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Some Flavonoids and the Diterpene Borjatriol from some Spanish *Sideritis* Species

FRANCISCO TOMÁS-LORENTE, FEDERICO FERRERES, FRANCISCO A. TOMÁS-BARBERÁN, DIEGO RIVERA* and CONCEPCIÓN OBON*

Laboratorio de Fitoquímica, Centro de Edafología y Biología Aplicada del Segura, C.S.I.C., Apdo. 195, Murcia, Spain; *Departamento de Botánica, Facultad de Ciencias Biológicas, Universidad de Murcia, Espinardo, Murcia, Spain

Key Word Index—*Sideritis*; Labiatae; excretory flavones; 8-hydroxyflavone glycosides; chemotaxonomy; phylogeny; ecology.

Abstract—An HPLC and TLC analysis of the vacuolar and epicuticular flavonoids from Spanish *Sideritis* species has been carried out, and the distribution of these compounds has been used as a chemotaxonomic approach to the systematic problems of this genus. Generally, a correlation between the flavonoid patterns and the morphological and genetic data has been found. Epicuticular flavonoids accumulate in the phyletically more advanced species while the primitive species were devoid of external flavonoids. Excretory flavonoids also seem to increase in species growing in semi-arid habitats.

Introduction

The section *Eusideritis* Bentham of the genus *Sideritis* L. (Labiatae) is widely represented in Spain where some 30 species have been described [1, 2]. The taxonomy of these species is rather difficult, and according to ref. [1] requires extensive experimental investigation. Interspecific hybrids have been recorded from many localities in Spain and elsewhere and it seems probable that much of the taxonomic difficulty is due to the occurrence of such hybrids.

In the last few years a number of papers have been published on the free flavonoid aglycones [3–6] and flavonoid glycosides [7–12] of species of section *Eusideritis*, showing that these plants produce unusual allose-containing 8-hydroxyflavone glycosides, which have also been found in some related genera of Labiatae, *Stachys* [13] and *Teucrium* [14], and in the Scrophulariaceae, *Veronica* [15].

In the present work, a chemotaxonomic approach has been made to the systematic problems of the section *Eusideritis* using HPLC to study free flavone aglycones and glycosides on the leaf surface and within the cells of more than 40 taxa of 27 species of this genus. Special attention has been devoted to some eastern taxa belonging to *S. angustifolia 'sensu lato'* (including *S. angustifolia, S. tragoriganum, S. saetabensis, S. mugronensis* and *S. funkiana*), a difficult group whose status is not clear. In the study of this complex, the occurrence of the diterpene borjatriol on leaf surfaces has also been assayed.

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Results

The different species analysed are listed in Table 1. The names suggested by different authors for several taxa are also shown and confirm the taxonomical difficulty of this section. Populations belonging to the complex *S. angustifolia 'sensu lato'* are listed with the accurate localities of collection in Table 4. A number is given to identify each one of these taxa which differentiate morphologically from *S. angustifolia 'sensu stricto'* in having more or less crowded verticillasters, longer leaves, or smaller bracts (often with distinct terminal teeth) and sometimes smaller flowers.

Epicuticular flavonoids

The occurrence of external flavonoids deposited as epicuticular layers usually dissolved in a lipid matrix, has been extensively described [16]. The presence of these lipophilic com-

TABLE 1. *SIDERITIS* TAXA OF SECTION *EUSIDERITIS* BENTHAM STUDIED.

