Essential Oil Chemical Composition of Wild Populations of Italian Oregano Spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): A Preliminary Evaluation of Their Use in Chemotaxonomy by Cluster Analysis. 1. Inflorescences

Mariateresa Russo,[†] Guido C. Galletti,*[,‡] Paola Bocchini,[§] and Alberta Carnacini[†]

Dipartimento di Scienza e Tecnologia Agro-Forestale ed Ambientale, Università degli Studi di Reggio, Calabria, Italy, Dipartimento di Chimica "G. Ciamician", Università degli Studi di Bologna, Bologna, Italy, and Centro di Studio per la Fisica delle Macromolecole, Consiglio Nazionale delle Ricerche, Bologna, Italy

Twenty-four steam-distilled samples of essential oils from inflorescences from *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart growing wild in Calabria, southern Italy, were analyzed by gas chromatography and gas chromatography/mass spectrometry. A total of 56 compounds were identified. The main components of the essential oil were thymol and carvacrol, while their biogenetic precursors, *p*-cymene and γ-terpinene, were the most aboundant monoterpenes. The relative amounts of the two main constituents were comparable to the literature data on this species. Four chemotypes were identified in Calabria on the basis of the phenolic content, i.e., thymol, carvacrol, thymol/carvacrol, and carvacrol/thymol chemotypes. The first chemotype was the most frequent. A significant variability of composition possibly correlated with the individual genotypes was observed.

Keywords: *Oregano; Origanum vulgare ssp. hirtum; essential oil composition; GC/MS*

INTRODUCTION

A large numbers of species, colloquially named "oregano or origanum", have achieved economic interest, although they belong to different botanical families and genera. Four main groups commonly used for culinary purposes can be distinguished, i.e., Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart); Spanish oregano (*Coridohymus capitatus* (L.) Hoffmanns & Link); Turkish oregano (*Origanum onites* L.); and Mexican oregano (*Lippia graveolens* HBK) (Lawrence, 1984).

In Europe and, in general, all over the world, the most commonly found oregano species belong to the botanical genus *Origanum*. Unfortunately, much confusion exists about the correct taxonomic classification of oregano despite of the fact that, in 1980, Ietswaart made a taxonomic revision of the genus. Before 1980, *O. vulgare* L. indicated indifferently the subspecies that Ietswaart identified as ssp. *hirtum* (Link) Ietswaart, ssp. *gracile* (C. Koch) Ietswaart, ssp. *vulgare*, and ssp. *viride* (Boiss.) Hayek.

It is not surprising that many analytical investigations about oregano essential oils did not discriminate the numerous subspecies that show subtle morphological and chemical differences. The yield in essential oils is the only character that shows a relative stability, thus being useful for subspecies identification.

O. vulgare L. ssp. *vulgare* and *O. vulgare* ssp. *hirtum* (Link) Ietswaart are commercial species. A few references have reported on the first species (Maarse and Van Os, 1973a,b, 1974; Baser et al., 1993), while more numerous works have dealt with the second (Fleisher et al. 1982; Lawrence, 1989; Baser et al., 1993, 1994; Sezik, 1993; Tucker and Maciarello, 1993; Melagari et al., 1995; Battistutta, 1995).

A survey of the literature reveals that *O. vulgare* ssp. *vulgare* L. has an extremely low essential oil yield. Its principal constituents are sabinene, (*Z*)-β-ocimene, β-caryophyllene, and germacrene D, while the phenols thymol and carvacrol are absent. By contrast *O. vulgare* ssp. *hirtum* has a high essential oil yield whose principal components are phenols, *p*-cymene, and γ-terpinene. The present chemical study is an attempt to throw light on the relationship between chemical composition and biotypes in *O. vulgare* ssp. *hirtum*.

EXPERIMENTAL PROCEDURES

Materials. Twenty-four oregano samples belonging to *O. vulgare* ssp. *hirtum* (Link) Ietswaart species were collected from populations growing wild in Calabria, southern Italy, differing for climatic conditions, altitude, and exposure (Table 1). One representative, homogeneous sample of each population was collected from June to July during the so-called "balsamic time" corresponding to flowering stage. Only sample 24 was collected post-flowering. The plant material used for the essential oil was air-dried at room temperature.

Oil Extraction. Fifty grams of dried inflorescences was hydrodistilled in triplicate for 2 h in a Clevenger-type apparatus. The isolated oils were dried over anhydrous sodium sulfate and stored at 4−6 °C. Moisture contents (European Pharmacopoeia I) and essential oil yield on a dry weight basis (% of dry matter) are reported in Table 1.

Analytical Method. Volatile oregano oil components were separated for identification by gas chromatography (GC-FID) and gas chromatography/mass spectrometry (GC/MS).

* To whom correspondence should be addressed. Tel, +39.51.259459; fax, +39.51.259456; e-mail, galletti@ciam.unibo.it.

[†] Università degli Studi di Reggio.

[‡] Università degli Studi di Bologna.

[§] Consiglio Nazionale delle Richerche.

