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COMPOSITION AND INFRASPECIFIC VARIABILITY OF ESSENTIAL OIL FROM *THYMUS CAMPHORATUS**

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Key Word Index—*Thymus camphoratus*; Lamiaceae; essential oil; intermedeol; homalomenol-D; multivariate analysis; infraspecific variability.

Abstract—The composition of the essential oils of seven populations of *Thymus camphoratus* from Portugal and their infraspecific variability were investigated by GC, GC-mass spectrometry and ^{13}C NMR. The results obtained from GC analyses of the volatile oils from individual plants were submitted to Principal Component and Chemometric Cluster analyses. 1,8-Cineole, linalool, borneol, α-pinene, camphene, *trans*-sabinene hydrate, and terpinen-4-ol were the main constituents. ^{13}C NMR spectra of the essential oil, previously fractioned by column chromatography, let to the identification of two new oxygenated sesquiterpenes for the genus *Thymus*: intermedeol, which is reported for the first time in the Lamiaceae, and homalomenol-D. Multivariate analysis enabled four different groups of essential oils to be distinguished: (i) linalool, (ii) borneol, (iii) 1,8-cineole and (iv) 1,8-cineole/borneol.

INTRODUCTION

Continuing our research on the composition and chemical polymorphism of the essential oils of the genus *Thymus* [1–5], we now report the results obtained in an exhaustive investigation of the volatile oil of *Thymus camphoratus*, an endemic species from Portugal, which belongs to the section *Thymus*, subsect. *Thymastra*. Some work dealing with the composition of this oil have been previously reported in the literature. Fernandes Costa [6, 7] and Palhinha [8] suggested the presence of carvacrol in the essential oil of this taxon. Later on, Adzet *et al.* [9] found terpinen-4-ol and γ-terpinene to be the major compounds of the oil of a sample collected in Cabo S. Vicente; Velasco-Negueruela and Pérez-Alonso [10, 11] reported 1,8-cineole, borneol, camphor, camphene, terpinen-4-ol and bornyl acetate as the main constituents of two different samples of essential oil of *T. camphoratus*.

In the present work, the composition and variability of the essential oil of seven populations of this species were investigated. Qualitative and quantitative analysis of the essential oils of representative samples of each population were carried out by GC, GC-mass spectrometery and ^{13}C NMR. Prior to ^{13}C NMR analysis, fractionation of the volatile oils by conventional column chromatography was performed. In a second step, to study the infraspecific variability, the oil of individual plants of each population was analysed by GC and the results obtained were submitted to Chemometric Cluster and Principal Component analyses.

RESULTS AND DISCUSSION

The air-dried aerial parts of representative samples of the seven populations of *T. camphoratus* investigated gave an average yield of essential oil of 1.4% (v/w). Qualitative and quantitative analytical results are shown in Table 1. In total, 92 compounds were identified, accounting for 92–98% of the essential oil. The essential oil of all populations was characterized by high percentages of monoterpenes, especially oxygenated ones. Nevertheless, important differences between the major constituents were found, particularly borneol (0.6–24.0%) and 1,8-cineole (3.9–20.0%), which are the main compounds of popu-

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Table 1. Constituents of essential oils of Portuguese populations of *Thymus camphoratus*

