Phytochemistry, Vol. 25, No. 12, pp. 2811–2816, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

A CHEMOTAXONOMIC STUDY OF FLAVONOIDS FROM EUROPEAN *TEUCRIUM* SPECIES

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(*Received* 6 *May* 1986)

Key Word Index—*Teucrium*; Labiatae; flavonoid aglycones and glycosides; chemotaxonomy; 6-hydroxyluteolin 7-rhamnoside; cirsimaritin 4′-glucoside.

Abstract—A survey of aerial tissues of 42 European taxa of the genus *Teucrium* has revealed the widespread presence of five surface flavonoids: cirsiliol, cirsimaritin, cirsilineol, salvigenin and 5-hydroxy-6,7,3′,4′-tetramethoxyflavone. The latter two compounds are useful taxonomic markers in that salvigenin is characteristic of species of section *Polium*, while 5-hydroxy-6,7,3′,4′-tetramethoxyflavone is completely confined to species of the other five sections surveyed. Eleven flavone glycosides, four flavonol glycosides and the glycoflavone vicenin-2 were found to occur as vacuolar constituents. One of the flavone glycosides, cirsimaritin 4′-glucoside, only occurs in the species *T. arduini*, while two others, hypolaetin and isoscutellarein 7-acetyl-allosylglucosides, are characteristic of the closely related *T. chamaedrys* and *T. webbianum*. 6-Hydroxyluteolin is widely present as the 7-glucoside and 7-rhamnoside, the latter compound being a new glycoside. In general, the chemical results are correlated with sectional classification and usefully indicate that at least one taxon, *T. compactum*, is misplaced within the genus. Phyletically, the restriction of flavonol glycosides mainly to section *Teucrium* suggests that this may be the basic group within the genus.

INTRODUCTION

The genus *Teucrium* is a taxonomically difficult genus of some 300 species of which 49 species are found in Europe [1], mostly in the Mediterranean basin. Much work has been published recently on the taxonomy of this genus based on morphological (inflorescences and calyx) [2, 3], micromorphological (trichomes) [4], and karyological [5] data, but relationships within the group still remain confusing. In the present work, a study of the flavonoid compounds (free aglycones and glycosides) of 42 European taxa of *Teucrium* has been carried out, and a chemotaxonomic approach to the taxonomic problems of the genus has been attempted. Previously, only flavonoids from *T. montanum*, *T. chamaedrys*, *T. botrys* [6], *T. pseudochamaepitys* [7] *T. polium* [8] and *T. gnaphalodes* [9] have been reported.

RESULTS

Free flavone aglycones (excretion flavonoids)

The free flavone aglycones present in the aerial parts of the different *Teucrium* species (Table 1) were analysed by TLC of extracts obtained by soaking a small leafy stem of each plant in chloroform for 2 min. Although herbarium material was used in the present study, analyses carried out with fresh material yielded the same results, indicating that these are excretion compounds located on the leaf and stem surfaces [10]. All the species studied showed excretion flavonoids in variable amounts, the highest being in those species growing in xeric habitats, in agreement with previous work [11]. All the excretion flavonoids found in the genus showed a trisubstituted A-ring (5-hydroxy-6,7-dimethoxyflavones) with different substitutions on the B-ring. The same substitution pattern in the A-ring has been found in the excretion flavonoids of *Salvia* and *Rosmarinus* species, and 5,6-dihydroxy-7-methoxyflavones occur in *Thymbra*, *Nepeta*, *Galeopsis* and *Ballota* species [12]. Other genera have additionally free flavone aglycones with a tetrasubstituted A-ring. Thus, *Sideritis*, *Ocimum* and *Satureia* produced 5-hydroxy-6,7,8-trimethoxyflavones, *Colebrookia*, *Pogostemon* and *Orthosyphon* 5,6,7,8-tetramethoxyflavones and *Thymus* and *Mentha* 5,6-dihydroxy-7,8-dimethoxyflavones [12]. The free flavone aglycones found in *Teucrium* were cirsiliol, cirsimaritin, cirsilineol, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone and salvigenin (Table 2). Cirsiliol and cirsimaritin were present in the majority of the species studied, while salvigenin was characteristic of species from section *Polium* and 5-hydroxy-6,7,3′,4′-tetramethoxyflavone was frequent in species of sections *Teucrium*, *Stachybotrys* and *Chamaedrys* (Table 3). Thus, from the chemotaxonomic point of view, salvigenin and 5-hydroxy-6,7,3′,4′-tetramethoxyflavone appear to be the most useful chemical markers at the sectional level.

