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ESSENTIAL OILS FROM FRUITS OF THREE TYPES OF *THAPSIA VILLOSA*

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Key Word Index—*Thapsia villosa*; *T. minor*; *T. laciniata*; Apiaceae; fruits; essential oils; geranyl acetate; chemotypes; ploidy.

Abstract—*Thapsia villosa* has been divided into five types and previous analyses of the essential oils from the fruits of two of these types showed that limonene and methyl eugenol were the major constituents. The composition of the essential oils from the fruits of the other three types of *T. villosa*, with the chromosome numbers $2n = 22$ ($2x$), $2n = 22$ ($2x$) and $2n = 44$ ($4x$), is reported here. The oil from all three types shows a similar chemical profile, with geranyl acetate as the main constituent accounting for 78–92% of the total oil. The composition of the essential oils from these plants is clearly different from the first two types of *T. villosa* mentioned, and also from the other species within the genus *Thapsia*. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Species of the Apiaceae are widely distributed in the Mediterranean area, where they are often used commercially as spices or drugs because of the presence of useful secondary metabolites. The most characteristic constituents are coumarins, essential oils and sesquiterpene lactones [1–3].

Among the genera belonging to the Apiaceae, *Thapsia* has, in recent years, been the subject of a great deal of interest. Intensive chemotaxonomic studies have been performed in order to investigate the distribution of specific bioactive polyoxygenated guaianolides, named thapsigargins. Thapsigargins have been proved to be selective and very potent $Ca(2^+)$-ATPase inhibitors; they are currently valuable tools in the study of calcium homeostasis [4–6].

According to *Flora Europaea* [7], the genus is divided into three species: *T. garganica* L. (syn. *T. transtagana* Brot.), *T. maxima* Miller and *T. villosa* L. However, another two species, *T. minor* Hoffgg. and Link and *T. laciniata* Rouy, were formerly described as being different from *T. villosa* [8].

Previous studies [9–15] on the secondary metabolites of the genus *Thapsia* have shown clear variations between, and also within the species. Chemotaxonomic studies have revealed that *T. garganica* and *T. transtagana* are separate species and that *T. maxima* includes two morphological types. The most pronounced heterogeneity was found among plants identified as *T. villosa*. In a recent publication [16], *T. villosa* was divided into two distinctly different groups, 1 and 2. Group 1 includes three types (1–3) with the chromosome numbers $2n = 22$ ($2x$), $2n = 22$ ($2x$) and $2n = 44$ ($4x$), respectively. None of the three types contain thapsigargins, whereas thapsigargins are characteristic constituents of the two types, 4 and 5, within group 2. Types 4 and 5 are polyploid plants with the chromosome number $2n = 44$ ($4x$) and $2n = 66$ ($6x$), respectively. The two diploid types within group 1 of *T. villosa* were considered as being identical to *T. minor* (type 1) and *T. laciniata* (type 2). Tetraploid type 3 was instead described with morphological characters similar to both types 1 and 2 [16]. Typical metabolites isolated from types 1–3 are different sesquiterpenes, especially hydroindene derivatives, named thapsans [11, 17–20]. Nevertheless, results from the analysis of the composition of the essential oils of the fruits from *T. garganica* [21] and *T. transtagana* [15], *T. maxima* [22] and *T. villosa*, group 2 [23] have also been found to be of chemotaxonomic value in separating the taxa within the genus.

As an extension of the chemical investigation of the genus *Thapsia*, we report the composition of the essential oils from the fruits of three types (1–3) within group 1 of *T. villosa*. The chemotaxonomic relationships between the different species and types of *Thapsia* is also discussed.

