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A chemosystematic investigation on the mono- and sesquiterpenoids in the genus *Origanum* (Labiatae)

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Abstract

We have examined the volatiles of *Origanum* species native to Crete together with two naturally occurring hybrids. The three groups of Ietswaart's classification were all represented. Group A, section *Amaracus* was represented by *Origanum calcaratum* and by *Origanum dictamnus*; group B, section *Chilocalyx* by *Origanum microphyllum*; section *Majorana* by *Origanum onites*; group C, section *Origanum* by *Origanum vulgare* ssp. *hirtum*. Two natural hybrids of *O. vulgare* ssp. *hirtum,* with *O. microphyllum* and *Origanum onites* L., *O.* x *minoanum* and *O.* x *intercedens* were also examined. The main volatiles found were compared with all the existing published analyses for *Origanum* and the taxonomic significance of volatile oil composition was assessed. Our own results and the existing data together show convincingly that most *Origanum* species are rich either in sabinyl compounds or cymyl compounds but never both. We found that the hybrid between sabinyl-rich *O. microphyllum* and cymyl-rich *O. vulgare* ssp. *hirtum* contains mainly cymyl compounds, the sabinyl being suppressed. We suggest that one or more components of the cymyl pathway act throughout the *Origanum* genus, whenever present, to suppress the sabinyl pathway, which however is never completely absent. Generally, volatile composition is in accord with Ietswaart's classification. Twelve of the 13 species in group A whose essential oil composition has so far been examined were found to be cymyl-rich and to lack significant amounts of acyclic compounds or sesquiterpenoids. Group B contains a similar number of cymyl-rich and sabinyl-rich species but is the only group with members rich in sabinyl compounds. Group C is the only group rich in acyclic compounds and/or sesquiterpenoids.

Keywords: Origanum; Labiatae; Oregano; Marjoram; Chemotaxonomy; Terpenoids; GC-MS; Hybrids

1. Introduction

Within the genus *Origanum,* Ietswaart (1980), based on morphological criteria, recognised 3 groups, 10 sections, 38 species, 6 subspecies and 17 hybrids. The members of the genus are mainly distributed along the Mediterranean region while 75% of them are restricted to the East Mediterranean. Eleven species occur in Greece, five of them are found in Crete (Greuter, Burdet & Long, 1986).

Origanum species are characterised by a wide range of volatile compounds, and in the present study we examined the volatiles of all taxa native to the island of Crete, and compare the profiles with all the other species whose volatile composition has been reported, in order to attempt to establish the taxonomic significance of volatile oil composition. We also examined the composition in two naturally occurring hybrids.

The Cretan *Origanum* taxa represent four different sections of the genus (Ietswaart, 1980). Group A, section *Amaracus* Bentham is represented by *Origanum calcaratum* Juss. (Syn. *Amaracus tournefortii* Bentham, *Origanum tournefortii* Aiton) endemic to Crete and the Aegean Islands and by *Origanum dictamnus* L. (Syn. *Amaracus dictamnus* Bentham) endemic to Crete. Group B, section *Chilocalyx* Ietswaart is represented by *Origanum microphyllum* Vogel (Syn. *Majorana microphylla* Bentham), endemic to Crete. Group B also includes section *Majorana* Bentham which is represented by *Origanum onites* L. endemic to Sicily and East Mediterranean region. Group C, section

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Origanum is represented by *Origanum vulgare* L. ssp. *hirtum* Ietswaart endemic to the east Mediterranean region. In addition, two natural hybrids of *O. vulgare* L. ssp. *hirtum* with *O. microphyllum* and *Origanum onites* L., namely *O.* x *minoanum* Davis and *O.* x *intercedens* Rech. are also examined.

The essential oils of some of these taxa have been studied to varying extents, as follows: *O. dictamnus* (Harvala, Menounos & Argyriadou, 1987; Sivropoulou, Papanikolaou, Nikolaou, Kokkini, Lanaras & Arsenakis, 1996); *Origanum onites* L. and *Origanum vulgare* L. ssp. *hirtum* (Arnold, Bellomaria, Valentini, & Arnold, 1993; Baser, Ozek, Kirkcuoglu & Tumen, 1994; Baser, Ozek, Tumen & Sezik, 1993; Kokkini & Vokou, 1989; Ruberto, Biondi, Meli & Piattelli, 1993; Vokou, Kokkini & Bessiere, 1988; Vokou, Kokkini & Bessiere, 1993); *O.* x *intercedens* (Bozabalidis & Skoula, 1998; Kokkini & Vokou, 1993); *O. calcaratum* and *O. microphyllum* (2–4 major compounds) (Karousou, 1995). The present study is the first in which the volatiles of *O. calcaratum, O. microphyllum* and *O.* x *minoanum,* have been studied extensively.

The volatile compounds of all these taxa have been examined by two extraction methods enabling a comparison of organic solvent extraction with headspace extraction. Both of these are considered much less destructive than the most commonly used distillation method which has been suspected for the creation of many artifacts (Coleman & Lawrence, 1997; Fischer, Nitz & Drawert, 1987). Additionally this is the first time that all these taxa have been compared in similar stages of development and by similar methods of analysis; this is important in order to eliminate differences that could arise from different times of collection or methods of analysis.

2. Results and discussion

Seventy five compounds have been identified in the volatile fraction of the five *Origanum* species and hybrids (Table 1). One of two major biochemically related groups of compounds seems to be present in all the *Origanum* examined. The first, the aromatic monoterpenes, are represented in oregano mainly by *p*-cymene, thymol, carvacrol, their precursor, γ-terpinene, and in much lower concentrations, by their derivatives, *p*-cymenene, *p*-cymen-8-ol, *p*-cymen-7-ol, thymoquinone (*p*-cymene-2,5-dione), thymohydroquinone (*p*-cymen-2,5-diol), thymol and carvacrol methyl ethers, and thymol and carvacrol acetates and possibly 4,(1,1)-dimethylethyl-1,2-benzendiol. For convenience, we refer to these compounds collectively as 'cymyl' compounds. The second group, the thujanes, are represented mainly by sabinene and by *cis*- and *trans*-sabinene hydrates, but also include their derivatives *cis*- and *trans*-sabinene hydrate acetates, *trans*-sabinol and sabina ketone, together with α-thujene. These are together referred to as 'sabinyl' compounds. Two more biochemical groups, generally of less significance quantitatively, are present in the genus: that of the acyclic monoterpenoids such as geraniol, linalool and β-myrcene and that of bornane type compounds. To the above, one or more sesquiterpenes, such as β-caryophyllene, β-bisabolene, germacrene-D, should also be added.

Thymoquinone and thymohydroquinone are reported for the first time in *O. dictamnus* in the present study; however thymoquinone has been found in *Origanum syriacum* L. (Syn. *Majorana syriaca*) (Hirobe, Qiao, Takeya & Itokawa, 1998) and thymohydroquinone in *O. compactum* Bentham (Bellakhdar, Passannanti, Paternostro & Piozzi, 1988). In fact, both were also found in minor quantities in some of the other taxa we examined.

