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Chemical Polymorphism of the Essential Oils from Populations of *Thymus caespititius* Grown on the Islands Pico, Faial and Graciosa (Azores)

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The compositions of the essential oils isolated from the aerial parts of 11 populations of *Thymus caespititius* collected during the flowering phase on Pico, Faial and Graciosa (Azores) were studied by GC and GC-MS. The monoterpene fraction was dominant in all the oils analysed (55–90%) and consisted mainly of oxygen-containing compounds (44–79%). Sesquiterpenes represented an important fraction of the oils from the populations grown on Graciosa (13–28%). In contrast, this fraction was rather small in the oils from the populations grown on Pico and Faial (6–11%). Despite this, oxygen-containing compounds (4–18%) were always dominant. Cluster analysis of all identified oil components grouped the oils into three main clusters that corresponded with their main components. The oils from the 11 populations studied showed a clear chemical polymorphism that, in some cases, was more evident among populations growing on the same island than among those from different islands. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: Essential oils; carvacrol; α-terpineol; chemotypes; *Thymus caespititius*; Azores.

INTRODUCTION

Thymus caespititius Brot. is an endemic species of the Lamiaceae that is characteristic of the Atlantic wet zones of the north-western Iberian peninsula, and of the Madeiran and Azorean archipelagos. This species is the only representative of the genus in the Azorean archipelago, where it is commonly known as 'erva-úrsula' (Fernandes Costa, 1945; Sjögren, 1984; Salgueiro, 1994; Pena and Cabral, 1997).

Previous studies have shown that the essential oils from populations of *T. caespititius* collected in the Portuguese mainland (Fernandes Costa, 1945, 1975; Salgueiro, 1994; Salgueiro *et al.*, 1997) were chemically different from those obtained from populations grown on the Azorean islands Pico and Faial, (Salgueiro *et al.*, 1997; Pereira *et al.*, 1999). However, the oils from the insular populations did not show noticeable differences with respect to their main components (Salgueiro *et al.*, 1997; Pereira *et al.*, 1999). As a part of our screening programme of the aromatic flora of Macaronesia, we here report on the compositions of the essential oils from 11 populations of *T. caespititius*: four grown on Pico, three on Faial and four on the island Graciosa (Azores), as indicated in Table 1.

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EXPERIMENTAL

Plant material. Samples of the aerial parts of *T. caespititius* were collected during the full flowering period of the plant (June 1999) from 11 populations growing on the islands Pico, Faial and Graciosa (Azores). A voucher specimen of each population has been deposited in the Herbarium of the Museu, Laboratório e Jardim Botânico de Lisboa (Table 1).

Isolation procedure. The essential oils were isolated from deep-frozen (–20°C) plant material by distillation-extraction for 3 h, using a Likens-Nickerson-type apparatus (Likens and Nickerson, 1964) with *n*-pentane as organic solvent, and by hydrodistillation for 3 h, using a Clevenger-type apparatus (Council of Europe, 1996). The oil samples isolated by hydrodistillation were used to estimate the oil yields whilst those isolated by distillation–extraction were used to determine percentage composition since the chance of artefact formation must be considered smaller when the latter extraction method is used.

Analyses. GC and GC-MS analyses were performed according to Pereira *et al.* (2000). Chiral GC analyses were performed using an FID instrument, a data handling system and a Cyclosil B (Agilent/J&W Scientific, Palo Alto, CA, USA) fused-silica column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The oven temperature was maintained at 105°C; the injector and detector temperatures were 280°C and 290°C, respectively; the carrier gas was hydrogen at a flow of 42 cm/s.

Cluster analysis. The percentage composition of the essential oil samples was used to determine the relationship

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Table 1. Sites of collection of the eleven populations of *Thymus caespititius* studied

Island	Collection site	Population	Altitude (m)	Voucher number
Pico	Fajã	P1	500	LISU:171716
	Pico	P2	1200	LISU:171719
	Testada Nova	P3	650	LISU:171718
	Testada Nova	P4	700	LISU:171721
Faial	Parque Capelo	F1	230	LISU:171730
	Parque Capelo	F2	300	LISU:171730
	Pico Gordo	F3	700	LISU:171731
Graciosa	Vertente da Caldeira	G1	250	LISU:171734
	Serra Branca	G2	350	LISU:171732
	Carapacho	G3	125	LISU:171733
	Gruta da Maria Encantada	G4	300	LISU:171735

between the different populations of *T. caespititius* by cluster analysis using the NTSYS software (Rohlf, 1992). Euclidean distance was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

RESULTS AND DISCUSSION

Essential oils isolated from the aerial parts of different populations of *T. caespititius*, collected during the flowering phase, were obtained in yields ranging from 0.2 to 0.8% (v/w). Thirty-four to 43 components were identified in the eleven oil samples, amounting to a total percentage of 82–96%, which are listed in Table 2 in order of their elution from a DB-1 column.

