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FURTHER STUDIES OF FLAVONOLS OF *CLIBADIUM* (COMPOSITAE)

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

Abstract—The flavonoids of an additional eight species of *Clibadium* have been determined. The compounds are derivatives of kaempferol, quercetin and quercetagetin. *O*-Methylated quercetagetin derivatives were found in several taxa with the possibility that 6-methoxykaempferol may also exist in one collection. Kaempferol and quercetin exist as 3-*O*-glucosides, galactosides, rhamnosides, rutinosides and diglucosides although not all glycosides occur in each taxon. Quercetagetin derivatives occur as 7-*O*-glucosides. Observations on these newly investigated species confirm previous work in the genus that three types of flavonoid profiles exist: (1) kaempferol and quercetin 3-glycosides; (2) kaempferol and quercetin 3-glycosides plus quercetagetin 7-glucoside; and (3) kaempferol and quercetin 3-glycosides plus quercetagetin 7-glucoside and *O*-methylated derivatives of quercetagetin.

Earlier studies in this series dealt with flavonol glycosides in *Clibadium* [1, 2], *Desmanthodium* [3] and *Ichthyothere* [4]. It is the purpose of this paper to record our observations on an additional eight species of *Clabadium*: one from Venezuela, three from Colombia, and four from Ecuador including the rare *C. sprucei* (Table 1).

All species exhibited 3-*O*-monoglycosides and 3-*O*-diglycosides of kaempferol and quercetin. The monosides identified were based on glucose, galactose and rhamnose and the diglycosides were either the rutinosides or diglucosides. However, not all of these glycosides were seen in each taxon. An eriodictyol 7-*O*-monoglycoside was chromatographically identified in *C. microcephalum*. *Clibadium glabrescens*, *C. pentaneuron* and *C. sprucei* exhibited profiles based solely upon glycosides of the simple flavonols.

The remaining five species examined produced, in addition to an array of simple derivatives, one or more derivatives of quercetagetin. The simplest of these was *C. mexiae*, two populations of which possessed quercetagetin 7-*O*-glucoside. *Clibadium laxum*, *C. pedunculatum* and *C. terebinthinaceum* have quercetagetin and its 3′-methyl ether, both of which occur as the 7-glucosides. Further *O*-methylation was seen in the flavonoids of *C. terebinthinaceum* in the form of quercetagetin 3,3′-dimethyl ether. *O*-Methylation occurs at the 6-position in *C. microcephalum* where patuletin 7-glucoside was found in all four populations sampled. A trace of patuletin 7-glucoside may also have been present in *C. pedunculatum* but lack of material prevented further study. One population of *C. microcephalum* (*SF* 5822) possessed a compound with UV absorption, chromatographic and colour characteristics of 6-methoxykaempferol 7-monoglycoside but limited quantities of plant material prevented unequivocal structural determination.

The finding that three types of flavonoid profile exist in the eight species examined in the present work agrees with our earlier observations on 12 other taxa of the genus (Table 2). The three types are: (1) kaempferol and quercetin 3-glycosides; (2) kaempferol and quercetin 3-glycosides plus quercetagetin 7-glucoside; and (3) kaempferol and quercetin 3-glycosides plus quercetagetin 7-glucoside and *O*-methylated derivatives of quercetagetin. Further distinctions can be made within the third profile group. Thus, *C. laxum* exhibited only *O*-methylation at the 3′-position. Other species yielded compounds with 3′-*O*-methylation but also produced 6-*O*-methylated derivatives; this was the largest subgroup with six representatives. Two species, *C. trianae* and *C. microcephalum*, appear to synthesize only 6-*O*-methylated compounds. However, the situation in several of these species is not straightforward. *Clibadium trianae*, for example, exhibits a considerable degree of variation in its flavonoid profile [4]. Six of the eight populations tested had only simple flavonol 3-monoglycosides, one had simple flavonol glycosides plus quercetagetin derivatives, and one had simple flavonol glycosides and large quantities of anthochlors, a type of compound not seen in other members of *Clibadium* [1, 4] or in related genera [2, 3]. Other instances of variation in production of *O*-methylated derivatives are discussed in an earlier paper [1]. In the case of the five species studied in the present work that were obtained from more than one population variation was restricted to minor differences in relative concentrations of individual compounds. The only exception to this was the observation of the tentatively identified 6-methoxykaempferol derivative in one collection of *C. microcephalum*.