Components	% in essential oil of populations						
	A	B	C	D	E	F	G
Monoterpene hydrocarbons	20.2	23.9	26.9	16.4	27.6	26.4	28.2
Tricyclen	0.1	0.3	0.2	t	0.6	0.1	0.2
α-Thujene	0.1	0.4	0.4	t	1.1	0.2	0.4
α-Pinene*	8.1	9.0	11.0	7.5	7.0	7.2	7.9
Camphene*	0.8	5.0	4.6	0.5	10.3	9.9	3.7
β-Pinene	0.4	1.4	1.1	0.6	1.8	1.7	0.5
Sabinene*	4.6	0.9	2.5	2.2	2.0	2.1	5.3
Myrcene	0.6	0.7	0.7	1.0	0.5	0.6	0.3
α-Phellandrene	t	0.2	0.1	0.1	0.1	0.1	0.1
α-Terpinene	0.2	0.6	0.5	0.1	0.5	0.5	2.0
Limonene*	1.0	2.0	1.8	1.4	1.4	1.6	1.0
cis-Ocimene	0.1	t	0.1	0.3	0.1	0.2	t
γ-Terpinene	0.4	1.1	1.0	0.2	0.8	0.9	3.9
trans-Ocimene*	3.0	1.0	1.2	1.5	0.4	0.5	0.2
p-Cymene	0.6	0.9	1.4	0.7	0.7	0.7	1.8
Terpinolene	0.1	0.3	0.3	0.1	0.2	0.1	0.8
α-*p*-Dimethylstyrene	t	t	t	0.1	0.1	t	t
Oxygenated monoterpenes	51.3	50.5	49.2	58.6	67.3	63.4	53.3
1,8-Cineole*	11.0	12.0	13.7	11.5	20.0	19.2	3.9
cis-Linalool oxide	0.5	0.4	0.3	0.6	0.2	0.2	0.4
trans-Sabinene hydrate	0.1	1.2	0.9	0.2	0.3	0.4	10.8
trans-Linalool oxide	0.5	0.4	0.2	0.5	0.2	0.1	0.1
Nerol oxide	t	t	t	t	—	—	t
Campholenal	0.1	0.4	0.2	0.1	0.2	0.1	0.2
Camphor*	0.7	6.3	3.7	0.2	6.2	6.8	2.9
Linalool*	21.0	5.5	4.0	19.8	4.2	3.0	3.0
cis-Sabinene hydrate	0.2	0.3	0.1	0.1	0.1	0.1	1.2
Linalyl acetate*	0.4	0.1	1.6	8.0	1.1	0.5	0.6
Pinocarvone	t	0.2	0.2	0.1	0.2	0.1	0.2
Bornyl formate	0.1	0.1	0.1	0.2	0.5	0.4	t
Bornyl acetate*	0.1	2.5	1.4	0.1	2.8	3.8	3.8
Terpinen-4-ol*	1.4	3.1	4.0	1.5	1.9	1.5	10.2
Myrtenal	0.1	0.3	0.2	0.1	0.2	0.2	0.2
Bornyl propionate	t	0.3	0.2	t	0.4	0.5	t
cis-Verbenol	t	0.1	0.1	t	t	t	0.2
trans-Pinocarveol*	0.5	0.6	0.9	1.2	0.6	0.4	0.6
δ-Terpineol	0.4	0.6	0.5	0.3	0.4	0.4	0.1
trans-Verbenol*	1.5	2.9	2.0	2.2	1.0	1.1	2.2
Neral	t	—	t	t	—	—	t
α-Terpineol*	1.6	1.0	1.1	5.5	1.1	1.0	2.5
α-Terpenyl acetate	t	0.1	0.1	0.6	0.2	0.3	2.0
Borneol*	1.0	8.0	9.4	0.6	24.0	22.1	6.1
Verbenone	0.5	1.0	0.7	0.4	0.3	0.2	0.5
Neryl acetate	t	—	t	0.5	t	t	0.2
Bornyl butyrate	0.1	0.1	0.2	0.1	0.2	0.1	0.1
Geranial	t	t	0.2	0.1	t	t	t
Bornyl isovalerate	t	—	0.1	t	t	0.1	t
Carvone	t	0.2	0.2	0.2	0.1	0.1	t
Geranyl acetate*	6.7	0.8	0.4	1.2	0.1	0.2	t
Myrtenol	—	0.3	0.2	0.2	0.1	t	0.3
Geranyl isobutyrate	t	0.3	0.1	0.3	—	—	t
Nerol	0.2	—	t	0.1	—	—	t
Geranyl propionate	0.1	t	t	t	—	—	t
trans-Carveol	0.2	0.4	0.5	0.3	0.2	0.1	0.3
Geraniol*	1.4	0.2	0.4	1.0	—	—	t
p-Cymen-8-ol	0.1	0.3	0.6	0.5	0.2	0.2	0.4
Geranyl butyrate	0.1	0.1	t	0.1	—	—	—
Geranyl isovalerate	0.3	0.2	0.2	0.1	—	—	t
Cuminic alcohol	0.1	t	0.1	0.1	t	t	—
Carvacrol	—	t	t	t	t	t	t