Flavonoid aglycones obtained after acidic hydrolysis of the naturally occurring glycosides

The 70% ethanol extracts of the different *Teucrium* species were hydrolysed with HCl and the aglycones extracted with ethyl acetate. The concentrated ethyl acetate extracts were redissolved in diethyl ether and chromatographed (TLC and PC) against authentic markers. The flavones luteolin, apigenin, diosmetin, 6-hydro-

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Table 1. *Teucrium* species: places of collection

Section	Species (and subspecies)	Place of collection
Teucrium (Sect. *Teucris*, Bentham)	*T. fruticans* L.	Cádiz, Spain
	T. brevifolium Schreber	Sitia, Crete
	T. pseudochamaepitys L.	Valencia, Spain
Stachybotrys Bentham	*T. arduini* L.	Cetinje, Yugoslavia
Scorodonia (Hill) Schreber	*T. scorodonia* L. ssp. *scorodonia*	Gerona, Spain
	T. massiliense L.	Corse, France
	T. salviastrum Schreber	S. Estrela, Portugal
	T. asiaticum L.	Mallorca, Spain
Scordium (Miller) Bentham	*T. scordium* L. ssp. *scordioides* Maire & Petitmegin	Huelva, Spain
	T. spinosum L.	Sevilla, Spain
	T. resupinatum Desf.	Sevilla, Spain
	T. botrys L.	Marne, France
Chamaedrys (Miller) Schreber	*T. chamaedrys* L.	C. de Vard, Switzerland
	T. webbianum Boiss.	Granada, Spain
	T. divaricatum Sieher ex Boiss, ssp. *divaricatum*	Corfu, Greece
	T. divaricatum ssp. *villosum* (Celak) Rech. fil.	Aegina, Greece
	T. flavum L.	Trieste, Italy
	T. intrincatum Lange	Almeria, Spain
	T. fragile Boiss.	Malaga, Spain
	T marum L.	Menorca, Spain
	T. subspinosum Pourret ex Willd.	Baleares, Spain
	T. compactum Clemente ex Lag.	S. Nevada, Spain
Polium (Miller) Schreber	*T. pyrenaicum* L.	Huesca, Spain
	T. rotundifolium Schreber	Albacete, Spain
	T. buxifolium Schreber	Alicante, Spain
	T. freynii Reverchon ex Willk.	Murcia, Spain
	T. montanum L.	Alpes, France
	T. thymifolium Schreber	Albacete, Spain
	T. cossoni D. Wood	Mallorca, Spain
	T. libanitis Schreber	Albacete, Spain
	T. carthaginense Lange	Murcia, Spain
	T. aragonense Loscos & Pardo	Zaragoza, Spain
	T. pumilum L. ssp. *pumilum*	Murcia, Spain
	T. pumilum ssp. *carolipaui* D. Wood	Alicante, Spain
	T. polium L. ssp. *polium*	Granada, Spain
	T. polium ssp. *aureum* (Schreber) Arcangeli	Alicante, Spain
	T. polium ssp. *capitatum* (L.) Arcangeli	Valencia, Spain
	T. polium ssp. *pii-fontii* Palau	Mallorca, Spain
	T. gnaphalodes L'Hér.	Albacete, Spain
	T. eriocephalum Willk.	Murcia, Spain
	T. haenseleri Boiss.	Córdoba, Spain
	T. charidemi Sandwith	Almeria, Spain

xyluteolin, hypolaetin, scutellarein, isoscutellarein and cirsimaritin (this also present free, see above), and the flavonols quercetin and isorhamnetin were detected (Table 2). The distribution of these flavonoid aglycones in the different species of European *Teucrium* (Table 3) showed remarkable infraspecific variation which is very interesting for chemotaxonomic purposes. Hence, luteolin and apigenin were found in almost all the species studied, in accordance with previous work [6], although sometimes only in trace amounts; diosmetin was found especially in species of section *Scordium;* 6-hydroxyluteolin in sections *Stachybotrys, Scorodonia* and *Polium,* and scutellarein only in *T. chamaedrys* and *T. webbianum.* In these last two species 6-hydroxyluteolin and scutellarein are produced from hypolaetin (5,7,8,3',4'-pentahydroxyflavone) and isoscutellarein (5,7,8,4'-tetrahydroxyflavone), respectively, by Wessely–Moser rearrangement carried out in acidic media during the hydrolytic process. Cirsimaritin was found as a product of hydrolysis only in *T. arduini* of section *Stachybotrys* which differentiates this species from all the other *Teucrium* species studied.

