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PHOTOACTIVE FUROCOUMARINS IN TWO POPULATIONS OF *SESELI ELATUM**

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Key Word Index—*Seseli elatum*, *Umbelliferae*; furocoumarins, within-plant distribution, secretory canals, ampullae.

Abstract—The furocoumarin content of wild plants of *Seseli elatum* aggregatum was examined in two populations. Xanthotoxol, phellopterin, seselin, isoimperatorin, isopimpinellin, xanthotoxin, oxypeucedanin hydrate, alloisoimperatorin and sphondin were isolated, identified, and their concentration quantified in different parts of the plants These compounds have not been reported previously. The phytochemical investigation has shown the two populations differ qualitatively and quantitatively. The furocoumarins are restricted to the secretory canals and to the ampullae. The furocoumarin content of fruits at different stages of maturity was also examined

INTRODUCTION

Furocoumarins are a well defined group of compounds contained in almost all the genera of *Umbelliferae* [3]. These substances show interesting photosensitizing properties on various biological substrates [4–7]. Skin photosensitization is the best known and most studied property and it is utilized in therapy for the cure of leucodermic spots of vitiligo [8] and for the treatment of psoriasis [9].

Recently furocoumarins isolated from *Peucedanum* species (*Umbelliferae*) were found to act as organic calcium antagonists [10, 11] and therefore are useful in the therapy of cardiovascular diseases Furocoumarins also display phototoxic properties toward viruses, bacteria, fungi, protozoans and insects [12, 13] by photobonding to the pyrimidine bases of DNA [14]

The present work is limited to the investigation of the furocoumarin components of two populations of *Seseli elatum* aggregatum of the Friuli-Venezia Giulia Region (northeast Italy) The chemistry of these populations has not been studied previously. In a recent publication we reported on the composition and the variation of the essential oil [2]

The populations of *S. elatum* agg. are widely distributed in the north-eastern part of Italy, from the Karst plateau near Trieste (*S. elatum* subsp. *gouanii* (Koch) P. W. Ball) to the Pre-Alps (*S. elatum* subsp. *elatum*) The population in the Alpine area close to Carinthia (Austria), may be attributed to *S. elatum* subsp. *austriacum* (G Beck) P. W Ball. This population is chemically scarcely different from the population of the Pre-Alps and therefore its chemical data are not given here.

*Part 3 in the series 'Taxonomical studies on *Seseli elatum* L and allied species' For part 1, see ref. [1], for Part 2, see ref [2].

RESULTS AND DISCUSSION

Chromatographic and spectrophotometric analysis led to the identification of seven linear furocoumarins (xanthotoxol, xanthotoxin, isopimpinellin, phellopterin, isoimperatorin, alloisoimperatorin, and oxypeucedanin hydrate), of one angular furanocoumarin (sphondin) and of one angular pyranocoumarin (seselin)

Psoralen (a 6-substituted 7-oxygenated coumarin), the fundamental unsubstituted furocoumarin, was not present. This compound is not accumulated because it is not a terminal product, but acts as a precursor for xanthotoxol, xanthotoxin, alloisoimperatorin (7,8-dioxygenated coumarins), oxypeucedanin hydrate (a 5,7-dioxygenated coumarin), isopimpinellin and phellopterin (5,6,7-trioxygenated coumarins) This agrees with the fact that all the 7-hydroxy-coumarins arise from umbelliferone. When nuclear prenylation of umbelliferone occurs at C-6, this leads eventually to linear furanocoumarins of the psoralene type. Alkylation at C-8, through the dihydrofuranocoumarin, gives rise to furanocoumarins of the angelicin class (and perhaps to sphondin), and via the isomeric dihydropyranocoumarin, to angular pyranocoumarins having the seselin skeleton [15].

It is interesting that both plant species elaborate two biogenetically related classes of coumarins, furanocoumarins and pyranocoumarins. This suggests that the biosynthetic patterns may be the same The two populations differ qualitatively as significant differences in relative concentrations of furocoumarins were found (Table 1).