The analyses of the leaf headspace of the above taxa revealed similar composition with that of the leaf extracts with some minor differences that are attributed to the method. In the headspace 'more volatile' constituents were present in higher quantities and 'less volatile' in lower quantities. Thus, in all samples *p*-cymene was more in the leaf headspace than in the leaf extract and carvacrol was less in leaf headspace and more in the leaf extract: often sesquiterpenes were less detectable by headspace analysis. The comparison of the leaf headspace with the inflorescence headspace showed that for the most part there were only insignificant quantitative differences. However, in *O. onites, O. vulgare* ssp. *hirtum, O.* x *intercedens* and *O.* x *minoanum*, the cymyl compounds were much more strongly biased towards carvacrol in the inflorescence compared with the leaf.

2.1. Volatile compounds in group A

The section *Amaracus* was represented here by *O. calcaratum* and *O. dictamnus*. The CH_2Cl_2 extract of *O. calcaratum* is characterised by *p*-cymene (45.7%), γ-terpinene (8.6%) and thymol (20.4%), followed by carvacrol (2.4%). The CH_2Cl_2 extract of *O. dictamnus* is characterised by *p*-cymene (26.0%), thymoquinone (22.9%) and carvacrol (6.3%) followed by sabinene (3.6%) and borneol (2.9%). The other members of this section, viz. *O. solymicum* P.H. Davis (Tumen, Ermin, Ozek & Baser, 1994), *O. saccatum* Davis (Tumen, Baser & Ozek, 1995) and *O. boissieri* Ietswaart (Baser & Duman, 1998) are *p*-cymene rich while *O. cordifolium* Vogel (Valentini, Arnold, Bellomaria & Arnold, 1991) is rich in α-terpineol, γ-terpinene, and *p*-cymene.

In section *Anatolicon* Bentham, the essential oil of *O. sipyleum* L., and *O. hypericifolium* O. Schwarz et

P.H.Davis were found rich in cymyl compounds (Baser, Ermin, Kirkcuoglu & Tumen, 1994; Baser, Ozek, Kirkcuoglu & Tumen, 1992). Thus, it seems that in both these sections taxa are characterised by the cymyl monoterpenoids. In section *Brevifilamentum* Ietswaart, *O. acutidens* Ietswaart and *O. bargyli* Mouterde were found rich in carvacrol and *p*-cymene (Baser & Duman, 1998; Baser, Tumen & Duman, 1997); *O. haussknechtii* Boiss. was rich in *p*-cymene and borneol (Baser, Ermin, Kirkcuoglu & Tumen, 1998), *O. leptocladum* Boiss. rich in cymyl compounds and borneol (Baser, Ermin, Ozek, Demircakmak, Tumen & Duman, 1996), whereas *O. rotundifolium* Boiss. contained mainly *cis*-sabinene hydrate, linalyl acetate, α-terpineol, β-caryophyllene, and *trans*-sabinene hydrate (Baser, Ozek & Tumen, 1995). So far, *O. rotundifolium* is the only analysed species within group A that lacks cymyl compounds as major constituents; instead it uniquely contains *cis*-sabinene hydrate as a major compound (Table 2).

2.2. Volatile compounds in group B

Group B was represented by *O. microphyllum* (Section *Chilocalyx*) and *O. onites* (Section *Majorana*). The volatiles of *O. microphyllum* were rich in sabinyl compounds and poor in the cymyl compounds. The CH_2Cl_2 extract of *O. microphyllum* is characterised by *cis*-sabinene hydrate (22.5%), *trans*-sabinene hydrate (26.3%), sabinene (14.2%), linalool (12.2%) followed by borneol (2.9%). Thus, *O. microphyllum* is almost completely dominated by sabinyl compounds and is very poor in the cymyl compounds, while acyclic monoterpenoids and borneol can also be observed. In contrast, the main volatile compounds of *O. onites* L. found in the CH_2Cl_2 extract were carvacrol (43.9%), β-bisabolene (18.9%), and geraniol (8.0%), while *p*-cymene and γ-terpinene were in lower amounts (2.4% and 2.2%, respectively). β-bisabolene content was found much higher than in the essential oil samples of this species reported previously.

Throughout both sections of group B there is compelling evidence of this clear division into sabinyl-rich and carvacrol rich plants with no intermediates observed. Within *Majorana* section, *O. syriacum* L. is either carvacrol or thymol rich (Baser, Ozek, et al., 1993; Beker, Dafni, Eisikowitch & Ravid, 1989; Halim, Mashaly, Zaghloul, Abd-El-Fattah & de Pooter, 1991; Ravid & Putievsky, 1983). Another member of this section, *O. majorana* L., is characteristic for its *cis*-and *trans*-sabinene hydrate content (Baser, Kirimer & Tumen, 1993; Arnold et al., 1993; Fischer et al., 1987; Franz, 1990); however samples collected from Turkey and Cyprus have been found poor in the sabinene derivatives and to contain mainly carvacrol instead (Arnold et al., 1993; Baser, Kirimer, et al., 1993). In the case of *O. onites,* besides the common carvacrol-rich type, an almost pure linalool-type has been recorded in Turkey (Baser, Ozek, et al., 1993). It seems that members in this section vary not only within the section level, but also within the species level; all three biochemical groups, the cymyl, sabinyl and the acyclic monoterpenoids can be distinguished within the *Majorana* section but (at significant concentrations) the sabinyl and the cymyl are mutually exclusive. Section *Chilocalyx* is similar; *O. micranthum* Vogel has very similar composition to the analysed *O. microphyllum* (Baser, Ozek, Kirkcuoglu & Tumen, 1996b). However, the other relatives, *O. minutiflorum* Schwarz & Davis and *O. bilgeri* Davis are rich in carvacrol and poor in sabinene compounds (Baser, Tumen & Duman, 1996; Baser, Tumen & Sezik, 1991).

2.3. Volatile compounds in group C

This group is represented by *O. vulgare* ssp. *hirtum* (section *Origanum,* which comprises only the subspecies of *O. vulgare*). The major volatile compounds found in the CH_2Cl_2 extract were carvacrol (53.2%) and *p*-cymene (14.7%). The volatiles reported in this section are rather variable. Subspecies *hirtum* is most commonly carvacrol-rich and less commonly thymol-rich (Baser, Ozek, et al., 1994; Kokkini & Vokou, 1989); subsp. *gracile* Ietswaart (syn. *O. tytthanthum* Gontsch.) has been found either rich in acyclic compounds and sesquiterpenoids or carvacrol/thymol rich (Baser, Demicakmak, Nuriddinov, Nigmatullaev & Aripov, 1997; Sezik, Tumen, Kirimer, Ozek & Baser, 1993;); subsp. *vulgare* (Kaul, Singh & Sood, 1996; Sezik et al., 1993) and subsp. *virens* Ietswaart (Alves-Pereira & Fernandes-Ferriera, 1998; Sezik et al., 1993) have been found to be rich in acyclic compounds and sesquiterpenoids, while subsp. *viride* Hayek was found to be rich in sabinyl compounds, and the acyclic and sesqui-terpenoids (Afsharypuor, Sajjadi & Efran-Manesh, 1997; Sezik et al., 1993).