RESULTS AND DISCUSSION

The results of GC and GC–EI-mass spectral analysis of the essential oils obtained by hydrodistillation of the

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Table 1. Composition (%) of essential oils from fruits of three types of *Thapsia villosa*

	Type 1 ($2n = 22$*)	Type 2 ($2n = 22$)			Type 3 ($2n = 44$)	
Components	88.30†	90.01	77.01	81.04	88.25	88.26
α-Pinene	–	0.02	t‡	t	t	t
β-Pinene	–	0.12	0.06	0.05	t	0.08
Sabinene	–	0.10	0.05	0.13	t	–
Myrcene	–	0.10	0.10	0.13	0.06	0.13
Limonene	3.12	0.15	5.88	8.30	0.19	0.38
β-Phellandrene	–	0.10	0.10	t	0.20	–
γ-Terpinene	–	0.47	1.05	0.53	0.18	0.79
p-Cymene	0.56	0.70	3.09	0.36	2.62	0.76
cis-Linalool oxide	0.60	0.20	0.03	0.03	4.82	0.05
trans-Linalool oxide	0.38	0.10	0.04	0.02	2.92	t
Benzaldehyde	–	0.06	–	–	–	–
Linalool	0.43	1.20	2.47	5.10	4.19	1.30
Terpinen-4-ol	–	0.03	0.02	0.03	–	–
Linalyl acetate	–	–	–	–	t	0.06
trans-p-Mentha-2,8-dien-1-ol	0.11	–	–	–	–	–
cis-p-Mentha-2,8-dien-1-ol	0.12	–	–	–	–	–
Geranial	0.06	–	–	–	t	–
Methylcarvacrol	–	–	–	–	t	0.10
Carvone	0.07	–	–	–	t	t
Neryl acetate	–	0.10	0.02	0.17	0.06	–
Geranyl acetate	92.19	88.47	85.63	81.72	78.55	88.38
Nerol	–	–	–	–	–	0.16
trans-Carveol	0.76	–	–	–	–	–
Geraniol	1.02	1.08	1.17	1.54	5.25	7.59
cis-Carveol	0.20	–	–	–	–	–
Caryophyllene oxide	–	0.10	0.26	t	0.73	0.11
Methyl eugenol	0.38	0.40	0.03	0.09	–	0.05
Methylionone	0.10	0.10	–	–	–	–
Elemicin	–	–	–	–	0.23	0.06
Unidentified	–	6.37	–	1.80	–	–

*Chromosome number.

†Sample identification number.

‡t: traces (≤0.01%); >0.01% quoted to nearest 0.01%.

fruits from the three types of *T. villosa* are shown in Table 1. Type 2 was collected at three distant locations and type 3 at two close locations; analysis of type 1 was performed only on material from one location.

The essential oil of all three types is composed mainly of monoterpenes. The dominant constituent in all samples is geranyl acetate, which makes up 78–92% of the oil. Substituted allylbenzenes are present only in negligible amounts. This general pattern is not noticeably affected by the different chromosome numbers of the types. However, the essential oil from the tetraploid plants contains a greater amount of geraniol, the biosynthetic precursor of geranyl acetate, than oil from the diploid plants (5–7% *vs* 1%, respectively; Table 1).

The constitution of the essential oils of types 1–3 is distinctly different from types 4 and 5 of *T. villosa*, and also from the oils of the other species of *Thapsia* [21–23]. Previously, it was found that the two main components of the essentail oil from *T. villosa* types 4 and 5 were limonene and methyl eugenol, accounting for 31–55% and 33–66%, respectively, of the total volatiles from type 4 and for 13–26% and 45–62%, respectively, of the oil from type 5. Furthermore, elemicin amounted to up to 30% of the total oil from some samples of type 5 [23]. These results are in agreement with data reported in a recent publication [24]. However, the karyotypes of the investigated plants are not specified in that study. Limonene and methyl eugenol also constitute 27–34% and 59–63%, respectively of the fruit volatiles from the two phenotypes of *T. maxima* [22], whereas the main compounds of the essential oils from *T. garganica* are *p*-vinylguaiacol (61.3%), linalool (8.6%) and 1,4-dimethylazulene (6.3%) [21], and from *T. transtagana*, 1,4-dimethylazulene (52.8%) and methyleugenol (47.2%) [15].