The population of *S gouanii* is less productive 0.879 g% dry wt). Seselin is the major constituent in all individual plants, xanthotoxin and sphondin are absent. The population of *S. elatum* is more productive (1.109 g% dry wt). Seselin is again the major constituent. The content of isoimperatorin is four times higher than in *S. gouanii* and isopimpinellin is three times higher. These differences are highly significant ($P < 0.001$, Wilcoxon 2-sample test).

Table 1 Average furocoumarin composition in whole plants of the two populations of *S elatum* agg

Coumarins	R_f (min)	Amount (g% dry wt) *S gouanii*	*S elatum*
Xanthotoxol	2 50	0 068	0 084
Phellopterin	2 67	0 252	0 094
Seselin	2 77	0 413	0 408
Isoimperatorin	3 00	0 093	0 375
Isopimpinellin	3 70	0 038	0 114
Xanthotoxin	4 17	–	0 005
Oxypeucedanin hydrate	4 93	0 006	0 015
Alloisoimperatorin	5 22	0 009	0 011
Sphondin	6 00	–	0 002
Total		0 879	1 108

There are also differences in furocoumarin composition and content in the various parts of the plants (Table 2) The richest organ of *S gouanii* is the inflorescence, which contains a very high percentage of phellopterin. In this organ two other furocoumarins (seselin and isopimpinellin) are present The fruits have the highest number of furocoumarins, the leaves the smallest The richest organ of *S elatum* is the fruit, which has a high content of isoimperatorin and seselin The largest number of furocoumarins is found in the roots

The furocoumarin content of fruits varied with ripening (Table 3), both qualitatively and quantitatively The absolute average amount is always higher in ripe fruits of *S elatum* The unripe fruits of the two populations differ for quantities of xanthotoxol, isoimperatorin, oxypeucedanin hydrate and alloisoimperatorin. The ripe fruits of the two populations present almost the same qualitative composition, but differ in furocoumarin contents. In the fruits of *S elatum* seselin and isoimperatorin are the major compounds while in the fruits of *S gouanii* isopimpinellin and oxypeucedanin hydrate predominate.

The chemical markers for the various parts of the plant and for the plants *in toto* are reported in Table 4 The two populations (*in toto*) are characterized by the absence or presence of xanthotoxin, the roots by the presence or absence of xanthotoxin, oxypeucedanin hydrate and sphondin, the stems by phellopterin, the leaves by isoimperatorin, the inflorescences by different quantities of phellopterin, the fruits by xanthotoxol

Some components seem to significatively characterize the two populations Some triangular graphs are shown in Fig 1 In contrast to previous reports for *Pastinaca sativa* [16], we found no trace of furocoumarins in the vittae of the two populations These compounds are localized exclusively in secretory canals and in ampullae The vittae of *S elatum* agg populations are the pool of the essential oil [1, 2]

If the furocoumarins were restricted to the vittae, they should occur only in the fruits, and not in other parts of the plant and would be not involved with contact photosensitization On the contrary, the two populations examined occasionally show biological properties The presence of xanthotoxin, oxypeucedanin hydrate and shondin may well explain the weak photoallergic response that sometimes appeared on the hands and arms of the authors during the collection of the plants

It is evident that in either of the populations during the ripening of fruits the quantity of some furocoumarins greatly decreases or increases Xanthotoxol, isopimpinellin, xanthotoxin decrease while seselin, isoimperatorin, oxypeucedanin hydrate and alloisoimperatorin increase. This behaviour can be explained by the fact that decreasing temperature favours the formation of some compounds (e g seselin, isoimperatorin and oxypeucedanin hydrate) through the interconversion of xanthotoxol and xanthotoxin

The fact that different concentrations of the same furocoumarins are found in different parts of the plants suggests that some of these compounds may have a specific biological role to play within each of the various organs The increased concentration of furocoumarins in ripe fruits may be due to increase selection pressure by herbivores, insects or pathogens, and could be related to their possible role as allelopathic agents or seed dormancy regulators [17] The significance of the within-plant variation has to be demonstrated with respect to defensive mechanism Since differences in furocoumarins distribution and composition correspond to functional and structural differences among plant parts, both ex-