Within group C, in the section *Elongatispica* Ietswaart, *O. elongatum* Emberger et Maire, and *O. floribundum* Munby contain mainly carvacrol (Benjilali, Richard & Baritaux, 1986; Houmani and Abed, 1999; Velasco-Negueruela, Perez-Alonso & Burzaco, 1991). *O. laevigatum* Boiss. from the section *Prolaticorolla* Ietswaart was found rich only in sesquiterpenoids (Baser, Ozek, Kirkcuoglu & Tumen, 1996a; Tucker & Marciarello, 1992), while in the same section *O. compactum* Bentham was found to be carvacrol/thymol rich (Benjilali et al., 1986; Van Den Broucke and Lemni, 1980). In the section *Campanulaticalyx* Ietswaart, *O. isthmicum* Danin has been described by smell as carvacrol-rich (Danin and Künne, 1996), whilst *O. ramonense* Danin was shown to contain terpinen-4-ol, α-terpineol and, rather surprisingly, either

Table 1
Qualitative and quantitative (% v/v) composition of the volatiles of *Origanum calcaratum* (Oc), *O. dictamnus* (Od), *O. microphyllum* (Om), *Origanum onites* (Oo). *O. vulgare* ssp. *hirtum* (Ovh), *O.* x *intercedens* (Oxi) and *O.* x *minoanum* (Oxm)

	Compounds	Scan[a]	RT[b]	Oc1[c]	Oc2	Oc3	Od1	Od2	Od3	Om1	Om2	Om3	Oo1	Oo2	Oo3	Ovh1	Ovh2	Ovh3	Oxi1	Oxi2	Oxi3	Oxm1	Oxm2	Oxm3
1	α-thujene	*307*	*8.39*	1.08	3.84	1.95	0.24	3.40	3.77	1.08	2.25	2.48	0.75	6.04	3.02	0.69	1.59	1.48	1.01	3.48	2.04	1.97	4.41	5.25
2	α-pinene	*319*	*8.66*	0.78	1.94	1.05	0.34	2.96	2.61	1.37	2.51	2.96	0.40	2.95	1.15	0.69	0.93	0.77	0.59	1.37	0.81	1.20	2.10	2.12
3	camphene	*340*	*9.37*	0.02	0.23	0.12	1.17	3.50	2.42	1.29	3.39	3.11	0.28	2.16	0.61	0.40	0.43	0.22	0.72	0.83	0.44	0.72	1.08	0.53
4	sabinene	*379*	*10.85*	1.06	0.78	8.45	3.59	7.41	8.68	14.24	14.12	24.23	0.10	0.51	0.16	1.35	0.98	1.45	0.35	0.56	0.25	1.62	2.01	1.11
5	β-pinene	*385*	*10.92*	t[d]	0.34	t	–	0.45	0.46	0.63	1.01	0.69	0.11	0.59	0.25	0.30	0.31	0.34	0.26	0.34	0.19	0.32	0.52	0.46
6	octanone-3	*397*	*11.37*	0.45	0.70	0.64	0.29	0.58	0.46	–	0.65	0.39	–	0.29	0.24	0.16	0.76	0.09	–	0.17	0.08	0.12	0.32	0.05
7	β-myrcene	407	12.01	1.12	3.89	1.56	0.02	1.03	0.99	1.4	1.54	2.79	0.78	6.33	3.06	0.62	1.46	1.50	1.26	3.85	2.37	1.61	4.29	4.69
8	3-octanol	*412*	*12.39*	–	0.02	–	0.03	0.18	0.11	–	0.15	0.04	–	0.01	–	–	0.05	–	–	–	–	–	0.04	–
9	α-phellandrene	*433*	*12.65*	0.02	0.52	0.12	–	0.16	0.18	0.12	0.17	0.19	–	0.74	0.33	–	0.13	0.12	–	0.43	0.26	0.17	0.51	0.59
10	δ-3-carene	*443*	*13.03*	–	0.18	t	–	0.15	0.14	–	0.03	0.02	–	0.26	0.15	–	0.11	0.10	–	0.15	0.11	0.11	0.15	0.18
11	α-terpinene	*455*	*13.48*	0.01	6.33	1.71	0.03	1.28	1.56	0.27	1.16	0.92	0.10	4.35	0.93	0.34	0.67	0.68	0.78	3.37	1.94	1.08	4.33	4.47
12	p-cymene	*471*	*14.04*	45.62	45.32	71.61	26.02	48.15	40.47	0.58	3.92	0.58	2.44	8.88	7.43	14.70	28.72	11.91	5.44	15.35	9.51	23.03	17.22	15.27
13	limonene	*480*	*14.30*	0.58	0.99	–	0.24	1.05	1.19	–	–	–	0.25	1.40	0.72	0.38	0.59	0.42	0.50	1.04	0.58	0.77	1.30	1.48
14	β-phellandrene	*482*	*14.40*	0.05	0.04	0.66	–	0.09	0.08	1.36	1.77	2.22	–	0.04	0.07	–	–	0.02	–	0.04	0.02	–	0.03	0.04
15	*Z*-β-ocimene	*498*	*14.69*	–	–	–	–	–	–	t	0.03	0.06	–	0.04	0.01	0.21	0.14	–	1.24	3.79	1.10	–	0.22	0.05
16	*E*-β-ocimene	*518*	*15.92*	–	0.06	–	–	0.01	0.02	t	0.04	0.07	–	0.29	0.10	–	0.02	0.01	–	0.29	0.16	–	0.18	0.16
17	γ-terpinene	*545*	*16.51*	8.62	26.53	8.48	0.32	2.93	5.16	1.01	2.39	1.79	2.24	18.80	2.43	3.20	5.16	2.67	11.20	26.28	13.75	11.59	28.16	19.52
18	*cis*-sabinene hydrate	*559*	*17.40*	0.87	0.79	1.26	1.17	1.65	2.13	22.45	31.09	23.56	1.11	1.60	2.57	2.73	0.61	1.37	2.49	2.34	2.19	11.79	12.90	19.95
19	α-terpinolene	*606*	*18.73*	0.02	0.09	t	–	0.07	0.09	0.18	0.47	0.43	–	0.43	0.22	–	0.10	0.09	–	0.29	0.18	0.05	0.24	0.30
20	*p*-cymenene	*607*	*18.82*	–	t	0.14	0.41	0.08	0.07	–	0.16	0.04	–	–	–	–	–	–	–	–	–	–	–	–
21	*trans*-sabinene hydrate	*628*	*19.67*	0.33	0.13	0.27	0.51	0.34	0.51	26.34	15.38	12.42	1.27	0.78	1.26	0.42	0.28	0.47	1.48	1.28	0.92	0.85	0.55	0.65
22	linalool	*635*	*19.90*	0.02	0.03	t	0.21	1.10	0.38	12.2	9.37	14.16	–	0.11	0.15	0.51	0.30	0.40	0.43	0.23	0.34	0.58	0.59	0.74
23	*cis-p*-menth-2-en-1-ol	*682*	*21.41*	–	–	–	0.02	0.02	0.05	0.06	0.13	0.04	–	0.12	0.07	–	–	–	–	0.11	–	–	0.09	0.11
24	*trans*-pino carveol	*724*	*22.10*	–	–	–	0.28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
25	*trans*-p-menth-2-en-1-ol	*725*	*22.93*	–	–	–	0.02	0.02	t	0.39	0.3	0.16	–	0.05	0.01	–	–	–	–	0.04	–	–	0.04	0.04
26	*trans*-sabinol	*726*	*23.06*	–	–	–	–	–	–	t	0.11	0.02	–	0.04	–	–	–	–	–	0.01	–	–	–	–
27	camphor	*735*	*23.35*	–	t	t	0.27	0.05	0.04	0.09	0.06	0.03	–	–	–	–	–	–	–	–	–	–	–	–
28	sabina ketone	*764*	*24.22*	0.02	0.02	0.19	–	–	–	0.04	0.06	0.02	–	–	–	–	–	–	–	–	–	–	–	–
29	β-pinene oxide	*769*	*24.48*	–	–	–	–	–	–	0.04	0.06	0.02	–	–	–	–	–	–	–	–-	–	–	–	–
30	borneol	*789*	*24.95*	0.02	0.06	t	2.92	1.55	1.60	2.94	2.91	1.86	1.68	2.97	2.51	0.85	0.51	0.45	2.00	1.26	2.80	1.26	0.92	0.40
31	terpinen-4-ol	*820*	*25.98*	0.31	0.13	0.38	0.43	0.23	0.32	0.44	1.04	0.40	0.24	0.35	0.50	0.32	0.25	0.38	0.33	0.28	0.43	0.48	0.36	0.47
32	*p*-cymen-8-ol	*837*	*26.89*	0.29	0.02	0.12	0.86	0.05	0.12	–	–	–	–	–	–	0.30	0.12	0.02	–	–	–	0.27	0.04	0.03
33	α-terpineol	*852*	*27.23*	0.02	0.04	t	0.02	0.06	–	1.08	0.4	0.36	–	0.14	0.17	–	0.07	0.11	–	0.09	0.14	0.33	0.19	0.24
34	*cis*-piperitol	*864*	*27.57*	–	–	–	0.02	–	0.10	0.02	0.05	0.04	–	0.02	–	–	–	–	–	–	–	–	–	–
35	*cis*-dihydrocarvone	*866*	*27.74*	–	–	–	0.02	0.09	0.12	0.08	0.07	0.10	–	0.04	0.08	–	0.12	0.15	–	–	0.14	–	0.05	0.06
36	*trans*-dihydrocarvone	*885*	*28.32*	–	–	–	0.02	0.05	0.06	0.08	0.03	0.05	–	0.01	–	–	0.09	0.12	–	–	0.07	–	0.03	0.03
37	*trans*-piperitol	*895*	*28.80*	–	–	–	0.02	–	–	0.02	0.02	0.02	–	–	–	–	–	–	–	–	–	–	–	–