Species	Place of collection
S. incana L. subsp. *incana*	La Toba, Cuenca
subsp. *virgata* (Desf.) Malagarriga	Grazalema, Cádiz
subsp. *sericea* (Pers.) P. W. Ball ex Heywood	Quesa, Valencia
var. *intermedia* Font-Quer	Sa. Almansa, Albacete
S. glauca Cav. [*S. incana* subsp. *glauca* (Cav.) Pau]	Sa. Orihuela, Alicante
S. stachydioides Willk.	Vélez Blanco, Granada
S. lacaitae Font-Quer	Relumbrar, Albacete
S. hirsuta L.	Zaida, Zaragoza
S. hirsuta L. var. *altilabra* Pau	Enguera, Valencia
S. endresii Willk. subsp. *laxespicata* (Degen & Debeaux) Heywood	Cazorla, Jaen
S. foetens Clemente ex Lag.	Bco. Caballar, Almeria
S. angustifolia Lag. (1)	Ayora, Valencia
(2)	Sa. Mariola, Alicante
(3)	Fte. La Higuera, Valencia
(4)	Alpera, Albacete
S. mugronensis Borja (1)	Los Llanos, Albacete
(2)	Mariquillas, Albacete
(3)	S. Mugrón, Albacete
S. tragoriganum Lag. (1)	Torreblanca, Castellón
(2)	Hoya de Altea, Alicante
S. saetabensis Rouy	Xativa, Valencia
S. funkiana Willk.	Pozo Lorente, Albacete
S. reverchonii Willk.	Ronda, Málaga
S. javalambrensis Pau [*S. hyssopifolia* L. subsp. *javalambrensis* (Pau) Rivera]	Sa. Javalambre, Valencia
S. hyssopifolia L. subsp. *hyssopifolia*	La Bonaigua, Lérida
subsp. *guillonii* (Timb.-Lagr.) Nyman [*S. guillonii* Timb.-Lagr.]	P. de Europa, Asturias
var. *pyrenaica*	S. Ao. Urquiola, Guipuzcoa
S. carbonellis Socorro [*S. hyssopifolia* L. subsp. *carbonellis* (Socorro) Rivera]	La Sagra, Granada
S. linearifolia Lam. [*S. pungens* Bentham]	Zaida, Zaragoza
S. glacialis Boiss.	Co. Almirez, Almeria
S. ovata Cav.	Valdegoira, Alava
S. spinulosa Barnades ex Asso	El Cerrato, Palencia
S. serrata Cav. ex Lag.	Tobarra, Albacete
S. ilicifolia Willd.	Barbastro, Huesca
S. leucantha Cav. var. *typica* F. Q.	Santomera, Murcia
var. *incana* (Willk.) F. Q. [*S. lineriaefolia* var. *incana* Willk.]	Albacete
var. *bourgaena* (Boiss, Reut.) F. Q. [*S. bourgeana* Boiss, Reut.]	Hellín, Albacete
var. *intermedia* F. Q.	Cieza, Murcia
S. pusilla (Lange) Pau [*S. scordioides* var. *pusilla* Lange. = *S. leucantha* var. *pusilla* Pau]	Adra, Almeria
var. *granatensis* (Pau) F. Q. [*S. hirsuta* var. *granatensis* Pau]	Nerja, Granada
var. *flavovirens* (Rouy) F. Q. [*S. leucantha* var. *flavovirens* Rouy]	Puertolumbreras, Murcia
var. *carthaginensis* F. Q. [*S. hirsuta* var. *carthaginensis* Pau]	Cartagena, Murcia
S. osteoxila Pau [*S. pusilla* var. *osteoxila* (Pau) F. Q.]	Cabo de Gata, Almeria
S. arborescens Salzm. ex Bentham	Kebdana, Morocco
S. grandiflora Salzm. ex Bentham	Tetouan, Morocco

pounds (free flavonoid aglycones) can be readily assessed by soaking a twig or leaf of the plant in lipophilic solvents such as $CHCl_3$, CH_2Cl_2, Me_2CO, etc. for 1 or 2 min (Wollenweber, personal communication). Previous work by our group has demonstrated that the flavone aglycones deposited externally on the leaves of *Sideritis* species include sideritoflavone (5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone), cirsiliol, cirsilineol, eupatorin, cirsimaritin, xanthomicrol, 8-methoxycirsilineol, 8-methoxyeupatorin, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone and 5-desmethylnobiletin (Table 2). HPLC and TLC conditions for the analysis of these flavonoids have been described previously [17, 18]. The two techniques are complementary, since HPLC affords a quantitative evaluation of the compounds present in the