Flavonol glycosides were found to be characteristic of section *Teucrium,* being absent from all other taxa except *T. botrys* (section *Scordium*), *T. compactum* (section *Chamaedrys*), *T. montanum, T. aragonense, T. polium* (in this last species only in trace amounts) and *T. haenseleri* (section *Polium*). Flavonols are rather infrequent in the Labiatae as a whole and tend to be found in the less evolved species [13]. This is the first time that isorhamnetin has been reported in this family.

Naturally occurring glycosides

These were studied from the 70% ethanolic extracts by means of 2-D PC using 15% HOAc and BAW and by elution of the different spots which were UV analysed and

Table 2. Flavonoid compounds from *Teucrium* species

Structures	Common names	*N*
Free flavone aglycones		
5,3',4'-Trihydroxy-6,7-dimethoxyflavone	Cirsiliol	1
5,4'-Dihydroxy-6,7-dimethoxyflavone	Cirsimaritin	2
5,4'-Dihydroxy-6,7,3'-trimethoxyflavone	Cirsilineol	3
5-Hydroxy-6,7,3',4'-tetramethoxyflavone	—	4
5-Hydroxy-6,7,4'-trimethoxyflavone	Salvigenin	5
Aglycones obtained after acidic hydrolysis of glycosides		
5,7,3',4'-Tetrahydroxyflavone	Luteolin	6
5,7,4'-Trihydroxyflavone	Apigenin	7
5,7,3'-Trihydroxy-4'-methoxyflavone	Diosmetin	8
5,6,7,3',4'-Pentahydroxyflavone	6-Hydroxyluteolin	9
5,6,7,4'-Tetrahydroxyflavone	Scutellarein	10
5,4'-Dihydroxy-6,7-dimethoxyflavone	Cirsimaritin	11
3,5,7,3',4'-Pentahydroxyflavone	Quercetin	12
3,5,7,4'-Tetrahydroxy-3'-methoxyflavone	Isorhamnetin	13
Flavonoid glycosides		
5,7,4'-Trihydroxyflavone 6,8-di-*C*-glucoside	Vicenin-2	14
5,7,4'-Trihydroxyflavone 7-*O*-glucoside	Cosmosiin	15
5,7,4'-Trihydroxyflavone 7-*O*-rutinoside	—	16
5,7,3',4'-Tetrahydroxyflavone 7-*O*-glucoside	Cynaroside	17
5,7,3'4'-Tetrahydroxyflavone 7-*O*-rutinoside	—	18
5,7,3',4'-Tetrahydroxyflavone 7-*O*-sambubioside	—	19
5,7,3'-Trihydroxy-4'-methoxyflavone 7-*O*-rutinoside	Diosmin	20
5,6,7,3',4'-Pentahydroxyflavone 7-*O*-glucoside	—	21
5,6,7,3',4'-Pentahydroxyflavone 7-*O*-rhamnoside	—	22
5,7,8,4'-Tetrahydroxyflavone 7-*O*-(6''-*O*-acetyl-2''-*O*-allosylglucoside)	—	23
5,7,8,3',4'-Pentahydroxyflavone 7-*O*-(6''-*O*-acetyl-2''-*O*-allosylglucoside)	—	24
5,4'-Dihydroxy-6,7-dimethoxyflavone 4'-*O*-glucoside	—	25
3,5,7,3',4'-Pentahydroxyflavone 3-*O*-glucoside	Isoquercitrin	26
3,5,7,3',4'-Pentahydroxyflavone 3-*O*-rutinoside	Rutin	27
3,5,7,4'-Tetrahydroxy-3'-methoxyflavone 3-*O*-glucoside	—	28
3,5,7,4'-Tetrahydroxy-3'-methoxyflavone 3-*O*-rutinoside	—	29

chromatographed against authentic samples. The glycosides identified are listed in Table 2, and their distribution in the *Teucrium* species studied is given in Table 4.