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Table 1. Places (Name, Altitude), Collection Date, Dry Matter (DM), and Essential Oil Yield (%) of the 24 Wild Populations of *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart[a]

	locality	collection date	altitude (mlm)	yield (%)	DM (%)
		1994			
1	Amaroni	June	400	2.25	88.80
2	Zagarise	June	500	5.07	88.79
3	Orti'	July	500	5.69	85.05
4	Melia	July	600	5.73	81.51
5	Filadelfia	July	800	5.00	87.09
		1995			
6	Amaroni	June	400	2.58	88.06
7	Ursini	July	400	3.45	85.85
8	Armo	July	400	3.73	87.78
9	Girifalco	June	450	2.25	88.70
10	Sersale	June	450	2.42	88.83
11	Gimigliano	June	500	3.97	87.82
12	Zagarise	June	500	4.05	87.51
13	Orti'	July	500	4.45	86.13
14	Sellia	July	500	4.56	87.18
15	Mesoraca	July	500	4.63	86.88
16	Bova Superiore	June	600	4.19	86.63
17	Melia	July	600	4.34	85.59
18	S. Roberto	July	600	4.50	88.46
19	S. Onofrio	June	700	5.34	87.71
20	Campoli	July	700	5.48	86.66
21	Molochio	July	700	5.61	84.60
22	S. San Bruno	July	800	5.58	86.55
23	Filadelfia	July	800	5.30	87.60
24	Pietrapennata	Sept	800	5.53	86.88

[a] Utilized part: flowers. Phenologic: full bloom.

(a) GC-FID. Gas chromatographic analyses were performed in duplicate by injecting 1 μL of oil solution (1% in ethyl ether) in the split mode into a Perkin-Elmer 8600 gas chromatograph equipped with a FID. A capillary column DB5 (J&W Scientific) (30 m × 0.25 mm i.d., film thickness 0.25 μm) was employed. The column temperature was programmed from 60 to 100 °C, at 3 °C/min; to 130 °C, at 2.5 °C/min; to 180 °C, at 3 °C/min (20 min hold); and finally up to 300 °C, at 5 °C/min (6 min hold). The injector was kept at 250 °C while FID was at 300 °C. The carrier gas was helium at a flow rate of 1 mL/min.

(b) GC/MS. Analyses were performed with a Varian 3400 gas chromatograph coupled with a Finnigan MAT model 800 ion trap detector mass spectrometer. One microliter of the solution was injected in the split mode (split ratio 1/100). The GC column was a SPB-5 (30 m × 0.32 mm i.d., 0.25 μm film thickness) and was operated from 50 to 290 °C at 5 °C/min, holding initial and final temperatures for 10 min. Mass spectra were recorded under electron impact at 70 eV, spectral range from 40 to 450 *m/z*, 1 scan/s. Chromatographic peaks were identified by retention times, mass spectra of authentic compounds when available, NIST Library of mass spectra, and published data (Adams, 1982).

DATA PROCESSING

Analytical data were treated by means of the statistical package STATISTICA/W release 4.5 (Statsoft Inc., USA). The percentage concentrations of the components in the different oils were used as matrix elements.

RESULTS AND DISCUSSION

The harvest times and the essential oil yield (percent of dry weight) of *O. vulgare* ssp. *hirtum* collected from 19 areas of Calabria, southern Italy, are reported in Table 1. The high yield in essential oils of Calabrian oregano biotypes is apparent. In particular, yields were higher as compared to those reported in the literature for samples of the oregano species coming from different

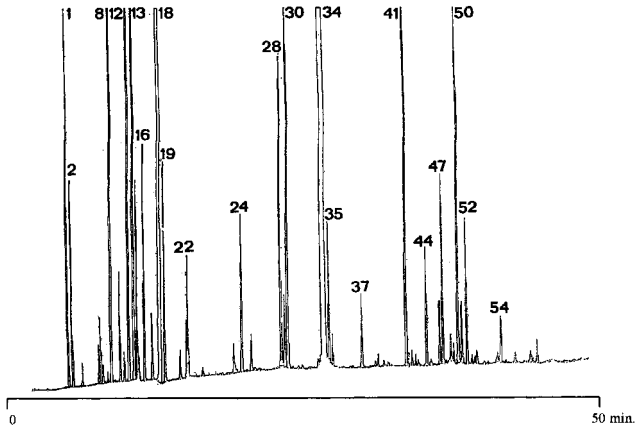

Figure 1. Gas chromatogram of oregano inflorescences essential oil (thymol chemotype). Conditions: 30 m × 0.25 mm fused silica capillary column (DB5, J&W Scientific); carrier gas, helium; injector 250 °C; FID 300 °C. Temperature was as follows: from 60 up to 100 °C, rate of 3 °C/min; up to 130 °C, rate of 2.5 °C/min; up to 180 °C, rate of 3 °C/min held 20 min, up to 300 °C, rate of 5 °C/min held 6 min. Perkin-Elmer 8600 gas chromatograph was used in this analysis.