TABLE 2. EPICUTICULAR FLAVONOIDS IN THE DIFFERENT *SIDERITIS* TAXA ANALYSED

		Flavonoid†								
Sideritis sp.	EXC.	1	2	3	4	5	6	7	8	9
S. foetens	+	+	+++	—	—	+	+	—	—	—
S. arborescens	—	—	—	—	—	—	—	—	—	—
S. hirsuta	—	—	—	—	—	—	—	—	—	—
S. hirsuta var.. *altilabra*	t	+	+++	+	+	++	++	—	—	—
S. endresii subsp.. *laxespicata*	t	+	+	+++	++	++	++	—	—	—
S. glacialis	t	—	+	+	++	+++	+	+	—	—
S. linearifolia	+	+	t	+	+++	+	++	—	—	—
S. javalambrensis	+	+	++	+	++	+	+++	—	—	—
S. spinulosa	+	—	t	t	t	+++	+++	—	—	—
S. serrata	+	t	+	t	+	+	+++	—	+	+
S. angustifolia (1)	++	+	+++	t	t	+	+	—	t	—
S. angustifolia (2)	++	+	+++	—	—	t	+	—	—	—
S. angustifolia (3)	++	+	+++	—	t	t	+	—	+	—
S. angustifolia (4)	++	+	+++	—	—	t	+	—	—	—
S. mugronensis (1)	++	+	+++	—	—	t	+	—	—	—
S. mugronensis (2)	++	+	+++	—	—	t	+	—	—	—
S. mugronensis (3)	++	t	+	—	t	—	+	t	+++	—
S. tragoriganum (1)	++	+	+++	—	t	+	++	—	—	—
S. tragoriganum (2)	++	+	+++	—	—	+	+	—	—	—
S. saetabensis	++	+	+++	—	t	t	+	—	—	—
S. funkiana	++	+	++	t	t	t	+	t	+++	—
S. reverchonii	++	+	+++	t	t	+	++	—	—	—
S. leucantha	++	+	+++	t	t	++	++	—	—	—
S. leucantha var. *incana*	++	+	+++	t	t	+	+	—	—	—
S. leucantha var. *bourgeana*	++	+	+++	—	—	+	+	—	—	—
S. leucantha var. *intermedia*	++	t	+++	—	—	++	++	—	—	—
S. pusilla	++	++	+++	+	+	+	++	—	—	—
S. pusilla subsp. *flavovirens*	++	++	+++	t	t	t	++	—	—	—
S. pusilla subsp. *carthaginensis*	++	+	+++	—	—	+	++	—	—	—
S. pusilla subsp. *osteoxila*	t	t	+++	—	—	++	+	—	—	—
S. pusilla subsp. *granatensis*	++	—	+	—	—	+++	+	—	—	—
S. ilicifolia	+	+	+++	—	—	+	—	—	—	—
S. lacaitae	t	+	—	+++	—	—	—	—	—	—
S. incana subsp. *incana*	—	—	—	—	—	—	—	—	—	—
S. incana subsp. *sericea*	—	—	—	—	—	—	—	—	—	—
S. incana subsp. *virgata*	—	—	—	—	—	—	—	—	—	—
S. incana subsp. *intermedia*	—	—	—	—	—	—	—	—	—	—
S. glauca	—	—	—	—	—	—	—	—	—	—
S. stachydioides	—	—	—	—	—	—	—	—	—	—
S. hyssopifolia subsp. *hyssopifolia*	—	—	—	—	—	—	—	—	—	—
S. hyssopifolia subsp. *guillonii*	—	—	—	—	—	—	—	—	—	—
S. hyssopifolia var. *pyrenaica*	—	—	—	—	—	—	—	—	—	—
S. carbonellis	—	—	—	—	—	—	—	—	—	—
S. ovata	—	—	—	—	—	—	—	—	—	—
S. grandiflora	—	—	—	—	—	—	—	—	—	—

*(EXC) Excretion levels; (++) abundant epicuticular flavonoids; (+) epicuticular flavonoids present; (t) epicuticular flavonoids in trace amounts; (—) epicuticular flavonoids not detected.

†Epicuticular flavonoids : **(1)** cirsiliol [5,3′,4′-trihydroxy-6,7-dimethxoxyflavone]; **(2)** sideritoflavone [5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone]; **(3)** cirsimaritin [5,4′-dihydroxy-6,7-dimethoxyflavone]; **(4)** cirsilineol [5,4′-dihydroxy-6,7,3′-trimethoxyflavonl]; **(5)** xanthomicrol [5,4′-dihydroxy-6,7,8-trimethoxyflavone]; **(6)** 8-methoxycirsilineol; **(7)** 5-hydroxy-6,7,3′,4′-tetramethoxyflavone; **(8)** 5-desmethylnobiletin [5-hydroxy-6,7,8,3′,4′-pentamethyoxyflavone]; **(9)** gardenin B [5-hydroxy-6,7,8,4′-tetramethoxyflavone].

Relative amounts: (+++) the main compound in the extract; (++) abundant but not the main compound; (+) present; (—) not detected.

extracts while TLC allows the detection with reagents (such as Naturstoffreagenz-A, 2-aminoethyldiphenylborinate) that give additional information which is useful in identification.

The HPLC and TLC analyses of the $CHCl_3$ cuticular extracts from the leaves of the different taxa studied in the present work have revealed that there are species which accumulate significant amounts of external flavonoids whereas others produce only trace amounts or are completely devoid of them (Table 2). Thus *S. angustifolia 'sensu lato'* (including *S. mugronensis, S. tragoriganum, S. saetabensis, S. funkiana*), *S. reverchonii, S. leucantha, S. pusilla, S. foetens, S. javalambrensis, S. linearifolia, S. spinulosa, S. serrata* and *S. ilicifolia* produce significant amounts of external flavonoids. On the other hand, other taxa produce only trace amounts, such as *S. hirsuta* var. *altilabra, S. lacaitae, S. endresii* subsp. *laxespicata* and *S. glacialis,* and yet other taxa were completely devoid of these substances such as *S. incana, S. glauca, S. stachydioides, S. hirsuta, S. ovata, S. hyssopifolia, S. arborescens* and *S. grandiflora.*

These results are of ecological and taxonomical interest. Ecologically, it has been suggested that these epicuticular flavonoids play an important role in the adaptation of plants to arid habitats [19]. Here, the taxa which produce significant amounts of external flavonoids certainly grow in xeric habitats, but there are some taxa that do not produce these compounds but which also grow under similar conditions. In this case we have observed that the plants possess other mechanisms of adaptation to the habitat, such as being covered with a dense mat of white hairs as in *S. incana* and *S. stachydioides,* or are glabrescent with a layer of wax covering stems and leaves as in *S. glauca.* The rest of the plants that do not produce external flavonoids grow in more or less wet habitats in the north of Spain, such as *S. hyssopifolia* and *S. ovata.*