All of the oils analysed were dominated by their monoterpene fraction (55–90%). In the oils obtained from the populations grown on Pico and Faial, this fraction ranged from 80 to 90% and 79 to 86%, respectively, whereas in those obtained from the populations grown on Graciosa it amounted to 55–80%. The proportion of sesquiterpenes in the oil samples from Pico and Faial (6–7% and 8–11%, respectively) was rather small compared with that recorded for the oils from the populations grown on Graciosa (13–28%).

The populations grown on Pico yielded carvacrol-rich oils (45–57%), but they differed in their second main components: thymol and carvacryl acetate in the oil from population P1 (10 and 9%, respectively), thymol in that from population P4 (12%), and carvacryl acetate in the oils from the populations P2 and P3 (15 and 17%, respectively). Although the populations P3 and P4 were collected from the same area (Testada Nova), the corresponding oils differed markedly not only in their second main components, the proportions of which varied considerably, but also in the proportion of α-terpineol. The sesquiterpene fraction represented only 6–7% of the total oils, T-cadinol (2–3%) being its main component. It is noteworthy that remarkable differences were observed with regard to the amounts of carvacrol, carvacryl acetate and thymol in the oil from population P2 (57, 15 and 2%, respectively) and that reported for an oil sample isolated from plants collected also during the flowering phase at the same site in 1994, (36, 8 and 16%, respectively; Salgueiro *et al.*, 1997).

The populations collected on Faial also yielded carvacrol-rich oils (51–54%); however, α-terpineol (12%) in population F1, *p*-cymene (10%) in population F2, and α-terpineol, *p*-cymene, and carvacryl acetate (5% each) in population F3, were their second main components. The sesquiterpenes attained 8–11% of the total oils, T-cadinol (3–4%) again being the major component of this fraction. Comparing the results obtained for plant material collected in 1999 (populations F1 and F3) with those recorded for two samples collected during the flowering phase of the plant at the same sites in 1998 (Pereira *et al.*, 1999), no remarkable differences could be seen.

The chemical compositions of the essential oils from the populations collected on Graciosa differed markedly from those found for the populations grown on Pico and Faial. In fact, although the oils from the populations G1 and G2 were dominated by carvacrol (35 and 32%, respectively), the relative amount of this component was lower than in the oils from the populations grown on the other two islands under study. In addition, α-terpineol was the second main component of oils G1 and G2 with a relative amount of 15 and 27%, respectively. Comparatively, the amount of α-terpineol in the oils from the populations of Pico and Faial was rather small, ranging from 0.4 to 12%. Moreover, the oils obtained from the populations G3 and G4 were dominated by α-terpineol (33 and 37%, respectively), with carvacrol (24%) and T-cadinol (11%) as the second main components, respectively. The composition of the oil from the population G4 was quite different from that found for the other populations grown either on Graciosa, Pico or Faial. Taking into account the high levels of α-terpineol (37%) and of the sesquiterpene fraction (28%), dominated by T-cadinol (11%), the composition of this oil resembled that reported for populations collected in the Portuguese mainland (Salgueiro *et al.*, 1997).

Cluster analysis of the identified components grouped the oils into three main groups (Fig. 1) corresponding with their main components: carvacrol (populations P1–P4 and F1–F3), carvacrol/α-terpineol (populations G1–G3) and α-terpineol/T-cadinol (population G4). The clusters formed by the populations P1–P4 and F1–F3 possessed carvacrol-rich oils, but the oils from the populations P1 and P4 may better be characterised as carvacrol/thymol-type oils. Likewise, population G1 is clearly separated from the populations G2 and G3, although all three are grouped in the same cluster (Fig. 1). This can be explained by the smaller relative amount of

Table 2. Percentage compositions of the essential oils isolated from aerial parts, collected during the flowering phase, of eleven populations of *Thymus caespititius* grown on Pico, Faial and Graciosa

