Pergamon

PII: S0031-9422(96)00872-2

Phytochemistry, Vol. 45, No. 2, pp. 307–311, 1997

0031-9422/97 $17.00+0.00

VARIABILITY OF ESSENTIAL OILS OF *THYMUS CAESPITITIUS* FROM PORTUGAL

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(*Received* 24 *September* 1996)

Key Word Index—*Thymus caespititius*; Lamiaceae; Portugal; essential oils; GC-MS; ^{13}C NMR; infraspecific variability.

Abstract—The composition and variability of the essential oils of several populations of *Thymus caespititius* from Portugal were investigated by GC, GC-mass spectrometry and ^{13}C NMR. All samples from NW Portugal were characterized by their high α-terpineol content, while the main components in the oil sample from Pico island (Azores archipelago) were carvacrol and thymol. The analytical data of individual samples from NW Portugal showed no chemical polymorphism in this area. The different areas of distribution (NW Portugal and Azores) that show great climatic and soil variation conditions may be the origin of the α-terpineol-type oil from NW Portugal and the carvacrol/thymol-type oil from the Azores. ^{13}C NMR spectra of the essential oil, previously fractionated by column chromatography, led to the identification of *trans*-dihydroagarofuran, a new oxygenated sesquiterpene for the genus *Thymus* and a characteristic compound of *T. caespititius*. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Thymus* widely distributed in the Iberian Peninsula is a taxonomically complex group of aromatic plants used for medical purposes, due to their antiseptic, antispasmodic and antitussive properties. *Thymus caespititius* is an endemic species of the NW Iberian Peninsula and of the Azores and Madeira archipelagos. This taxon belongs to the Section *Micantes* and grows wild in the Atlantic wet areas. The essential oil of *T. caespititius* from Portugal has been studied only by Fernandes Costa [1, 2], who found 60% alcohols (α-terpineol as a main constituent), 10% esters, pinene, cadinene and a small amount of phenols; more precise data were not given. Moreover, in the first reports on the samples of Spanish origin, Seoane *et al.* [3, 4] found α-terpineol as a main constituent with large amounts of borneol, *p*-cymene and linalool. The sesquiterpenes, α- and β-cubebene, α-muurolene and γ-cadinene, were also identified. Later, Morales [5] reported α-terpineol and *p*-cymene as the major constituents of the essential oil from one sample from Pontevedra (Spain).

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Continuing our research on the composition of the essential oils of Portuguese *Thymus* species [6–12], in the present work, we report on the composition and the variability of the essential oils of several populations of *T. caespititius* collected from NW Portugal and of one sample collected at Pico island (Azores archipelago). The composition of the oils was investigated by GC, GC-mass spectrometry and ^{13}C NMR. A study on the chemical polymorphism was also performed using the oils of individual plants from each population from NW Portugal.

RESULTS AND DISCUSSION

The air-dried aerial parts of representative samples from NW Portugal and fresh plant material from the Azores gave an average essential oil yield of 1.1% and 0.2% (v/w), respectively. The analytical data are shown in Table 1, where the compounds are listed in order of elution on a DB-1 column. More than 91% of the total oil was identified in each sample and 70 compounds were assigned. Oxygenated monoterpenes were the main group of constituents in all samples (36.7–73.1%). Nevertheless, the main components in all samples from NW Portugal (samples 1–7) were α-

