

Analysis of the Volatile Components of *Onosma echioides* (L.) L. var. *columnnae* Lacaita Growing in Central Italy

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Abstract

Volatile components obtained by hydrodistillation from the aerial parts (leaves and flowers) of *Onosma echioides* (L.) L. var. *columnnae* Lacaita (Boraginaceae family) growing wild in central Italy (Marche, Umbria and Abruzzo regions) were investigated by GC and GC/MS for the first time. Sixty-four volatile components were identified: hexadecanoic acid (3.2–33.8%) and phytol (7.3–15.2%) were predominant in the flower oils, while phytol (36.5–54.5%) and hexahydrofarnesyl acetone (7.1–12.1%) were the major components in the leaf oils. Alkanes, fatty acids and aldehydes constituted the major fraction in the flower oils, while oxygenated diterpenes and ketones were predominant in the leaf oils. Phytol was isolated by flash chromatography and its identity was confirmed by IR, ¹H-NMR and ¹³C-NMR spectra. Variation of the volatile components in the six populations of *O. echioides* var. *columnnae*, due to genetic and geographic factors, was analyzed by cluster analysis.

Key Word Index

Onosma echioides var. *columnnae*, Boraginaceae, essential oil composition, phytol, hexadecanoic acid, hexahydrofarnesyl acetone.

Introduction

Genus *Onosma*, belonging to Boraginaceae family, comprises 150 species occurring in dry, cliffy and sunny habitats, and is distributed mainly in Eurasia and Mediterranean area, having its center of distribution and maximum concentration of species in Iran (1). It is represented by biennial or perennial, hispid herbs, with flowers in terminal cymes, calyx accrescent, stamens inserted at the middle of the corolla, four nutlets flat at the base, and an indumentum composed of specific trichomes, called stellate setae (2). About nine species of this genus occur in Italy (3). *Onosma echioides* (L.) L. (tribe Lithospermeae DC) is an endemic plant of Italy, distributed from Sicily to the southern border of the eastern Alps, on xeric and rocky habitats, and its area is prolonged also to Dalmatia (4,5). According to Jávorka (6), *O. echioides* belongs to the section *Asterotricha* Boiss., characterized by several stellate setae on the leaves and stems. From a taxonomical point of view, it

is a critical taxon owing to its morphological polymorphism, which in the past led some authors to consider local races as independent taxa (7,8). Nowadays, according to previous investigations (4,5), some botanists believe that the *O. echioides* group occurs exclusively in Italy with five vicarious taxa (9). Therefore, some Asian *Onosma* taxa which were erroneously called “*echioides*” in the past (10,11), are to be considered taxonomically different from the species growing in Italy. In particular the variety *columnnae* Lacaita, investigated in this work, occurs in central Italy.

During the last years the majority of scientific works concerning *Onosma* species focused mainly on chemistry and biology of naphthoquinone derivatives (12–15) and on pyrrolizidine alkaloids (16–18). On the other hand, only one study concerning volatile constituents of genus *Onosma* and related to *O. microcarpum* from Iran has been previously published (19). Thus, the authors decided to investigate the volatile constituents of *O. echioides* for the first time.

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Experimental

Plant material and isolation of volatile components:

Six populations of *O. echioides* var. *columnnae* were collected during flowering period in May 2006 from three regions of central Italy (Marche, Umbria and Abruzzo; see Table I). Voucher specimens were identified by Dr. F. Maggi and deposited in the Herbarium Camerinensis, Dept. of Environmental Sciences, Sect. of Botany and Ecology, University of Camerino, Italy, under the following accession codes: CAME 7988, CAME 7989, CAME 7992, CAME 7993, CAME 7996 and CAME 7997; they are also available at the following website: <http://erbariitaliani.unipg.it>. Air-dried and powdered leaves and flowers (100 g and 50 g, respectively) from each population were subjected separately to hydrodistillation for 5 h, using a Clevenger-type apparatus with hexane (10 mL) as collector solvent, yielding 0.01–0.03% of a yellowish oil. After evaporation of the solvent under a N₂ flow, the oil was dried over anhydrous sodium sulphate and stored in sealed vials at -20°C before GC and GC/MS analyses. Six oil samples (three from flowers and three from leaves) for each population were obtained by hydrodistillation and subsequently analyzed by GC and GC/MS. The percentage values of volatile components were the mean of three determinations.

Gas chromatography: GC analysis of the volatile components was carried out using an Agilent 4890D instrument with FID detector and a HP-5 column (25 m × 0.32 mm, 0.17 μm film thickness), working with the following temperature program: 5 min at 60°C, subsequently at 4°C/min up to 220°C, then 11°C/min up to 280°C, and held for 15 min; injector and detector temperatures, 280°C; carrier gas, He (1.4 mL/min); injection volume of 1 μL, split ratio, 1:34.