Table 2 Average furocoumarin composition (g% dry wt) in roots (r), stems (s), leaves (l), inflorescences (i), and fruits (f) of the two populations of *S elatum* agg

Coumarins	*S gouanii* r	s	l	i	f	*S elatum* r	s	l	i	f
Xanthotoxol	0 133	—	–	—	0 020	0 124	–	0 295	–	–
Phellopterin	—	–		1 260		–	0 189		0 279	–
Seselin	0 329	0 467	0 685	0 202	0 380	0 138	0 326	0 529	0 332	0 713
Isoimperatorin	0 056	0 099	–	–	0 685	0 060	0 043	0 506	0 143	1 250
Isopimpinellin	0 022	0 016	0 014	0 061	0 078	0 029	0 114	0 324	0 102	0 003
Xanthotoxin	—	–	–	–		0 021				–
Oxypeucedanin hydrate	—	–	—		0 062	0 055	–	–	—	0 023
Alloisoimperatorin	0 016	–		—	0 055	0 004	–		—	0 052
Sphondin	–	—	–			0 008	–		—	–
Total	0 056	0 582	0 699	1 523	0 863	0 439	0 672	1 654	0 856	2 041

Table 3 Average furocoumarin composition in unripe and ripe fruits

	Amount (g% dry wt)			
	S. gouanii		*S elatum*	
Coumarins	Unripe	Ripe	Unripe	Ripe
Xanthotoxol	0 209	0 020	0 679	—
Phellopterin	—	—	—	—
Selenin	0 301	0 380	0 387	0 713
Isoimperatorin	0 183	0 685	0 321	1 250
Isopimpinellin	0 109	0 078	0.134	0 003
Xanthotoxin	—	—	0 030	—
Oxypeucedanin hydrate	0 032	0 062	—	0 023
Alloisoimperatorin	0 029	0 055	—	0 052
Sphondin	—	—	—	—
Total	0 863	1 280	1 551	2.041

Table 4 Furocoumarins as markers in the two populations of *S elatum* agg

	In toto		Roots		Stems		Leaves		Inflores		Fruits	
	SeG	SeE	SeG	SeE	SeG	SeE	SeG	SeE	SeG	SeE	SeG	SeE
Xanthotoxin	0	+										
Xanthotoxin			0	+								
Oxypeucedanin hydrate			0	+								
Sphondin			0	+								
Phelloperin					0	+						
Isoimperatorin							0	+				
Phellopterin									+ + + +	+		
Xanthotoxol											+	0

trinsic factors such as insects, herbivores, and/or environmental factors and intrinsic factors (such as physiological processes relating to fruit ripening) may be important in determining the observed distribution

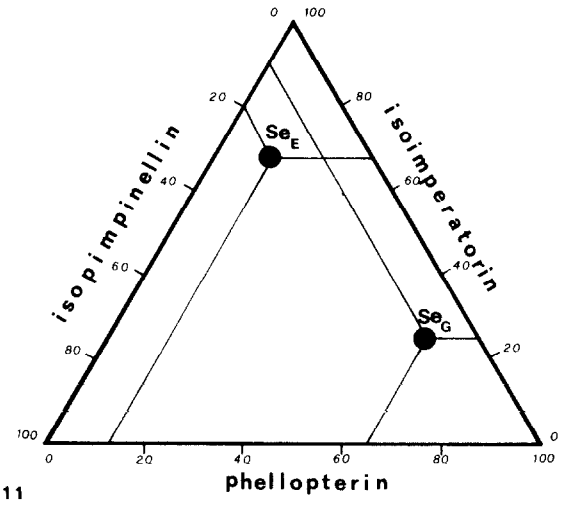
The two populations are a good source of some biologically active compounds. Isopimpinellin is lethal to snails [18], and perhaps also active against parasitic worms and has diuretic properties [19], a mixture of bergapten, xanthotoxin and sphondin is used in spasmolytic preparations [20]; xanthotoxin, sphondin, isopimpinellin and oxypeucedanin hydrate shown photobiological properties.