The glycoflavone vicenin-2, which was found previously to be a chemotaxonomic marker within *Origanum* and related genera [14], was detected in all the sections except *Scordium*. Apigenin 7-glucoside and 7-rutinoside were also detected in all sections with the exception of section *Teucrium*. Luteolin glycosides (as 7-glucoside, 7-rutinoside and 7-sambubioside) were found in all species, although sometimes in very small amounts. Luteolin 7-neohesperidoside was also detected, but it was impossible to differentiate this compound from the 7-rutinoside in the 2-D PC chromatograms. Diosmetin 7-rutinoside was especially frequent in section *Scordium*, and 6-hydroxyluteolin 7-glucoside and/or 7-rhamnoside (a new naturally occurring glycoside) were found in 100% of the species of section *Stachybotrys*, and 75% of the species of sections *Scorodonia* and *Polium*. The 8-hydroxyflavone glycosides hypolaetin 7-*O*-(6'''-*O*-acetyl-2''-*O*-allosylglucoside) and isoscutellarein 7-*O*-(6'''-*O*-acetyl-2''-*O*-allosylglucoside) were exclusively found in *T. chamaedrys* and *T. webbianum* of section *Chamaedrys*. These peculiar acetylated allose-containing 8-hydroxyflavones were found previously in several Scrophulariaceae and Labiatae species; in *Veronica* [15, 16], *Stachys* [17] and *Sideritis* species [18, 19]. The presence of allose was established by chromatographic analysis of the acid hydrolysis products. The structures of these compounds were confirmed by chromatographic comparisons against authentic samples isolated from *V. filiformis* and *V. persica*. Another unusual constituent, cirsimaritin 4'-glucoside, was found only in *T. arduini*. This compound was found for the first time in *Cirsium maritimum* (Compositae) [20], and this is only the second time that it has been reported in nature. This is the principal flavonoid component of *T. arduini*, and differentiates this species from the rest of the genus.

Flavonol glycosides such as isoquercitrin, rutin, isorhamnetin 3-glucoside and 3-rutinoside (isorhamnetin glycosides were found in smaller amounts than the quercetin glycosides) were detected as the principal components in species of section *Teucrium*, which clearly differentiates this section from remaining sections of the genus. However, they were present in *T. botrys*, *T. compactum*, *T. montanum*, *T. aragonense*, *T. haenseleri* and all the subspecies of *T. polium*. Flavonol triglycosides of quercetin and isorhamnetin (with sugars in the 3 and 7 positions) were found in *T. pseudochamaepitys* and *T. botrys* in too small an amount to allow their complete characterization.

DISCUSSION

While most *Teucrium* species can be distinguished by their flavonoid glycoside and/or excretion flavonoids, the