Gas chromatography/mass spectrometry: GC/MS analysis was performed using an Agilent 6890N-5973N GC/MS system operating in the EI mode at 70 eV, using a HP-5MS (30 m × 0.25 mm, 0.1 μm film thickness) capillary column which was programmed at 40°C for 5 min, then ramped at 4°C/min to 220°C, then 11°C/min up to 280°C, held for 15 min, finally 11°C/min up to 300°C, and held for 5 min; carrier gas: He; flow rate: 1.0 mL/min; injector and transfer line temperatures: 280°C; injection volume: 2 μL; split ratio: 1:50; scan time: 75 min; acquisition mass range: 29–400.

Identification of the components: The identification of the components of the oils was based on GC retention times, retention indices relative to n-alkanes and computer matching with the WILEY275 and NIST02 libraries, as well as by comparison of the mass spectra with those reported in the

literature (20–22). Relative area percentages of the volatile constituents were obtained electronically from the GC-FID response without any correction factor.

Further, the main volatile component of the leaf oil was isolated by flash chromatography column on Silica gel 60F Baker; 150 mg of the oil was eluted with hexane-ethyl acetate 90:10 to obtain 40 fractions of 30 mL. Separation was monitored by TLC and GC. Fractions 23–30 gave phytol (26.4 mg). The IR spectrum of phytol was recorded on a Perkin Elmer Spectrum 1FT-IR system using the Spectrum v 5.3.1 software that confirmed the presence of hydroxylic group and double bond. In addition, its structure was confirmed by ¹H-NMR and ¹³C-NMR spectra, which were performed on a Varian Gemini 200 Spectrometer at 200 MHz and 50 MHz, respectively, using TMS as internal standard and CDCl₃ as solvent. The NMR data (see below) are in agreement with those reported in literature (23).

3,7,11,15-Tetramethylhexadec-2-en-1-ol (phytol), yellowish oil (26.4 mg), EIMS, *m/z* (rel. int. %) [M⁺] 296 C₂₀H₄₀O; 71 (999), 123 (394), 43 (316), 81 (304), 57 (293), 55 (274), 69 (264), 41 (246), 95 (211), 68 (194). IR: ν = 3368 (OH) cm⁻¹, ν = 1669 (C=C) cm⁻¹, ν = 1002 (C-O) cm⁻¹. ¹H-NMR (CDCl₃): δ 5.4 (m, 1H, H₂), 4.16 (d, 2H, J = 7.14, H₁), 1.9 (t, 2H, J = 7.55, H₄), 1.76 (br s, 1H, OH), 1.7 (s, 1H, H₁₇), 1.0–1.6 (br m, 19H, H₅₋₁₅), 0.8 (m, 12H, H_{16, 18, 20}). ¹³C-NMR: δ 140.24 (C₃), 123.43 (C₂), 59.54 (C₁), 40.05 (C₄), 39.56 (C₁₄), 37.61 (C₁₀), 37.55 (C₈), 37.47 (C₁₂), 36.85 (C₆), 32.97 (C₁₁), 32.87 (C₇), 28.14 (C₁₅), 25.33 (C₅), 24.96 (C₁₃), 24.64 (C₉), 22.87 (C₁₇), 22.79 (C₁₆), 19.92 (C₁₉), 19.89 (C₁₈), 16.34 (C₂₀).

Statistical analysis: The normalized percentage of the compounds, as recorded in each individual sample, was submitted to numerical cluster analysis (24) (centroid clustering of mean squared Euclidean distances) using the SPSS 13.0 software package. Figures 1 and 2 show the dendrograms that were derived. The percentages of the compounds were normalized using the followed formula: $(x_i/x_{j_{\max}})$ where x_i is the *i*-th percentage for *i*-th compound and $x_{j_{\max}}$ is the maximum percent value from the *i*-th compound.

Results and Discussion

Sixty-four volatile components were identified (55 in the flower, 49 in leaf oils, respectively) in the six populations of *O. echioides* var. *columnnae*, representing 90.18–95.76% of the total composition of the volatile fraction (Tables II and III). The most abundant components were hexadecanoic acid (3.19–33.82%) and phytol (7.33–15.19%) in the flower

Table I. Collection localities of the investigated populations of *Onosma echioides*

<i>O. echioides</i> population	Voucher specimen	Locality	Elevation (m)	GPS coordinates
1	CAME 7993	Genga (Marche)	320	N 43°26'56.0" EO 12°56'35.2"
2	CAME 7992	Esanatoglia (Marche)	494	N 43°19'57.8" EO 12°56'08.8"
3	CAME 7996	Camerino (Marche)	516	N 43°07'53.6" EO 13°07'39.4"
4	CAME 7988	Cerreto di Spoleto (Umbria)	516	N 42°49'226" EO 12°55'31.5"
5	CAME 7989	Norcia (Umbria)	647	N 42°46'13.1" EO 13°06'12.2"
6	CAME 7997	Ofena (Abruzzo)	410	N 42°17'52.3" EO 13°45'34.2"

O. echioides

Table II. Volatile constituents (%) in flowers of six populations of *O. echioides* growing in central Italy. The values are the mean of three determinations for each population, with standard deviation (RSD).