Table 1. *Continued.*

Sesquiterpene hydrocarbons	7.6	5.3	4.8	6.3	0.8	1.3	2.7
α-Cubebene	—	0.2	0.1	t	—	—	—
β-Bourbonene	0.2	t	0.1	t	t	t	0.1
β-Caryophyllene	0.6	0.2	0.2	1.0	0.3	0.3	0.3
allo-Aromadendrene	0.4	0.4	0.5	1.4	t	t	0.5
β-Cubebene	0.1	t	t	t	t	t	t
D-Germacrene	0.3	0.3	0.4	0.4	t	t	0.2
β-Bisabolene	t	t	t	t	t	t	—
Bicyclogermacrene	0.3	0.1	0.1	0.6	0.3	0.2	t
δ-Cadinene	0.5	0.5	0.8	0.5	t	0.1	1.0
γ-Cadinene*	0.6	2.7	2.4	1.8	t	0.5	0.5
cis-α-Bisabolene*	4.5	0.5	0.1	0.5	t	t	t
Cuparene	t	0.3	t	t	t	t	t
Oxygenated sesquiterpenes	13.4	13.2	13.8	10.1	1.6	4.9	8.6
β-Caryophyllene oxide*	1.2	0.4	0.4	3.0	0.5	0.8	3.5
Ledol*	0.1	0.3	0.6	0.9	t	0.1	0.8
Cubenol*	0.3	1.5	0.9	0.1	0.1	0.2	0.6
β-Elemol	t	t	0.2	—	0.1	t	t
Viridiflorol*	0.7	0.5	1.0	0.4	t	0.4	0.9
10-*epi*-γ-Eudesmol	1.3	0.5	1.7	0.3	t	t	0.6
Spathulenol*	0.8	t	0.1	2.0	—	t	0.6
T-Cadinol*	2.0	8.3	5.7	1.2	t	2.2	0.4
10-*epi*-Cadinol	—	0.2	0.4	0.4	—	t	0.3
α-Bisabolol	0.1	0.2	0.2	—	—	—	—
α-Cadinol	0.5	1.4	0.2	1.8	t	t	0.9
β-Eudesmol	—	—	t	—	t	t	—
Intermedeol*	6.5	0.1	2.6	t	0.8	1.0	t
Others	0.5	1.3	0.5	0.7	0.3	0.3	0.3
Ethyl 2-methylbutyrate	t	0.2	t	t	0.1	0.1	t
Ethyl isovalerate	t	t	—	—	t	t	—
6-Methylhept-5-en-2-one	t	t	t	0.1	t	t	t
Oct-1-en-3-yl acetate	—	t	t	0.1	—	t	—
Hexyl butyrate	t	0.1	t	t	t	t	t
Oct-1-en-3-ol	t	t	t	t	t	—	t
Decanol	0.2	0.2	0.1	0.3	0.1	0.1	0.1
Eugenol	t	0.1	t	t	—	—	t
Eugenyl acetate*	0.2	0.6	0.2	t	t	t	t
Total identified:	93.0	94.2	95.2	92.1	97.6	96.3	93.1

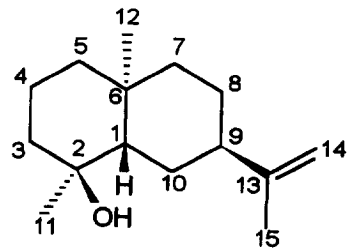
t: trace (≤0.05%).

* Constituents selected for the multivariate analysis.

lations E and F, linalool (3.0–21.0%) which had the highest percentage in population A, and *trans*-sabiene hydrate (0.1–10.8%) and terpinen-4-ol (1.4–10.2%), the major components of population G. Populations B and C had 1,8-cineole and α-pinene as the main constituents. The latter was present in all populations in percentages ranging from 7.0 to 11.0%.