Chemotaxonomically these results are of interest since they support the subdivision of section *Eusideritis* into (at least) two subsections in agreement with ref. [2]. For example, subsection *Gymnocarpae* is generally devoid of external flavonoids (*S. incana, S. glauca, S. stachydioides*) while subsection *Carpostegiatae* as a general rule contains such compounds, with the exceptions of *S. hyssopifolia, S. ovata, S. arborescens, S. grandiflora* and *S. hirsuta.* The latter species deserve a special mention since the samples from wetter areas in northern Spain are devoid of external flavonoids, while varieties from the south, such as the variety *altilabra,* contain trace amounts of external compounds presumably as an adaptation to the more arid habitats.

Sideritoflavone (5,3',4'-trihydroxy-6,7,8-trimethoxyflavone) is the main external flavonoid in these species which have the greater amounts of these excretion products, and this is in fact the main external compound in *S. angustifolia 'sensu lato', S. leucantha, S. pusilla, S. reverchonii, S. foetens* and *S. ilicifolia.* On the other hand, *S. javalambrensis, S. spinulosa* and *S. serrata* produced mainly 8-methoxycirsilineol, *S. glacialis,* xanthomicrol, *S. linearifolia,* cirsilineol, and *S. endresii* var *laxespicata* and *S. lacaitae* cirsimaritin (Table 2).

Vacuolar flavonoids

The vacuolar flavonoids in *Sideritis* species include the 7-allosyl(1→2)glucosides of 5,8-dihydroxyflavones with different substitution patterns on the B ring (i.e. hypolaetin, hypolaetin 3'-methyl ether, hypolaetin 4'-methyl ether, isoscutellarein and isoscutellarein 4'-methyl ether) (Table 3). The presence of 7-allosyl(1→2)glucosides of the common phloroglucinol-like flavones, chrysoeriol, luteolin and apigenin has been also described in *S. grandiflora* [8] and in North African *Sideritis* (Tomás-Barberán, F. A., in preparation). The *Sideritis* species also contain flavone monoglucosides, including luteolin 7-glucoside, hypolaetin 8-glucoside, hypolaetin 7-glucoside and hypolaetin 3'-methyl ether 7-glucoside, these compounds being less widely distributed within the genus and therefore of a great chemotaxonomic interest, especially hypolaetin 8-glucoside.

The distribution of these flavonoids within section Eusideritis of genus *Sideritis* has been studied by HPLC of the MeOH–H_2O (7:3) extracts of the leaves and the results obtained are summarized in Table 3. The accumulation of hypolaetin 8-glucoside is noteworthy in the species closely related to *S. angustifolia* (*S. tragoriganum, S. reverchonii, S. funkiana, S. mugronensis* and *S. saetabensis*) and is also

TABLE 3. DISTRIBUTION OF VACUOLAR FLAVONOIDS IN THE DIFFERENT *SIDERITIS* TAXA ANALYSED

	Flavonoid*					
Sideritis sp.	**A**	**B**	**C**	**D**	**E**	**F**
S. foetens	—	+++	t	t	++	—
S. arborescens	—	++	—	t	+	—
S. hirsuta	—	++	—	++	+++	t
S. hirsuta var. *altilabra*	—	+++	—	++	++	t
S. endresii subsp.. *laxespicata*	—	+++	—	++	+++	+
S. glacialis	—	++	—	++	+	+++
S. linearifolia	—	—	—	+	+	+++
S. javalambrensis	—	t	—	+	+	+++
S. spinulosa	—	++	t	++	+++	+
S. serrata	—	+	—	++	t	+++
S. angustifolia (1)	—	+	+++	—	+++	—
S. angustifolia (2)	—	++	++	—	+++	—
S. angustifolia (3)	—	++	++	+	t	—
S. angustifolia (4)	—	++	++	—	+	—
S. mugronensis (1)	—	++	+++	+	t	—
S. mugronensis (2)	—	++	+++	—	++	—
S. mugronensis (3)	—	+	++	++	++	—
S. tragoriganum (1)	—	++	+++	t	+	—
S. tragoriganum (2)	—	++	—	+++	t	—
S. saetabensis	—	+	++	+++	+	—
S. funkiana	—	+++	++	+	+++	t
S. reverchonii	—	+	+++	—	t	—
S. leucantha	—	+++	+	++	++	—
S. leucantha var. *incana*	—	+	+	+++	++	t
S. leucantha var. *bourgeana*	—	+	t	+++	+	—
S. leucantha var. *intermadia*	—	++	+	++	+++	t
S. pusilla	—	+	—	+++	—	—
S. pusilla subsp. *flavovirens*	—	t	t	+++	+	—
S. pusilla subsp. *osteoxila*	—	t	t	+++	t	—
S. pusilla subsp. *carthaginensis*	—	+++	—	+	+	—
S. pusilla subsp. *granatensis*	—	+	—	+++	+	t
S. ilicifolia	—	++	—	—	+++	+
S. lacaitea	—	+++	—	+	+++	+
S. incana subsp. *incana*	+	+++	—	+	++	t
S. incana subsp. *sericea*	++	+++	—	t	+	t
S. incana subsp. *intermedia*	++	+++	—	t	++	t
S. incana subsp. *virgata*	t	+++	—	+	++	—
S. glauca	+	+	—	+++	+	—
S. stachydioides	—	+	—	—	—	—
S. hyssopifolia subsp. *hyssopifolia*	—	—	—	+++	++	—
S. hyssopifolia subsp. *guillonii*	—	—	—	+	+++	+
S. hyssopifolia var. *pyrencaica*	—	—	—	+++	—	+
S. carbonellis	—	+	—	+++	+	—
S. ovata	—	t	—	+++	—	—
S. grandiflora†	—	t	—	—	t	—