38	1-octanol acetate	*908*	*29.31*	–	–	–	–	–	–	–	–	t	–	–	–	0.25	–	–	–	–	–	–	0.03	0.04
39	*cis*-sabinene hydrate acetate	*929*	*29.89*	–	–	–	–	–	–	0.04	0.02	0.04	–	–	–	–	–	–	–	–	–	–	–	–
40	thymolmethylether	*965*	*31.39*	–	–	–	–	–	–	0.07	0.12	0.02	0.16	–	–	0.16	–	–	0.76	0.06	–	0.09	t	–
41	cuminaldehyde	*979*	*31.41*	–	–	–	0.28	t	t	–	–	–	–	–	–	–	–	–	–	–	–	–	0.04	–
42	carvone	*987*	*31.85*	–	t	–	0.03	0.06	0.07	–	0.04	0.06	0.11	0.11	0.22	–	–	–	–	0.01	–	–	–	–
43	carvacrol methyl ether	*989*	*32.06*	–	–	–	–	t	–	0.28	0.19	0.18	–	0.06	0.08	–	–	–	–	0.06	–	–	0.04	0.05
44	thymoquinone	*1004*	*32.46*	1.04	0.27	0.20	22.90	13.00	16.94	t	t	t	0.86	0.38	0.59	3.56	1.74	1.04	1.40	0.66	0.92	1.69	0.31	0.38
45	*trans*-sabinene hydrate acetate	*1012*	*32.80*	–	–	–	–	–	–	0.45	0.02	0.30	0.47	0.04	0.14	–	–	–	0.61	0.17	–	–	–	–
46	geraniol	*1018*	*33.40*	1.49	0.14	–	–	–	t	–	–	0.05	8.06	3.59	4.94	–	–	–	4.14	3.64	1.42	–	–	–
47	isobornyl acetate	*1098*	*35.57*	–	–	–	0.75	t	–	0.15	0.04	0.37	–	–	–	–	–	–	–	–	–	–	0.02	–
48	*p*-cymen-7-ol	*1102*	*36.21*	0.51	0.04	–	–	–	–	–	–	–	–	–	–	0.36	0.13	0.08	–	0.05	–	0.26	–	–
49	thymol	*1110*	*36.70*	20.38	5.26	t	0.26	t	0.18	0.5	0.02	0.02	0.15	0.10	0.24	0.62	0.24	0.28	1.70	0.11	0.22	2.67	1.66	2.21
50	thujyl acetate	*1121*	*36.90*	–	–	–	–	–	–		0.02	0.15	–	–	–	–	0.13	0.02	–	0.02	–	–	–	–
51	carvacrol	*1140*	*37.48*	2.43	0.38	0.27	6.25	2.85	5.57	1.37	0.05	0.04	43.88	29.18	59.13	53.19	51.64	72.04	39.03	23.60	54.28	27.55	13.21	17.10
52	δ-elemene	*1230*	*40.57*	–	–	–	–	–	–	0.09	–	t	–	–	–	–	–	–	–	–	–	–	–	–
53	α-cubebene	*1262*	*41.03*	–	–	–	0.82	0.21	0.22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
54	thymol acetate	*1278*	*41.83*	1.31	0.02	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
55	carvacrol acetate	*1320*	*43.00*	0.02	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–	–
56	α-copaene	*1334*	*43.11*	–	–	–	5.45	1.99	1.10	–	–	0.02	–	0.02	–	–	–	–	–	–	–	–	–	–
57	β-bourbonene	*1353*	*43.75*	–	–	–	0.48	0.07	–	–	–	–	0.65	0.13	–	–	–	–	–	0.19	–	–	–	–
58	β-cubebene	*1368*	*44.46*	–	–	–	2.06	1.15	0.52	–	–	–	–	0.02	–	–	–	–	–	–	–	–	–	–
59	β-elemene	*1373*	*44.55*	–	–	–	–	–	–	–	–	0.02	–	0.03	–	–	–	–	–	–	–	–	–	–
60	β-gurjunene	*1419*	*45.80*	–	–	–	–	–	–	0.13	0.05	0.10	–	–	–	–	–	–	–	–	–	–	0.02	0.03
61	β-caryophyllene	*1440*	*46.54*	1.59	0.66	0.18	0.92	0.43	0.20	3.23	1.44	1.77	2.82	0.92	0.59	0.81	0.13	0.24	1.28	0.47	0.35	0.62	0.32	0.43
62	*trans*-bergamotene	*1489*	*48.44*	–	–	–	–	–	–	t	t	t	0.46	0.08	0.17	0.12	0.02	–	0.11	0.05	t	0.14	0.03	t
63	4,(1,1)-dimethylethyl-1,2-benzendiol	*1502*	*49.20*	5.86	–	–	1.19	–	–	–	–	–	–	–	–	5.15	–	–	–	–	–	1.93	–	–
64	α-humulene	*1525*	*49.44*	–	–	–	–	–	–	0.15	0.05	0.07	0.18	0.04	–	–	–	–	–	–	–	–	–	–
65	*allo*-aromadendrene	*1545*	*49.96*	–	–	–	–	–	–	0.11	0.06	0.05	–	–	–	–	–	–	–	–	–	–	–	–
66	germacrene-D	*1593*	*51.81*	–	t	t	3.16	0.58	0.31	0.03	–	0.02	6.59	1.15	1.12	–	0.12	0.14	2.96	0.94	0.39	–	0.10	0.06
67	*cis*-β-guaiene	*1620*	*52.45*	–	–	–	–	–	–	0.08	0.04	0.05	–	0.04	–	–	–	–	–	–	–	–	–	–
68	bicyclogermacrene	*1633*	*53.14*	–	–	–	–	–	–	1.13	0.12	0.46	0.58	0.14	0.08	–	–	–	–	0.08	–	0.25	0.12	0.09
69	α-muurolene	*1642*	*53.43*	–	–	–	–	–	–	0.09	0.02	0.02	–	–	–	–	–	–	–	–	–	–	–	–
70	β-bisabolene	*1667*	*54.55*	0.76	0.07	–	–	–	–	–	t	0.02	18.91	2.46	3.17	1.52	0.26	0.25	6.84	1.94	0.66	2.27	0.81	0.33
71	δ-cadinene	*1700*	*55.55*	–	–	–	1.75	0.19	–	–	t	0.02	–	0.01	–	–	–	–	–	–	–	–	–	–
72	thymohydroquinone	*1772*	*58.75*	–	–	–	1.42	–	–	–	–	–	–	–	–	1.71	–	–	2.17	–	–	1.42	–	–
73	D-germacren-4-ol	*1823*	*59.15*	–	–	–	–	–	–	0.82	–	–	–	–	–	–	–	–	–	–	–	–	–	–
74	caryophyllene-oxide	*1840*	*59.56*	–	–	–	–	–	–	0.34	–	–	–	–	–	–	–	–	–	–	–	–	–	–
75	β-thujopsan-2-ol	*1845*	*59.87*	1.32	–	–	1.45	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.32	–	–