Component	Retention index[a]	Populations[b]										
		Pico				Faial			Graciosa			
		P1	P2	P3	P4	F1	F2	F3	G1	G2	G3	G4
α-Thujene	924	2.7	1.9	2.0	2.1	1.5	1.9	2.0	1.5	1.5	0.6	0.3
α-Pinene	930	0.8	0.6	0.7	0.6	0.7	0.6	0.7	0.5	0.5	0.3	0.1
Camphene	938	0.1	0.1	0.1	0.1	0.1	0.1	0.1	t[c]	t	t	t
Sabinene	958	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.5	0.4	0.5	1.0
Oct-1-en-3-ol	961								0.9	0.2	0.8	
β-Pinene	963	0.3	0.2	0.3	0.2	0.6	0.6	0.4	t	0.9	t	0.6
Dehydro-1,8-cineole[d]	974			0.1	t	0.1	t	0.1	0.1	0.1	0.1	
β-Myrcene	975			0.5		t	t	t				
α-Phellandrene	995	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	t
Δ3-Carene	1000	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	t	t	t
α-Terpinene	1002	0.9	0.7	0.7	1.0	0.7	0.9	0.8	1.1	1.0	0.7	0.5
***p*-Cymene**	1003	**5.5**	**4.0**	**4.2**	**4.5**	**5.0**	**9.6**	**5.1**	**9.5**	**4.4**	**6.6**	**2.6**
β-Phellandrene	1005	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1
Limonene	1009	0.4	0.2	0.4	0.3	0.4	0.4	0.4	0.6	0.9	0.7	1.1
γ-Terpinene	1035	**2.8**	**2.7**	**2.1**	**2.7**	**2.9**	**3.1**	**3.0**	**8.3**	**6.8**	**4.2**	**4.4**
Terpinolene	1064	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3
cis-Sabinene hydrate	1066			t								
Linalool	1074	t	t					t	0.1	0.1	0.1	0.2
Oct-1-en-3-yl acetate	1086	t	t	0.2	0.1		0.5	0.3	0.4	0.3	0.3	0.1
Borneol	1134		t	0.1	t		0.1	0.1	0.1	0.1		
Terpinen-4-ol	1148	0.8	0.8	0.8	0.8	0.9	1.0	0.7	1.1	0.8	0.9	1.2
α-Terpineol	1159	**4.0**	**2.7**	**0.4**	**4.6**	**11.8**	**6.3**	**5.3**	**15.3**	**27.2**	**32.5**	**36.7**
Carvone	1206	0.1	0.1	0.2	0.1	0.2	0.4	0.2	t	0.1	t	t
Carvacrol methyl ether	1224		t	t		1.0	0.3		0.4	0.2		0.2
Thymol	1275	**9.7**	**2.4**	**1.4**	**12.1**	**0.7**	**4.6**	**0.1**	**0.1**	**0.6**	**1.1**	**1.4**
Carvacrol	1286	**48.1**	**56.8**	**48.5**	**44.9**	**51.1**	**51.3**	**54.0**	**35.0**	**32.0**	**24.3**	**3.3**
Thymyl acetate	1330	2.9	0.6	0.3	3.6	0.5	1.1			0.2	0.2	0.2
Carvacryl acetate	1348	**9.1**	**15.4**	**17.0**	**1.6**	**3.2**	**3.2**	**4.9**	**4.2**	**1.6**	**2.5**	**0.4**
α-Copaene	1375								t		t	0.1
β-Elemene	1388	0.1	0.1	0.1	0.1		t	0.1	t		t	0.1
β-Caryophyllene	1414	0.1	0.1	0.1	t	t	t	0.1	0.1	1.1	0.1	0.2
α-Humulene	1447								0.1		t	0.1
allo-Aromadendrene	1454					0.3			0.5	0.3	0.7	1.3
Germacrene-D	1474						t	t	0.1	t	t	t
trans-Dihydroagarofuran	1489	0.8	0.6	0.9	0.7	2.0	2.2	2.7	1.7	1.0	2.0	3.4
α-Muurolene	1494	t		0.1	t				0.4	0.3	0.3	0.3
γ-Cadinene	1496	0.7	0.6	1.0	0.7	1.3	0.9	1.3	1.5	2.2	2.0	3.7
Calamenene	1505	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.3
δ-Cadinene	1505	0.3	0.3	0.5	0.4	0.3	0.3	0.5	1.5	1.1	1.0	1.9
Kessane[d]	1517	0.3	0.3	0.3	0.3	0.9	1.1	1.0	0.8	0.4	0.9	1.3
α-Cadinene	1529	0.3	t			0.1	t	t	0.1	0.1	0.1	0.2
Elemol	1530	0.1	0.3		0.3				t		t	0.2
epi-Cubenol	1600								0.1	t	0.1	0.1
T-Cadinol	1616	**2.3**	**2.2**	**3.3**	**2.5**	**4.1**	**3.0**	**4.4**	**4.3**	**6.4**	**7.0**	**11.2**
δ-Cadinol	1618			t					0.2	0.1		t
β-Eudesmol	1620	0.2	0.3		0.3						t	0.2
α-Cadinol	1626	0.7	0.8	0.9	1.0	0.5	0.8	0.7	1.3	1.1	1.2	3.0
Identified components (%)		95.2	95.6	88.2	86.5	91.9	95.2	90.0	93.2	94.7	92.4	82.4
Grouped components (%)												
Monoterpene hydrocarbons		14.5	11.1	11.9	12.3	12.7	18.0	13.4	22.6	16.9	14.0	11.0
Oxygen-containing monoterpenes		74.7	78.8	68.8	67.7	69.5	68.3	65.3	56.4	63.0	61.7	43.7
Sesquiterpene hydrocarbons		1.9	1.5	2.2	1.6	3.1	2.4	3.2	5.3	5.7	5.3	9.5
Oxygen-containing sesquiterpenes		4.1	4.2	5.1	4.8	6.6	6.0	7.8	7.6	8.6	10.3	18.1
Others				0.2	0.1		0.5	0.3	1.3	0.5	1.1	0.1
Oil yield (%, v/w)		0.5	0.7	0.2	0.5	0.8	0.5	0.3	0.4	0.4	0.6	nd[e]