Among the sesquiterpenes, oxygenated ones were detected in higher concentrations than the hydrocarbons in all populations analysed, some of them showing a certain variability, especially T-cadinol (trace to 8.3%) and intermedeol (trace to 6.5%).

The use of GC and GC-mass spectrometry together with ^{13}C NMR allowed the identification of all the components listed in Table 1. A special case was that of intermedeol (**1**), an oxygenated sesquiterpene not reported previously in the Lamiaceae. This constituent was present in all populations, especially in that from Vila do Bispo (A), which contained 6.5%. Its retention indices and mass spectrum suggested it was an oxygenated sesquiterpene with a $[M]^+$ at *m/z* 222, which could not be identified from the data available in our library. For this reason, the essential oil of population A was submitted to a fractionation by column chromatography and ^{13}C NMR spectra of all fractions were subsequently recorded. In the ^{13}C NMR spectrum of the fraction eluted with pentane–diethyl ether (19:1) several oxygenated monoterpenes and sesquiterpenes were identified. In the same spectrum, 15 signals corresponding with an unknown sesquiterpene were detected. Its chemical shifts were in accordance with those previously reported for intermedeol [12, 13]. The multiplicity of each carbon was assessed from a sequential spin-echo ^{13}C NMR spec-



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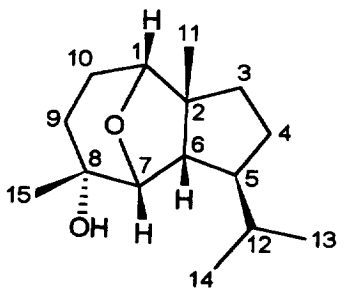

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trum. Furthermore, mass spectral data of the unknown sesquiterpene were also in accordance with those reported for intermedeol [13]. Intermedeol has also been found by us in the essential oil of *T. lotocephalus* (unpublished data).

Intermedeol was also found in the fraction eluted with diethyl ether. ^{13}C NMR of this fraction showed also the presence of several oxygenated compounds, as well as 15 signals which were coincident with those reported for homalomenol-D (**2**) [14]. This is the first time that this sesquiterpene has been recorded in the genus *Thymus*.

The ^{13}C NMR of the total oil of population A showed intense signals corresponding with intermedeol, while those for homalomenol-D indicated that the percentage of this constituent was less than 1%.

Results obtained from Principal Component Analysis (PCA) and Cluster Analysis showed the existence of a high infraspecific variability within the essential oils of *T. camphoratus*. Figure 1 shows the relative position of the essential of the individuals analysed, in relation to an axial system originated in the PCA. Four different types of essential oils, whose mean chemical composition is presented in Figs 2–5, were found: cluster I, characterized by a high content of linalool, cluster II, in which borneol was the major constituent, cluster III, with substantial percentages of 1,8-cineole, and cluster IV, which shows an inter