European countries (Marzi et al., 1992). Calabrian ecotype oil yields varied between 2.25% and 5.73% and increased with altitude (Table 1).

Climatic factors, intensity of plant metabolism, differentiation, and secretory activity of glandular hairs affect synthesis and secretion of oils (Falchi Delitalia et al., 1983). As essential oils are secondary products of plant metabolism, a more active metabolism may be associated with a larger production of oils (Wilkins, 1969). Climatic data relative to localities of interest demonstrate that Calabrian high hills and mountains receive more rainfall and that the highest oil yield is that from the sample collected in areas where precipitation is most evenly distributed across all the year while the lowest oil yield is from the sample collected in areas where precipitation is concentrated in the period from September to April (Ser. Idr. CZ, 1996; Russo, 1996).

A typical GC-FID profile of essential oils from oregano is shown in Figure 1. Table 2 shows the results of gas chromatographic analysis (area percentage) (Russo, 1996). Compounds are listed in order of their elution time. A total of 56 compounds have been identified. Eight of them, tentatively identified by GC/MS, were not previously reported in the oil of *O. vulgare* ssp. *hirtum* (Link) Ietswaart, i.e., 3-octanone, *trans-p*-menth-2-en-1-ol, thymoquinone, thymol acetate, cuminyl alcohol, valencene, *allo*-aromadendrene, germacrene-D, and germacrene-D-4-ol.

Significant quantitative differences among different essential oils were apparent only between the two isomeric phenols, carvacrol or noncrystallizable phenol, and thymol or crystallizable phenol and their biosynthetic precursors γ-terpinene and *p*-cymene. The concentration of these components varied greatly among the samples, in particular those of carvacrol (0.12−56.63%) and thymol (7.91−53.62%). In all cases, however, the sum of the two phenols and their precursors constituted the bulk of each essential oil (72.08−82.86%).

Using hierarchical cluster analysis, four main groups of samples were observed (Figure 2). These can be classified in two main chemotypes (thymol and carvacrol chemotypes) and two intermediate chemotypes (thymol/carvacrol (T/C) and carvacrol/thymol (C/T)) in which the

Table 2. Total Area Percentage Composition of Essential Oils of 24 Wild Populations of *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart

	compounds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	α-thujene	1.37	1.19	1.06	1.01	1.32	1.50	1.38	1.43	0.76	1.31	1.29	0.92	1.50	1.65	1.56	1.41	1.44	1.48	1.53	1.39	2.04	1.77	1.49
2	α-pinene[a]	0.61	0.63	0.53	0.47	0.62	0.62	0.60	0.57	0.37	0.57	0.86	0.48	0.55	0.67	0.63	0.62	0.54	0.61	0.57	0.55	0.78	0.62	0.62
3	camphene[a]	0.09	0.09	0.08	0.21	0.10	0.09	0.10	0.09	0.07	0.15	0.07	0.11	0.08	0.11	0.14	0.11	0.12	0.09	0.08	0.08	0.10	0.09	0.12
4	sabinene[a]	0.09	0.11	0.05	0.10	0.11	0.16	0.12	0.35	0.23	0.12	0.16	0.06	0.09	0.53	0.19	0.10	0.11	0.14	0.26	0.13	0.19	0.17	0.12
5	β-pinene[a]	0.18	0.30	0.28	0.13	0.21	0.16	0.14	0.17	0.18	0.14	0.21	0.11	0.14	0.23	0.15	0.16	0.14	0.16	0.14	0.14	0.19	0.16	0.16
6	octen-3-ol[a]	0.07	0.03	0.12	0.13	0.22	0.11	0.06	0.12	0.07	0.08	0.08	0.12	0.18	0.13	0.26	0.24	0.12	0.15	0.09	0.15	0.22	0.13	0.14
7	3-octanone[a]	0.04		0.05	0.08	0.07	0.10	0.04	0.05	0.07	0.06	0.08	0.06	0.07	0.04	0.11	0.07	0.04	0.07	0.05	0.06	0.05	0.05	0.04
8	myrcene[a]	2.25	1.99	2.05	1.82	2.09	2.49	2.28	2.28	1.38	2.17	2.32	1.72	2.20	2.49	2.36	2.17	2.00	2.37	2.18	2.13	2.69	2.18	2.37
9	3-octanol										0.02	0.04				0.02								
10	α-phellandrene[a]	0.29	0.25	0.30	0.37	0.30	0.39	0.37	0.35	0.21	0.34	0.34	0.22	0.32	0.38	0.35	0.35	0.31	0.31	0.32	0.40	0.40	0.32	0.35
11	δ3-carene[a]	0.09	0.08	0.08	0.13	0.09	0.10	0.10	0.09	0.06	0.08	0.12	0.08	0.08	0.09	0.09	0.10	0.08	0.09	0.08	0.07	0.11	0.08	0.10
12	α-terpinene[a]	2.65	1.87	3.08	2.11	2.83	4.11	2.94	3.50	2.23	3.69	3.29	1.96	3.55	3.70	3.07	3.24	2.65	2.52	3.50	3.09	3.84	3.16	3.69
13	*p*-cymene[a]	6.22	4.35	6.54	3.63	8.89	4.47	5.41	4.31	4.91	4.39	3.70	3.82	5.90	5.61	5.85	9.74	3.74	4.38	3.57	4.62	5.76	4.31	5.17
14	limonene[a]	0.57	0.44	0.92	0.50	0.74	0.56	0.57	0.59	0.70	0.58	0.54	0.43	0.55	0.66	0.61	0.58	0.43	0.50	0.56	0.62	0.67	0.48	0.58
15	1,8-cineole[a]	0.02	0.15			0.11	0.10		0.12	0.07	0.04	0.16			0.18								0.09	
16	(*Z*)-β-ocimene[a]	0.62	0.16	0.79	0.26	0.40	0.44	0.55	0.54	0.76	0.48	0.47	0.31	0.20	0.24	0.48	0.30	0.19	0.22	0.67	0.30	0.27	0.22	0.44
17	(*E*)-β-ocimene[a]	0.22	0.15	0.22	0.23	0.36	0.33	0.19	1.00	0.57	0.21	1.47	0.22	0.16	1.28	0.16	0.12	0.15	0.19	0.22	0.52	0.43	0.38	0.18
18	γ-terpinene[a]	30.47	12.64	21.72	16.14	15.98	31.07	20.14	24.24	22.25	32.59	25.94	13.33	25.37	23.08	17.14	18.71	17.31	17.61	27.43	19.78	22.17	21.24	24.90
19	*cis*-*p*-menth-2-en-1-ol	0.68	1.10	0.84	1.02	0.86	0.63	0.73	0.39	0.84	0.68	0.81	0.07	0.85	1.31	0.84	0.70	0.72	0.88	0.72	0.81	0.88	0.74	0.89
20	terpinolene[a]	0.11	0.15	0.12	0.13	0.12	0.14	0.08	0.12	0.09	0.17	0.12	0.10	0.08	0.11	0.08	0.11	0.09	0.09	0.10	0.10	0.12	0.09	0.14
21	*trans*-*p*-menth-2-en-1-ol[b]	0.11	0.18	0.10		0.05	0.14	0.08	0.08	0.09	0.13	0.15	0.02	0.14	0.17	0.07	0.12	0.06	0.13	0.06	0.10	0.16	0.10	0.17
22	linalool[a]	0.30	0.07	0.71	0.60	1.45	0.21	0.21	0.37	0.18	0.30	0.19	0.23	1.56	0.54	0.39	0.09	0.24	0.09	1.62	0.33	1.73	0.25	0.42
23	borneol[a]	0.16	0.21	0.16	0.15	0.29	0.15	0.10	0.06	0.02	0.15	0.11	0.08	0.01	0.16	0.09	0.09	0.11	0.15	0.08	0.40	0.13	0.22	0.18
24	terpinen 4-ol[a]	0.51	0.53	0.44	0.37	0.40	0.43	0.36	0.42	0.59	0.40	0.44	0.44	0.34	0.16	0.40	0.35	0.44	0.39	0.34	0.39	0.35	0.35	0.37
25	*p*-cymen-8-ol[a]																							
26	α-terpineol[a]	0.17	0.96	0.13	1.79	1.03	0.37	0.20	0.26	0.25	0.08	0.27	0.25	0.10	0.34	0.21	0.51	0.09	0.10	1.42	0.40	0.54	0.28	0.28
