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EXTERNAL AND VACUOLAR FLAVONOIDS FROM IBERO–NORTH AFRICAN *SIDERITIS* SPECIES. A CHEMOSYSTEMATIC APPROACH

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Key Word Index—*Sideritis*; Lamiaceae; external flavones; flavone 7-allosylglucosides; chemotaxanomy.

Abstract—The flavonoids, both external aglycones and vacuolar flavonoid glycosides, have been identified in the aerial parts of endemic North African *Sideritis* species. The flavonoids produced by these African species are very similar to those produced by Spanish species, being mainly 7-allosylglucosides of 8-hydroxyflavones. Two new flavonoid glycosides, apigenin and luteolin 7-allosylglucosides, have been identified in these plants. Species of the section *Sideritis* are characterized by the accumulation of the 7-glycosides of 8-hydroxyflavones (isoscutellarein, hypolaetin and their methyl ethers) whereas those of section *Hesiodia* accumulate 7-glycosides of the common flavones (apigenin, luteolin and chrysoeriol).

INTRODUCTION

The genus *Sideritis* L., consists of some 140 species [1] distributed throughout the Mediterranean region (including the Canary Islands and Madeira), where along with *Micromeria*, *Phlomis* and *Rosmarinus* they constitute a characteristic component of the maquis and the garrigue. This is a taxonomically difficult genus, still requiring extensive experimental investigation; in particular, the specific limits of section *Sideritis* are often obscure [2].

In the last few years the phytochemistry of this genus has been developed, and various di- and triterpenoids [3, 4], essential oils [5], sterols, coumarins and lignans [4] and flavonoid aglycones [6–8] and glycosides [9–12] have been identified. While the flavonoids from species growing in the Iberian Peninsula [6, 8–11] and in the Canary Islands [7] have been extensively studied revealing unusual structures both for aglycones and glycosides, the species from North Africa, including several interesting endemics from Morocco and Algeria, have not been studied so far.

Therefore, the flavonoids, of these North African *Sideritis* species have been investigated, with the aim of providing new data for the systematic revision of this genus. Wherever possible, populations of the same species growing in Spain have been analysed, for comparative purposes. An additional reason to study the flavonoids of these plants is their pharmacological interest since extracts of the aerial parts of these species are used in folk medicine for their anti-inflammatory and antirheumatic properties; several flavonoids isolated from these extracts have been shown to be responsible for these pharmacological activities [13, 14].

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RESULTS AND DISCUSSION

The different taxa analysed are listed in Table 1. In all the samples, the aerial parts were firstly soaked in chloroform to obtain a rinse which contained the external lipophilic flavonoids (excretion compounds) [15], and subsequently, the plant material was extracted with aqueous methanol to recover the vacuolar, hydrophilic flavonoid glycosides.

External flavonoids

These lipophilic flavonoids are externally deposited in a resin which mainly consists of terpenoids (diterpenoids, sesquiterpenoids). This resin covers the leaves and young stems of several of these plants. The study of the free flavone aglycones revealed the existence of highly substituted flavones with ring A tetrasubstituted (5-hydroxy-6,7,8-trimethoxyflavones) or trisubstituted (5-hydroxy-6,7-dimethoxyflavones) and ring B either mono- or disubstituted (Table 2). These seven compounds (sideritoflavone, cirsiliol, xanthomicrol, cirsilineol, 8-methoxycirsilineol, gardenin B, and 5-demethylnobiletin) have been found to occur widely in *Sideritis* species from Spain (section *Sideritis*) [6] but are not present in *Sideritis* species growing in the Canary Islands (section *Marrubiastrum* Bentham) [7]. There is thus a close chemical relationship between the North African species and the Spanish species. However, only two Moroccan species, *S. jahandiezii* and *S. briquetiana*, accumulate these compounds to the same extent as the Spanish species of subsection *Carpostegiatae* which grow in (semi-)arid habitats, i.e. *S. leucantha*, *S. angustifolia*, *S. serrata*, *S. pusilla*, etc. [6]. By contrast, the other Moroccan species either contain only trace amounts of surface flavonoids (i.e. *S. hirsuta*, *S. ochroleuca* var *maroccana*, *S. maireana* and *S. maura*) or lack them altogether.