Table 3. Flavonoid aglycone distribution in *Teucrium* species

Section	Species	Excretion flavonoids						Aglycones after acidic hydrolysis: A		B	C	D		E	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Teucrium	*fruticans*	+	−	−	++	−	t	−	−	−	−	+	+	−	−
	brevifolium	+	+	+	+	−	t	−	−	−	−	+	+	−	−
	pseudochamaepitys	+	+	+	+	−	t	−	−	−	−	+	+	−	−
Stachybotrys	*arduini*	+	+	−	+	−	+	+	−	t	+	−	−	−	−
Scorodonia	*scorodonia*	++	+	−	−	−	+	−	−	+	−	−	−	−	−
	massiliense	+	+	+	−	++	+	+	−	+	−	−	−	−	−
	salviastrum	++	−	−	+	−	+	−	−	−	−	−	−	−	−
	asiaticum	++	+	−	−	−	+	+	−	+	−	−	−	−	
Scordium	*scordium*	+	−	+	+	−	+	+	+	−	−	−	−	−	−
	spinosum	++	+	−	−	−	+	+	t	−	−	−	−	−	−
	resupinatum	−	−	+	−	−	+	+	t	−	−	−	−	−	−
	botrys	++	−	−	−	−	+	+	+	−	−	+	−	−	−
Chamaedrys	*chamaedrys*	++	+	−	−	−	+	+	t	−	−	−	−	+	+
	webbianum	++	+	−	−	−	−	+	−	−	−	−	−	+	+
	divaricatum	++	+	+	+	+	+	+	−	−	−	−	−	−	−
	villosum	++	+	+	+	+	+	+	−	−	−	−	−	−	−
	flavum	++	−	−	+	−	+	+	+	+	−	−	−	−	−
	intrincatum	−	+	−	−	−	+	t	−	−	−	−	−	−	−
	fragile	++	+	−	+	−	+	t	−	−	−	−	−	−	−
	marum	+	+	++	++	−	+	+	−	−	−	−	−	−	−
	subspinosum	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	compactum	++	+	+	+	−	−	−	+	−	−	+	+	−	−
Polium	*pyrenaicum*	+	+	+	−	+	+	+	−	−	−	−	−	−	−
	rotundifolium	+	+	+	−	+	+	+	−	t	−	−	−	−	−
	buxifolium	+	+	+	−	+	+	−	−	+	−	−	−	−	−
	freynii	+	+	+	−	−	+	+	−	−	−	−	−	−	−
	montanum	+	+	+	−	−	+	−	−	−	−	+	−	−	−
	thymifolium	+	+	+	−	++	+	−	−	+	−	−	−	−	−
	cossoni	+	+	−	−	−	+	+	−	−	−	−	−	−	−
	libanitis	+	+	+	−	+	+	+	−	+	−	−	−	−	−
	cartaginensis	++	+	+	−	+	+	+	−	+	−	−	−	−	−
	aragonense	++	+	+	−	+	t	−	−	−	−	+	+	−	−
	pumilum	++	+	+	−	+	+	+	−	+	−	−	−	−	−
	carolipaui	++	+	+	−	+	+	+	−	+	−	−	−	−	−
	polium	++	+	+	−	−	+	−	+	−	−	t	−	−	−
	aureum	+	+	+	−	+	+	−	−	+	−	t	−	−	−
	capitatum	++	+	+	−	+	+	+	−	+	−	+	−	−	−
	pii-fontii	+	+	+	−	+	+	+	−	−	−	t	−	−	−
	gnaphalodes	+	+	+	−	+	+	+	−	+	−	−	−	−	−
	eriocephalum	++	+	+	−	+	+	+	−	+	−	−	−	−	−
	haenseleri	++	+	+	−	+	+	+	−	t	−	+	−	−	−
	charidemi	−	+	+	−	−	+	+	−	+	−	−	−	−	−

(A) 5,7-Dihydroxyflavones; (B) 6-hydroxyflavones; (C) 6-methoxyflavones; (D) flavonols; (E) 8-hydroxyflavones; (1) cirsiliol; (2) cirsimaritin; (3) cirsilineol; (4) 5-hydroxy-6,7,3′,4′-tetramethoxyflavone; (5) salvigenin; (6) luteolin; (7) apigenin; (8) diosmetin; (9) 6-hydroxyluteolin; (10) cirsimaritin; (11) quercetin; (12) isorhamnetin; (13) hypolaetin; (14) isoscutellarein; (++) the principal flavonoid present in this extract; (+) present; (t) present in trace amounts; (−) not detected.

present results are probably most useful at the sectional level. Thus, the free flavone aglycone salvigenin is a good chemical marker for section *Polium* and 5-hydroxy-6,7,3′,4′-tetramethoxyflavone is present in 70% of the species studied in section *Chamaedrys*, and all three taxa in section *Teucrium*. Perhaps the most clearly chemically defined section is in fact *Teucrium*, where all three species had very similar flavonoid glycoside patterns and flavonols were universally present. Elsewhere, in the Labiatae, flavonols have been found in what are considered less evolved species [12] so the present evidence suggests that section *Teucrium* is a basic group within the genus. Section *Scordium* on the other hand was characterized by diosmetin 7-rutinoside and the absence of vicenin-2. Only one species of section *Stachybotrys*, *T. arduini*, was available for study but this plant was distinguished from all the other *Teucrium* species surveyed by the presence of the rare flavone glycoside cirsimaritin 4′-glucoside. 5-Hydroxy-6,7,3′,4′-tetramethoxyflavone was also found free in this taxon. *Teucrium chamaedrys* and *T. webbianum* from section *Chamaedrys* showed very similar flavonoid patterns characterized by the presence of acetylated