Components	RI ^a	Populations						Method of identification
		1	2	3	4	5	6	
hexanal	802	0.10±0.01	0.31±0.01	tr	0.11±0.01	tr	0.21±0.01	1,2
isopropyl butyrate	850	0.34±0.01	1.61±0.01	0.31±0.01	2.64±0.01	0.97±0.01	1.65±0.03	1,2
(E)-2-hexenal	854	-	0.97±0.01	-	0.29±0.01	-	1.02±0.02	1,2
hexanol	870	0.24±0.02	0.80±0.09	0.21±0.02	0.57±0.06	0.32±0.03	0.67±0.07	1,2
2-pentylfuran	996	tr	0.22±0.01	-	0.21±0.01	-	-	1,2
octanal	1006	0.29±0.01	0.75±0.01	0.31±0.01	0.54±0.01	0.37±0.01	0.52±0.01	1,2
phenylacetaldehyde	1053	0.19±0.01	0.43±0.01	-	0.38±0.01	0.27±0.01	0.37±0.01	1,2
undecane	1100	0.21±0.01	0.25±0.01	0.32±0.01	0.34±0.01	0.22±0.01	0.46±0.01	1,2
nonanal	1106	3.87±0.06	6.67±0.1	3.74±0.05	6.32±0.09	4.05±0.07	7.63±0.18	1,2
nonanol	1179	-	0.51±0.01	-	-	-	-	1,2
safranal	1195	-	0.20±0.01	-	-	-	-	1,2
dodecane	1200	0.59±0.02	1.15±0.03	0.69±0.01	1.25±0.03	0.50±0.01	0.91±0.02	1,2
decanal	1207	1.07±0.03	1.76±0.05	1.55±0.04	1.60±0.04	0.85±0.03	1.55±0.04	1,2
<i>trans</i> -pulegol	1220	-	0.16±0.01	-	0.13±0.01	-	0.33±0.01	1,2
geraniol	1265	-	-	-	-	-	0.40±0.01	1,2
1H-indole	1293	0.38±0.01	0.99±0.01	-	0.55±0.01	0.79±0.01	1.24±0.02	1,2
tridecane	1300	0.42±0.01	0.38±0.01	0.39±0.01	0.51±0.01	-	0.42±0.01	1,2
3'-methoxy-acetophenone	1320	0.49±0.01	0.74±0.01	0.61±0.01	1.69±0.01	0.25±0.01	1.43±0.03	1,2
(E,E)-2,4-decadienal	1328	0.49±0.01	tr	-	tr	-	tr	1,2
eugenol	1363	1.70±0.04	4.89±0.13	2.71±0.08	3.58±0.1	1.58±0.04	1.05±0.03	1,2
dodecanal	1409	2.44±0.02	3.44±0.02	3.65±0.02	3.01±0.01	2.25±0.02	3.47±0.02	1,2
geranyl acetone	1457	0.35±0.03	0.50±0.05	0.36±0.04	0.45±0.04	0.35±0.03	0.67±0.08	1,2
β-selinene	1483	-	-	-	0.38±0.01	-	-	1,2
(E)-β-ionone	1486	0.41±0.01	0.90±0.01	0.65±0.01	0.76±0.01	0.57±0.01	1.09±0.02	1,2
pentadecane	1500	0.37±0.01	0.41±0.01	0.42±0.01	0.51±0.01	0.34±0.01	0.58±0.01	1,2
bornyl angelate	1521	1.54±0.01	3.26±0.04	1.40±0.02	3.55±0.04	1.80±0.02	4.54±0.12	1,2
caryophyllene oxide	1580	-	0.38±0.01	-	0.95±0.01	-	0.22±0.01	1,2
hexadecane	1600	-	0.38±0.01	-	-	-	0.38±0.01	1,2
humulene epoxide II	1607	-	-	-	0.79±0.01	-	-	1,2
pentadecanal	1613	1.58±0.01	1.70±0.01	1.59±0.01	1.91±0.01	1.18±0.01	2.02±0.04	1,2
megastigmatrienone*	1626	-	0.26±0.01	-	-	-	0.21±0.01	1,2
β-eudesmol	1650	-	-	-	0.50±0.01	-	-	1,2
heptadecane	1700	0.31±0.01	0.49±0.01	0.35±0.01	0.39±0.01	0.27±0.01	0.49±0.01	1,2
hexadecanal	1816	1.28±0.01	1.37±0.02	1.36±0.02	1.58±0.02	1.04±0.01	1.95±0.06	1,2
hexahydrofarnesyl acetone	1844	1.97±0.06	3.16±0.09	2.36±0.06	2.45±0.06	2.63±0.08	3.24±0.11	1,2
nonadecane	1900	-	0.17±0.01	-	0.13±0.01	-	-	1,2
farnesyl acetone	1916	1.61±0.02	1.52±0.01	0.95±0.01	1.07±0.01	0.83±0.01	2.24±0.05	1,2
methyl hexadecanoate	1924	0.42±0.01	0.79±0.01	0.50±0.01	0.70±0.01	0.62±0.01	0.55±0.01	1,2
hexadecanoic acid	1970	33.82±0.14	5.60±0.03	27.89±0.16	18.47±0.12	30.73±0.15	3.19±0.07	1,2
eicosane	2000	-	-	-	-	-	0.24±0.01	1,2
geranyl linalool isomer*	2031	3.80±0.12	0.94±0.03	2.81±0.08	3.24±0.09	2.59±0.07	2.70±0.09	1,2
methyl linoleate	2096	0.56±0.01	0.55±0.01	0.55±0.01	0.51±0.01	0.39±0.01	0.38±0.01	1,2
methyl linolenate	2101	2.08±0.02	1.77±0.01	1.92±0.01	2.73±0.01	2.84±0.02	2.30±0.05	1,2
phytol	2115	7.33±0.08	15.19±0.19	9.27±0.11	9.21±0.12	8.73±0.10	13.35±0.34	1,2,3
oleic acid	2140	2.44±0.25	2.05±0.21	0.90±0.10	2.09±0.22	2.54±0.26	0.42±0.05	1,2
linoleic acid	2145	1.77±0.01	2.60±0.02	0.80±0.01	1.56±0.01	2.92±0.02	1.16±0.02	1,2
stearic acid	2167	0.27±0.01	-	0.14±0.01	-	0.14±0.01	-	1,2
docosane	2200	0.33±0.01	0.33±0.01	0.26±0.01	-	-	0.31±0.01	1,2
tricosane	2300	1.80±0.01	1.80±0.01	2.73±0.02	1.21±0.01	1.12±0.01	2.07±0.05	1,2
tetracosane	2400	0.53±0.01	0.49±0.01	0.63±0.01	0.35±0.01	0.44±0.01	0.84±0.02	1,2
pentacosane	2500	8.00±0.01	8.45±0.03	10.72±0.04	4.92±0.02	7.78±0.02	12.57±0.27	1,2
hexacosane	2600	0.47±0.01	0.48±0.01	0.58±0.01	0.32±0.01	0.46±0.01	0.93±0.02	1,2
heptacosane	2700	5.14±0.01	5.15±0.01	7.12±0.01	3.75±0.01	5.21±0.01	7.19±0.14	1,2
octacosane	2800	0.26±0.01	0.35±0.01	0.35±0.01	0.25±0.01	0.32±0.01	0.60±0.01	1,2
nonacosane	2900	3.20±0.05	4.16±0.07	4.04±0.07	3.25±0.05	4.01±0.07	6.03±0.15	1,2
Total identified (%)		94.50±0.09	91.45±0.13	95.13±0.07	91.69±0.12	92.25±0.13	95.76±0.11	
Grouped components								
Aldehydes		11.31±0.11	17.42±0.14	12.21±0.11	15.73±0.13	10.00±0.11	18.73±0.32	
Ketones		4.33±0.05	6.08±0.04	4.32±0.02	4.72±0.02	4.38±0.05	7.24±0.15	
Aliphatic alcohols		0.24±0.02	1.31±0.09	0.21±0.02	0.57±0.06	0.32±0.03	0.67±0.08	
Alkanes		21.62	24.45	28.59	17.18	20.68	34.01	
Fatty acids		38.30±0.39	10.24±0.23	29.73±0.26	22.12±0.33	36.33±0.38	4.77±0.12	