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

		Per cent in samples							
RI	Components	1	2	3	4	5	6	7	8
921	Tricyclene	t	t	t	0.1	t	—	t	—
924	α-Thujene	0.8	1.1	0.9	1.0	0.7	0.3	0.7	2.6
930	α-Pinene	0.7	1.1	0.6	1.2	0.4	0.4	0.3	1.9
938	Camphene	1.1	1.6	0.9	1.8	0.4	0.6	0.5	0.1
958	Sabinene	1.9	1.4	0.9	0.8	0.8	0.6	0.4	0.4
961	1-Octen-3-ol	0.7	0.8	0.9	1.2	1.7	0.5	1.5	—
963	β-Pinene	0.2	0.2	0.2	0.3	0.1	0.2	0.1	0.8
975	Myrcene	2.5	2.4	2.1	1.5	1.8	1.0	1.2	—
1000	α-Phellandrene	0.1	0.1	0.1	0.1	0.1	t	0.1	0.2
1002	α-Terpinene	1.0	0.9	0.5	1.1	0.5	0.4	0.4	0.7
1003	*p*-Cymene	6.0	7.4	9.1	8.5	6.5	6.8	7.8	6.8
1005	β-Phellandrene	0.1	t	0.1	0.2	—	t	t	—
1005	1,8-Cineole	—	—	—	—	—	—	—	1.2
1009	Limonene	2.0	1.8	1.7	1.4	1.4	1.1	1.3	0.4
1017	*cis*-β-Ocimene	0.1	0.2	0.1	0.1	0.1	t	t	—
1027	*trans*-β-Ocimene	0.4	0.4	0.2	0.5	0.1	0.1	t	—
1035	γ-Terpinene	3.8	5.2	4.8	6.9	4.2	3.7	3.8	1.7
1037	*trans*-Sabinene hydrate	0.1	0.1	0.2	0.1	t	—	t	0.3
1059	α-*p*-Dimethylstyrene	t	t	t	t	t	—	t	—
1064	Terpinolene	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.2
1066	*cis*-Sabinene hydrate	0.1	0.1	0.1	t	0.1	0.2	0.1	0.1
1074	Linalool	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.2
1086	1-Octen-3-yl acetate	0.1	0.3	0.4	0.3	0.4	0.2	0.5	—
1134	Borneol	1.2	2.0	2.0	2.1	1.0	2.5	1.3	0.1
1148	Terpinen-4-ol	2.0	1.0	1.5	1.2	0.9	1.1	0.9	0.9
1148	*p*-Cymene-8-ol	0.2	0.3	0.2	0.1	0.1	0.1	t	—
1159	α-Terpineol	39.6	33.5	36.2	30.6	40.5	39.0	40.4	5.1
1189	*trans*-Carveol	0.1	0.1	0.1	0.2	0.1	0.1	0.1	—
1206	Carvone	—	—	—	—	—	—		0.2
1224	Carvacrol methyl ether	0.3	0.3	0.5	0.3	0.4	0.3	0.5	0.1
1260	Cuminic alcohol	t	t	t	t	—	0.1	t	—
1265	Bornyl acetate	0.6	1.2	1.6	1.4	0.4	1.7	0.4	0.1
1275	Thymol	0.1	t	—	0.2	—	t	t	16.1
1286	Carvacrol	t	t	—	0.1	—	—	t	36.3
1330	Thymol acetate	—	—	—		—	—	—	4.0
1334	α-Terpenyl acetate	0.2	0.2	0.3	0.2	0.3	0.1	0.4	0.1
1345	α-Cubebene	t	t	t	t	0.1	0.1	t	—
1348	Carvacrol acetate	—		—	—	—	—		8.3
1375	α-Copaene	0.2	0.1	0.2	0.1	0.2	0.2	0.2	—
1379	β-Bourbonene	0.3	0.3	0.2	0.3	0.4	0.4	0.6	—
1385	β-Cubebene	0.2	0.3	t	0.3	0.3	0.4	0.2	—
1388	β-Elemene	0.2	0.2	0.1	0.4	0.3	0.2	0.2	—
1400	α-Gurjunene	0.1	0.1	0.2	0.1	0.2	0.2	0.2	—
1414	β-Caryophyllene	1.1	2.3	0.8	2.0	3.6	1.7	2.4	—
1414	*trans*-Cinnamyl acetate	—	—		—	—	—		0.6
1447	α-Humulene	0.2	0.3	0.1	0.2	0.4	0.3	0.4	—
1454	allo-Aromadendrene	0.6	0.7	0.5	0.7	0.7	0.9	0.5	—
1469	γ-Muurulene	0.2	0.2	0.2	0.1	0.2	0.2	0.2	—
1469	γ-Cadinene	2.6	3.0	2.0	2.6	2.9	3.7	2.5	0.5
1474	β-Gurjunene	0.1	0.1	t	t	0.1	0.1	0.1	—
1474	γ-Gurjunene	0.1	t	0.1	0.1	t	—	t	—
1474	Germacrene-D	1.0	1.4	0.6	0.9	1.7	1.2	1.5	—
1484	Cadina-1,4-diene	0.7	0.5	t	0.1	t	t	0.6	—
1487	Bicyclogermacrene	0.1	0.2	0.1	t	t	t	t	—
1489	*trans*-Dihydroagarofuran	1.5	1.0	3.0	2.0	1.2	2.1	2.9	1.7
1491	Cuparene	0.3	0.3	0.2	0.2	0.1	0.1	0.1	—
1505	δ-Cadinene	0.9	0.9	1.3	1.2	0.9	1.1	1.0	0.2
1506	Calamenene	—	—	—	—		—	—	0.1
1530	β-Elemol	0.9	1.5	0.8	1.0	2.0	1.1	0.6	0.4
1561	β-Caryophyllene oxide	0.1	0.2	t	0.1	0.1	0.1	0.1	—