The phytochemical variations in the composition of the essential oils from different species and types of *Thapsia* are in good agreement with the variations found in the secondary metabolites of the roots [16]. Thus, thapsigargins have not been isolated from types 1–3 (group 1), whereas they are characteristic constituents of types 4–5 (group 2) of *T. villosa*. Combined with the morphological differences it proves that there is a distinction between groups 1 and 2, justifying a taxonomic separation.

Although the two diploid types 1 and 2 show great similarity in the composition of their essential oils, morphological and other phytochemical characters separate the two types [16]. Type 1 is identical to *T. minor* and type 2 is considered as identical to *T. laciniata*. The origin of the tetraploid type 3 is still an unknown.

Chromosome studies in the Apiaceae have shown that plants of the subfamily Apioideae most frequently have the basic haploid number $x = 11$ and that polyploid variation is quite common [25]. In the genus *Thapsia*, polyploid types have been detected only within *T. villosa* [26]. On the basis of the earlier morphological and phytochemical results combined with the present data, the origin of the tetraploid type 3 is probably by evolution from the diploid type 1. Nevertheless, allopolyploidy is reported to be more common than autopolyploidy in the Apiaceae [25]. In particular, tetraploid populations are considered to be allotetraploids. In view of the above and the similarity of chemical composition between types 1 and 2, hybridization of the two may also be considered as a possible mechanism of evolution of the tetraploid plants.

EXPERIMENTAL

General. TLC: silica gel 60 F254. Elution systems: (1) toluene–EtOAc (93:7); (2) CH_2Cl_2; (3) hexane–EtOAc (4:1). Components were visualized by spraying with vanillin (1% EtOH)–H_2SO_4 (5% EtOH) reagent and heating at 120°. FID-GC: Supelcowax 10 fused silica capillary column (60 m × 0.32 mm i.d., 0.25 μm film thickness) was used with the following programme: 60° (5 min), 4° min^{-1} to 270° (15 min). Cold on-column injection mode was used. Detector port was maintained at 280°. The carrier gas was H_2; air and H_2 flow rates were adjusted to yield optimum separation. Data were processed with the aid of a computing integrator. GC-EIMS: samples were analysed with a Supelcowax 10 capillary column (as above) inserted directly into the ion source. Analytical conditions were as follows: from 90° (10 min) to 210° (30 min) at 5° min^{-1}; injector, 250°. Carrier gas was He (0.8 ml min^{-1}). MS: 40–450 amu, 1 scan sec^{-1}; ionization electron energy, 70 eV; ion source, 200°; electron current, 220 μA; vacuum 10^{-5} torr. The splitless injection mode (1 μl) was used.

Plant material. Specimens of the three types of *T. villosa* were classified and collected by UWS at the following locations. Type 1 ($2n = 22$), Portugal: 88.30, *ca* 20 km north of Monchique road no. 266. Type 2 ($2n = 22$), France: 90.01, *ca* 12 km from Fréjus road no. N 7; Portugal: 77.01, *ca* 5 km from Capo Espicel on road no. 379 from Santana; 81.04, *ca* 4 km west of Tavira road no. E.N. 270. Type 3 ($2n = 44$), Portugal: 88.25, *ca* 2 km from 88.26; 88.26, *ca* 7 km west of Colares, road no. 247. Fruits were collected at the ripe stage and air-dried before chemical investigations. Voucher specimens are deposited at the Department of Pharmacognosy, Royal Danish School of Pharmacy, Copenhagen.

Determination of chromosome numbers. Chromosome numbers were determined on seedling root tips with the conventional Feulgen squash method, after pretreatment with 0.1% colchicine for 1.5–2 hr at 20–25°.

Isolation of essential oils. Fruits (20 g) from each of the samples were submitted to hydrodistillation for 3 hr. The distillate was extracted with Et_2O, dried overnight (Na_2SO_4) and then concd under vacuum. Oils were kept at 4° until analysed.

Identification of essential oils components. Authentic ref. compounds, MS data base, as well as published mass spectra [27–29] were used for identification.

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