One of the Moroccan species, *S. jahandiezii*, can be separated from all others by its having the rare external

Table 1. *Sideritis* species and localities of collection

Plant name	Place of collection
Section *Sideritis* Heywood (*Eusideritis* Bentham)	
S. incana L.	
subsp. *incana* Heywood (1)	Albacete (Spain)
subsp. *incana* Heywood (2)	Ourzazate (Morocco)
subsp. *incana* Heywood (3)	Ayachi, Grand Atlas (Morocco)
subsp. *sericea* (Pers.) P. W. Ball ex Heywood	Valencia (Spain)
subsp. *intermedia* F. Q.	Murcia (Spain)
subsp. *tomentosa* Batt. & Pitard	Col du Zad, Moyen Atlas (Morocco)
subsp. *guyoniana* F. Q.	Oran (Algeria)
S. subatlantica Doum.	Bocaya, Hoceima (Morocco)
S. subatlantica var. *heterostachya* Sennen	Hidum (Morocco)
S. ochroleuca De Noé	
var. *maroccana* F. Q.	Guercif (Morocco)
var. *antiatlantica* Maire	Ourzazate (Morocco)
S. arborescens Salzm.	Kebdana (Morocco)
S. hirsuta L. (1)	Zaragoza (Spain)
S. hirsuta L. (2)	Murcia (Spain)
S. hirsuta L. (3)	Asri, Grand Atlas (Morocco)
S. hirsuta L. (4)	Hichlifen, Moyen Atlas (Morocco)
S. jahandiezii F. Q.	Almis de Guigou (Morocco)
S. maireana F. Q.	Bu-Merziat (Morocco)
S. maura Cosson	Dahra, Oran (Algeria)
S. imbricata Lindb.	Tirarine, Djebel Aunsitene (Morocco)
*S. sp.**	Agard, Ourzazate (Morocco)
S. briquetiana F. Q. & Pau	Djebel Kerker (Morocco)
S. grandiflora Salzm.	Tetouan (Morocco)
Section *Hesiodia* Bentham	
S. romana L. (1)	Ibiza (Spain)
S. romana L. (2)	Tetouan (Morocco)
S. montana L. (1)	Teruel (Spain)
S. montana L. (2)	Ourzazate (Morocco)
S. cossoniana Ball	Agadir (Morocco)
S. villosa Cosson	Bekrit, Meium Atlas (Morocco)
S. gossypina F. Q.	Ourzazate (Morocco)

*The description this new species is now in progress (Rejdali, unpublished results). Voucher specimens of the different species have been deposited in the Herbaria of the Universities of Rabat, Murcia and Reading. The numbers between brackets identify the different populations of the same species.

flavonoids, gardenin B and 5-desmethylnobiletin. It may be taxonomically significant that these two rare markers occur in *S. serrata* [8], a Spanish species which according to Font-Quer [16] is morphologically similar to *S. jahandiezii*.

It is generally assumed that external flavonoids act as a UV-screen and play a role in the adaptation of plants to (semi-)arid and alpine habitats [17]. It is noteworthy that those North African species of section *Sideritis* which grow in arid habitats, do not excrete flavonoids as a general rule (see above), but they do produce external resins of a terpenoid nature. Exceptionally, the different taxa of *S. incana sensu lato* lack both flavonoids and terpenoids at the leaf surface. However, they do appear to be protected from harmful UV irradiation since their leaves are completely covered with a dense mat of white hairs. This is nicely illustrated by the electron micrographs of the leaf surface of *S. jahandiezii* (hairless, but contains external flavonoids) and *S. incana* (hairy but lacking excretion flavonoids) (Fig. 1).

