

A New *cis*-Sabinene Hydrate Chemotype Detected in Large Thyme (*Thymus pulegioides* L.) Growing Wild in Denmark

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Abstract

Foliage was collected from flowering individuals of large thyme (*Thymus pulegioides* L.) at seven localities in southern Denmark. The leaf material was soaked in 96% ethanol and subsequently analyzed for terpenes using GC and GC/MS. Five chemotypes were detected: carvacrol, linalool, geraniol and thymol, all of which are known to appear in large thyme. In addition, individuals containing the monoterpene *cis*-sabinene hydrate as the dominant component of their essential oil were found. A sabinene hydrate chemotype has not previously been detected in large thyme, although it does occur in other thyme species.

Key Word Index

Thymus pulegioides, Lamiaceae, extract composition, *cis*-sabinene hydrate, chemotype.

Plant Name

Thymus pulegioides L. (Lamiaceae).

Source

Large thyme occurs in Denmark at the Northwestern limit of its distribution range. It is a rare species in Denmark restricted to the Southeastern part of the country, where the climate is more continental than is the case for the rest of Denmark. We chose seven localities representative of that area, with three in Jutland, two on Funen, one on Zealand and one on Moenen. The collections were made in 2005, during full flowering.

Plant Material

Terpene determinations were made using the leaves from large thyme. Shoot tips (upper 1 cm) were collected from 20 individuals from each of the seven sites, except Jutland 1 and 3, where population sizes were too small for this number to be reached. Terpenes were extracted by submersing each sample in 1.5 mL of 96% ethanol for up to 30 days prior to analysis.

Previous Work

Six different chemotypes have been described in large thyme growing wild in Europe. An overview of the literature

reveals that only the thymol chemotype is found in southern Europe, although it also occurs elsewhere – it is absent only in Lithuania (1–3). A rarer α -terpineol/ α -terpinyl acetate chemotype has been found only in Lithuania and Serbia (1,4,5). The linalool/linalyl acetate, carvacrol and geraniol chemotypes are widespread in most of Europe (1) and the fenchone chemotype has only been found in Slovakia (1,6,7).

Present Work

Initial analyses of the extracted samples were carried out with a Hewlett Packard 6890 gas chromatograph (GC), equipped with a flame ionization detector (FID) and helium as the carrier gas at a flow rate of 1.3 mL/min. Injector and detector temperatures were set at 260°C and 250°C, respectively. A DB-Wax capillary column (15 m \times 0.25 mm I.D., film thickness 0.25 μ m; J&W Scientific) was installed in the GC. The oven profile consisted of an isothermal hold at 50°C for 4 min, followed by a ramp of 8°C/min to 68°C, then a second ramp to 240°C at 25°C/min. Five μ L of each sample was injected in the split mode with a split flow ratio of 50:1. Compounds were identified by retention time comparisons with injections of known pure standards (all standards from Sigma-Aldrich).

Additional analyses were done on the two *cis*-sabinene hydrate containing plants using an Agilent 6890N GC coupled with an Agilent 5975 inert mass selective detector with an ion

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source of 70.0 eV at 230°C. Helium was also used as the carrier gas at a flow rate of 1.0 mL/min, with the injector temperature set at 260°C. One µL of each sample was injected in the splitless mode using two columns with different polarities. The first column was an EC-Wax capillary column (30 m × 0.25 mm I.D., film thickness 0.25 µm; Alltech Associates, Inc.) with oven conditions that included an isothermal hold at 60°C for 5 min, followed by a ramp of 10°C/min to 250°C. Next, both samples were also analyzed using a HP-5MS capillary column (30 m × 0.25 mm I.D., film thickness 0.25 µm; Aligent Technologies, Inc.) with an initial oven temperature of 60°C, followed by a ramp of 3°C/min to 246°C. Terpenes were identified using retention times of standards, the NIST 2005 mass spectral library and/or Adams (8).

