Phytochemistry, Vol. 20, No. 5, pp. 1053–1055, 1981.
Printed in Great Britain.

0031-9422/81/051053-03 $02.00/0

FLAVONOL DERIVATIVES OF THE GENUS *CLIBADIUM* (COMPOSITAE)

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(*Received* 19 *July* 1980)

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

Abstract—The flavonoids of eleven species of *Clibadium* are all based on the flavonols kaempferol, quercetin and quercetagetin. Glucose and galactose sugar substitutions occur in all taxa, while rhamnosides, arabinosides, xylosides, rutinosides and diglucosides are less widely distributed. *O*-Methylated compounds provide the most meaningful taxonomic information and serve to divide the species into two groups. Infraspecific variation of flavonoids is commonplace. This is the first report of flavonoids in *Clibadium*.

INTRODUCTION

As part of a biosystematic study of the subtribe Milleriinae of the Heliantheae we have undertaken an examination of the flavonoid chemistry of several species of *Clibadium* L. The genus, comprising some 40 species, occurs in Central and South America [1]. Recent attention has been drawn to the occurrence of the polyacetylenic compounds ichthyothereol and its acetate [2] in *C. sylvestre* (Aubl.) Baill. (= *C. asperum* (Aubl.) DC. [3] and to the distribution of sequiterpenes and acetylenic compounds in seven species of *Clibadium* [4]. No previous reports of flavonoids in *Clibadium* have been made.

RESULTS AND DISCUSSION

Eleven species of *Clibadium* were examined for their flavonoid constituents (Table 1). All compounds identified were based upon kaempferol, quercetin and quercetagetin. Kaempferol and quercetin were ubiquitous occurring in all taxa as the 3-glucosides and 3-galactosides. Quercetin and kaempferol 3-rhamnosides were identified in about half of the species, while monoarabinosides and monoxylosides were present in two and one species, respectively. All other monoglycosides were linked through the 7-position; these included quercetagetin and isorhamnetin in one species (*C. sessile*) and all the quercetagetin derivatives discussed below.

Eight of the species exhibited small diglycoside fractions; *C. anceps*, *C. arboreum*, and *C. pittieri* had none. Only kaempferol and quercetin diglycosides were seen. 3-Rutinosides were consistently the major diglycosides with lesser amounts of kaempferol and/or quercetin 3-diglucosides in six species. Neither the stereochemistry nor the point of attachment of the outer sugar was determined owing to the limited amount of material obtained, even after pooling the samples. Total hydrolysis of the rutinosides consistently yielded a small amount of galactose in addition to glucose and rhamnose. Since diglycosides consisting of glucose and galactose have higher R_f values than the corresponding rutinosides, it seems likely that a small amount of the flavonol 3-rhamnosylgalactosides also occurs in these plants. This is not surprising considering the occurrence of monogalactosides in all of these plants.

Seven of the species examined have, in addition to the compounds mentioned above, a series of flavonols based upon quercetagetin (6-hydroxyquercetin). The simplest situation, i.e. the presence of quercetagetin 7-glucoside alone, was seen in two species *C. arboreum* and *C. anceps*. The other five species possessed one or more *O*-methylated derivatives of quercetagetin. *O*-Methylated compounds identified from these species were the 7-glucosides of 6-methylquercetagetin (patuletin), 3′-methylquercetagetin, and 6,3′-dimethylquercetagetin.

In addition to differences in flavonols between species, variation *within* taxa of *Clibadium* is common (Table 1). In the case of *C. surinamense* where fourteen collections were analysed, the differences in flavonoid profiles were relatively minor consisting of, in SG 4461, the absence of rhamnosides and the total absence of diglycosides, and in seven other samples (H 3930; SG 4451, 4489, 4491, 4504, 4506, 4516), the absence of kaempferol 3-diglucoside. In the latter case quercetin 3-diglucoside was sometimes seen in trace quantities only (H 3930; SG 4506, 4516) which suggests that the enzymatic equipment is present but that very little of these compounds are being made. The only difference in the three specimens of *C. glomeratum* was the presence of kaempferol and quercetin 3-rutinoside in SG 4492 and 4519 and absence in the third. The three collections of *C. grandifolium* showed variation in the occurrence of the 3′-methylquercetagetin 7-glucoside: present in H 3936, absent from the other two. Differences in the amounts of quercetagetin 7-glucoside range from apparently none in SG 4534 to trace quantities in SG 4533 and to major proportions in H 3936. Since both the 6-methyl and 6,3′-dimethyl derivatives are present in all three collections, the enzymes necessary for the formation of the presumed precursor, viz. quercetagetin, must also be present.