*Vacuolar flavonoids: (**A**) hypolaetin 7-glucoside [hypolaetin = 5,7,8,3′,4′-pentahydroxyflavone]; (**B**) hypolaetin 7-allosyl(1→2)glucoside; (**C**) hypolaetin 8-glucoside; (**D**) isoscutellarein 7-allosyl(1→2)glucoside [isoscutellarein = 5,7,8,4′-tetrahydroxyflavone]; (**E**) hypolaetin 3′-methyl ether 7-allosyl(1→2)glucoside; (**F**) isoscutellarein 4′-methyl ether 7-allosyl(1→2)glucoside. Codes as in Table 2.

†This contains mainly chrysoeriol 7-allosyl(1→2)glucoside and other 7-glycosides of luteolin and apigenin.

present, although in smaller amounts, in the different subspecies of *S. leucantha*. This compound has not been detected in the rest of taxa analysed in any appreciable quantity. The isomeric compound hypolaetin 7-glucoside, that was recently described from *S. incana* [12], has been exclusively detected in the different subspecies of *S. incana* but we were unable to detect it in the closely related *S. glauca*. The presence of phloroglucinol-like flavone glyco-

sides was especially remarkable in *S. grandiflora, S. arborescens, S. pusilla* var. *carthaginensis* and *S. pusilla* var. *granatensis.*

The group of species accumulating hypolaetin (and its methyl ethers) 7-allosylglucosides includes *S. foetens, S. arborescens, S. leucantha, S. lacaitae, S. incana* (with the different subspecies), *S. hirsuta, S. hirsuta* var. *altilabra* and *S. endresii* subsp. *laxespicata* (Table 3). Onyl a few species accumulate isoscutellarein 4'-methyl ether 7-allosylglucoside, these being *S. glacialis, S. linearifolia, S. javalambrensis* and *S. serrata* and, producing it in a smaller amount, *S. spinulosa.* The accumulation of this compound can be considered as an advanced character from the phyletic point of view. Other species accumulate isoscutellarein 7-allosylglucoside such as *S. leucantha* (including all the different varieties), *S. pusilla* (including different varieties), *S. hyssopifolia, S. ovata, S. carbonellis* and *S. glauca.*

Discussion

The analysis of the epicuticular and vacuolar flavonoids of selected taxa of section *Eusideritis* by means of HPLC and TLC, shows different flavonoid patterns, characterized by the accumulation of one kind of vacuolar flavonoid (isoscutellarein-based, hypolaetin-based or luteolin-based) or by the presence or absence of epicuticular flavonoids. These results give us a valuable information to support one of the different taxonomical treatments of this genus. The group of *Sideritis* species that has received most attention from taxonomists in the last few years has been species related to *S. angustifolia* which includes *S. angustifolia 'sensu stricto', S. mugronensis, S. saetabensis, S. tragoriganum* and *S. funkiana.* This group is taxonomically very difficult, and was treated as a single species by Heywood [1]. Their vacuolar flavonoids have confirmed this homogeneity since they all accumulate hypolaetin 8-glucoside, a flavonoid which is absent in the rest of the species studied with the exception of *S. reverchonii* (which accumulates this compound in a similar amount to those found in *S. angustifolia* supporting the close relationship between these two species) and *S. leucantha* which produces this compound although in a much smaller amount. The presence of hypolaetin 8-glucoside in *S. angustifolia* and *S. leucantha* supports that the former species originates from the latter by translocation in accordance with genetical and morphological data [20].