The evolutionary significance of these flavonoid patterns within the genus, especially of the three major types, is still uncertain. Such a synthesis awaits completion of morphological studies on the genus and the related genera *Desmanthodium* and *Ichthyothere* which are now in progress by the junior author. The results should be interesting because two of the most presumptively advanced species, characterized by congested synflorescences, exhibit very different flavonoid profiles: *Clibadium sprucei* has a simple profile while that of *C. trianae* is complex.

Table 1. Flavonoids in some species of *Clibadium*

Taxon (number of populations analysed)	Flavonoid glycosides*														
	Kaempferol					Quercetin							Methylated compounds		
	3-Glucoside	3-Galactoside	3-Rhamnoside	3-Rutinoside	3-Diglucoside	3-Glucoside	3-Galactoside	3-Rhamnoside	3-Rutinoside	3-Diglucoside	Erio 7-glycoside	Qt 7-glucoside	Pat 7-glucoside	Qt 3'-ME 7-glucoside	Qt 3,3'-DIME 7-glucoside
C. glabrescens (1)	+	+		+	+	+	+		+	+					
C. laxum (3)	+	+		+	+	+	+	+	+	+		+		+	
C. mexiae (2)	+	+	+	+		+	+	+	+			+			
C. microcephalum (4)	+	+	+	+	+	+	+	+	+	+	+	+	+		
C. pedunculatum (3)	+	+	+	+	+	+	+	+	+	+		+	+	+	
C. pentaneuron (1)	+	+	+		+	+	+	+		+					
C. sprucei (1)	+	+	+			+	+	+	+	+					
C. terebinthinaceum (3)	+	+	+	+	+	+	+	+	+	+		+	+	+	+

*Erio = eriodictyol; Qt = quercetagetin; Pat = patuletin; ME = methyl ether; DIME = dimethyl ether.

Table 2. Aglycone types in *Clibadium*

Taxon	Kaempferol and quercetin	Quercetagetin	Quercetagetin*				Ref.†
			6 ME	3'ME	6,3'-DIME	3,3'-DIME	
C. cf. *glomeratum*	+	−	−	−	−	−	[1]
C. glabrescens	+	−	−	−	−	−	
C. pentaneuron	+	−	−	−	−	−	
C. pilonicum	+	−	−	−	−	−	[1]
C. pittieri	+	−	−	−	−	−	[1]
C. sprucei	+	−	−	−	−	−	
C. surinamense	+	−	−	−	−	−	[1, 4]
C. anceps	+	+	−	−	−	−	[1]
C. arboreum	+	+	−	−	−	−	[1]
C. mexiae	+	+	−	−	−	−	
C. asperum	+	+	+	+	−	−	[1]
C. grandifolium	+	+	+	+	+	−	[1]
C. laxum	+	+	−	+	−	−	
C. leiocarpum	+	+	+	+	+	−	[1]
C. microcephalum	+	+	+	−	−	−	
C. pedunculatum	+	+	+	+	−	−	
C. peruvianum	+	+	+	+	−	−	[1]
C. sessile	+	+	−	+	+	−	[1]
C. terebinthinaceum	+	+	+	+	−	+	
C. trianae	+	+	+	−	−	−	[4]

*ME = monomethyl ether; DIME = dimethyl ether.
†Lack of reference = data from present paper.