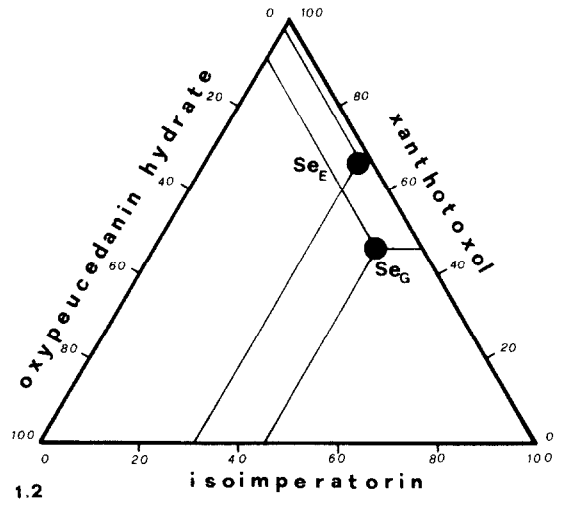
From the botanical point of view, knowledge of the qualitative and quantitative within-plant variation of furocoumarins in the two entities of *S. elatum* agg. may eventually contribute to a better understanding of the chemotaxonomic classification of this difficult group but further data are required.

EXPERIMENTAL

Mps are uncorrected The identity of the unknown products was established by comparison of their chromatographic (TLC, HPLC) and physical (analytic values, mp, uv, and/or MS, IR) data with those of authentic samples. *Biological materials* The plant materials were collected at random with unripe and with ripe fruits in September and October 1985 and 1986. The quantitative data are means of two years, the variations from year-to-year was within ±1 2%, for a content of 1 0% The specimens were identified by Prof L Poldini and deposited in the TBS Herbarium (no 6042) The plants were subdivided into roots, stems, leaves, inflorescences and fruits We have examined the plants either *in toto* or as individual parts

Isolation of furocoumarins. The dried, ground, plant materials (100 g dry wt) were extracted (×3) with MeOH at 45° with occasional shaking for 6 hr The combined filtrates were concentrated *in vacuo*, dissolved in water–MeOH (2 1) and extracted in a continuous liquid extractor with *n*-hexane (24 hr) to remove chlorophylls and other fatty materials The H_2O–MeOH mixture was concentrated and back extracted with Et_2O in a continuous liquid extractor for 24 hr to remove furocoumarins The furocoumarin mixture was separated on a silica gel H column with *n*-hexane–petrol (bp 40–50°) (7 3, 4 1, 9 1, 1.0) and *n*-hexane–EtOAc (49 1; 9 1, 4:1) 70 fractions (100 ml each) were collected, fractions with similar material (based on UV fluorescence at 254 nm TLC, colour tests and R_f) were combined, concentrated, applied in bands, and separated on silica gel prep. TLC (cyclohexane–EtOAc, 13.7) The silica gel areas, identified by co-chromatography with authentic samples, were scraped off and eluted with MeOH. The analytical separation was performed by HPLC on a Lichrosorb–SI 100 (250 × 4 mm) column fitted with a CSK I Whatman guard column (Co· Pell ODS 30–38 μm, 50 × 4 mm i.d.) and with a solvent consisting of cyclohexane–EtOAc (7:3, 1 ml/min) Detection was by UV absorbance (254 nm) Quantification was by peak-area integration and peak-height All work was carried out at room temp