As mentioned above, the existence of several populations of *S. mugronensis* which produce different epicuticular flavonoid patterns (the one from 'Sierra del Mugrón' [Albacete] produces mainly 5-desmethylnobiletin while the one from 'Los Llanos' [Albacete] produced mainly sideritoflavone as the rest of taxa included in *S. angustifolia 'sensu lato'*) prompted us to study the epicuticular flavonoids present in different populations of this species. In addition the presence of the diterpene borjatriol (7*S*,14*R*, 15-trihydroxy-8α-13-epoxylabdane), reported for the first time from *S. mugronensis* collected in 'Sierra del Mugrón', was studied. The analyses of these external compounds (Table 4) show the existence of two well defined groups; the group I which excretes mainly flavones with two free hydroxyls on B-ring (sideritoflavone and cirsiliol) and without borjatriol and the group II which accumulate their 3',4'-dimethyl ethers (5-desmethylnobiletin and 5-hydroxy-6,7,3',4'-tetramethoxyflavone) and with the diterpene borjatriol. These results suggest that the taxa from the second group are phyletically more advanced than those included in the first, and have helped to a new taxonomical treatment of this complex species [21].

Another species which is very rich in epicuticular flavonoids, *S. pusilla* (including *S. flavovirens, S. carthaginensis, S. granatensis* and *S. osteoxila*), also accumulates flavones with two free hydroxyls on the B-ring (sideritoflavone and cirsiliol), but which clearly differ in vacuolar flavonoids. Thus, the *S. pusilla* group accumulates isoscutellarein 7-allosylglucoside which is nearly absent in *S. angustifolia* and present only in a small amount in *S. leucantha,* which accumulates mainly hypolaetin 7-allosylglycoside, supporting the separation of this species from *S. leucantha,* from which it certainly evolved. *Sideritis carthaginensis* shows, in addition, 7-glycosides of phloroglucinol-like flavones (luteolin derived) which differentiate this taxa from the rest of *S. pusilla* varieties. *Sideritis pusilla* var. *osteoxila* is the only taxa of this complex that accumulate epicuticular flavonoids in a small amount, and this fact contrasts with the very arid habitat of this taxa, but again, as we

TABLE 4. DISTRIBUTION OF EPICUTICULAR SECONDARY METABOLITES IN DIFFERENT SELECTED POPULATIONS OF *S. ANGUSTIFOLIA 'SENSU LATO'* FROM THE SOUTH-EAST OF SPAIN

	Compounds*									
Populations	*EXC.*	1	2	3	4	5	6	7	8	B
Group I										
1. Cassas de San Pedro, Albacete	++	+	+++	—	—	t	+	—	t	—
2. Los Llanos, Albacete	++	+	+++	—	—	t	+	—	—	—
3. Mariquillas, Albacete	++	+	+++	—	—	t	+	—	—	—
4. Agost, Alicante	++	+	+++	—	—	t	t	—	t	—
5. Sa. Mariola canteras, Valencia	++	+	+++	t	t	+	+	—	+	—
6. Sa. Mariola, Valencia	++	+	+++	—	—	—	t	—	t	—
7. Sa. Mariola base, Valencia	++	+	+++	—	—	t	+	—	—	—
8. Ayora, Valencia	++	+	+++	—	—	t	+	—	t	—
9. F. la Higuera, Valencia	++	+	+++	—	—	+	+	—	—	—
10. Balsa Ves, Albacete	++	+	+++	t	t	+	+	—	—	—
11. Alpera, Albacete	++	+	+++	—	—	t	+	—	—	—
12. Agost, Aligante	++	+	+++	—	t	t	+	—	—	—
13. Castalla, Alicante	++	+	+++	—	—	+	+	—	+	—
14. F. la higuera, Valencia	++	+	+++	—	t	t	+	—	+	—
15. Castillo de Jativa, Valencia	++	+	+++	—	t	t	+	—	—	—
16. Miralcampo, Albacete	++	+	+++	t	t	+	++	—	—	—
GROUP II										
17. Saladar, Albacete	++	+	++	t	+	t	++	+	+++	+
18. Caudete, Albacete	++	+	t	t	+	t	t	++	+++	+
19. Sa. de la Oliva, Albacete	++	+	++	—	t	—	+	t	+++	+
20. Cerro del Rosario, Albacete	++	+	+	—	+	t	++	+	+++	+
21. Yecla, Murcia	++	+	+	—	+	t	+	+	+++	+
22. Bonete, Albacete	++	+	+	t	+	t	+	+	+++	+
23. Jodar, Albacete	++	+	+	—	t	—	+	t	+++	+
24. Sa. del Mugron, Albacete	++	t	+	—	t	—	+	t	+++	+
25. Meca, Albacete	++	++	+++	—	t	—	++	t	++	+
26. Pozo Lorente, Albacete	++	++	++	t	t	t	+	+	+++	+

*Code as for Table 2, (B) borjatriol.

described above in the case of *S. incana, S. osteoxila* is completely covered with a dense mat of white hairs that play the same role as the excretion flavonoids in the adaptation of the plants to semi-arid habitats.