Vacuolar flavonoid glycosides

Previous studies of the flavonoid glycosides from *Sideritis* species have revealed that a number of unusual compounds are present, especially the 7-allosyl(1 → 2)glucosides of the four 8-hydroxyflavones hypolaetin, hypolaetin 3′-methyl ether, isoscutellarein and isoscutellarein 4′-methyl ether [9–11] and of chrysoeriol [12]. The 6″-acetylated analogues have been found in the closely related genus *Stachys* [18] and recently in *Teucrium chamaedrys* and *T. webbianum* [19], and in *Veronica filiformis* (Scrophulariaceae) [20]. The present study now shows that all these compounds are widespread in the North African *Sideritis* species (Table 2).

The flavonoid glycosides of several endemic species (*S. jahandiezii*, *S. maura* and *S.* sp.) were studied in more detail, since the 2D-PC survey revealed the presence in these species of several distinctive components. Thus, *S. jahandiezii* was found to contain mainly isoscutellarein and its 4′-methyl ether as their 7-allosyl(1 → 2)glucosides;

Table 2. External and vacuolar flavonoids from North African *Sideritis* species

	Excretion flavonoids							Vacuolar flavonoids				
								8-OH-flavones		5,7-OH-flavones		
Plant name	1	2	3	4	5	6	7	ISOSC	HYP	CHRY	LUT	API
Section *Sideritis*												
S. incana												
subsp. *incana* (1)	–	–	–	–	–	–	–	+	+ + +	–	–	–
subsp. *incana* (2)	–	–	–	–	–	–	–	+	+ + +	–	–	–
subsp. *incana* (3)	–	–	–	–	–	–	–	+	+ + +	–	–	–
subsp. *sericea*	–	–	–	–	–	–	–	+	+ + +	–	–	–
subsp. *intermedia*	–	–	–	–	–	–	–	+	+ + +	–	–	–
subsp. *tomentosa*	–	–	–	–	–	–	–	+	+ + +	–	–	–
subsp. *guyoniana*	–	–	–	–	–	–	–	+	+ + +	t	–	t
S. subatlantica	–	–	–	–	–	–	–	t	+ + +	–	–	–
S. ochroleuca var. *maroccana*	+	+	+	–	–	–	–	t	+ + +	+	–	–
S. hirsuta (1)	–	–	–	–	–	–	–	+ +	+ +	t	–	–
S. hirsuta (2)	+	+	+	–	+	–	–	+ +	+ +	t	–	–
S. hirsuta (3)	+	+	+	–	+	–	–	+ +	+ +	t	t	–
S. hirsuta (4)	+	+	+	–	+	–	–	+ +	+ +	–	t	–
S. ochroleuca var *antiatlantica*	–	–	–	–	–	–	–	+ + +	t	t	t	–
S. jahandiezii	t	t	+	t	+	+	+	+ + +	t	–	–	–
S. maireana	+	+	–	–	–	–	–	+ + +	t	t	t	–
S. maura	–	–	+	–	+	–	–	+ + +	+	+	+	–
S. imbricata	–	–	–	–	–	–	–	+ + +	t	–	–	–
S. subatlantica var. *heterostachya*	–	–	–	–	–	–	–	+ + +	+	+	t	–
S. sp.	–	–	–	–	–	–	–	+ + +	t	t	t	+ +
S. arborescens	–	–	–	–	–	–	–	t	+ +	+ +	t	–
S. briquetiana	+	+	+	–	+	–	–	t	+	+ + +	+	+
S. grandiflora	–	–	–	–	–	–	–	–	t	+ + +	+	+
Section *Hesiodia*												
S. romana (1)	–	–	–	–	–	–	–	–	t	+ + +	+ +	+
S. romana (2)	–	–	–	–	–	–	–	–	–	+ + +	+	t
S. montana (1)	–	–	–	–	–	–	–	–	t	+ + +	+ +	+
S. montana (2)	–	–	–	–	–	–	–	–	–	+ + +	+	t
S. cossoniana	–	–	–	–	–	–	–		+	+ + +	+	t
S. villosa	–	–	–	–	–	–	–	–	–	+ + +	+	t
S. gossypina	–	–	–	–	–	–	–	–	–	+ + +	t	+

Excretion compounds: [1] cirsiliol (5,3′,4′-trihydroxy-6,7-dimethoxyflavone); [2] sideritoflavone (5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone); [3] xanthomicrol (5,4′-dihydroxy-6,7,8-trimethoxyflavone); [4] cirsilineol (5,4′-dihydroxy-6,7,3′-trimethoxyflavone); [5] 8-methoxycirsilineol (5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone); [6] 5-demethylnobiletin (5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone); [7] gardenin-B (5-hydroxy-6,7,8,4′-tetramethoxyflavone).