[a] Scan No. of the identified compounds by GC-MS.
[b] Retention times referring to the analysis of the quantitative composition by GC.
[c] 1: CH_2Cl_2 leaf extract; 2: leaf headspace; 3: flower headspace.
[d] t: trace, $<0.01\%$.

Table 2
Distribution of cymyl-rich, sabinyl-rich, acyclic-rich and sesquiterpene-rich species within their infrageneric categories of Ietswaart's classification

Group	Section	Taxon	Cymyl-rich	Sabinyl-rich	Acyclic-rich	Sesquiterpene-rich
A	*Amaracus*	***O. calcaratum***	+			
		O. dictamus	+			
		O. solymicum	+			
		O. saccatum	+			
		O. boissieri	+			
		O. cordifolium	+			
	Anatolicon	*O. sipyleum*	+			
		O. hypericifolium	+			
	Brevifilamentum	*O. acutidens*	+			
		O. bargyli	+			
		O. haussknechtii	+			
		O. leptocladum	+			
		O. rotundifolium		+		
B	*Chilocalyx*	***O. microphyllum***		+		
		O. micranthum		+		
		O. minutiflorum	+			
		O. bilgeri	+			
	Majorana	***O. onites***	+			
		O. syriacum	+			
		O.majorana I		+		
		O. majorana II	+			
C	*Campanulaticalyx*	*O. ramonense*		(+)		
		O. isthmicum	+			
	Elongatispica	*O. floribundum*	+			
		O. elongatum	+			
	Origanum	***O. vulgare*** ssp. ***hirtum***	+			
		O. vulgare ssp. *gracile I*	+			
		O. vulgare ssp. *gracile II*			+	+
		O. vulgare ssp. *vulgare*			+	+
		O. vulgare ssp. *virens*			+	+
		O. vulgare ssp. *viride*		+	+	+
	Prolaticorolla	*O. compactum*	+			
		O. laevigatum				+

sabinene hydrates or 1,8-cineole and α- and γ-terpinene, depending on the method of analysis (Danin, Ravid, Umano & Shibamoto, 1997). Thus, with the exception of subsp. *viride,* sabinene compounds are either absent or their presence is uncertain in any unambiguously identified group C taxa (Table 2). However, there are also reports of sabinyl-rich plants of *Origanum vulgare* in which the subspecies is unidentified (Chalchat and Pasquier, 1998; Lawrence, 1980). It does seem that group C is unique in containing plants with only minor amounts of both cymyl and sabinyl compounds. The taxonomic difficulties with subspecies of *O. vulgare* seem to extend to the volatiles too.