In 1924, Font-Quer, in a study of the *Sideritis* from Spain [2] divided the section into two subsections, namely *Carpostegiatae,* including all the species having a ring of hairs inside the calyx (carpostegium), and *Gymnocarpae,* including the species without a ring of hairs inside the calyx. Subsection *Carpostegiatae* includes the majority of the Spanish *Sideritis* with the exception of *S. incana, S. glauca, S. lacaitae* and *S. stachydioides* which are included in subsection *Gymnocarpae.* From the chemical point of view, subsection *Gymnocarpae* is characterized by the lack of epicuticular flavonoids with the exception of *S. lacaitae* which produces these compounds in trace amounts, meanwhile taxa from section *Carpostegiatae* accumulate epicuticular flavonoids as a general rule, although *S. hyssopifolia* and *S. hirsuta* are devoid of these compounds. If we consider the production of external flavonoids as an advanced character gained with the evolution as an adaptation mechanism to xeric habitats, the results found here support a recent phyletical study of the section based on genetical and morphological data [20]. If we examine a phyletical scheme built based on genetical data, it is clear that the basic species are devoid of external flavonoids and the more evolved species are those which produce these compounds in greater amounts (Fig. 1). Thus, *S. lacaitae* which evolved from *S. incana,* produces trace amounts of epicuticular flavonoids: *S. serrata* which evolved from *S. hirsuta* produces epicuticular flavonoids, etc.

The vacuolar flavonoids of species included in subsection *Gymnocarpae,* show that the different subspecies of *S. incana,* produce a very

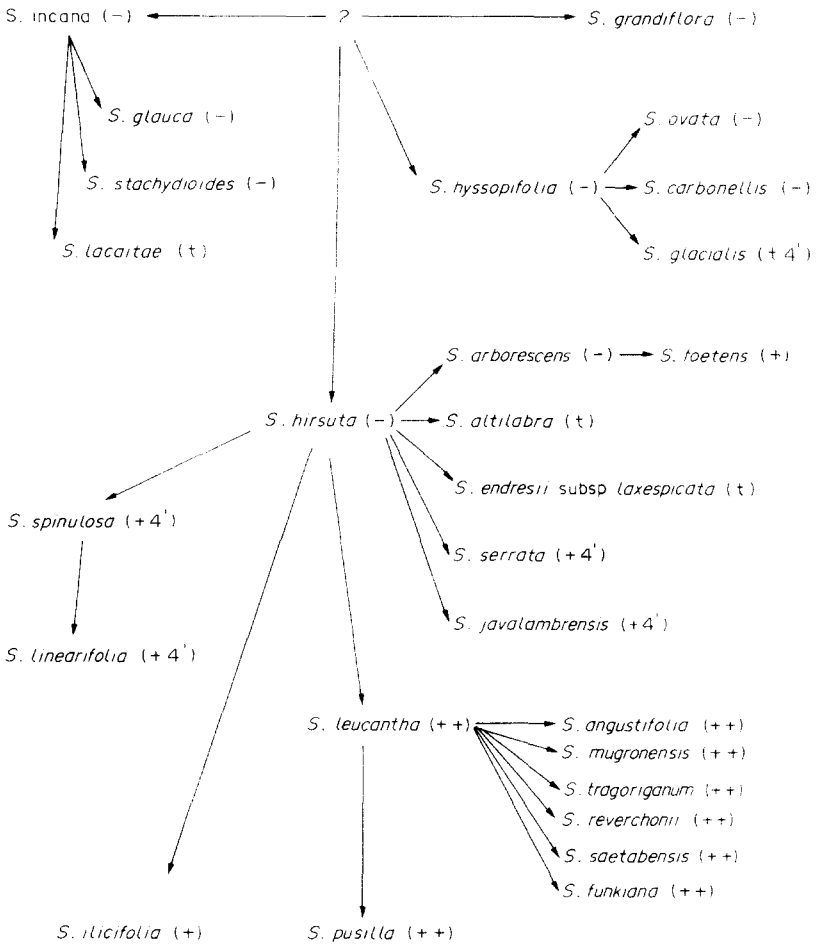

FIG. 1. CORRELATIONS BETWEEN PHYLOGENY OF SECTION *SIDERITIS* AND THE PRODUCTION OF EPICUTICULAR FLAVONOIDS. (—) Epicuticular flavonoids not detected; (t) detected in trace amounts; (+) present; (++) abundant. The species which accumulate the vacuolar flavonoid isoscutellarein 4'-methyl ether 7-allosyl(1→2)glucoside, have been also marked with 4'. To build this scheme, genetical, morphological and chemical data have been considered. Note that the more advanced species accumulate epicuticular flavonoids whereas the more primitive species are devoid of these compounds.

constant flavonoid pattern, characterized by accumulation of hypolaetin-based (hypolaetin and its methyl ethers) 7-glycosides, and they produce hypolaetin 7-glucoside [12], this compound being only detected in these taxa and absent in the rest of species studied. *S. lacaitae* produced the same kind of compounds supporting its close relationship with *S. incana,* but *S. glauca* (considered by some authors as a subspecies of *S. incana*) and *S. stachydioides* show very different flavonoid patterns, the former accumulating isoscutellarein 7-glycosides and the later producing luteolin and apigenin 7-*p*-coumaroyl-glucosides that are absent in the rest of the species of the section and are characteristic compounds from *Phlomis* [22] *Marrubium, Ballota* and several *Sideritis* endemic to the Canary Islands (Tomás-Barberán, F. A., unpublished work).

