The Main Citral−Geraniol and Carvacrol Chemotypes of the Essential Oil of *Thymus pulegioides* L. Growing Wild in Vilnius District (Lithuania)

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Thymus pulegioides L. with lemon and carvacrol odor form the major part of plants growing wild in all 10 investigated localities during 1995−1997. The main components of the citral-geraniol chemotype of lemon-scented essential oil are the following (%): geraniol (14.9−30.8), geranial (*trans*-citral, 9.7−19.7), β-caryophylene (6.0−11.4), nerol (4.1−11.8), and neral (*cis*-citral, 0.1−9.5). The essential oil of carvacrol chemotypes contain more compounds that are characteristic of the thyme genus (%): carvacrol (16.0−22.2), β-bisabolene (11.1−20.2), β-caryophyllene (11.1−19.1), γ-terpinene (5.8−16.2), *p*-cimene (5.5−10.4), thymol (3.3−9.8), and carvacrol methyl ether (5.6−8.6). The correlation between the odor and composition of the essential oil will help the users of wild thyme to choose the necessary chemotype for their purposes.

Keywords: *Thymus pulegioides L.; chemotypes of essential oil; citral−geraniol chemotype; carvacrol chemotype*

INTRODUCTION

Some examples of chemical polymorphism of *Thymus pulegioides* L. essential oil has been reviewed (Stahl, 1991). Thymol and carvacrol chemotypes of the essential oil were found in Norway and Romania and thymol, citral, linalyl acetate, and fenchone chemotypes in Czeckoslovakia. Thymol and carvacrol chemotypes of essential oil were later found in Yugoslavia (Kustrak et al., 1990). Geraniol (44.6%) and linalool (28.61%) were the main components of the essential oil from plants grown wild in Croatia (Mastelic et al., 1992). Five chemotypes of the essential oil were found in Slovakia (Màrtonfi, 1992; Màrtonfi et al., 1994). Only in 2 of the 12 localities investigated in Slovakia grew plants forming essential oils of carvacrol, thymol, citral−geraniol, linalool, and fenchone chemotypes. Thymol chemotype was found in Italy (Senatore, 1996) and citral−geraniol in Lithuania (Mockute et al., 1998). The above data showed that more chemotypes of *T. pulegioides* L. essential oil were found in central Europe than in southern and northern Europe. The growing area of chemotypes could not be determined from the available data. Further investigations in different countries would reveal the necessary conditions for growth of *T. pulegioides* L. of every chemotype.

The essential oils of thyme are used for their aromatic and medicinal values (Senatore, 1996). Thyme plants are widely used as a culinary herb. The essential oil of the thyme is used for flavoring and preserving different food products (Senatore, 1996; Reddy et al., 1998). It may be used as a natural antioxidant and for the prevention of lipid radiolysis (Lacroix et al., 1997; Zygadio et al., 1995). Carvacrol and the essential oil of carvacrol chemotype are natural insecticides (Ahn et al., 1998; Karpouhtsis et al., 1998) and have a strong antibacterial activity (Sivropolou et al., 1996). The above properties depend on the composition of the essential oil. Investigation of the essential oil composition will help to determine the range of *T. pulegioides* L. for diverse applications.

EXPERIMENTAL PROCEDURES

Materials. The odor of 40−100 *T. pulegioides* L. plants was investigated in 10 localities. Plants with lemon and carvacrol odor were collected in 1995−1997 in four localities. The weight of the collected sample was ~1 kg. The essential oil was prepared by hydrodistillation of 100 g of air-dried plants (Senatore, 1996).

GC and GC-MS. A Hewlett-Packard (HP) model 5890 II gas chromatograph equipped with an integrator HP-3396, a flame ionization detector, and fused silica capillary column (25 m × 0.2 mm) coated with FFAP stationary phase was used for routine analysis. The GC oven temperature was 70 °C for 10 min and then programmed from 70 to 210 °C at a rate of 3 °C/min using He as the carrier gas (0.7 mL/min.). Injector and detector temperatures were 200 and 250 °C, respectively.

The HP 5890 II GC was interfaced to a HP 5971A mass spectrometer (ionization voltage 70 eV) and used for MS identification of the GC components using a capillary column (30 m × 0.25 mm) coated with DB-5 stationary phase. The GC-MS oven temperature was 40 °C for 1 min and then programmed from 40 to 230 °C at a rate of 4 °C/min using He as the carrier gas (0.8 mL/min.). Injector and detector temperatures were 250 and 280 °C, respectively.