[a] Relative to C_9-C_{17} *n*-alkanes on a DB-1 column.
[b] Collection sites as in Table 1.
[c] t = trace (<0.05%).
[d] Identification based on MS only.
[e] nd = not determined.

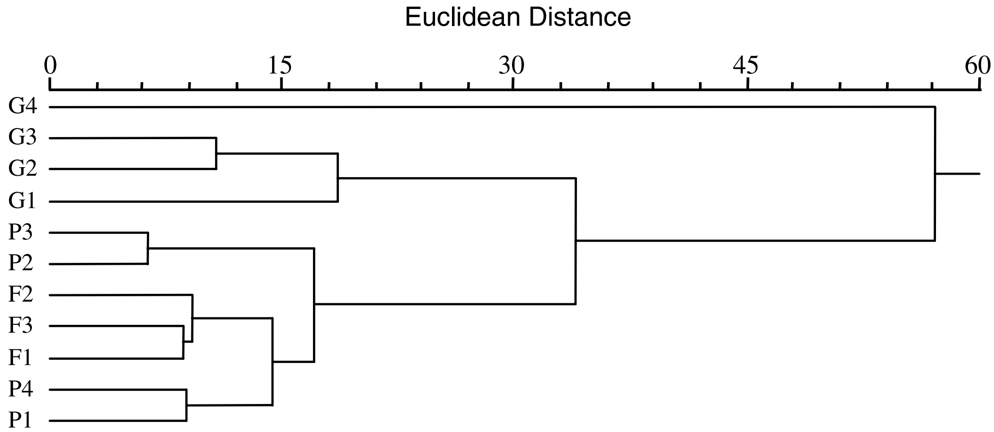

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from 11 populations of *Thymus caespititius*, based on Euclidean distance and using the unweighted pair-group method with arithmetic average (UPGMA).

Table 3. Enantiomeric percentage composition of α-terpineol in the essential oils from populations of *Thymus caespititius* grown on Pico, Faial and Graciosa

	Populations									
Component	P1	P2	P4	F1	F2	F3	G1	G2	G3	G4
(−)-α-Terpineol	3.4	3.3	3.2	3.0	3.0	3.1	2.9	11.7	14.4	25.0
(+)-α-Terpineol	96.6	96.7	96.8	97.0	97.0	96.9	97.1	88.3	85.6	75.0

α-terpineol in the oil from population G1 than in those from the other two populations in question.

Of the chiral compounds present in the oils, only α-terpineol was investigated with respect to its enantiomeric ratio because of its high concentration in the oil samples from some populations. Although (+)-α-terpineol was the predominant enantiomer in all oils analysed (Table 3), its purity showed a considerable variation (75–97%). In the oils from the populations grown on Pico and Faial and in that from population G1, collected on Graciosa, this compound was detected with a high enantiomeric purity (97%), whereas in the oils from the populations G2, G3 and G4 the (+)-enantiomer attained only 88, 86 and 75%, respectively (Table 3).

Although the populations of *T. caespititius* show a clear chemical polymorphism, no correlation was found between the altitudes of the collection sites (Table 1) and the chemical compositions of the oils. In view of this, the polymorphism recorded in the present study may result either from the genetic variability of the populations or from the influence of edaphic factors.

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