine, was present in relatively small quantities. It was characterized as a tetraose derivative (**1**) of tomatidine, the tetraose moiety being identical to the



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