The percentage composition of the essential oils was computed from GC peak areas without using correction factors. Qualitative analysis was based on a comparison of the retention times and of the mass spectra with the corresponding data of components of reference oils and data in the literature (Chung et al., 1993; Zenkevich, 1996, 1997). Mass spectra were compared with those of mass spectra libraries (RPL.L; HPPEST.L; NBS 75K.L).

RESULTS AND DISCUSSION

The major part of *T. pulegioides* L. plants have lemon or carvacrol odor in 10 investigated localities of the Vilnius district. The ratio of plants with different odors depended on growing conditions. Lemon-scented plants

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Table 1. Compounds (%) Found in Essential Oil from Lemon-Scented *Thymus pulegioides* L. Growing Wild in Vilnius District (Lithuania)

				location								
		I		Sapiegyne			Rokantishkes			A[b] Virshupis		
peak no.[a]	compound	FFAP	DB-5	1995	1996	1997	1995	1996	1997	1995	1996	1997
1	γ-terpinene	1274	1060	0.3	2.8	0.1	0.1	1.0	1.6	1.3	1.1	0.3
2	*p*-cymene	1301	1025	1.7	2.2	0.2	0.1	1.8	1.1	1.5	1.8	0.7
3	linalool	1548	1102	0.8	9.9	0.8	9.1	13.7	0.4	5.8	6.6	1.1
6	β-caryophyllene	1667	1425	7.6	11.4	6.0	7.6	9.0	9.3	7.4	10.9	11.4
7	neral (*cis*-citral)	1695	1234	0.5	0.1	8.8	1.1	3.1	9.5	3.5	8.9	7.5
8	geranial (*trans*-citral)	1752	1263	19.7	18.4	19.5	9.7	13.9	16.1	12.4	16.8	16.9
9	geranyl acetate	1785	1375	0.4	4.3	2.9	2.6	3.0	2.1	6.6	1.9	3.0
10	β-bisabolene	1798	1507	tr	0.1	7.5	tr	<0.1	6.8	<0.1	tr	5.9
11	nerol	1818	1226	4.4	4.1	8.5	5.7	4.2	5.6	4.7	5.2	11.8
12	geraniol	1864	1250	30.8	14.9	27.0	23.4	16.3	29.2	23.2	16.1	24.2
13	caryophyllene oxide	2068	1580	2.3	3.8	4.4	8.1	5.0	2.4	3.5	5.5	4.3
14	thymol	2196	1293	0.6	0.5	0.2	1.1	0.3	1.1	0.6	0.2	0.7
15	carvacrol	2235	1303	2.8	2.8	2.0	5.6	1.9	4.4	2.7	3.7	3.4
	sum			71.9	75.3	87.9	74.2	73.2	89.6	73.3	78.7	91.2
A	geranial + neral			20.2	18.5	28.3	10.8	17.0	25.6	15.9	72.5	24.4
B	geraniol + nerol			35.2	19.0	35.5	29.1	20.5	34.8	27.9	21.3	36.0
	A + B			55.4	37.5	63.8	39.9	37.5	60.4	43.8	47.0	60.4
	oxygen-containing monoterpenes +14+15			60.0	55.0	69.7	58.3	56.4	68.4	59.5	59.4	68.2

[a] Peak number refers to Figure 1. [b] Antakalnis.