Table II. Continued

Components	RI ^a	Populations						Method of identification
		1	2	3	4	5	6	
Esters		5.42±0.05	8.73±0.12	5.28±0.08	11.82±0.01	6.85±0.07	10.85±0.25	
Oxygenated monoterpenes		1.70±0.02	5.24±0.15	2.71±0.04	3.71±0.09	1.58±0.02	1.78±0.02	
Sesquiterpene hydrocarbons		-	-	-	0.38±0.01	-	-	
Oxygenated sesquiterpenes		-	0.38±0.01	-	2.24±0.03	-	0.22±0.01	
Oxygenated diterpenes		7.33±0.08	15.19±0.20	9.27±0.11	9.21±0.12	8.73±0.10	13.35±0.34	
Others		4.18±0.02	2.41±0.01	2.81±0.01	4.00±0.02	3.38±0.01	4.15±0.02	

tr = trace, < 0.1%; ^a Calculated retention indices, relative to n-alkane series on a HP5 capillary column; 1: mass spectra, 2: Retention Index 3: IR, ¹H-NMR and ¹³C-NMR spectra; * correct isomer not identified.

Table III. Volatile constituents (%) in leaves of six populations of *O. echioides* growing in central Italy. The values are the mean of three determinations for each population, with standard deviation (RSD).