2.4. Volatile compounds in the hybrids

The composition of the volatile fraction of *O.* x *intercedens* is intermediate to its parental species, *O. vulgare* ssp. *hirtum* and *O. onites*. The major compounds were carvacrol (39.0%), γ-terpinene (11.2%), β-bisabolene (6.8%), *p*-cymene (5.4%) and geraniol (4.1%); the carvacrol and *p*-cymene being characteristic of *O. vulgare* ssp. *hirtum,* and β-bisabolene and geraniol typical for *O. onites*. All contain only low levels of the sabinyl compounds. While the content of some of the major compounds such as γ-terpinene and carvacrol do not appear to be intermediate in this hybrid but are either higher or lower than the parental species, Fig. 1 shows that cumulatively, the content of sabinyl, cymyl, acyclic and sesquiterpenoid compounds is always intermediate in the hybrid, if compared with the two parental species.

Origanum x *minoanum,* the hybrid between *O. vulgare* ssp. *hirtum* and *O. microphyllum*, has an interesting and unexpected composition of its volatiles (Fig. 2). The composition of the volatiles of the two parental species differ extremely, the former dominated by the cymyl compounds (accounting for 77%), the latter by the sabinyl compounds (totalling 64%). In this

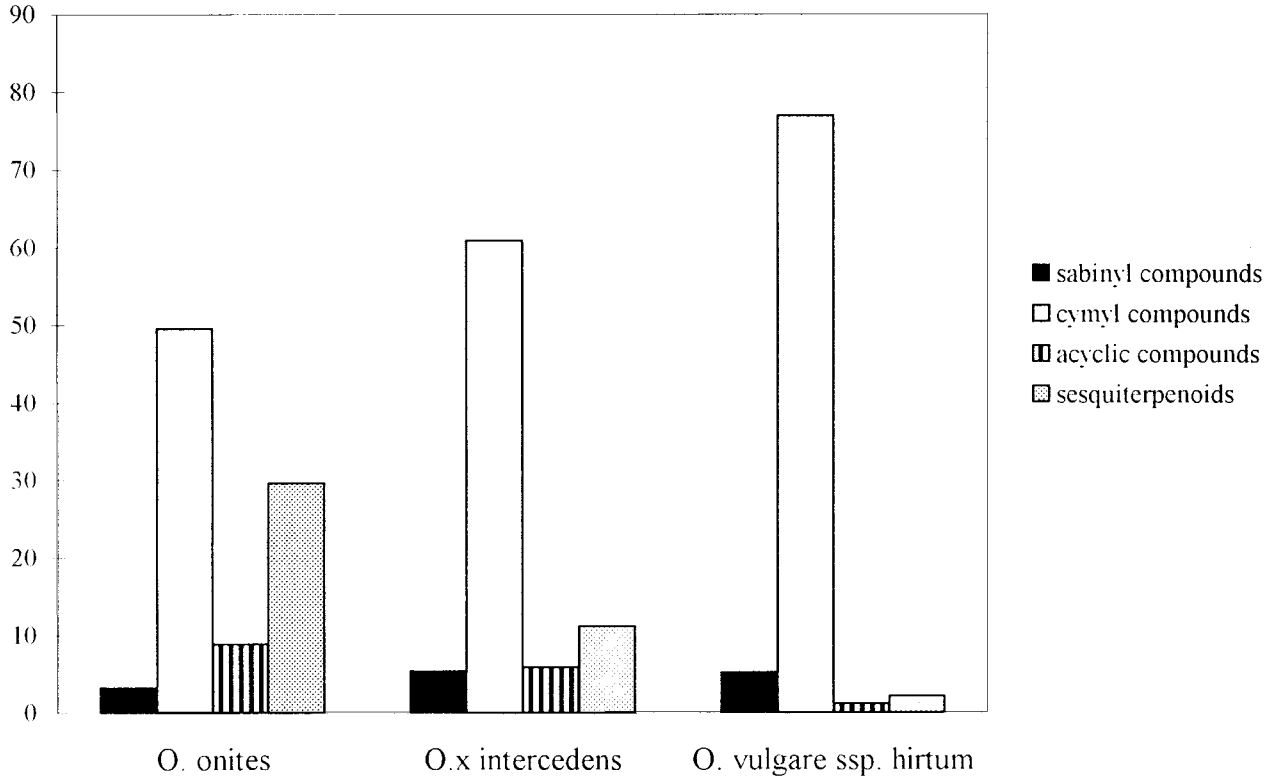

Fig. 1. Cumulative quantitative presentation of the major biochemical groups of volatile compounds found in the parental species *Origanum onites*, *O. vulgare* ssp. *hirtum* and their hybrid.

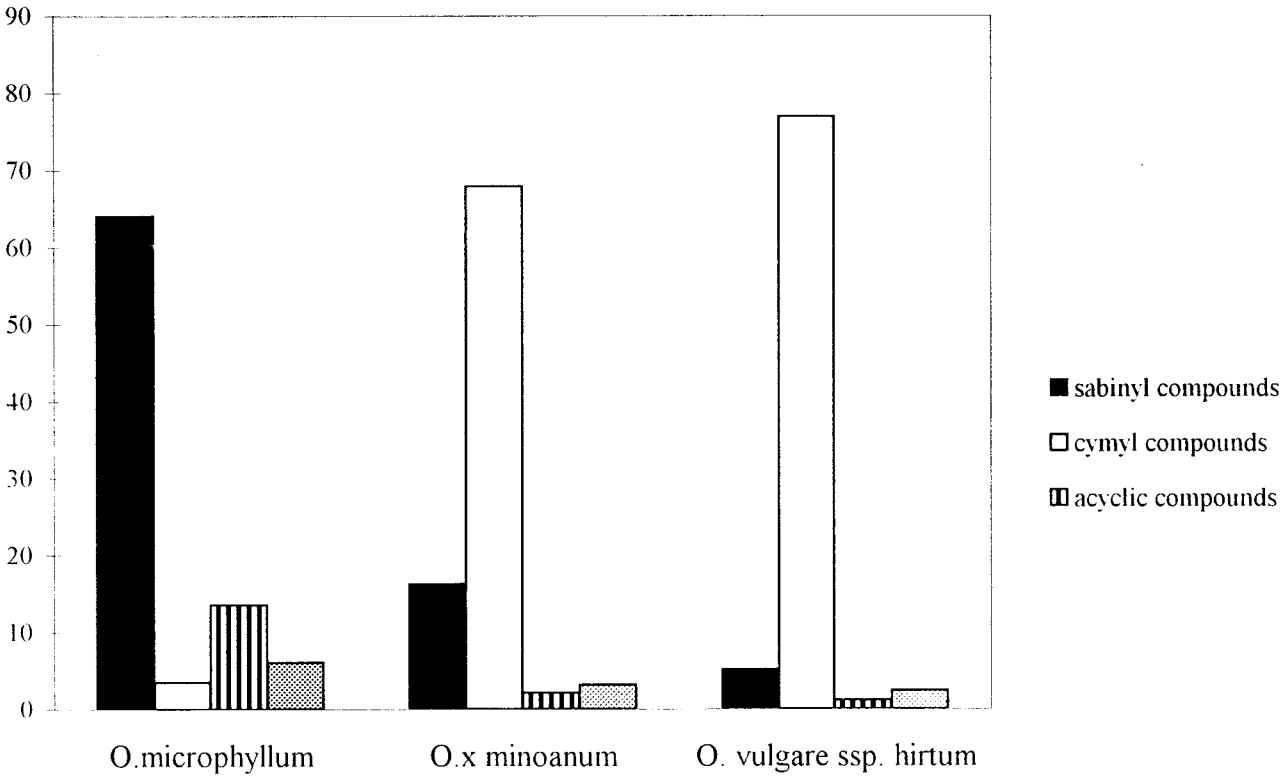

Fig. 2. Cumulative quantitative presentation of the major biochemical groups of volatile compounds found in the parental species *Origanum microphyllum*, *O. vulgare* ssp. *hirtum* and their hybrid.