Table 4. Flavonoid glycoside distribution in *Teucrium* species

		Flavone glycosides												Flavonol glycoside			
		A			B				C		D		E	F			
Section	Species	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Teucrium	*fruticans*	+	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+
	brevifolium	+	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+
	pseudochamaepitys	+	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+
Stachybotrys	*arduini*	+	+	−	+	+	−	−	+	−	−	−	+	−	−	−	−
Scorodonia	*scorodonia*	−	t	−	+	+	−	−	+	+	−	−	−	−	−	−	−
	massiliense	+	+	+	+	+	−	−	+	+	−	−	−	−	−	−	−
	salviastrum	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−
	asiaticum	+	+	+	+	+	−	−	+	−	−	−	−	−	−	−	−
Scordium	*scordium*	−	t	−	t	+	+	−	−	−	−	−	−	−	−	−	−
	spinosum	−	t	−	+	+	t	+	−	−	−	−	−	−	−	−	−
	resupinatum	−	t	+	+	+	t	+	−	−	−	−	−	−	−	−	−
	botrys	−	+	−	+	t	+	−	−	−	−	−	−	+	+	−	−
Chamaedrys	*chamaedrys*	+	+	+	+	+	+	−	−	−	+	+	−	−	−	−	−
	webbianum	t	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−
	divaricatum	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	villosum	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	flavum	−	+	−	+	−	+	−	+	−	−	−	−	−	−	−	−
	intrincatum	+	t	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	fragile	+	t	t	+	+	−	−	−	−	−	−	−	−	−	−	−
	marum	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	subspinosum	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	compactum	+	−	−	−	−	+	−	−	−	−	−	−	+	+	+	+
Polium	*pyrenaicum*	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−
	rotundifolium	−	t	−	t	+	+	−	+	−	−	−	−	−	−	−	−
	buxifolium	+	t	t	+	+	−	−	+	+	−	−	−	−	−	−	−
	freynii	t	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	montanum	+	−	−	+	+	−	−	−	−	−	−	−	+	+	−	−
	thymifolium	−	t	−	+	+	−	−	+	+	−	−	−	−	−	−	−
	cossoni	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	libanitis	+	+	t	+	+	−	−	+	+	−	−	−	−	−	−	−
	cartaginensis	t	+	+	+	+	−	−	+	+	−	−	−	−	−	−	−
	aragonense	+	−	−	−	+	−	−	t	t	−	−	−	+	+	+	+
	pumilum	+	t	t	+	+	−	−	+	−	−	−	−	−	−	−	−
	carolipaui	+	t	t	+	+	−	−	+	+	−	−	−	−	−	−	−
	polium	+	−	−	t	+	+	+	t	−	−	−	−	t	−	−	−
	aureum	+	−	−	+	+	−	−	+	−	−	−	−	t	−	−	−
	capitatum	+	+	−	+	+	−	−	+	−	−	−	−	+	−	−	−
	pii-fontii	+	+	+	−	−	+	−	−	−	−	−	−	t	−	−	−
	gnaphalodes	−	+	−	+	+	−	+	+	+	−	−	−	−	−	−	−
	eriocephalum	−	t	−	+	+	−	+	+	+	−	−	−	−	−	−	−
	haenseleri	t	−	−	t	+	−	+	−	+	−	−	−	+	+	−	−
	charidemi	+	+	−	+	+	−	+	−	+	−	−	−	−	−	−	−

(A) *C*-Glycosides (vicenin-2); (B) 5,7-dihydroxyflavone glycosides; (C) 6-hydroxyflavone glycosides; (D) 8-hydroxyflavone glycosides; (E) 6-methoxyflavone glycosides; (F) flavonol glycosides; (**15**) apigenin-7-glucoside; (**16**) apigenin 7-rutinoside; (**17**) luteolin 7-glucoside; (**18**) luteolin 7-rutinoside; (**19**) luteolin 7-sambubioside; (**20**) diosmetin 7-rutinoside; (**21**) 6-*OH*-luteolin 7-glucoside; (**22**) 6-*OH*-luteolin 7-rhamnoside; (**23**) isoscutellarein 7-allosylglucoside; (**24**) hypolaetin 7-allosylglucoside; (**25**) cirsimaritin 4′-glucoside; (**26**) quercetin 3-glucoside; (**27**) quercetin 3-rutinoside; (**28**) isorhamnetin 3-glucoside; (**29**) isorhamnetin 3-rutinoside; the rest of the symbols as for Table 3.