Table 1.—*Continued*

RI	Components	Per cent in samples 1	2	3	4	5	6	7	8
1569	Viridiflorol	0.5	0.7	0.5	1.6	0.5	0.9	0.4	—
1580	Ledol	0.2	0.2	0.2	0.1	0.2	0.1	0.1	—
1593	epimer of guaiol	2.1	1.4	2.5	2.5	2.4	2.5	2.4	2.4
1600	Cubenol	0.5	1.0	1.0	1.0	0.9	1.6	2.0	—
1616	T-Cadinol	7.5	8.7	7.0	6.8	6.2	8.1	7.6	1.3
1620	β-Eudesmol	1.5	1.0	1.0	1.8	1.2	1.1	0.7	—
1626	α-Cadinol	1.0	0.8	0.7	1.0	0.4	0.6	0.7	0.3
1629	Intermedeol	0.8	0.2	t	0.1	—	0.1	0.1	—
1634	α-Eudesmol	1.1	1.1	1.0	1.1	1.0	1.0	0.7	—
1656	α-Bisabolol	0.3	0.4	0.4	0.1	0.1	0.4	0.3	—
Monoterpene hydrocarbons		21.3	24.3	22.4	25.8	17.5	15.7	17.1	15.8
Oxygenated monoterpenes		44.7	39.0	43.0	36.7	43.9	45.4	44.3	73.1
Sesquiterpene hydrocarbons		9.0	11.0	6.8	9.4	12.2	10.9	10.8	0.8
Oxygenated sesquiterpenes		18.0	18.2	18.2	19.2	16.2	19.5	18.6	6.1
Others		0.8	1.1	1.3	1.5	2.1	0.7	2.0	0.6
Total identified		93.8	93.6	91.7	92.6	91.9	92.2	92.8	96.4

t: traces $\leqslant 0.05\%$.
RI: retention indices relative to C_9–C_{17} *n*-alkanes.

terpineol (30.6–40.5%), *p*-cymene (6.0–9.1%) and T-cadinol (6.2–8.7%), whereas the major components in the oil sample from the Azores were carvacrol (36.3%), thymol (16.1%), carvacrol acetate (8.3%) and *p*-cymene (6.8%). Thus, the phenolic compounds, carvacrol and thymol, were the most abundant constituents of the Azores oil, in contrast to the other populations of NW Portugal, in which the higher percentage of both compounds attained only 0.2%.

The essential oils isolated from plant material collected at NW Portugal were characterized by a high number of sesquiterpenes (31 compounds identified) comprising 25.0–30.4% of the essential oil, while the oil of the sample from the Azores attained a percentage of 6.9%. All these results show a marked difference between the essential oils from NW Portugal and that from the Azores.

The use of GC and GC-mass spectrometry, together with ^{13}C NMR, allowed the identification of all constituents shown in Table 1. A special mention should be made of the oxygenated sesquiterpene, *trans*-dihydroagarofuran, not previously reported in the genus *Thymus*. This constituent was present in all samples analysed (1.0–3.0%) of *T. caespititius*, so it is a characteristic compound of this species. Its mass spectrum strongly suggested that it was dihydroagarofuran, but more precise data of this compound was not available. For this reason, essential oil of sample 3 was fractionated by column chromatography on silica gel and the fractions obtained were analysed by ^{13}C NMR. Fifteen signals corresponding to an oxygenated sesquiterpene were recorded in the spectrum of the fraction eluted with pentane–diethyl ether (49:1). Its chemical shifts agreed with those previously reported for *trans*-dihydroagarofuran [13, 14]. In the ^{13}C NMR spectrum of the fraction eluted with pentane–diethyl ether (9:1), 15 signals with chemical shifts very similar to those of guaiol, were recorded. This led us to consider the compound as an epimer of guaiol, that is also supported by the mass spectral data.

The analytical results from the essential oils of individual plants from NW Portugal showed that there is no chemical polymorphism in this area. All samples were characterized by their high α-terpineol content. That is in accordance with the results found in the analysis of the representative samples of each population from this area. Thus, a defined group of essential oil was established (Fig. 1).

In conclusion, an α-terpineol-type oil was found to be characteristic of NW Portugal samples, whereas a carvacrol/thymol-type was characteristic of Azores (Pico island) sample. These chemical differences could be related, in part, to the different soil and climatic conditions that characterize both geographical areas.