Components	RI ^a	Populations						Method of identification
		1	2	3	4	5	6	
hexanal	802	0.27±0.01	0.14±0.01	tr	-	tr	0.46±0.01	1,2
(E)-2-hexenal	854	-	0.88±0.01	tr	-	0.10±0.01	4.16±0.04	1,2
hexanol	870	-	-	-	-	-	0.23±0.02	1,2
nonane	900	-	-	-	tr	-	0.26±0.01	1,2
α-pinene	933	-	-	-	1.36±0.01	-	-	1,2
β-pinene	976	-	-	-	2.35±0.01	-	-	1,2
2-pentylfuran	996	-	-	-	-	-	0.10±0.01	1,2
octanal	1006	-	0.3±0.016	-	-	tr	0.56±0.01	1,2
2,4 heptadienal*	1020	-	-	-	-	-	0.30±0.01	1,2
limonene	1031	-	-	-	0.68±0.01	-	-	1,2
undecane	1100	0.16±0.01	-	-	-	-	1.08±0.02	1,2
nonanal	1106	1.52±0.02	1.46±0.02	0.53±0.01	0.32±0.01	0.69±0.01	1.91±0.03	1,2
nonanol	1179	0.57±0.01	0.78±0.01	0.48±0.01	0.35±0.01	0.35±0.01	0.57±0.01	1,2
safranal	1195	tr	tr	tr	tr	tr	tr	1,2
dodecane	1200	0.88±0.02	0.95±0.03	0.75±0.02	0.74±0.02	0.76±0.02	1.25±0.03	1,2
decanal	1207	2.15±0.05	2.28±0.06	1.28±0.03	0.89±0.02	1.23±0.03	3.55±0.11	1,2
trans-pulegol	1220	1.38±0.02	2.01±0.03	0.97±0.02	1.15±0.02	0.69±0.01	3.19±0.06	1,2
geraniol	1265	-	-	-	-	-	0.14±0.01	1,2
1H-indole	1293	-	-	-	-	-	0.24±0.01	1,2
tridecane	1300	-	-	-	-	-	0.24±0.01	1,2
3'-methoxy-acetophenone	1320	1.12±0.01	2.58±0.02	0.85±0.01	0.80±0.01	0.75±0.01	1.19±0.01	1,2
(E,E)-2,4-decadienal	1328	-	-	-	-	-	tr	1,2
eugenol	1363	4.06±0.11	3.54±0.10	2.95±0.09	0.41±0.02	3.11±0.09	1.44±0.04	1,2
tetradecane	1400	0.28±0.01	-	0.27±0.01	-	0.25±0.01	-	1,2
dodecanal	1409	1.31±0.01	2.38±0.01	1.05±0.01	1.05±0.01	1.59±0.01	2.30±0.01	1,2
α-humulene	1451	-	-	-	0.15±0.01	-	-	1,2
geranyl acetone	1457	1.58±0.16	2.34±0.24	1.69±0.17	1.27±0.12	1.63±0.16	2.44±0.25	1,2
6-demethoxy-ageratochromene	1480	0.37±0.01	tr	tr	0.25±0.01	tr	tr	1,2
(E)-β-ionone	1486	2.81±0.03	3.68±0.03	3.81±0.03	2.74±0.02	3.55±0.04	3.56±0.03	1,2
pentadecane	1500	0.44±0.01	0.38±0.01	0.32±0.01	0.30±0.01	0.52±0.01	0.48±0.01	1,2
bornyl angelate	1521	1.95±0.03	2.45±0.03	1.63±0.02	2.01±0.02	2.51±0.03	4.18±0.04	1,2
caryophyllene oxide	1580	1.92±0.01	2.80±0.01	1.20±0.01	0.91±0.01	1.48±0.01	0.96±0.01	1,2
hexadecane	1600	0.39±0.01	-	tr	0.36±0.01	0.38±0.01	-	1,2
pentadecanal	1613	1.09±0.01	1.31±0.01	0.73±0.01	0.76±0.01	0.97±0.01	1.18±0.01	1,2
megastigmatrienone*	1626	1.66±0.03	2.47±0.04	1.12±0.02	0.88±0.02	1.44±0.02	1.07±0.02	1,2
heptadecane	1700	0.55±0.01	0.52±0.01	-	-	-	0.39±0.01	1,2
hexadecanal	1816	0.98±0.01	1.11±0.01	0.72±0.01	1.02±0.01	0.96±0.01	1.43±0.01	1,2
hexahydrofarnesyl acetone	1844	10.01±0.28	8.70±0.25	11.32±0.33	7.14±0.23	12.10±0.35	7.56±0.23	1,2
hexadecanol	1849	0.41±0.01	-	0.43±0.01	0.29±0.01	0.59±0.02	-	1,2
farnesyl acetone	1916	4.50±0.05	4.80±0.06	3.32±0.04	3.00±0.04	3.30±0.04	4.15±0.06	1,2
methyl hexadecanoate	1924	0.40±0.01	0.56±0.01	0.61±0.01	0.27±0.01	0.71±0.01	0.42±0.01	1,2