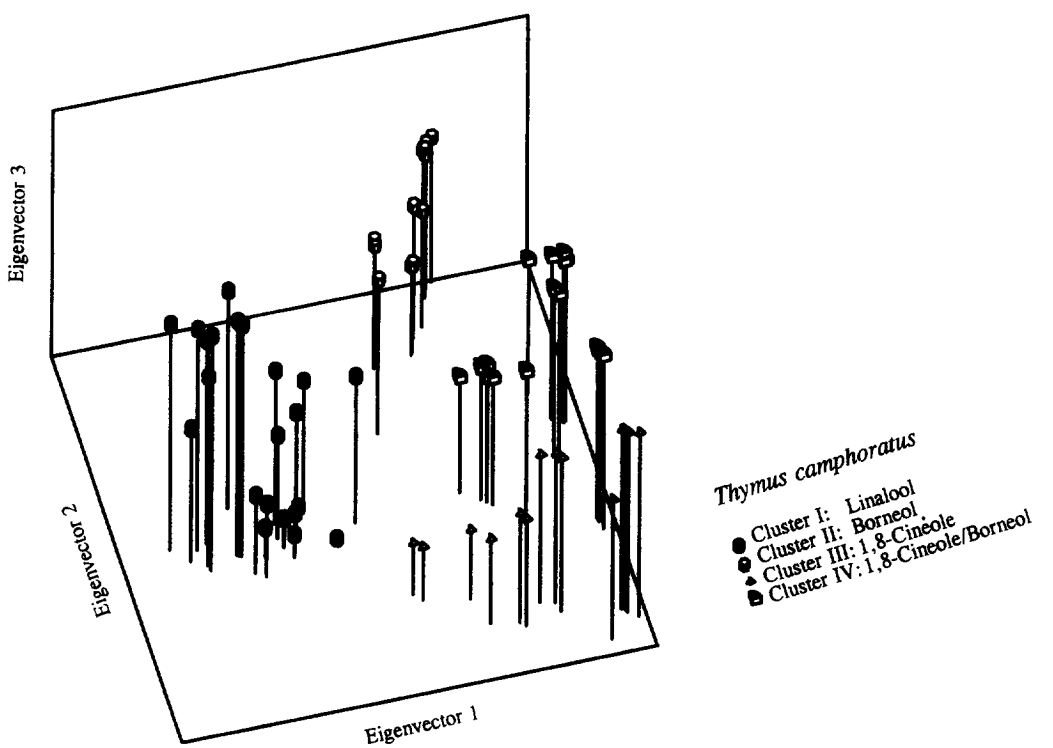

Fig. 1. Relative position of samples (individuals) in the space defined by the first three Principal Components.

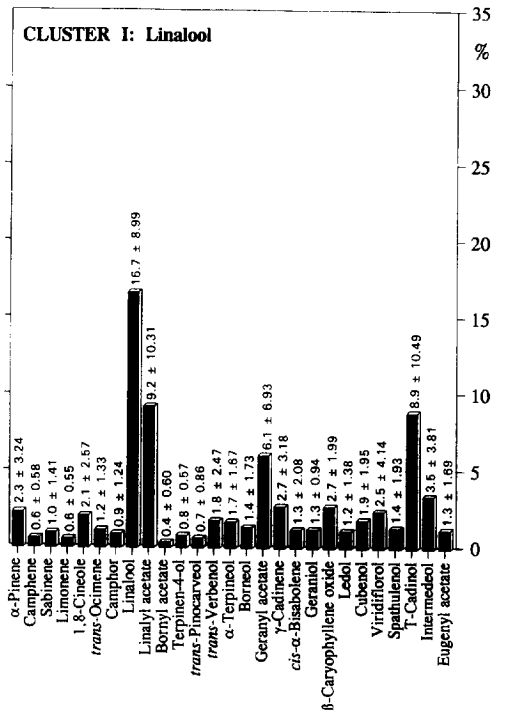

Fig. 2. Mean chemical composition of essential oil of cluster I (n = 26). Vertical: mean percentage in the essential oil. For each constituent, mean ±S.D. is given.

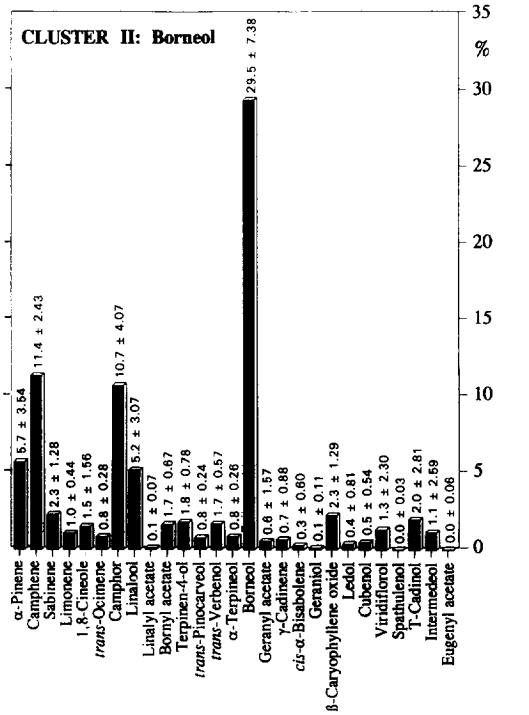

Fig. 3. Mean chemical composition of essential oil of cluster II (n = 14). Vertical: mean percentage in the essential oil. For each constituent, mean ±S.D. is given.

