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VERIFICATION OF *SIDERITIS INCANA* X *S. ANGUSTIFOLIA* HYBRIDS BY FLAVONOID ANALYSIS

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Abstract—The occurrence of interspecific hybrids of *Sideritis angustifolia* and *S. incana* has been biochemically verified by TLC and HPLC analyses of external and internal flavonoids. A correlation between the excretion of flavonoids and the presence of glands in the taxa studied has been observed. The hybrids produce flavonoid patterns which contain the substances typical of both parents.

INTRODUCTION

Interspecific hybrids of the genus *Sideritis* have been recorded from many localities in Spain and elsewhere and it seems that most of the taxonomic difficulties within this genus are due to the occurrence of such hybrids. Therefore it is important to be able to recognize which plants are of hybrid origin and this is often difficult on the basis of morphological characters only. For this reason, a chemical or biochemical approach to this problem was thought to be useful as the genus *Sideritis* is very rich in flavonoids and these have been used recently for chemotaxonomial purposes [1–3].

The presence of interspecific hybrids of *Sideritis incana* and *S. angustifolia* have been previously reported from Quesa (Valencia, Spain) [4]. These hybrids are especially interesting since the parents belong to two different subsections of section *Sideritis*, namely subsection *Gymnocarpae* (*S. incana* var. *edetana*) and *Carpostegiatae* (*S. angustifolia*). Recently these hybrids have been studied morphologically by means of a modified Anderson's hybrid index [5], and several degrees of hybridization were observed between the two parents. In the present work, internal and external flavonoids have been studied by HPLC and TLC analyses to prove the existence of these hybrids biochemically.

RESULTS AND DISCUSSION

Internal (vacuolar glycosides) and external (lipophilic methylated aglycones) flavonoids have been analysed after selective extraction by means of HPLC and TLC techniques. Selected individuals of both parents and hybrids were analysed. The results are shown in Table 1. External flavonoids were absent from *S. incana* var *edetana* as expected [1–3]. On the other hand, the other parent, *S. angustifolia*, produced a profuse exudate which, after HPLC analysis, showed the typical flavonoid pattern for members of section *Sideritis* subsection *Carpostegiatae* [1]. The hybrids excrete flavonoids but in a smaller amount than *S. angustifolia*. The flavonoid patterns of the different hybrids analysed showed no significant differences. The major external flavonoid was sideritoflavone (5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone) with cirsiliol (5,3′,4′-trihydroxy-6,7-dimethoxyflavone), xanthomicrol (5,4′-dihydroxy-6,7,8-trimethoxyflavone) and 8-methoxycirsilineol (5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone) as other prominent constituents.

The morphological analysis of the leaf hairs of the parents and hybrids agreed with the observed biochemical results. Thus, the leaves of *S. incana* var *edetana* were completely covered with white dead hairs and lacked glands while *S. angustifolia* had many glands and the hybrids had both hairs and glands but in smaller number than either parent (Table 2). In these species, the occurrence of external flavonoids is associated with the presence of glands.

Sideritis species are characterized by the production and accumulation in the cell sap of 8-hydroxyflavone glycosides (hypolaetin, isoscutellarein and their methyl ethers) [2, 3]. The analysis of the internal flavonoids in these taxa showed that *S. incana* var *edetana* contained mainly hypolaetin 7-glycosides and isoscutellarein 7-allosylglucosides but the most typical flavonoid in this species was isoscutellarein 4′-methyl ether 7-allosylglucoside. The latter compound was absent from *S. angustifolia*, whose flavonoid pattern was characterized instead by the presence of hypolaetin 8-glycosides which are absent from *S. incana*. The hybrids, as could be expected, showed flavonoid patterns which contained both hypolaetin 8-glycosides and isoscutellarein 4′-methyl ether 7-allosylglucoside, the typical compounds for both parents (Table 1). However, one of the hypolaetin 7-glycosides (R_t 4.05 min) is only present in *S. incana*, this compound not being detected in the hybrids nor in the other parent.