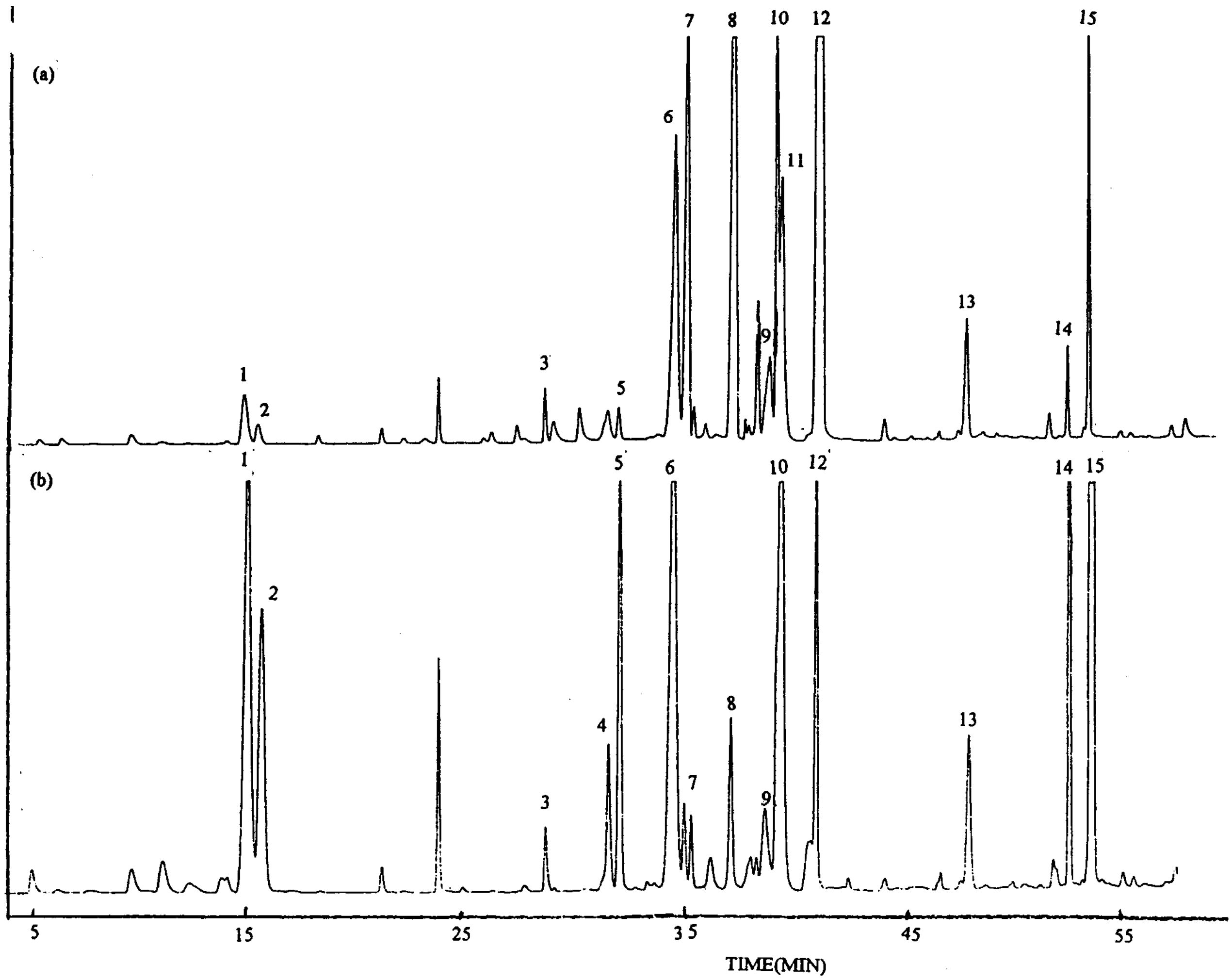

Figure 1. Gas chromatograms (column FFAP) of *Thymus pulegioides* L. essential oils with (a) lemon and (b) carvacrol odor. Peak numbers correspond to those in Tables 1 and 3.

preferred sunny and windless localities. This preference has also been reported in Slovakia (Màrtonfi et al., 1994).

Thirteen compounds given in Table 1 (Figure 1a) were found in all of the investigated lemon-scented essential oils. The main component of six essential oils from plants collected in the sunny season of 1995 and 1997 was geraniol (Figure 1a, peak 12, Table 1, 23.2−30.8%). Nearly equal quantities of aliphatic alcohols (geraniol + nerol) and aliphatic aldehydes (geranial + neral) were