Vacuolar glycosides: 7-O-glycosides of luteolin (LUT),apigenin (API), chrysoeriol (CHRY), isoscutellarein (5,7,8,4′-tetrahydroxyflavone) and its 4′-methyl ether (ISOSC), hypolaetin (5,7,8,3′,4′-pentahydroxyflavone) and its 3′- and 4′-methyl ethers (HYP).

Relative abundances: (+ + +) the main compounds in the extract; (+ +) significative amounts; (+) present; (t) trace amount; (–) not detected.

these were identified by standard UV procedures and chromatographic comparison with authentic samples isolated from Spanish *Sideritis* species. The presence of allose was confirmed after acid hydrolysis by cochromatography. In addition, other 7-glycosides of isoscutellarein were detected. One of these compounds gave allose and glucose after acid hydrolysis, and its higher R_f values (*n*-BuOH–HOAc–H_2O 4:1:5, upper phase and HOAc 15%) compared with isoscutellarein 7-allosyl(1 → 2)glucoside suggest that this compound is the 6‴-acetate. The glycosylflavone vitexin (apigenin 8-*C*-glucoside) was also detected in the same plant; this is the second time that this compound has been found in this family. Previously this compound was reported from *Majorana hortensis* [21]. Here again, as in the case of the excretory flavones, the flavonoid glycosides identified in *S. jahandiezii* support its close relationship to *S. serrata* since this species also produces 7-allosyl(1 → 2)glucosides of isoscutellarein and its 4′-methyl ether (Tomás-Barberán, unpublished results).

S. maura, an endemic from Algeria, contains mainly isoscutellarein 7-allosyl(1 → 2)glucoside, and 7-glycosides of hypolaetin and its 3′-methyl either in smaller amounts. In addition, this plant produces chrysoeriol and luteolin 7-allosyl(1 → 2)glucosides, the latter being a new naturally occurring compound.