27	*cis*-dihydrocarvone	0.11	0.10			0.02						0.06	0.13					0.05	0.08					
28	methyl thymyl ether[a]	0.99	0.06	0.54	0.52	0.81	0.70	1.00	0.48	1.26	0.95	0.68	0.12	0.33	0.46	0.67	0.73	0.15	0.19	0.40	0.56	0.35	0.45	0.54
29	carvone[a]	0.39	0.08			0.12						0.02	0.04											
30	methyl carvacryl ether[a]	2.60	2.56	1.91	3.20	3.47	3.24	3.16	3.35	2.90	2.64	4.20	4.15	2.81	3.77	3.75	3.53	2.89	4.67	2.61	2.73	2.90	3.20	3.38
31	thymoquinone[b]																							
32	linalyl acetate[a]													0.02										
33	bornyl acetate[a]	0.03		0.04		0.10				0.08		0.28	0.05	0.01										
34	thymol[a]	40.49	9.24	53.62	19.00	48.74	40.33	53.16	49.09	44.45	38.76	24.66	9.03	47.39	44.20	45.69	30.49	27.03	7.91	44.67	55.46	49.52	41.17	42.07
35	carvacrol[a]	0.47	56.63	0.40	36.43	2.54	0.42	0.43	0.52	0.47	0.27	18.66	54.90	0.64	5.33	10.02	21.00	32.53	50.40	0.38	0.57	0.66	11.73	8.43
36	cuminyl alcool[b]	0.14																						
37	carvacryl acetate	0.30		0.13		0.19	0.48	0.50		1.24		0.30	0.18	0.09	0.11	0.16	0.63	0.09	0.07		0.41	0.12	0.26	0.20
38	thymyl acetate[b]	0.03	0.26		0.26	0.02			0.21	0.03	0.90	0.50	0.46	0.02		0.09	0.08	0.25	0.35	0.27			0.06	
39	α-copaene	0.04	0.03	0.02		0.06				0.04		0.04	0.20	0.06			0.06	0.06					0.05	
40	β-bourbonene	0.04								0.04														
41	β-caryophyllene[a]	2.14	1.11	0.87	2.63	1.48	1.86	1.46	1.45	4.40	2.89	2.09	1.66	1.55	0.75	1.45	0.07	1.86	1.17	2.00	1.09	0.89	1.79	0.85
42	β-cubebene	0.06	0.03	0.01	0.13	0.03		0.16		0.05		0.10	0.07	0.03		0.02		0.10				0.05		
43	aromadendrene	0.02		0.01	0.21	0.02							0.09	0.03										
44	α-humulene[a]	0.39	0.20	0.19	0.80	0.33	0.44	0.27	0.30	0.82	0.56	0.38	0.40	0.27	0.13	0.30	0.25	0.41	0.29	0.43	0.34	0.17	0.30	0.26
45	*allo*-aromadendrene[b]	0.05				0.09		0.06		0.12		0.25		0.06		0.02			0.02	0.05		0.01		
46	γ-muurolene	0.23	0.12	0.08	0.39	0.28	0.20	0.25	0.14	0.17	0.21	0.44	0.25	0.13	0.04	0.09	0.16	0.24	0.06	0.06	0.10	0.07	0.13	0.10
47	germacrene-D	0.64	0.08	0.11	0.32	0.25	0.94	0.78	0.88	1.86	0.95	0.75	0.23	0.67	0.51	1.04	0.40	0.65	0.69	1.16	0.55	0.28	1.27	0.27
48	valencene[b]	0.13	0.11	0.07	0.26	0.24	0.26	0.17	0.17	0.13	0.18	0.19	0.18	0.13	0.08	0.13	0.21	0.20	0.15	0.14	0.13	0.10	0.13	0.10
49	α-muurolene	0.05	0.03	0.02	0.13	0.06	0.10	0.07	0.05	0.07	0.09	0.29	0.06	0.04		0.03	0.07	0.08	0.04	0.05	0.08	0.03	0.07	0.04
50	β-bisabolene[a]	1.69	0.82	0.96	1.55	1.23	1.24	0.80	0.85	3.00	1.59	2.09	1.29	0.92	0.37	0.75	1.36	1.37	0.63	1.14	0.95	0.57	1.08	0.58
51	γ-cadinene	0.22	0.13	0.12	0.51	0.29	0.16	0.22	0.09	0.18	0.10	0.23	0.25	0.13	0.05	0.07	0.16	0.19	0.04	0.07	0.07	0.05	0.10	0.08
52	δ-cadinene	0.45	0.34	0.31	0.26	0.42	0.51	0.42	0.25	0.62	0.54	0.41	0.68	0.30	0.12	0.23	0.53	0.47	0.15	0.23	0.23	0.16	0.40	0.19
53	germacrene-D-4-ol[b]	0.03	0.03	0.03	0.13	0.15	0.05	0.06	0.36	0.05	0.14	0.04		0.08	0.18	0.13	0.04	0.11	0.18	0.26	0.06	0.12	0.08	
54	caryophyllene oxide	0.19	0.25	0.14	0.91	0.22	0.05	0.08	0.02	0.44	0.15	0.06	0.21	0.07	0.07	0.02	0.08	0.05	0.04	0.07	0.06	0.04	0.09	
55	torrejol[b]	0.07	0.09	0.03	0.47	0.13	0.08	0.11	0.22	0.21	0.05	0.03	0.21	0.07			0.10	0.05	0.09	0.14	0.04	0.05	0.08	
56	α-cadinol	0.10	0.07	0.01	0.53	0.07	0.10	0.08	0.09	0.44	0.11	0.04	0.04	0.07		0.12	0.07	0.04	0.08	0.28	0.06	0.06	0.10	