Table III. Continued

Components	RI ^a	Populations						Method of identification
		1	2	3	4	5	6	
hexadecanoic acid	1970	0.47±0.01	0.48±0.01	0.55±0.01	-	0.62±0.01	1.09±0.01	1,2
methyl linolenate	2101	0.88±0.01	1.29±0.01	1.44±0.01	0.38±0.01	1.57±0.01	0.85±0.01	1,2
phytol	2115	41.40±0.52	36.52±0.44	47.95±0.53	54.52±0.47	47.82±0.54	36.82±0.39	1,2,3
docosane	2200	0.27±0.01	-	0.28±0.01	-	-	-	1,2
tricosane	2300	0.38±0.01	0.21±0.01	0.41±0.01	0.12±0.01	0.22±0.01	0.50±0.01	1,2
pentacosane	2500	0.53±0.01	0.51±0.01	0.50±0.01	0.59±0.01	0.41±0.01	0.24±0.01	1,2
heptacosane	2700	3.76±0.01	2.50±0.01	4.20±0.01	2.25±0.01	1.55±0.01	1.98±0.01	1,2
nonacosane	2900	1.07±0.01	0.94±0.02	1.09±0.02	0.87±0.02	0.67±0.01	1.07±0.02	1,2
Total identified (%)		91.62±0.12	90.91±0.14	92.47±0.13	90.18±0.19	92.50±0.13	93.32±0.11	
Grouped components								
Aldehydes		7.33±0.07	9.92±0.07	4.31±0.05	4.04±0.04	5.54±0.06	15.85±0.12	
Ketones		18.90±0.14	19.52±0.03	20.15±0.17	14.15±0.12	20.57±0.19	17.72±0.01	
Aliphatic alcohols		0.99±0.01	0.78±0.01	0.9±0.011	0.65±0.01	0.95±0.02	0.80±0.02	
Alkanes		8.73±0.03	6.01±0.04	7.83±0.05	5.22±0.06	4.74±0.02	7.51±0.10	
Fatty acids		0.47±0.01	0.48±0.01	0.55±0.01	-	0.62±0.01	1.09±0.01	
Esters		4.72±0.02	6.88±0.05	4.53±0.02	3.71±0.01	5.53±0.04	6.65±0.05	
Monoterpene hydrocarbons		-	-	-	4.39±0.01	-	-	
Oxygenated monoterpenes		5.44±0.02	5.55±0.02	3.92±0.01	1.57±0.01	3.80±0.01	4.77±0.02	
Sesquiterpene hydrocarbons		-	-	-	0.15±0.01	-	-	
Oxygenated sesquiterpenes		1.92±0.01	2.80±0.01	1.20±0.01	0.91±0.01	1.48±0.01	0.96±0.01	
Oxygenated diterpenes		41.40±0.52	36.52±0.44	47.95±0.53	54.52±0.47	47.82±0.54	36.82±0.39	
Others		1.66±0.01	2.47±0.02	1.12±0.01	0.88±0.01	1.44±0.01	1.17±0.01	

tr = trace, < 0.1%; ^a Calculated retention indices, relative to n-alkane series on a HP5 capillary column; 1: mass spectra, 2: Retention Index 3: IR, ¹H-NMR and ¹³C-NMR spectra; * correct isomer not identified.