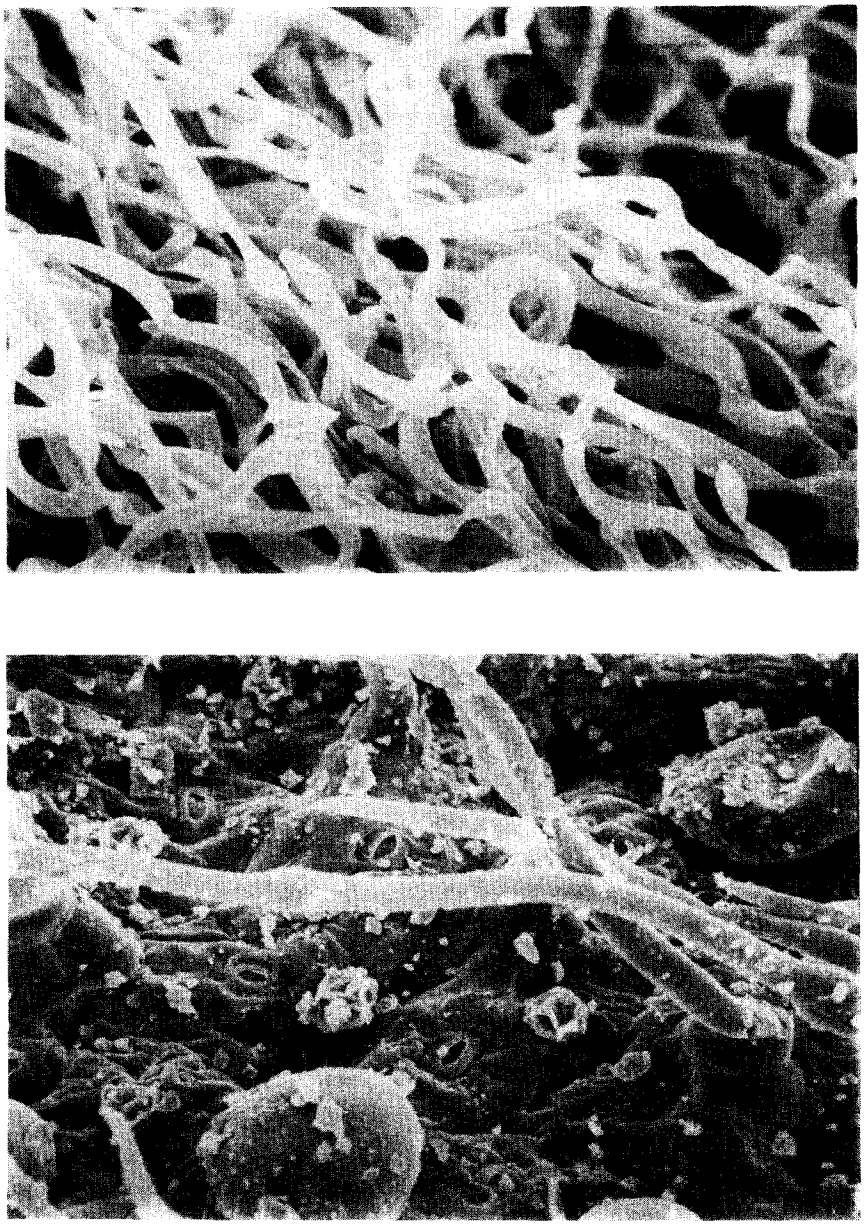

Fig. 1. Leaf surfaces of *Sideritis incana* (top) and *S. jahandiezii* (bottom).

The third species studied in detail was collected in Agard (Ourzazate, Morocco), and is a new taxon, the affinities of which is still being determined (Rejdali, unpublished work). This plant contains mainly isoscutellarein allosyl(1 → 2)glucoside and a small amount of the 7-allosyl(1 → 2)glucosides of hypolaetin and its 3'-methyl ether. In addition, it produces apigenin 7-allosyl(1 → 2)glucoside. This is a new naturally occurring compound which is not present in any of the other *Sideritis* species so far examined.

This study has revealed that the taxa studied can be divided broadly into two groups on the basis of the accumulation of 7-allosyl(1 → 2)glucosides of 8-hydroxyflavones (isoscutellarein, hypolaetin) or of phloroglucinol-based flavones (apigenin, luteolin, chrysoeriol) (Table 2). The first group includes all the species of section *Sideritis*, with the exception of *S. briquetiana* and *S. grandiflora* which accumulate chrysoeriol glycosides. The second group include all the species of section *Hesiodia* and the latter two species of section *Sideritis*. This second group is quite homogeneous from the chemical point of view. This is in line with the classification of Bentham who treated all these plants as a single section; it is at variance with the system of Briquet, which places these plants into two sections: *Hesiodia* (*S. montana*) and *Burgsdorfia* (*S. romana*, *S. cossoniana*, *S. villosa*).

The first group of plants can be further divided chemically into three subgroups on the basis of the predominance of isoscutellarein-based or hypolaetin-based glycosides. The first subgroup which accumulate hypolaetin glycosides includes all the taxa of *S. incana*

sensu lato, *S. subatlantica* and *S. ochroleuca* var *maroccana*. The second subgroup which accumulate isoscutellarein glycosides includes *S. ochroleuca* var *antiatlantica*, *S. jahandiezii*, *S. maireana*, *S. maura*, *S. imbricata*, *S. subatlantica* var *heterostachya* and the new species. There are minor chemical differences at the subspecific level in the case of *S. subatlantica* and *S. ochroleuca*, whereas subspecies of *S. incana* and of *S. hirsuta* collected variously in Spain and North Africa are more or less uniform in chemical content.