[a] Identified injecting standard compound. [b] Tentatively identified.

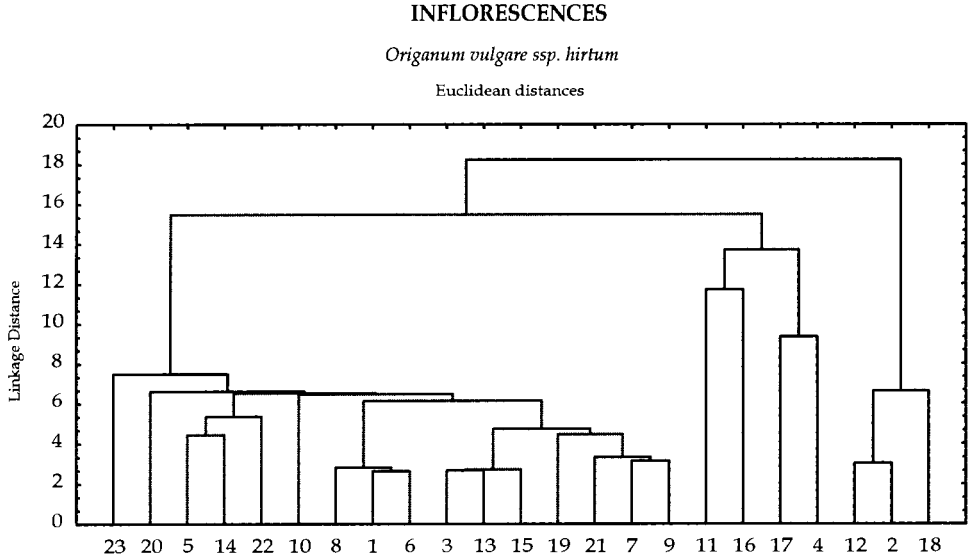

Figure 2. Cluster analysis of GC essential oils data.

predominant compound is thymol or carvacrol, respectively. Carvacrol chemotypes are represented by samples 18, 2, and 12. The last two samples were collected in the same locality but in two different years: 1994 and 1995. T/C chemotype contains samples 11 and 16, while C/T chemotype contains samples 4 and 17 collected, respectively, in the same locality during 1994 (11, 4) and 1995 (16, 17). The others samples belong to thymol chemotype, which is also the most numerous. These results are in agreement with those of Battistutta et al. (1995), who reported that in southern Italy the most common chemotype was of the thymol type. Others, however, (Kokkini et al., 1989) have reported carvacrol to be the main component of *O. vulgare* ssp. *hirtum* essential oil, and no one has previously reported an intermediate chemotype.

The observed increase in thymol percentage with decreasing carvacrol content in all samples indicates a biosynthetic correlation between the two compounds. Chemotypes form "biochemical varieties" or "physiologic forms" in botanical species, each of which with specific genetically codified enzymatic equipment directs biosynthesis to the preferential formation of definite compounds. In the case of phenolic compounds, the metabolic pathway is through the autoxidative conversion of γ-terpinene to *p*-cymene followed by hydroxylation of *p*-cymene to thymol, carvacrol, *p*-cymene-8-ol, and cuminyl alcohol. The last two are present only in trace amounts in samples examined and might be synthesized by a similar mechanism involving the hydroxylation at C-2 of the *p*-cymene aromatic ring or at the side chain (Poulose and Crotau, 1978).

In carvacrol chemotype, noncrystallizable phenol amounts to 56.63% with appreciable amounts of thymol (7.91−9.24%) present, while in thymol chemotype the percentages of crystallizable phenol varied from 38.76% to 55.46% and in every case the isomer is present only in trace amounts. The total amount of phenolic compounds and monoterpene hydrocarbons, mainly represented by γ-terpinene and *p*-cymene, was constant in all chemotypes (Table 3), but thymol biosynthetic pathway is favored with altitude and becomes more efficient than the carvacrol biosynthetic pathway.

As noted before, the characterization of habitat is of fundamental importance to understanding species distribution. In a specific geographical area, the factors that weigh heavily on chemotypes differentiation are mainly related to intrinsic factors such as sexual polymorphism or genetic mechanism, but for the phenolic essences, environmental conditions are able to influence which biosynthetic pathway may be dominant. With regard to intermediate chemotypes, the phenomenon might be related exclusively to genetic factors. Then plants belonging to different chemotypes hybridize and give rise to intermediate chemotypes (Granger and Passet, 1973).

Table 3. Percentage of Monoterpene Hydrocarbons and Phenols in Oregano Essential Oils from Different Homogeneous Localities

altitude		monterpene hydrocarbons	phenols (thymol + carvacrol)	sum
400−600	cluster thymol	40.11	47.62	87.73
	cluster carvacrol	24.13	64.90	89.03
600−700	cluster thymol	38.69	50.66	89.35
	cluster carvacrol	30.77	58.31	89.08
> 700	cluster thymol	36.61	51.56	88.17

From the observations of Table 2, except for thymoquinone, most of the chemical compounds of oregano essential oils are common in all samples analyzed, even if they are in different percentages. Thymoquinone, together with a large amount of γ-terpinene and a low amount of *p*-cymene and thymol, was present only in sample 24. This, apparently, anomalous sample was collected during post-bloom, and the observed differences could be due to the phenologic phase. In fact, the result could be related with essential oils migration into the glandular hairs.