EXPERIMENTAL

Plant material. Thymus caespititius Brot. was collected at seven different localities from some provinces of NW Portugal: Trás-os-Montes (samples 2 and 7), Minho (samples 3 and 4), Beira Alta (samples 1 and 6) and Beira Litoral (sample 5) and one at Azores-Pico mountain at 1200 m altitude (sample 8). One representative, homogeneous sample of each population was collected at the flowering period of the plant (May–August) to perform the qualitative and quantitative analysis of the essential oil. Voucher specimens are deposited in the Herbaria of the Botanical Institutes of the Universities of Coimbra (COI) and Lisbon (LISU). In order to study infraspecific

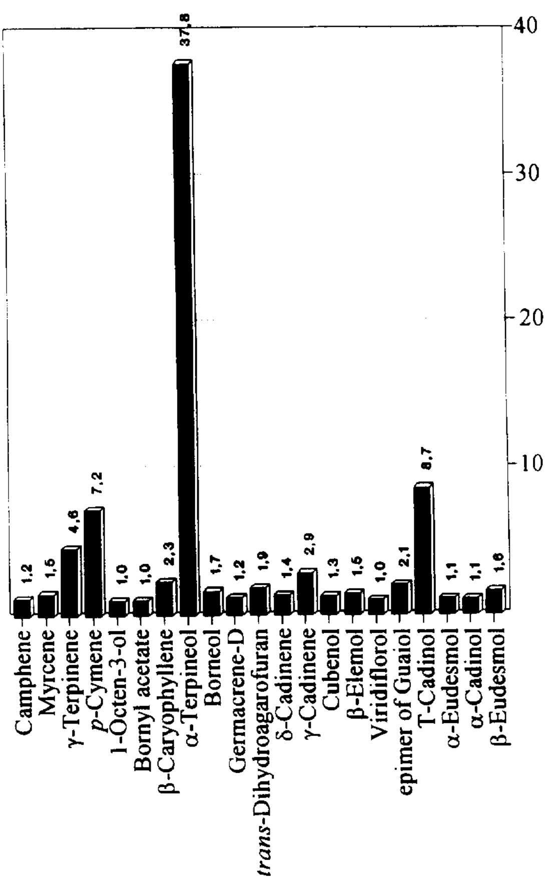

Fig. 1. Mean chemical composition (%) of α-terpineol-type essential oil.

variability, 91 individual plants were also collected at the localities in NW Portugal at the same time.

Analysis of essential oils. The oil yield of air-dried plant material and fresh plant material (only from the Azores) were determined according to the European Pharmacopoeia method [15]. Isolation of the essential oils was carried out by hydrodistillation and samples were analysed by GC and GC-MS on two fused-silica capillary columns with stationary phases of different polarities [10, 16, 17]. ^{13}C NMR were recorded at 50 MHz in $CDCl_3$, using TMS as int. standard [10]. Components were identified by comparison of their retention indices, relative to a homologous series of fatty acid Me esters or to C_9–C_{17} *n*-alkanes, and mass spectra, with those of authentic samples. Identification of compounds with a percentage equal or higher than 1% was also performed by ^{13}C NMR, [18, 19]. The percentage composition of essential oils was computed from the GC peak areas on the two columns, without using correction factors.

Fractionation of essential oil. Essential oil of sample 3 and some individual samples were fractionated by CC on silica gel (Kieselgel 60, 0.2–0.5 mm), using pentane–Et_2O as mobile phase (gradient from 1:0 to 0:1). Frs obtained were analysed by GC-MS and ^{13}C NMR.

trans-*Dihydroagarofuran.* EI-MS, *m/z* (rel. int): 222 $[M]^+$ (9), 207 (100), 189 (30), 149 (31), 137 (76), 109 (53), 55 (40), 43 (45). ^{13}C NMR (50 MHz $CDCl_3$): δ 87.9 (C-1), 40.4 (C-2), 29.4 (C-3), 16.9 (C-4), 37.4 (C-5), 38.5 (C-6), 38.0 (C-7), 25.0 (C-8), 44.6 (C-9), 38.4 (C-10), 17.8 (C-11), 22.9 (C-12), 81.0 (C-13), 30.6 (C-14), 23.6 (C-15).

Epimer of guaiol. EI-MS, *m/z* (rel. int.): 222 $[M]^+$ (6), 204 (60), 189 (68), 161 (100), 105 (60), 59 (55), 43 (33). ^{13}C NMR (50 MHz, $CDCl_3$): δ 19.2, 19.6, 26.2, 27.2 (CH_3); 27.1, 29.0, 31.2, 33.3, 33.8 (CH_2); 34.8, 45.1, 49.8, (CH); 73.8, 140.0, 140.9 (C).

Acknowledgements—The authors are grateful to the 'Ministerio de Educación y Ciencia' (Spain), and Conselho de Reitores das Universidades Portuguesas' (Portugal), the 'Junta Nacional de Investigação Científica e Tecnológica' (Portugal), the 'Services pour le Science et la Technologie de l'Ambassade de France' in Lisbon and in Madrid, that gave financial support under different Portugese–Spanish, the French–Spanish and the French–Portugese Cooperation Programs and to the Centro de Biotecnologia Vegetal-Instituto de Biotecnologia e Química Fina (IBQF)

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