There is an intermediate group of plants based on *S. hirsuta* which accumulate both isoscutellarein and hypolaetin glycosides in approximately the same amounts. Previously, the 7-allosyl(1 → 2)glucoside of 6-hydroxydiosmetin (5,6,7,3′-tetrahydroxy-4′-methoxyflavone) was reported from *S. hirsuta* [22], but we have been unable to detect this compound in this species, finding instead the 8-hydroxy isomer. This result could be attributed to the difficulty of identifying flavonoids with an extra substituent on A-ring only on the basis of NMR evidence [23]. *S. arborescens* occupies an intermediate position between the two main groups of plants, since it produces chrysoeriol glycosides and hypolaetin glycosides in a similar amount. Finally, it is apparent that *S. grandiflora* and *S. briquetiana* can be separated from all other species of section *Sideritis* by the fact that they contain the flavonoids typical of plants of section *Hesiodia*. Thus, they may well represent bridge species between the two sections.

EXPERIMENTAL

Plant material. the different *Sideritis* species were collected at flowering in Spain, Morocco and Algeria, and voucher specimens are deposited in the Herbaria at Reading, Rabat and Murcia Universities.

Extraction of flavonoids. Aerial parts of the different species were first dipped in $CHCl_3$ for 2 min. to obtain a rinse in which the excretion flavonoids were analysed, and second, after removing the excess of $CHCl_3$, with EtOH–H_2O (7:3) overnight at room temp. to extract the flavonoid glycosides. Both extracts were taken to dryness and dissolved in MeOH and MeOH–H_2O (4 : 1) respectively.

External flavonoids analyses. The excretion flavonoids were analysed by TLC on silica gel with toluene–HOAc (4:1) and with the solvents previously described [24], and their structures were confirmed by chromatographic comparisons against authentic markers isolated previously from other species of *Sideritis* and by standard UV spectrophotometric procedures.

Flavonoid glycosides analyses. The hydroalcoholic extracts were analysed by 2D-PC on Whatman No. 1 with *n*-BuOH–HOAc–H_2O (4:1:5, upper phase) and 15% HOAc, and the different flavonoids were detected under UV light (366 nm). The different spots were eluted with MeOH–H_2O (4:1) and their UV spectra run in the same solvent and after addition of the classical shift reagents. The original extracts were also hydrolysed with 2 N HCl (90°) for 30 min and the aglycones obtained were extracted with EtOAc and TLC analysed on cellulose TLC with 50% HOAc against the expected aglycone markers (luteolin, chrysoeriol, apigenin, 6-hydroxyluteolin, 6-hydroxychrysoeriol, scutellarein and scutellarein 4′-methyl ether) giving a complementary information to the 2D-PC analysis. The presence of flavonoid glucuronides in the original extracts was also analysed by paper electrophoresis in 0.1 N acetate buffer pH 4.4. The structures of the isolated compounds were confirmed by chromatographic comparisons against authentic markers isolated from known sources of Spanish *Sideritis* species. The presence of the uncommon sugar allose in these glycosides was evidenced by paper chromatographic comparisons against a marker with PhOH–H_2O (4:1) a system which has proved useful for the differentiation of this sugar from the rest of the hexoses [9–11, 18, 19]. The flavonoid compounds which after this survey remained unknown, were subsequently thoroughly studied in order to establish their structures.

Identification of apigenin and luteolin 7-allosylglucosides. These two new compounds were isolated from *Sideritis* sp. and *S. maura* respectively. Their UV spectra in MeOH and after addition of the classical shift reagents clearly demonstrated that these are 7-glycosides of luteolin and apigenin. After prolonged acid hydrolysis (1 hr, 90°, 2 N HCl), allose, glucose and the respective aglycones (luteolin and apigenin) were detected. Mild acid hydrolysis [9–11] produced a small amount of the monoglucosides showing the sugar sequence in the natural glycosides.

Electron micrographs. Whole specimens were mounted on brass SEM stubs by means of double sided adhesive tape. The stubs were then coated with gold in an atmosphere of argon for 3–4 min, and examined in a JEOL TSM T2o instrument [25].

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