Table 1. Distribution of flavonoids in some *Clibadium* species

Taxon (number of populations analyzed)	Flavonoid glycosides																			
	Kaempferol							Quercetin						Methylated compounds						
	3-*O*-Glucoside	3-*O*-Galactoside	3-*O*-Rhamnoside	3-*O*-Arabinoside	3-*O*-Xyloside	3-*O*-Rutinoside	3-*O*-Diglucoside	3-*O*-Glucoside	3-*O*-Galactoside	3-*O*-Rhamnoside	3-*O*-Arabinoside	3-*O*-Rutinoside	3-*O*-Diglucoside	7-*O*-Glucoside	Isorhamnetin 7-*O*-Glucoside	Quercetagetin 7-*O*-Glucoside	Patuletin 7-*O*-Glucoside	3′-*O*-Methylquercetagetin 7-*O*-Glucoside	6,3′-Di-*O*-Methylquercetagetin 7-*O*-Glucoside
Group One																				
C. anceps (1)	5	5	5					5	5	5						5				
C. arboreum (1)	5	5		5	5			5	5	5						5				
C. cf. *glomeratum* (3)	5	5	5			3		5	5	5		3								
C. pilonicum (1)	5	5				5		5	5		5	5								
C. pittieri (1)	5	5						5	5	5										
C. surinamense (14)	5	5	4			4	4	5	5	4		4	2							
Group Two																				
C. asperum (8)	5	5				5	4	5	5			5	4			4	3	3		
C. grandifolium (3)	5	5				5	5	5	5			5	5			3	5	2	5	
C. leiocarpum (9)	5	5	1			5	4	5	5	2		5	4			1	1	1	2	
C. peruvianum (1)	5	5	5			5	5	5	5	5		5	5			5	5	5		
C. sessile (1)	5	5				5	5	5	5			5	5	5	5	5		5	5	

* Numbers indicate the percentage of populations analysed in each taxon that have the particular compound: 5 = 100%, 4 = 75–99%, 3 = 50–74%, 2 = 25–49%, 1 = less than 25%; absence of a number represents absence of compound.

However, the most extreme flavonoid variation was seen in *C. leiocarpum*. The collection chosen at random for flavonoid isolation (SG 4474) exhibited glucosides, galactosides, rhamnosides (of quercetin only) and rutinosides of kaempferol and quercetin. Screening of seven other collections showed the presence of *O*-methylated quercetagetin derivatives as major constituents of H 3923. 6,3′-Dimethylquercetatgetin 7-glucoside was observed as a trace constituent of a further three collections (SG 4456, 4462, 4494). Diglycoside variation was also observed and rhamnosides were seen only sporadically.

Despite the acknowledged populational variation of flavonols in *Clibadium*, some comments on the taxonomic utility of the data can be made. First, the occurrence of kaempferol and quercetin and their sugar derivatives seem to have little taxonomic value. Both kaempferol and quercetin 3-glucoside and 3-galactoside are found in all the populations of every species, which does not help in assessing taxonomic relationships within the genus. The other sugar substitutions of these two structural types are distributed apparently at random, except that the occurrence of -rutinosides and -diglucosides are found more commonly in *C. asperum*, *C. grandifolium*, *C. leiocarpum*, *C. peruvianum* and *C. sessile* than in the other species. Unique occurrences of sugar substitutions are seen in *C. arboreum* (kaempferol 3-arabinoside and kaempferol 3-xyloside), *C. pilonicum* (quercetagetin 3-arabinoside), and in *C. sessile* (quercetin 7-glucoside). Because these species were studied from single collections, it is possible that wider sampling might reveal additional substitutions.

Second, the flavonoids other than quercetin and kaempferol seem to contain most of the information of taxonomic value in *Clibadium*. Isorhamnetin 7-glucoside is found only in *C. sessile*, which serves as a specific marker. This species is rare, being confined to a single mountain (Cerro Horqueto) in Panama [3]. Quercetagetin 7-glucoside occurs in seven of the 11 species examined, and therefore seems less valuable as a taxonomic marker. The most significant flavonol variables taxonomically are the methylated compounds (patuletin 7-glucoside; 3′-methylquercetagetin 7-glucoside; 6,3′-dimethylquercetagetin 7-glucoside) which occur in only five species (listed as 'Group Two' in Table 1). Studies are continuing to determine if these groups should be recognized taxonomically as sections of the genus.