hybrid the cymyl compounds were still predominant (68%) with a slightly different composition: carvacrol content intermediate to the parental species (27.6%) but *p*-cymene and γ-terpinene contents much higher than in both parental species (23.0% and 11.6%, respectively). The sabinyl compounds were much suppressed compared with *O. microphyllum*: sabinene and *trans*-sabinene hydrate were reduced to one tenth of the level in *O. microphyllum* or less with *cis*-sabinene hydrate suppressed rather less (11.8% c/w 22.5%). This surprising finding suggests that the presence of the cymyl pathway exerts a strong suppressive effect on the sabinyl pathways. The phenomenon bears obvious similarities with another concerning terpenoid biosynthesis, first reported in 1970 (Lincoln, Murray & Lawrence, 1986; Murray and Lincoln, 1970). These authors described in *Mentha citrata* a dominant gene *I*, epistatic to genes involved in the synthesis of monoterpenoids other than linalool. They propose that the gene product acts by utilizing the precursor to all the other monoterpenoids normally present in related species, consequently preventing synthesis of significant amounts of these compounds. Indeed, a similar gene could possibly play a role in *Origanum* where its action would explain the high linalool type of *O. onites* from Turkey discussed above.

The suppression of the sabinene pathways by one or more components of the cymene pathway offers a simple explanation of the otherwise remarkable observation that taxa of *Origanum* appear able to be either sabinyl-rich or cymyl-rich, but never intermediate, a generalization which appears to be true with regard to all the *Origanum* species whose volatiles have so far been examined (Table 2). The presence of low levels of sabinyl compounds in most of the species examined suggests that this pathway is suppressed rather than absent in *Origanum*. The role of dominant suppressive genes in determining essential oil chemotypes may thus be a more widespread one than has hitherto been presumed.

The distribution of terpenoids also proves to be remarkably in accord with Ietswaart's taxonomic revision of the genus *Origanum*. Thus, with the exception of *O. rotundifolium*, all the 13 species in group A whose essential oil composition has so far been examined have been found to be cymyl-rich and to lack significant amounts of acyclic compounds or sesquiterpenoids. Group C (11 species examined) is distinguished by being the only group rich in acyclic compounds and/or sesquiterpenoids. Most of the species lack sabinyl compounds, but these compounds are not altogether absent from the section. Group B contains a similar number of cymene rich and sabinyl-rich species but is the only group with members rich in the sabinene compounds.

3. Experimental

Plant material was collected in August 1997, whilst flowering. *O. calcaratum* was collected from the area of Roussa Ekklisia, ca. 400 m, north-east Crete; *O. dictamnus* from the gorge of Tripiti ca. 100 m, south-west Crete; *O. microphyllum* from the mountain area of Sfakia, ca. 1000 m, south-west Crete; *O. onites* from Kavousi, ca. 50 m, north-east Crete; O. *vulgare* ssp. *hirtum* from the area of Lakki, ca. 500 m, north-west Crete; *O.* x *minoanum* from the area of Kallergi, ca. 1400 m, north-west Crete and *O.* x *intercedens* from the area of Episkopi, ca. 200 m, north-west Crete. *O.* x *minoanum* was found within a dense *O. microphyllum* population with sparse individuals of O. *vulgare* ssp. *hirtum* and *O.* x *intercedens* was found within a dense population of *O. onites* with sparse individuals of O. *vulgare* ssp. *hirtum*. Herbarium specimens of all the above samples are kept in the Herbarium of MAICh, while clone plants are established in the field gene bank of MAICh.

1 g of dry leaves were extracted with 10 ml of CH_2Cl_2 for 10 min in an ultrasonic bath. Extracts were filtered and then condensed over liquid N_2 to 2 ml. Qualitative composition was carried out using a HP-GC 5890 II coupled with an MS VG TRIO 2000, using a HP-5MS column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness; injector temp. was 230°C, oven temp. was programmed 60–230°C at 3°C min^{-1} and isothermal 20 min, according to Adams (1995). 1 μl of extract was injected, carrier gas was He at 1 ml min^{-1} flow rate and split ratio 1:20. Mass spectra were taken at 70 eV, with 1 scan s^{-1} from 35 until 320 m/z. Components were identified by comparing retention indices and spectral data from the Adams and Wiley electronic libraries (Adams, 1995; Wiley Library, 1989). Quantitative composition was carried out by a HP-GC, 5890 II, equipped with a FID; column was the same as above, injector and detector were set at 230 and 250°C, respectively, carrier gas was He at a flow rate of 1.8 ml min^{-1}, calculated at 45°C, with a constant pressure programme at 127 kpa; split ratio was 1:30 and oven temp. was programmed at 45–150°C at 1.5°C min^{-1}, then to 220°C at 40°C min^{-1} and then isothermal for 3 min. This slower oven programme was chosen for better peak resolution.

The same GC, with FID equipped with an HP-7694 Headspace sampler was used for the headspace analysis; 0.1–0.2 g dry plant material (leaves and flowers separately), depending on the volatile richness of the plant material, were put in a 20 ml vial, and remained in the oven for 30 min at 90°C; then it was extracted with carrier gas, kept in the 1 ml loop at 100°C for 1 min, and finally was transferred to the GC with a transfer line at 100°C. GC conditions were the same as described above on the quantitative composition.

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References

Adams, R. (1995). *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, IL: Allured Publishing.

Afsharypuor, S., Sajjadi, E. S., & Efran-Manesh, M. (1997). *Planta Medica*, *63*, 179.

Alves-Pereira, I. M., & Fernandes-Ferriera, M. (1998). *Phytochemistry*, *48*, 795.

Arnold, N., Bellomaria, B., Valentini, G., & Arnold, H. J. (1993). *Journal of Essential Oil Research*, *5*, 71.

Baser, K. H. C., & Duman, H. (1998). *Journal of Essential Oil Research*, *10*, 71.

Baser, K. H. C., Tumen, G., & Sezik, E. (1991). *Journal of Essential Oil Research*, *3*, 445.