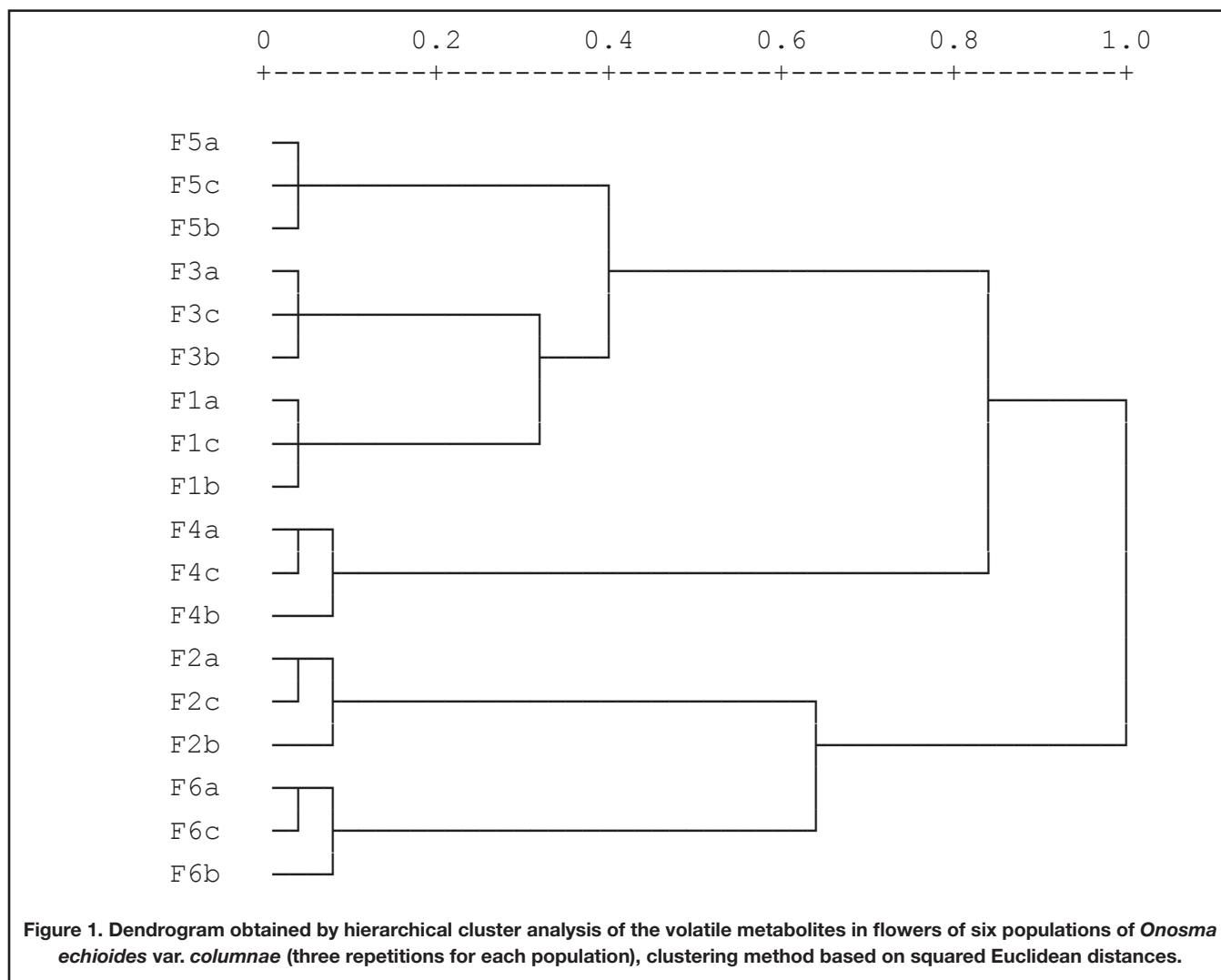
oils, and phytol (36.52–54.52%) and hexahydrofarnesyl acetone (7.14–12.10%) in leaf oils. Phytol is a natural linear diterpene alcohol by balsamic olfactive note which is used in manufacturing synthetic vitamins E and K and in cosmetic applications, such as soap, detergent and beauty care products. It is also a decomposition product of chlorophyll. It has been demonstrated that phytol possesses anti-*Staphylococcus* and anti-mycobacterial activity (25,26) and may be useful for the prevention of vitamin A teratogenicity (27). This volatile compound may be synthesized by plants as a repellent agent against several parasite arthropods (28).

The most abundant classes of volatile components occurring in the flowers were alkanes (17.18–34.01%), fatty acids synthesized (4.77–38.30%) and aldehydes (10.00–18.73%), with pentacosane (4.92–12.57%), hexadecanoic acid (3.19–33.82%) and nonanal (3.74–7.63%) as major components, respectively; oxygenated diterpenes (36.52–54.52%) and ketones synthesized (14.15–20.57%) were the main fraction in leaves, with phytol (36.52–54.52%) and hexahydrofarnesyl acetone (7.14–12.10%) as dominant compounds, respectively. All samples showed a low content of monoterpenes and sesquiterpenes, which ranged from 1.58% (sample 5 flowers) to 8.35% (sample 2 leaves).

From comparison of the percentage composition of volatile components in the six populations of *O. echioides* var. *columnnae*, more quantitative than qualitative differences, due to genetic and geographic factors, were noticed. The hierarchical cluster analysis (Figures 1 and 2) showed that the variation between one population was lower than that between different popula-

tions. Samples from Genga [1], Camerino [3] and Norcia [5] clustered closely at a distance (ds) = 0.40 or 0.47, according to the computational data of the volatile compounds from the flower and leaf oil compositions, respectively, indicating that the volatile compounds of these populations were quite similar. The populations from Esanatoglia [2], Cerreto di Spoleto [4] and Ofena [6] were different from the others; among them, Esanatoglia [2] and Ofena [6] clustered closely at a ds = 0.64 or 0.68 (according to the data from the flower or leaf oil compositions, respectively). The population from Cerreto di Spoleto [4] clustered at a ds = 0.68 or 0.84 (according to the data from the leaf and flower oil compositions, respectively), indicating that this population was quite dissimilar from the others or otherwise quite similar to the Genga [1], Camerino [3] and Norcia [5] ones. In particular, populations 1, 3 and 5 were characterized by the presence of high concentrations of hexadecanoic acid (33.82%, 27.89% and 30.73%, respectively) in the flower oils, and high amounts of oxygenated diterpenes (phytol) in the leaf oils (41.40%, 47.95% and 47.82%, respectively), with the exception of the population 4. Surprisingly, neighbor populations, such as those from Cerreto di Spoleto [4] and Norcia [5], showed significant differences, meaning that genetic, in addition to geographic factors, may play an important role in influencing the composition of volatile fraction, supporting a previous genetic investigation of *O. echioides* (29).