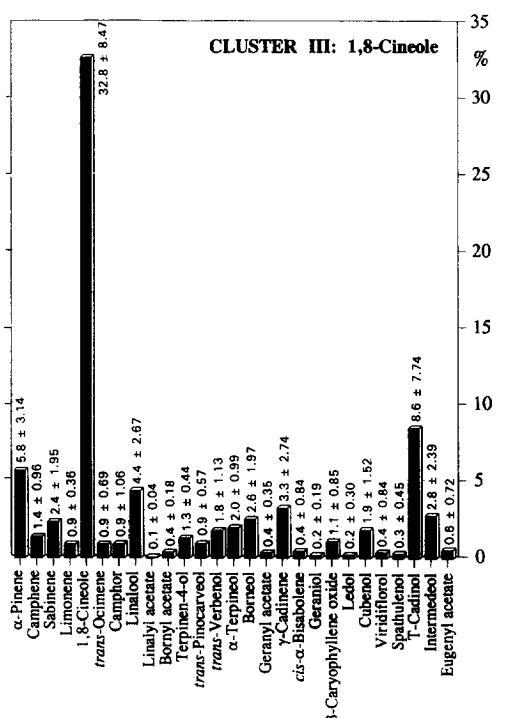

Fig. 4. Mean chemical composition of essential oil of cluster III (n = 15). Vertical: mean percentage in the essential oil. For each constituent, mean ±S.D. is given.

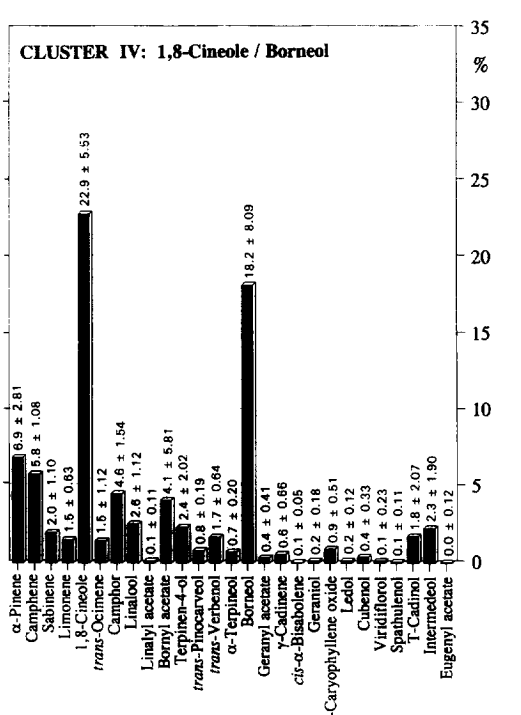

Fig. 5. Mean chemical composition of essential oil of cluster IV (n = 17). Vertical: mean percentage in the essential oil. For each constituent, mean ±S.D. is given.

Table 2. Percentages of individuals of each locality belonging to each cluster

Locality	N	Cluster I	II	III	IV
A	16	75.0	12.5	12.5	—
B	14	28.6	14.2	28.6	28.6
C	10	30.0	10.0	30.0	30.0
D	11	63.6	—	18.2	18.2
E	12	—	41.7	16.6	41.7
F	9	—	44.5	22.2	33.3

mediate composition between cluster II and III, its main constituents being 1,8-cineole and borneol.

Within the essential oils of cluster I some subgroups could be detected: linalool/T-cadinol, linalool/linalyl acetate and linalool/geranyl acetate. Essential oils of Cluster II, which showed borneol as major constituent, were also characterized by high percentages of camphene and camphor. All of these constituents are biogenetically related.