Two different kinds of glandular hairs are present in oregano: peltate or long-term glandular hairs and capitate or short-term glandular hairs (Bosabadils, 1982, 1984; Werker, 1985, 1993). In peltate trichomes, the secretory materials are gradually secreted in young tissue, accumulate under an epidermic bag, and used by the plant as a protection of its mature organs, while capitate trichomes start and end secretion rapidly with the aim of young organs protection. Hence, the differences in chemical composition of sample 24 as compared to the others might be correlated with the fact that during post-bloom (harvest time for the sample) plant tissue were already senescent. The essential oil con-

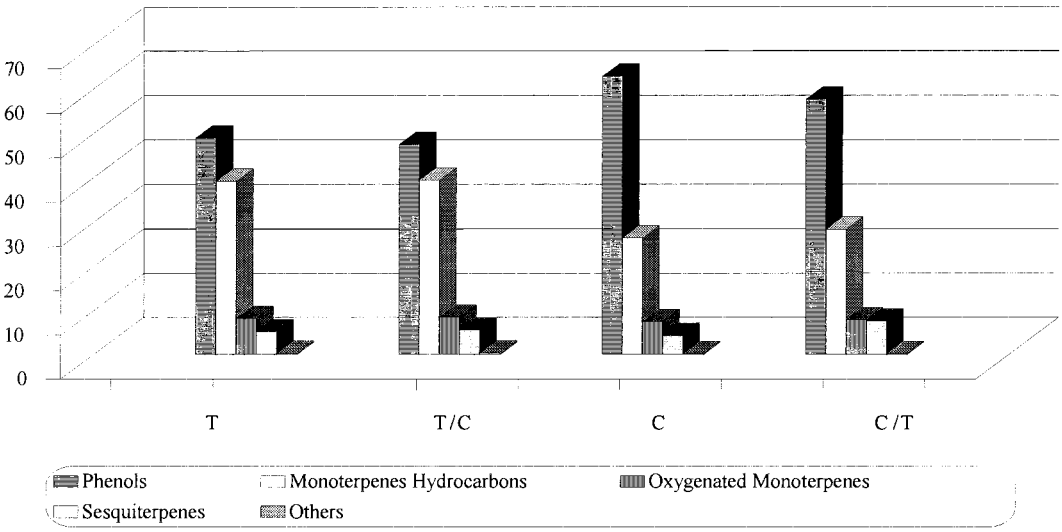

Figure 3. Histograms of the percentage composition of five classes of components of the essential oils of oregano chemotypes: i.e., thymol (T), thymol/carvacrol (T/C), carvacrol (C), and carvacrol/thymol (C/T). Bars from left to right: phenols, monoterpenes hydrocarbons, oxygenated hydrocarbons, sesquiterpenes, and others.

tained in epidermic bags might have undergone chemical modifications with a decomposition of phenols, which are well-known as natural antioxidants. This hypothesis could explain the low content of phenols in sample 24. The increase of oxygenated compounds and the lower content of hydrocarbon compounds can be due to environmental factors (namely, xerofilic and altitude). However a large number of substances are present in oregano essential oils, 80−90% is represented by 10 main substances, namely, thymol, carvacrol, γ-terpinene, *p*-cymene, myrcene, thymyl methyl ether, carvacryl methyl ether, *trans*-β-caryophyllene, and β-bisabolene. It is plain that there are substantial similarities between thymol and thymol/carvacrol chemotypes on one hand and carvacrol and carvacrol/thymol chemotypes on the other.

The histograms in Figure 3 show the different contents of essential oil compounds as subdivided in different chemical classes and chemotypes. Overall the phenols were the main part of the essential oils for all chemotypes even if C and C/T types showed a higher content of these compounds than T and T/C types. While the monoterpene hydrocarbons were important constituents of carvacrol main chemotype (C and C/T) and nearly equaled phenolic content in the thymol main chemotype (T and T/C), no significant difference exists for the sesquiterpenic fraction among the chemotypes.

The chemical composition of oregano essential oils is of great importance because of its biological activity. Essential oils rich in phenolic compounds and oregano essential oils particularly are reported to possess high levels of antimicrobial activity. Thymol has been reported to possess a stronger activity than carvacrol against Gram-negative bacteria even if the antibacterial activity of both phenols was variable between different strains of the same bacterial species. Also synergistic effects were attributed at least in part to other oils components such as 1,8-cineole, linalool, α-terpineol, etc. Carvacrol also has been reported to possess high levels of antifungal activity and antioxidant activity (Sivropoulou et al., 1996).

The previous consideration of high biological acitivity coupled with the putative anticancer potential of the oregano essential oil (Sivropoulou et al., 1996) shows the potential of these aromatic plants in food preservation as well as in the cosmetical and pharmaceutical industry.

LITERATURE CITED

Adams, R. P. *Identification of essential oils by ion trap mass spectroscopy*; Accademic Press: New York, 1989.

Baser, K. H. C.; Ozek, T.; Tumen, G.; Sezik, E. Composition essential oil of Turkish *Origanum* species with commercial importance. *J. Essent. Oil Res.* **1993**, *5*, 619−623.

Baser, K. H. C.; Ozek, T.; Kurkcuoglu, M.; Tumen, G. The essential oil of *Origanum vulgare* subsp. *hirtum* of Turkish origin. *J. Essent. Oil Res.* **1994**, *6*, 31−36.