Finally, among *Onosma* species investigated so far, it can be concluded that the composition of volatile fraction of *O. echioides* is very different from that of *O. microcarpum*, which was found to be rich in thymol, carvacrol and heptane (19).



The results of the present study contribute to a better knowledge of the *O. echioides* group and encourage further deep and multidisciplinary studies on its intraspecies variability.

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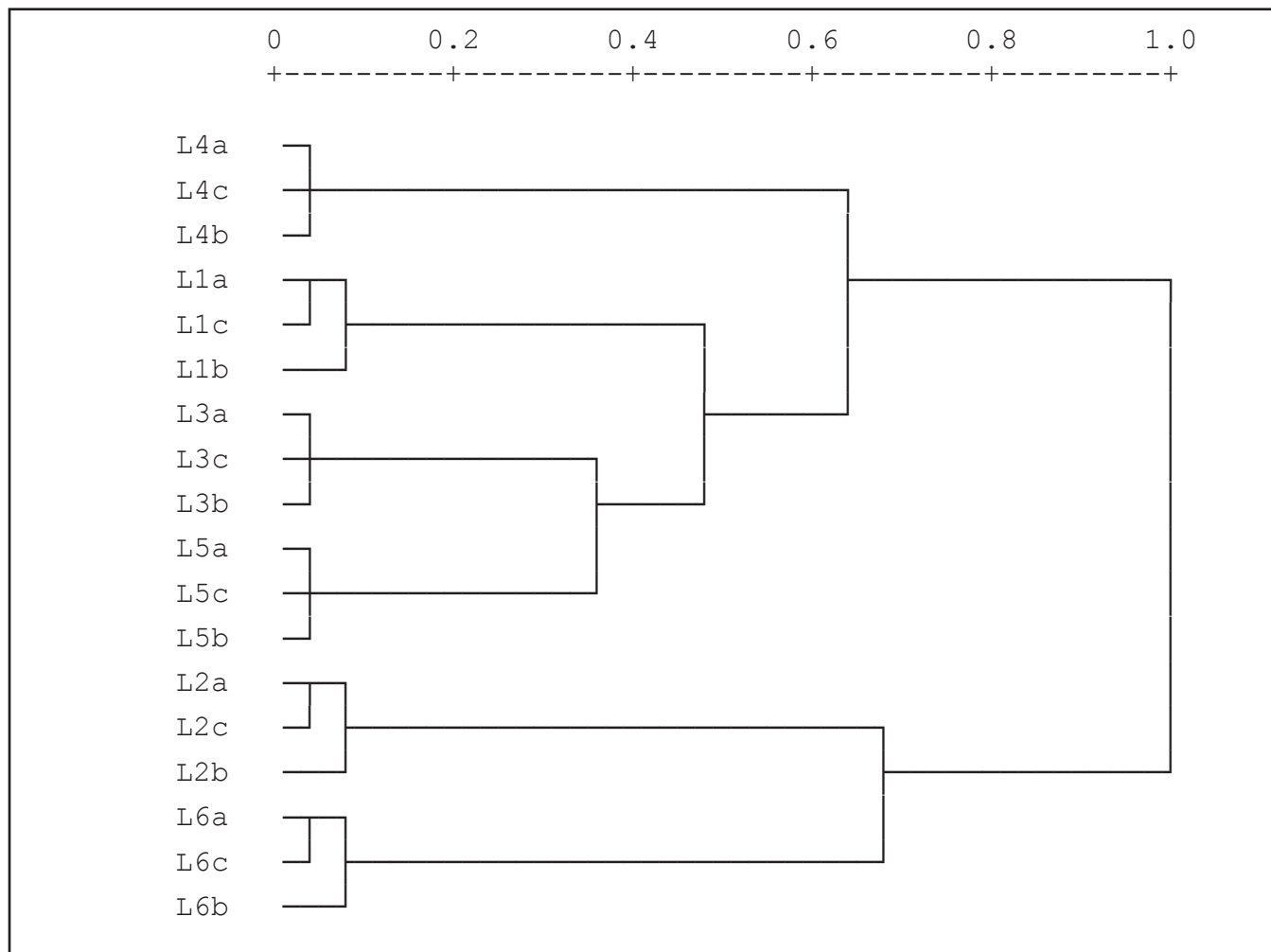


Figure 2. Dendrogram obtained by hierarchical cluster analysis of the volatile metabolites in leaves of six populations of *Onosma echioides* var. *columnae* (three repetitions for each population), clustering method based on squared Euclidean distances.

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