Sideritis grandiflora shows a very different flavonoid pattern, characterized by the accumulation of 7-allosyl(1→2)glucosides of chrysoeriol and luteolin, supporting that this species is quite different from the rest of the section, and that probably should be more related to section *Hesiodia.*

Sideritis arborescens accumulates hypolaetin-based glycosides, as well as *S. hirsuta,* and its

flavonoid pattern is very similar to that of *S. foetens* supporting their close relationship and the presence of epicuticular flavonoids in *S. foetens* support the fact that this taxa evolved from *S. arborescens* in accordance to genetical data [20]. An accumulation of the 4'-methyl ether of isoscutellarein 7-allosyl(1→2)glucoside has been observed in species that can be considered as phyletically advanced such as *S. glacialis, S. linearifolia, S. javalambrensis* and *S. serrata* (Fig. 1). *S. hyssopifolia* and related species (*S. ovata* and *S. carbonellis*) are characterized by the accumulation of isoscutellarein 7-allosyl(1→2)glucoside, with the exception of *S. hyssopifolia* subsp. *guillonii* that accumulates hypolaetin 3'-methyl ether 7-allosyl(1→2)glucoside instead, supporting the independence of this subspecies.

Recently, the epicuticular flavonoids have been used with success in a chemotaxonomic study of the genus *Thymus* [23], and the present work supports the usefulness of excreted flavonoids as taxonomic markers. In addition, the complementary study of both epicuticular and vacuolar flavonoids by HPLC and TLC has proved to be quite useful for chemotaxonomic purposes.

Experimental

Plant material. Aerial parts of *Sideritis* sp. were collected from typical localities and voucher specimens are deposited in the Herbarium of the Department of Botany at Murcia University (Table 1).

Extraction of epicuticular flavonoids and borjatriol. The air-dried aerial parts of the different species were rinsed in $CHCl_3$ for 2 min. The extracts were filtered, $CHCl_3$ removed and the residue redissolved in MeOH and re-filtered.

Extraction of vacuolar flavonoids. The flavonoids within the cells were extracted overnight with cold EtOH–H_2O (7:3). The EtOH was removed under red. pres. and the remaining aqueous extract was extracted successively with Et_2O and *n*-BuOH. The *n*-BuOH was removed under red. pres. and the residue redissolved in MeOH.

Analyses. The flavonoids present on the leaf surfaces (lipophilic extracts) were first analysed by TLC on silica gel as described previously [18] and the different spots visualized under UV light (360 nm) before and after spraying with Naturstoffreagenz-A. Another set of TLC plates were run in parallel under the same conditions to test the presence of the diterpene borjatriol (using an authentic marker), after spraying with vanillin and H_2SO_4 and heating at 120°. These extracts were also analysed by HPLC on reversed-phase column (C-18) as described previously [17]. The different flavonoids were identified by comparisons with authentic samples. Quantitative studies were carried out by means of a Sigma 15 data treatment station.

The vacuolar flavonoids analysed by TLC on cellulose with 30% HOAc, and the different spots visualized under UV light (360 nm) before and after spraying with Naturstoffreangenz-A. The extracts were run against authentic markers isolated previously. The same extracts were analysed by HPLC on a Perkin–Elmer Liquid Chromatograph, equipped with a 2/2 pump module and a Model LC85B UV-visible variable-wavelength detector. HPLC were run on a reversed-phase column Spherisorb C-8, 5 μm (25×0.46 cm). Runs were carried out for 25 min. The elution solvents were H_2O–HCOOH (19:1) from pump B and acetonitrile from pump A. The flow-rate was 1.5 ml/min with a gradient at a rate of 1%/min acetonitrile. Samples of 6 μl were injected, and peaks were detected at 340 nm. Retention times for the different flavonoid glycosides. Hypolaetin 7-allosyl(1→2)glucoside (10.0 min), hypolaetin 8-glucoside (11.7 min), isoscutellarein 7-allosyl(1→2)glucoside (12.7 min), hypolaetin 3'-methyl ether 7-allosyl(1→2)glucoside (13.7 min), isoscutellarein 4'-methyl ether 7-allosyl(1→2)glucoside (18.5 min).

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