Third, the close biosynthetic relationships of the flavonols in *Clibadium* offer the possibility of viewing the data in a phylogenetic way. If one assumes that the general pathway of flavonoid biosynthesis in *Clibadium*, based upon mechanistic analysis, mirrors the ancestral evolution of the system within the genus (really a form of

argument of Earliest Ontogenetic State [5]), then those taxa that have methylated compounds would be regarded as more advanced; i.e., the 'Group Two' taxa would be more advanced evolutionarily than those of 'Group One'. The determination of primitive and advanced character states and taxa requires the evaluation of many sets of data, especially morphology, before acceptable judgments can be made. Nonetheless, it is anticipated that the flavonoid data in *Clibadium* will prove very helpful in the final interpretation of evolutionary relationships.

EXPERIMENTAL

Source of plants. All vouchers at OS. *Clibadium anceps* Greenm.: Costa Rica, Puntarenas, Stuessy & Gardner (SG) 4518. *C. arboreum* J. D. Smith: Mexico, Oaxaca, SG 4574. *C. asperum* (Aubl.) DC.: Costa Rica, Cartago, SG 4493, Puntarenas, SG 4507, 4508, 4509, 4541, Panama, Darién, Hartman & Duke 3868, Panamá, Hartman (H) 3937, 3964. *C.* cf. *glomeratum* Greenm.: Costa Rica, Cartago, SG 4492, Puntarenas, SG 4517, 4519. *C. grandifolium* S. F. Blake: Panama, Coclé, H 3936, SG 4533, 4534. *C. leiocarpum* Steetz in Seemann: Costa Rica, Alajuela, SG 4456, 4462, 4474, 4475, 4478, 4479, Cartago, SG 4494, 4495, Panama, Chiriquí, H 3923. *C. peruvianum* Poepp. ex DC.: Peru, San Martin, Johns & Ramirez-R239. *C. pilonicum* Stuessy: Panama, Coclé, H 3963. *C. pittieri* Greenm.: Costa Rica, Alajuela, SG 4465. *C. sessile* S. F. Blake: Panama, Chiriqui, H 3916. *C. surinamense* L.: Costa Rica, Alajuela, SG 4451, 4461, Cartago, SG 4489, 4491; Puntarenas, SG 4506, 4516; San José, SG 4504. Panama; Coclé, H 3930, Los Santos, H 3942, Panamá, H 3939, SG 4524, 4538, 4540, Veraguas, H 3932.

Isolation and identification of flavonoids. Plant material was extracted continuously with MeOH, the extracts were taken to dryness, and the phenols extracted with boiling H_2O (filtration with Celite filter aid). *n*-Butanol extraction yielded the phenolic fraction which was resolved into its component flavonoids by Sephadex LH-20 and partition column chromatography followed by PLC as described by Wilkins and Bohm [6]. UV and proton NMR methods were those described by Mabry *et al.* [7].

Glycosylation at position 7 of the flavonols was evidenced by the absence of bathochromic shifts in band II of the UV spectra on addition of NaOAc and the stability of the glycosidic link at that position to acid hydrolysis relative to 3-*O*-glycosides. ^{1}H NMR spectra of quercetagetin and its *O*-methylated derivatives gave the general pattern expected for flavonols having protons at positions 8, 2′, 5′ and 6′. Integral spectra of the *O*-methylated compounds gave the anticipated number of protons for either 0, 1 or 2 such groups. Location of the *O*-methyl groups was determined by colour reactions with diphenylboric acid ethanolamine complex and by UV spectral data. In all cases structural assignments were consistent with appropriate model compounds described by Mabry *et al.* [7].

Acknowledgements—Appreciation is expressed to James Duke, Robert Gardner, and Ronald Hartman for help with field work; The National Geographic Society and the National Science Foundation (Grant DEB-7520819) for financial support to T.F.S.; The NRCC (now NSERC) for operating and equipment grants to B.A.B. and D. S. Eckt for insights on morphological relationships.

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