allosylglucosides of 8-hydroxyflavones which were absent from the rest of the species of this and the other sections confirming the close morphological relationship between these two species, as other authors previously suggested [1], although differing in geographical origin. *Teucrium compactum* has been classified in sections *Chamaedrys* [1], *Polium* [4, 5] and *Scordium* [21] but its flavonoid pattern which contained mainly flavonols and 5-hydroxy-6,7,3′,4′-tetramethoxyflavone suggests it is out of place in any of these sections. Chemically, it fits into the section *Teucrium*. A survey of *Teucrium* species from other parts of the world would obviously prove valuable in the study of the genus but was not possible at the present time because of lack of plant material.

EXPERIMENTAL

Plant material. Samples of each specimen studied are deposited in the University of Reading herbarium (RNG). Several populations of some species were studied, and no significant dif-

ferences were found in the flavonoid (aglycones and glycosides) patterns.

Extraction of flavonoids. The aerial parts of each species were first extracted for 2 min by dipping the plant material in $CHCl_3$ to give the free flavone aglycones (excretion flavonoids). The $CHCl_3$ was decanted off and extracted with EtOH–H_2O (7:3) overnight. The ethanol extracts were concd and one part dissolved in MeOH and 2 N HCl added (1:1). Hydrolysis was achieved by heating at 90° for 30 min. The aglycones were extracted with EtOAc.

Identification of free flavone aglycones. These were identified from the $CHCl_3$ extracts by means of chromatographic comparisons on silica gel TLC against authentic samples isolated previously from *Teucrium* species. The solvents used were toluene–HOAc (4:1), $CHCl_3$–*n*-hexane–MeOH (40:40:3) and toluene–MeOH–HOAc (45:3:2) as described previously [22].

Identification of flavonoid glycosides. The ethanolic extracts were 2D chromatographed on Whatman No. 1 paper in 15% HOAc and *n*-BuOH–HOAc–H_2O (4:1:5, upper phase), and the different fractions visualized under UV-light + NH_3. Fractions were eluted and subjected to UV spectroscopy and chromatographic comparison against authentic markers.

Identification of 6-hydroxyluteolin 7-rhamnoside. This compound appeared dark under UV light and gave similar UV λ_{max} in MeOH and after addition of the classical shift reagents to 6-hydroxyluteolin 7-glucoside, but with higher R_f values in BAW (0.26) and 15% HOAc (0.29) than that compound (0.18 and 0.11, respectively) suggesting the presence of rhamnose. After acidic hydrolysis 6-hydroxyluteolin and rhamnose were detected. When a mild acidic hydrolysis was carried out no intermediate compound was found confirming that this compound is a monoglycoside in accordance with the chromatographic behaviour. This glycoside remained unaltered after enzymic treatment with β-D-glucosidase, and yielded the aglycone 6-hydroxyluteolin by enzymic hydrolysis with naringinase and anthocyanase (30°, acetate buffer pH 4.4). It is thus characterized as 6-hydroxyluteolin 7-rhamnoside.

Identification of cirsimaritin 4'-glucoside. This compound showed a dark colour under UV light and after addition of NH_3. The R_f values on 15% HOAc (0.59) and BAW (0.47) suggested that it was a monoglycoside of a highly methylated flavone. After acidic hydrolysis cirsimaritin and glucose were identified. Under controlled acidic hydrolysis, it yielded the aglycone directly without any intermediates confirming the existence of a monoglucoside and also not rearranged. The UV spectra of the glycoside in MeOH and after addition of the classical shift reagents were identical to those of salvigenin, showing that the sugar was linked in the 4' position.

Identification of flavonoid aglycones obtained after acidic hydrolysis. These compounds were identified by means of chromatographic comparisons against authentic samples on cellulose TLC in 50% HOAc and BAW.

Acknowledgement—F.A.T.B. is grateful to the Spanish C.S.I.C. for the fellowship.

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