Battistutta, F.; Conte, L. S.; Zironi, R.; Carruba, A.; Leto, C.; Tuttolomondo, T. Caratteristiche compositive di oli essenziali da diversi ecotipi di *Origanum vulgare* L. di provenienza siciliana. *Atti Congr. Chim. Alimenti* **1995**.

Bosabadils, A. M.; Tsekos, I. Glandular scale development and essential oil secretion in *Origanum dictamnus* L. *Planta* **1982**, *156*, 496−504.

Bosabadils, A. M.; Tsekos, I. Glandular hair formation in *Origanum* species; *Ann. Bot.* **1984**, *53*, 559−563.

Falchi Delitalia, L.; Solinas, V.; Gessa, C. Variazioni stagionali quantitative e qualitative di olio essenziale e dei suoi fenoli in *Thymus capitatus* Hofmgg. Et Lk. Ed in *Thymus herba barona* Loisel. *Fitoterapia* **1983**, *64*, 87−96.

Fleisher, A.; Sneer, N. Oregano species and Origanum chemotypes. *J. Sci. Food Agric.* **1982**, *33*, 441−446.

Granger, R.; Passet, J. Thymus vulgaris spontane de France: races chimiques et chemotaxonomie. *Phytochemistry* **1973**, *12*, 1683−1691.

Ietswaart, J. M. A taxonomic revision of genus *Origanum*. Leiden University Press: Leiden, 1980.

Kokkini, S.; Vokou, D. Carvacrol rich plants in Greece. *Flavour Fragrance J.* **1989**, *4*, 1−7.

Lawrence B. M. The botanical and chemical aspects of Oregano. *Perfum. Flavor.* **1984**, 9, 41−51.

Lawrence, B. M. *Origanum* oil (Greek type). *Perfum. Flavor.* **1989**, *14*, 38−40.

Maarse, H.; Van Os, F. H. L. Volatile oil of *Origanum vulgare* L. ssp. vulgare. II. Quantitative composition of the oil. *Flavour Ind.* **1973a**, *4*, 48−484,.

Maarse, H.; Van Os, F. H. L. Volatile oil of *Origanum vulgare* L. ssp. vulgare. I. Qualitative composition of the oil. *Flavour Ind.* **1973b**, *4*, 477−481.

Maarse, H.; Van Os, F. H. L. Volatile oil of *Origanum vulgare* L. ssp. vulgare. III. Changes in composition during maturation. *Flavour Ind*. **1974**, *4*, 278−281.

Marzi, V.; Morone Fortunato, I.; Circella, G.; Picci, V.; Melegari, M. Origano. *Agric. Ricerca* **1992**, *132*, 71−80.

Melagari, M.; Severi, F.; Bertoldi, M.; Benvenuti, S.; Circetta, G.; Morone Fortunato, I.; Bianchi, A.; Leto, C.; Carubba, A. Chemical characterization of essential oils of some *Origanum vulgare* L. sub-species of various origin. Riv. *Ital. EPPOS* **1995**, *16*, 21−28.

Poulose, A. J.; Croteau, R. Biosynthesis of aromatic monoterpenes. Conversion of γ terpinene to p cimene and thymol in *Thymus vulgaris* L. *Arch. Biochem. Biophys*. **1978**, *187*, 307−314.

Russo, Mt. Caratterizzazione dell'origano presente sul territorio calabrese. Individuazione dei principi attivi utili per l'industria agro-alimentare. Ph.D. Thesis, University of Reggio Calabria, Italy, 1996.

Servizio Idrografico Sez. Genio Civile CATANZARO Ministero dei Lavori Pubblici. Precipitazioni e Temperature, 1995.

Sezik, E.; Tumen, G.; Kirimer, N.; Ozek, T.; Baser, K. H. C. Essential oil composition of four *Origanum vulgare* subspecies of Anatolian origin. *J. Essent. Oil Res*. **1993**, *5*, 425−431.

Sivropoulou, A.; Papanikolaou, E.; Nikolaou, C.; Kokkini, S.; Lanaras, T.; Arsenakis, M. Antimicrobial and cytotoxic activities of *Origanum* essential oil. *J. Agric. Food Chem*. **1996**, *44*, 1202−1205.

Tucker, A. O.; Maciarello, M. J. Oregano: botany and chemistry. In *Spices, Herbs and Edible Fungi*; Charalambous, G., Ed.; Elsevier Science B.V.: Amsterdam, 1994; pp 439−456.

Werker, E. Function of essential oil secreting glandular Hairs in Aromatic plants of Lamiaceae, A Review. *Flavour Fragrance J*. **1993**, *8*, 249−255.

Werker, E.; Putievsky, E.; Ravid, U. The essential oils and glandular hairs in different chemotypes of *Origanum vulgare* L. *Ann. Bot.* **1985a**, *55*, 793−801.

Werker, E.; Ravid, U.; Putievsky, E. Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiate. *Isr. J. Bot.* **1985b**, *34*, 31−45.

Wilkins, M. B. *The physiology of plant and development*; McGraw-Hill: London, 1969.

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