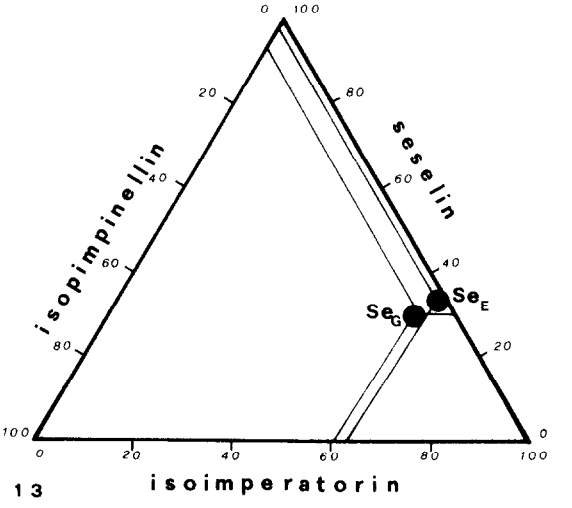


Fig 1 Numerical analysis of the furocoumarinic compounds, taken three by three Furocoumarins discriminating the two populations 1.1, plants *in toto*, 1 2, unripe fruits, 1 3, ripe fruits SeG = *S gouanii*, SeE = *S elatum*

Relative standard deviations of R_t were in the range 0 4–1 0% The reproducibility of peak-area measurements in a five replicate analysis with standard compounds was found to be better than that for peak-height measurements A calibration curve (by peak-height) was prepared for concentration ranging from 0 0005 to 0 0065% (w/v) The relative angular coefficients (log α) were obtained by the least-squares regressions method The relative standard deviation of peak-area for the available standards was in the range 1 9–3 1% The isolated compounds were identified as follows

Xanthotoxol Yellow fluorescence in UV light R_f 0 72 R_t 2 50 min Yellow needles Mp 245 log α 2 737 Anal (found C, 65 56, H, 3 20, calc for $C_{11}H_6O_4$ C, 65 35, H, 2 99)

Phellopterin Yellow fluorescence in UV light R_f 0 25 R_t 2 67 min Colourless needles Mp 102 log α 2 862 Anal (found C, 67 90, H, 5 25, MeO–, 10 40, calc for $C_{17}H_{16}O_5$ C 68 0 H 5 37, MeO–, 10, 33)

Seselin Blue fluorescence in UV light R_f 0 60 R_t 2 77 min Colourless needles Mp 119 5 log α 2 829

Isoimperatorin Greenish-yellow fluorescence in UV light R_f 0 60 R_t 3 00 min Colourless prisms Mp 110 log α 2 897 Anal (found C, 70 87, H, 5 18, calc for $C_{16}H_{14}O_4$ C, 71 10, H, 5 22)

Isopimpinellin Brown-yellow fluorescence in UV light R_f 0 20 R_t 3 70 min Yellow needles Mp 150 , log α 2 742 Anal (found C, 63 2, H, 4 36, MeO–, 24 9 calc for $C_{13}H_{10}O_5$ C 63 4, H, 4 09, 2MeO–, 25 1)

Xanthotoxin Yellow fluorescence in UV light R_f 0 68 R_t 4 17 min Colourless needles Mp 147 log α 2 542 Anal (found C, 66 16, H, 3 89 MeO–, 14 47 Calc for $C_{12}H_8O_4$ C, 66 67 H, 3 73, MeO–, 14 36)

Oxypeucedanin hydrate Blue fluorescence in UV light R_f 0 08 R_t 4 93 min Light-tan prisms Mp 133 , log α 2 648 Anal (found C, 62 9, H, 5 29, calc for $C_{16}H_{16}O_6$ C, 63 2, H, 5 30)

Alloisoimperatorin Blue fluorescence in UV light R_f 0 57, R_t 5 22 min Slightly yellow needles Mp 230 5 , log α 2 858 Anal (found C, 70 91, H, 5 34, calc for $C_{16}H_{14}O_4$ C 71 10 H, 5 22) This compound is probably an artefact of isoimperatorin

Sphondin Strong blue fluorescence in UV light R_f 0, 64 R_t 6 0 min Colourless needles Mp 190–192 , log α 2 794

The physical constants (UV, IR MS) were in accord with those reported in literature [21–32] The analytical data (HPLC) were submitted to numerical analysis taking into account at the time, three components of furocoumarinic mixture

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