Baser, K. H. C., Ozek, T., Kirkcuoglu, M., & Tumen, G. (1992). *Journal of Essential Oil Research*, *4*, 139.

Baser, K. H. C., Kirimer, N., & Tumen, G. (1993). *Journal of Essential Oil Research*, *5*, 577.

Baser, K. H. C., Ozek, T., Tumen, G., & Sezik, E. (1993). *Journal of Essential Oil Research*, *5*, 619.

Baser, K. H. C., Ermin, N., Kirkcuoglu, M., & Tumen, G. (1994). *Journal of Essential Oil Research*, *6*, 631.

Baser, K. H. C., Ozek, T., Kirkcuoglu, M., & Tumen, G. (1994). *Journal of Essential Oil Research*, *6*, 31.

Baser, K. H. C., Ozek, T., & Tumen, G. (1995). *Journal of Essential Oil Research*, *7*, 95.

Baser, K. H. C., Ermin, N., Ozek, T., Demircakmak, B., Tumen, G., & Duman, H. (1996). *Journal of Essential Oil Research*, *8*, 675.

Baser, K. H. C., Ozek, T., Kirkcuoglu, M., & Tumen, G. (1996a). *Journal of Essential Oil Research*, *8*, 203.

Baser, K. H. C., Ozek, T., Kirkcuoglu, M., & Tumen, G. (1996b). *Journal of Essential Oil Research*, *8*, 185.

Baser, K. H. C., Tumen, G., & Duman, H. (1996). *Journal of Essential Oil Research*, *8*, 217.

Baser, K. H. C., Demircakmak, B., Nuriddinov, K. R., Nigmatullaev, A. M., & Aripov, K. N. (1997). *Journal of Essential Oil Research*, *9*, 611.

Baser, K. H. C., Tumen, G., & Duman, H. (1997). *Journal of Essential Oil Research*, *9*, 91.

Baser, K. H. C., Ermin, N., Kirkcuoglu, M., & Tumen, G. (1998). *Journal of Essential Oil Research*, *10*, 227.

Beker, R., Dafni, A., Eisikowitch, D., & Ravid, U. (1989). *Oecologia*, *79*, 446.

Bellakhdar, J., Passannanti, S., Paternostro, M. P., & Piozzi, F. (1988). *Planta Medica*, *1*, 94.

Benjilali, B., Richard, H. M. J., & Baritaux, O. (1986). *Lebensmittel Wissenschaft and Technologie*, *19*, 22.

Bozabalidis, A., & Skoula, M. (1998). *Journal of Essential Oil Research*, *10*, 277.

Chalchat, J. C., & Pasquier, B. (1998). *Journal of Essential Oil Research*, *10*, 119.

Coleman III, W. M., & Lawrence, B. M. (1997). *Flavour and Fragrance Journal*, *12*, 1.

Danin, A., & Künne, I. (1996). *Willdenowia*, *25*, 601.

Danin, A., Ravid, U., Umano, K., & Shibamoto, T. (1997). *Journal of Essential Oil Research*, *9*, 411.

Fischer, N., Nitz, S., & Drawert, F. (1987). *Flavour and Fragrance Journal*, *2*, 55.

Franz, C. (1990). *Herba-Hungarica*, *29*, 79.

Greuter, W., Burdet, H. M., & Long, G. (1986). *Med-Checklist*. In *Editions de Conservatoire de Jardin Botaniques de la Ville de Geneve, (Vol. 3)*.

Halim, A. F., Mashaly, M. M., Zaghloul, A. M., Abd-El-Fattah, H., & de Pooter, H. L. (1991). *International Journal of Pharmacognosy*, *29*, 183.

Harvala, C., Menounos, P., & Argyriadou, N. (1987). *Planta Medica*, *53*, 107.

Hirobe, C., Qiao, Z. S., Takeya, K., & Itokawa, H. (1998). *Natural Medicines*, *52*, 74.

Houmani, Z., & Abed, L. (1999). In V. H. Heywood, & M. Skoula, *Proceedings of the 3rd Mediterranean workshop on wild food and non-food plants: from information to practice: case studies, Cahier Options Meditérranéennes*. CIHEAM/MAICh (in press).

Ietswaart, J. H. (1980). *A taxonomic revision of the genus Origanum* (Labiatae). In *Leiden Botanical Series, (Vol. 4)*. The Hague: Leiden University Press.

Karousou, R. (1995). Taxonomic studies on the Cretan *Labiatae*: distribution, morphology and essential oils. Ph.D. thesis, University of Thessaloniki, Thessaloniki (in Greek, English summary).

Kaul, V. K., Singh, B., & Sood, R. P. (1996). *Journal of Essential Oil Research*, *8*, 101.

Kokkini, S., & Vokou, D. (1989). *Flavour and Fragrance Journal*, *4*, 1.

Kokkini, S., & Vokou, D. (1993). *Biochemical Systematics and Ecology*, *21*, 397.

Lawrence, B. M. (1980). In *Essential Oils 1979–1980* (pp. 79–80). Carol Stream, IL: Allured Publishing.

Lincoln, D. E., Murray, M. J., & Lawrence, B. M. (1986). *Phytochemistry*, *25*, 1857.

Murray, M. J., & Lincoln, D. E. (1970). *Genetics*, *65*, 457.

Ravid, U., & Putievsky, E. (1983). *Planta Medica*, *49*, 248.

Ruberto, G., Biondi, D., Meli, R., & Piattelli, M. (1993). *Flavour and Fragrance Journal*, *8*, 197.

Sezik, E., Tumen, G., Kirimer, N., Ozek, T., & Baser, K. H. C. (1993). *Journal of Essential Oil Research*, *5*, 425.

Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T., & Arsenakis, M. (1996). *Journal of Agricultural and Food Chemistry*, *44*, 1202.

Tucker, A. O., & Marciarello, M. J. (1992). *Journal of Essential Oil Research*, *4*, 419.

Tumen, G., Ermin, N., Ozek, T., & Baser, K. H. C. (1994). *Journal of Essential Oil Research*, *6*, 503.

Tumen, G., Baser, K. H. C., & Ozek, T. (1995). *Journal of Essential Oil Research*, *7*, 175.

Valentini, G., Arnold, N., Bellomaria, B., & Arnold, H. J. (1991). *Journal of Ethnopharmacology*, *35*, 115.

Van Den Broucke, C. O., & Lemni, J. A. (1980). *Planta Medica*, *38*, 264.

Velasco-Negueruela, A., Perez-Alonso, M., & Burzaco, A. (1991). *Anales de Bromatologia*, *43*, 395.

Vokou, D., Kokkini, S., & Bessiere, J. M. (1988). *Economic Botany*, *42*, 407.

Vokou, D., Kokkini, S., & Bessiere, J. M. (1993). *Biochemical Systematics and Ecology*, *21*, 287.

Wiley Library (1989). John Wiley and Sons.