Table 2 shows the percentages of essential oil of each type found in every locality. It should be noted that borneol and/or 1,8-cineole are the major constituents of oils obtained from individuals of *T. camphoratus* collected in the north western localities studied (Rogil and Odemira), while linalool is present mainly in the oils obtained from plants collected in the southern areas. This trend was also observed in the case of *T. carnosus*, whose linalool chemotype is mainly located in the region of Estremadura (Portugal) [3].

Individual plants from Cabo S. Vicente (population G) were not available for this study. Nevertheless, provided that the major constituents found in the essential oil of the population, terpinen-4-ol and *trans*-sabinene hydrate, are different from those of the other populations investigated, the study of its variability should be the object of further research.

EXPERIMENTAL

Plant material. Aerial parts of *T. camphoratus* Hoffmanns. and Link (Lamiaceae) were collected at the flowering stage, in April–June 1992, in the Algarve and Baixo Alentejo regions: Vila do Bispo (A), Colinas Verdes, Lagos (B), Barao de S. Miguel (C), Planalto de Sagres (D), Rogil (F), Cabo de S. Vicente (G) (Algarve) and surroundings of Odemira (E) (Baixo Alentejo). Voucher specimens of each population are deposited in the Herbarium of the Instituto Botanico of the University of Coimbra. In order to study variability in essential oils, individual plants from populations A–F were collected from each place at the same time as homogeneous samples of the corresponding populations.

Analysis of essential oils. Essential oil contents of air-dried plant material of the general sample of each population was determined according to the European Pharmacopoeia method [15]. Analysis of volatile oils obtained by hydrodistillation were carried out by GC and GC-MS using fused silica capillary columns with two different stationary phases, as previously described [3, 16]. NMR spectra were recorded at 200 MHz for ^1H and 50 MHz for ^{13}C, in $CDCl_3$, with all shifts ref. to int. TMS [3]. Identification of components was made on the basis of their retention indices, in relation to an homologous series of fatty acid Me esters and their MS, which were compared with those of our own library, literature data and authentic samples [17, 18]. Compounds with a percentage equal or higher than 1% were also identified by ^{13}C NMR [19, 20].

Fractionation of essential oil of population C. Essential oil of population C was submitted to fractionation by CC on silica gel (Kieselgel 60, 0.2–0.5 mm). Elution was carried out with pentane–Et_2O (gradient from 1:0 to 0:1).

Infraspecific variability. In order to investigate chemical polymorphism, essential oils of 72 individual plants obtained by hydrodistillation were analysed by GC and, when necessary, by GC-MS, using the same analytical conditions indicated above. Identification of components was made by comparison with the chromatograms of the essential oil of the population collected at the same place. From all the volatile constituents, 27 (27 variables × 72 individuals = 1944 data) were selected to be included in the multivariate analysis (Principal Component Analysis and Cluster Analysis) using PARVUS [21] and ESTATS [22] chemometric software packages, as previously reported [2, 3]. Selected constituents are shown in Table 1.

Intermedeol (**1**). EI-MS, *m/z* (rel. int.): 222 $[M]^+$ (2), 204 (50), 189 (47), 161 (45), 81 (60), 71 (66), 43 (100). ^{13}C NMR (50 MHz, $CDCl_3$): δ 49.08 (C-1), 72.07 (C-2), 43.53 (C-3), 20.17 (C-4), 41.42 (C-5), 35.21 (C-6), 40.39 (C-7), 23.53 (C-8), 39.39 (C-9), 22.70 (C-10), 22.31 (C-11), 18.46 (C-12), 146.88 (C-13), 110.82 (C-14), 22.64 (C-15).

Homalomenol-D (**2**). ^{13}C NMR (50 MHz, $CDCl_3$): δ 82.2 (C-1), 54.71 (C-2), 32.97 (C-3), 35.95 (C-4), 45.75 (C-5), 61.78 (C-6), 85.57 (C-7), 70.23 (C-8), 30.34 (C-9), 24.33 (C-10), 31.57 (C-11), 34.88 (C-12), 22.62 and 20.49 (C-13 and C-14, may be reserved), 25.31 (C-15).

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