Table 2. Comparison of Essential Oil Composition (%) of *Thymus pulegioides* L. Growing Wild in (A) Eastern Slovakia (Màrfonfi, 1992) and (B) Vilnius District (Lithuania)

	chemotypes			
	citral−geraniol		carvacrol	
compound	A	B	A	B
γ-terpinene	0.0−0.7	0.1−2.8	14.7−19.7	5.8−16.2
p-cymene	0.0−1.1	0.1−2.2	7.3−10.7	5.5−10.4
linalool	2.6−5.6	0.4−13.7	0.2−2.2	0.3−0.7
β-caryophyllene	11.3−17.9	6.0−11.4	14.4−18.8	11.1−19.1
citral	23.0−35.2	10.8−28.3	8.0−16.6	0.8−3.1
geraniol	16.8−28.0	14.9−30.8	0.0−0.2	1.3−5.7
thymol	0.0−1.0	0.2−1.1	0.2−1.4	3.3−9.8
carvacrol	0.0−7.1	1.9−5.6	28.9−36.9	15.0−22.2

found in essential oils from plants collected in the colder season of 1996 (Table 1). The lemon odor of plants and essential oil was due to geranial (*trans*-citral, peak 8, 9.7−19.7%) and neral (*cis*-citral, peak 7, 0.1−9.5%). The essential oil of *T. pulegioides* L. with the main components geraniol and citral was named as citral−geraniol chemotype in Slovakia (Màrtonfi, 1992, Table 2). All nine investigated lemon-scented essential oils may be attributed to citral−geraniol chemotype (Tables 1 and 2). The compounds given in Table 1 make up 73.2−91.2% of the lemon-scented essential oil. The aliphatic aldehydes (geranial + neral) make up 10.8−28.3% and aliphatic alcohols (geraniol + nerol) about 19.0−36.0%. The sum of the main aliphatic aldehydes and alcohols may be from 37.5% to 63.8%. Oxygen-containing monoterpenes and phenols form more than one-half of the lemon-scented essential oil.

The quantities of essential oil components from plants with lemon and carvacrol odors were very different (Tables 1 and 3), except for β-caryophyllene (Figure 1, peak 6) and caryophyllene oxide (peak 13). The above sesquiterpene and its oxide in lemon-scented essential oil make up 6.0−11.4% and 2.3−8.1%, and in essential oil with a carvacrol odor, they make up 11.1−19.1% and 2.6−5.4% (Tables 1 and 3). The quantities of β-bisabolene (peak 10) and other identified sesquiterpene hydrocarbons are lower than those of β-caryophyllene in lemon-scented essential oil. The quantity of β-bisabolene in essential oil with carvacrol odor exceeded the quantity of β-caryophyllene in seven samples from the nine investigated (Table 3). Four hydrocarbons, two monoterpenes (γ-terpinene, *p*-cimene), and two sesquiterpenes (β-caryophyllene, β-bisabolene) form 40−50% of the above essential oil. The quantity of all terpene hydrocarbons makes up >50% of the essential oil with carvacrol odor (Table 3), while the major part (55−70%) of lemon-scented essential oil was formed from oxygen-containing monoterpenes and phenols (Table 1). The oxygen-containing monoterpenes and phenols make up <45% of the essential oil with a carvacrol odor (Table 3). Carvacrol forms 16.0−22.2% of essential oil (Figure 1, peak 15). The other aromatic compounds found are thymol (peak 14, 3.3−9.8%), ethers of phenols (peaks 4 and 5, 7.3−10.4%), and *p*-cymene (peak 2, 5.0−10.4%). γ-Terpinene (peak 1, 5.2−16.6%) is the precursor of both *p*-cymene and phenols. The sum of phenols, ethers, and precursors of phenols (γ-terpinene + *p*-cymene) is 44.2−64.3%. Only in three samples of the nine investigated did the above compounds exceed 50% (Table 3). Twelve compounds given in Table 3 formed 80.4−96.5% of essential oil. All nine samples of essential oil from plants with carvacrol odor may be attributed to carvacrol chemotype (Tables 2 and 3). The investigated plants contained less carvacrol and more thymol than plants of Eastern Slovakia (Màrtonfi, 1992, Table 2). The antimicrobial properties of these essential oils and plants depend on both monoterpene phenols carvacrol and thymol (Senatore, 1996; Sivropoulou et al., 1996).

Besides the 15 compounds given in Tables 1 and 3, 16 more were positively identified and 12 were tentatively identified. The number of peaks in essential oil chromatograms varied between 35 and 75. Only one essential oil of citral−geraniol chemotype contains ≥106 compounds (Sapieginë, 1996).