Results And Discussion

We found the *cis*-sabinene hydrate chemotype individuals in populations from two of the Jutland localities in southern Denmark, which were otherwise composed of all carvacrol chemotypes. The compounds detected in the two *cis*-sabinene hydrate chemotype plants are shown in Table I. In the remaining five localities, we found carvacrol, thymol, linalool and geraniol chemotypes (Table II), all of which are already known from this species (1). Besides our new finding in *T. pulegioides*, other thyme species with sabinene hydrate chemotypes are known to

Table II. Chemotypes detected in leaves of *Thymus pulegioides* at seven different sites in Denmark

Site	N	Chemotypes
Jutland 1	2	carvacrol (1), <i>cis</i> -sabinene hydrate (1)
Jutland 2	20	carvacrol (19), <i>cis</i> -sabinene hydrate (1)
Jutland 3	1	carvacrol (1)
Funen 1	19	carvacrol (17), thymol (2)
Funen 2	20	carvacrol (18), linalool (2)
Zealand	20	carvacrol (19), geraniol (1)
Moenen	20	thymol (18), geraniol (2)

N: number of plants analyzed. In brackets: number of plants of each chemotype.

occur in other parts of Europe, including in Portugal (*T. carnosus*, *T. villosus*), Spain (*T. x arundanus*, *T. baeticus*, *T. vulgaris*, *T. willkommii*, *T. zygis*), France (*T. vulgaris*), Great Britain (*T. praecox*), Austria (*T. praecox*), Macedonia (*T. macedonicus*, *T. tosevii*), and Turkey (*T. fedtschenkoi*, *T. striatus*) (1). Our study revealed that not only does the compound *cis*-sabinene hydrate occur as the major component of the oil in yet another thyme species; it also occurs in the northern part of continental Europe, where it has not been found previously.

Table I. Percentage composition of the compounds found in the two *Thymus pulegioides cis*-sabinene hydrate chemotype plants

Compound	Locations	
	Jutland 1	Jutland 2
δ-3-carene	1.9	2.9
myrcene	3.9	8.7
unknown A	1.9	3.7
limonene	2.4	3.5
β-phellandrene	1.8	3.3
γ-terpinene	3.6	6.8
<i>cis</i> -sabinene hydrate	63.2	62.1
terpinen-4-ol	0.4	-
β-caryophyllene	4.1	-
aromadendrene	2.1	-
α-terpineol	2.6	2.2
β-bisabolene	6.5	2.3
germacrene D-4-ol	3.3	4.5
thymol	1.4	tr
carvacrol	1.1	tr

Mass spectral fragmentation pattern for unknown A: GC/MS, 70 eV, 230°C, m/z (rel. int.): 136[M]⁺ (53), 121(100), 93(79), 91(45), 79(26), 77(31), 75(23), 45(23), 31(26), 18(29); tr = trace amount

References

1. E. Stahl-Biskup, *Essential oil chemistry of the genus Thymus – a global view*. In: *Thyme: the genus Thymus*. Edits., E. Stahl-Biskup and F. Saez, pp. 75–124, Taylor and Francis: London. (2002)
2. L. Salgueiro, R. Vila, X. Tomas, F. Tomi, S. Canigueral, J. Casanova, A.P. Dacunha, and T. Adzet, *Chemical polymorphism of the essential oil of Thymus carnosus from Portugal*. *Phytochemistry*, **38**, 391–396 (1995).
3. F. Senatore, *Influence of harvesting time on yield and composition of the essential oil of a thyme (Thymus pulegioides L.) growing wild in Campania (southern Italy)*. *J. Agric. Food Chem.*, **44**, 1327–1332 (1996).
4. D. Mockute and G. Bernotiene, *The α-terpinyl acetate chemotype of essential oil of Thymus pulegioides L.* *Biochem. Syst. Ecol.*, **29**, 69–76 (2001).
5. L. Karuza-Stojakovic, *Composition and yield of essential oils of various species of the genus Thymus L.* *Arh. Farm.*, **39**, 105–111 (1989).
6. P. Martonfi, *Polymorphism of essential oil in Thymus pulegioides subsp. chamaedrys in Slovakia*. *J. Essent. Oil Res.*, **4**, 173–179 (1992).
7. P. Martonfi, A. Grejtovsky, and M. Repcak, *Chemotype pattern differentiation of Thymus pulegioides on different substrates*. *Biochem. Syst. Ecol.*, **22**, 819–825 (1994).
8. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th Ed. Allured Publishing Corp., Carol Stream, IL (2007).