EXPERIMENTAL

Source of plants. All vouchers are at OS. *SF* = Stuessy and Funk; *SN* = Stuessy and Nesom; *SRA* = Stuessy, Ricardi and Adamo. *Clibadium glabrescens* S. F. Blake: Colombia, Santander, 15 km NE of Bucaramanga, *SF* 5606. *C. laxum* S. F. Blake: Ecuador, Chimborazo, 2 km NW of Bucay, *SN* 5851; El Oro, 10.5 km W of Piñas, *SN* 5867, 40.6 km ENE of La Avanzda, *SN* 5870. *C. mexiae* S. F. Blake: Ecuador, Pastaza, W edge of Puyo, *SN* 5817, 3.6 km W of Puyo, *SN* 5819. *C. microcephalum* S. F. Blake: Ecuador, Tungarahua, 3.3 km E of Río Topo on rd to Puyo, *SN* 5814, 4.8 km E of Río Topo on rd to Puyo, *SN* 5816, 10.4 km W of Mera, *SN* 5822, 11.9 km W of Mera, *SN* 5823. *C. pedunculatum* Aristeg.: Venezuela, Mérida, La Carbonera, *SRA* 6034, just W of La Carbonera, *SRA* 6038, 5 km W of La Carbonera, *SRA* 6042. *C. pentaneuron* S. F. Blake: Colombia, Antioquía, 3 km SE of Santa Elena, *SF* 5709. *C. sprucei* S. F. Blake: Ecuador, Tungarahua, slopes of Volcán Tangarahua, *SN* 5811. *C. terebinthinaceum* DC.: Colombia, Valle, just S of Cordoba, *SF* 5725, 2 km W of Queremal, *SF* 5737, 6 km W of Queremal, *SF* 5742.

Isolation of flavonoids. Flavonoids were isolated by procedures described in Wilkins and Bohm [5] and Gornall and Bohm [6].

Identification of flavonoids. Structures were established using standard UV [7], NMR [7] and MS techniques [8]. The unidentified eriodictyol 7-monoglycoside was chromatographically identical to eriodictyol 7-glucoside isolated and identified by these methods in earlier studies. The nature of the sugar in the unknown compound was not determined.

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FLAVONOL GLYCOSIDES FROM *ASPLENIUM BULBIFERUM*

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Key Word Index—*Asplenium bulbiferum*; Aspleniaceae; flavonol glycosides; kaempferol 3,7-diglucoside; kaempferol 3-*O*-rhamnoside-7-*O*-glucoside; kaempferol 3-*O*-β-glucoside-7-*O*-β-galactoside.

Abstract—From aerial parts of the fern *Asplenium bulbiferum*, besides kaempferol 3,7-diglucoside and kaempferol 3-*O*-rhamnoside-7-*O*-glucoside, the new glycoside kaempferol 3-*O*-β-glucoside-7-*O*-β-galactoside has been characterized.

Although chemical investigations of some *Asplenium* species have led to interesting results [1, 2] in connection with the identification of hybrid plants, the chemistry of most species of *Asplenium* is not well known. Previous work [3, 4] on the flavonoids of *Asplenium bulbiferum* Forster f. showed the presence of kaempferol 3,7-diglucoside, 3-*O*-rhamnoside-7-*O*-glucoside and 3-*O*-glucoside-7-*O*-rhamnoside. In the present work two flavonoid bands (A and B) were isolated from an ethanolic extract of aerial parts of *A. bulbiferum*.

Colour reactions (dull ochre to yellow in UV + NH_3) and UV spectral data: λ_{max}^{MeOH} nm 266, 320 (sh), 343; + NaOAc 266, 355, 393 (sh); + NaOAc–H_3BO_3 268, 347; + $AlCl_3$ 273, 298 (sh), 345, 390; + $AlCl_3$–HCl 272, 297 (sh), 340, 389; + NaOMe 270, 300 (sh), 390 (increase in intensity) suggest [5] that band A is a 3,7-disubstituted flavonol glycoside with free hydroxyl groups at the 5 and 4′ positions. Both total acid hydrolysis and treatment with β-glucosidase gave kaempferol, D-glucose and D-galactose; controlled acid hydrolysis gave, in addition to the products of total acid hydrolysis, kaempferol 7-glucoside, kaempferol 7-galactoside and a small amount of kaempferol 3-glucoside. On hydrogen peroxide oxidation [6] band A gave glucose. Methylation followed by acid hydrolysis gave 5,4′-di-*O*-methylkaempferol, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-