Table 1. Vacuolar and external flavonoids from *Sideritis* taxa

Vacuolar flavonoid glycosides Structures R_t	Relative concentrations *S. incana edetana*	x	x	*S. angustifolia*
3, 12 Hypolaetin 7-glycoside	10. 4	12. 8	9. 7	19. 7
4, 05 Hypolaetin 7-glycoside	7. 5	—	—	—
4, 38 Hypolaetin 7-allosylglucoside	26. 4	24. 2	26. 4	10. 5
4, 69 8-HC 7-allosylglucoside*	19. 5	24. 0	26. 8	10. 3
6, 40 Isoscutellarein 7-allosylglucoside	34. 4	3. 9	3. 7	4. 4
10, 42 Hypolaetin 8-glycoside	—	3. 5	5. 1	15. 7
11, 63 Hypolaetin 8-glucoside	—	30. 1	21. 5	39. 4
12, 40 Iso 4'-Me 7-allosylglucoside*	1. 8	1. 5	6. 8	—
External flavonoid aglycones.				
2, 35 Cirsiliol	—	13. 6	11. 9	26. 1
2, 87 Sideritoflavone	—	47.8	49.6	39.5
3, 34 Cirsimaritin	—	0. 4	5. 9	10. 6
3, 91 Cirsilineol + eupatorin	—	0. 6	1. 6	5. 3
4, 63 Xanthomicrol	—	15. 0	20. 1	3. 3
5, 11 8-Methoxycirsilineol	—	8. 2	1. 0	8. 6
5, 92 5-Hydroxy-6,7,3',4'-methoxyflavone	—	2. 2	0. 6	1. 9
6, 88 —	—	—	—	2. 0
— Other substances	—	12. 2	9. 3	2. 7
Excretion level	—	+	+	+ + +

Numbers are % of absorbance (340 nm) in the HPLC chromatogram.
R_t are HPLC retention times (for HPLC analytical conditions see Experimental).
*8-HC = 8-hydroxychrysoeriol; Iso 4'-Me = isoscutellarein 4'-methyl ether.
Excretion level: (—) excretion absent; (+) excretion present; (+ + +) abundant excretion.

Table 2. Hair-covering of the different *Sideritis* taxa analysed

Types	Inflorescence's axis			Calyx			Bracts			Leaves			Stem's base		
	INC	X	*ANG*	*INC*	X	*ANG*	*INC*	X	*ANG*	*INC*	X	*ANG*	*INC*	X	*ANG*
Hoary	100	42. 8	—	100	—	—	100	57. 1	—	100	28. 6	—	100	57. 1	—
Hoary & glandular	—	14. 3	—	—	42. 8	—	—	28. 6	12. 5	—	71. 4	—	—	—	—
Hoary & hooklets	—	28. 5	—	—	—	—	—	—	—	—	—	—	—	42. 9	—
Hoary & shaggy	—	14. 3	—	—	—	—	—	—	—	—	—	—	—	—	—
Shaggy (arched)	—	—	37. 5	—	—	—	—	—	—	—	—	—	—	—	—
Shaggy & glandular	—	—	37. 5	—	—	87. 5	—	—	37. 5	—	—	100	—	—	—
Shaggy (patent)	—	—	25. 0	—	—	—	—	—	—	—	—	—	—	—	—
Hairy	—	—	—	—	—	—	—	14. 3	—	—	—	—	—	—	—
Hairy & hooklets	—	—	—	—	—	—	—	—	—	—	—	—	—	—	25. 0
Hairy & hooklets & glandular	—	—	—	—	—	—	—	—	—	—	—	—	—	—	62. 5
Hairy & glandular	—	—	—	—	28. 6	—	—	—	50. 0	—	—	—	—	—	12. 5
Downy & glandular	—	—	—	—	28. 6	12. 5	—	—	—	—	—	—	—	—	—

Numbers are % of individuals showing each character, *INC* = *S. incana* var. *edetana*; X = hybrids; *ANG* = *S. angustifolia*.

EXPERIMENTAL

Plant material. Samples of the two parents, *S. angustifolia* Lag. and *S. incana* var *edetana* Pau ex Font Quer, and the hybrids *S. incana* var *edetana* × *S. angustifolia* = *S.* × *viciosoi* nothovar *stricta* Font Quer were collected on June 1987 in Valencia (Spain) between Quesa and Bicorp. Parents and hybrids grow together in this area.

For the morphological study 7 individuals of *S. incana* var *edetana*, 7 hybrids and 8 *S. angustifolia* individuals were studied. The flavonoid analyses were carried out in four selected individuals, two hybrids and one of each parent.

Flavonoid analysis. Flavonoids were extracted as reported previously [3]. External flavonoids were analysed by TLC and HPLC as described [3] and vacuolar flavonoids were analysed by TLC on cellulose with 30% HOAc, and the different spots visualized under UV light (360 nm) before and after spraying with Naturstoffreagenz-A. The extracts were run against authentic markers isolated previously. HPLC were run on a reversed-

phase column Spherisorb C-8, 5 μm (25 × 0.46 cm). Runs were carried out isocratically with a mixture of THF–ACN–MeOH–H_2O as described previously [6]. The flow-rate was 1 ml/min. Samples of 6 μl were injected, and peaks were detected at 340 nm. The UV spectra of the different flavonoids were recorded by a photodiode detector coupled to the HPLC equipment.

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