The quantity of monoterpene hydrocarbons without γ-terpinene and *p*-cymene makes up 0.5−6.0% of essential oils. Nine positively identified hydrocarbons were the following (%): α-tujene (0−0.9), α-pinene (0−0.5), camphene (0−0.1), β-pinene (0−0.5), mircene (0−0.9),

Table 3. Compounds (%), Characteristic for Thyme Genus, Found in Essential Oil from *Thymus pulegioides* L. with Carvacrol Odor Growing Wild in Vilnius District (Lithuania)

				location								
		RI		Sapiegine			Rokantishkes			Virshupis		
peak no.[a]	compound	FFAP	DB-5	1995	1996	1997	1995	1996	1997	1995	1996	1997
1	γ-terpinene	1274	1060	9.2	5.8	16.6	5.9	14.5	9.1	5.8	7.2	6.1
2	*p*-cymene	1301	1025	5.0	5.9	7.1	6.1	10.1	10.4	5.5	10.0	5.0
3	linalool	1548	1102	0.7	0.3	0.5	0.6	0.4	0.5	0.3	0.4	0.5
4	thymol methyl ether	1595	1229	1.4	3.1	1.7	2.4	1.1	2.1	2.5	2.7	2.4
5	carvacrol methyl ether	1614	1236	7.0	7.3	8.6	7.2	6.2	5.9	7.0	6.6	5.6
6	β-caryophyllene	1667	1425	12.1	15.2	11.1	15.8	11.4	12.4	19.1	15.6	15.4
8	geranial	1752	1263	1.6	1.4	0.8	2.4	1.1	3.1	1.1	1.4	1.0
10	β-bisabolene	1798	1507	16.3	18.9	12.5	11.1	15.2	11.4	20.2	18.4	17.5
12	geraniol	1864	1250	5.1	3.5	1.7	2.7	1.3	5.7	3.6	3.3	4.3
13	caryophyllene oxide	2068	1580	2.6	4.5	2.7	3.6	2.6	5.4	3.9	2.9	2.9
14	thymol	2196	1293	5.5	6.2	9.8	6.6	4.3	8.3	6.4	3.3	7.5
15	carvacrol	2235	1303	19.4	16.4	20.5	16.0	25.5	22.2	17.2	19.3	21.5
	sum			85.9	88.5	93.6	80.4	93.7	96.5	92.6	91.1	89.7
A	carvacrol + thymol			24.9	22.6	30.3	22.6	29.8	30.5	23.6	22.6	29.0
B	ethers of phenols			8.4	10.4	10.3	9.6	7.3	8.0	9.5	9.3	8.0
C	γ-terpinene + *p*-cymene			14.4	11.7	23.7	12.0	24.6	19.5	11.3	17.2	11.1
	A + B + C			47.5	44.7	64.3	44.2	61.7	58.0	44.4	49.1	48.1
D	β-bisabolene + β-caryophyllene			28.4	34.3	23.6	26.9	26.5	23.8	39.3	34.0	32.9
	C + D			42.6	46.0	47.3	38.9	51.1	43.3	50.6	51.2	44.0
A + B + oxygen-containing monoterpenes				39.1	36.8	42.8	35.5	38.8	44.7	37.0	35.6	41.8

[a] Peak numbers refer to Figure 1.

δ-3-carene (0−0.1), and α-terpinene (0−1.8). Sabinene, β-phellandrene, and terpinolene were only tentatively identified. Their content did not exceed 0.1%.

Two aliphatic alcohols, two monoterpene alcohols, and three sesquiterpene hydrocarbons were positively identified (%): 1-octen-3-ol (0−1.9), octan-3-ol (0−0.4), borneol (0−0.9), terpinen-1-ol (0−0.5), α-humulene (0−0.7), β-cubebene (0−2.3), and δ-cadinene (0−0.7). The following compounds were tentatively identified (%): α-terpineol (0−0.2), linalyl propionate (0−0.3), α-copaene (0−0.4), aromadendrene (0−0.1), spathulenol (0−1.3), nerolidol (0−0.9), and cadinol (0−0.2). All 43 identified compounds have been found earlier in thyme essential oils (Stahl, 1991; Màrtonfi, 1992; Senatore, 1996).

The composition of essential oils of plants collected according to their odor in different localities of the Vilnius district in 1995−1997 was similar (Tables 1 and 3). The correlation between the odor of *T. pulegioides* L. plants and composition of essential oils will help the users of wild thyme to choose the necessary chemotype for diverse industrial applications.

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