The Genus *Thapsia* as a Source of Petroselinic Acid

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ABSTRACT: We describe the results from the isolation and structural identification of the acylglycerol constituents of fruits from wild plants belonging to different species of *Thapsia* (Apiaceae). The isolated lipid fractions were analyzed and characterized by chemical, chromatographic, and spectroscopic means. In particular, ^{13}C nuclear magnetic resonance data allowed the identification of petroselinic acid as the major fatty acid esterified to glycerol in the fruit oils from all the plant samples. This was also confirmed by gas chromatography (GC) and GC–mass spectrometry analyses of fatty acid methyl and butyl esters derivatives from *Thapsia* oil. The genus *Thapsia* should be regarded as a useful source for the extraction of petroselinic acid, which represents an important oleochemical raw material.

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The Apiaceae (Umbelliferae) represent one of the best-known plant families, widely distributed in temperate climate regions where they are often used as spices or drugs owing to the presence of useful secondary metabolites such as coumarins, essential oils and sesquiterpenes (1–3).

In addition, more recently the plant family has attracted attention for the high level of petroselinic acid (*cis*-6-octadecenoic acid) produced by some of the genera belonging to it. This unsaturated fatty acid, isomer of oleic acid, represents in fact an interesting oleochemical for the food, cosmetics, and pharmaceutical industries (4–7). In particular, petroselinic acid can be oxidatively split at the double bond into lauric (12:0) and adipic (6:0) acids, both with important applications in the manufacture of soaps and plastics (4–7).

Attempts to engineer industrial oilseed crops, e.g., by introducing genes from coriander, to increase the yield of petroselinic acid have not yet been very successful (4–7). Therefore, we believe that the identification of new plants which can provide high amounts of petroselinic acid is still appropriate.

In recent years, within the Apiaceae family, the genus *Thapsia* has been the subject of several scientific investigations (8–10), especially for the medical properties of *T. garganica*. Phytochemical studies of plants belonging to different species of *Thapsia* have shown that they are an important source of bioactive compounds, and may be useful in providing new insights into the present taxonomy of the genus (11–17). In the present paper we report the results from the isolation and structural identification of the acylglycerol components of fruits from wild plants of different *Thapsia* species.

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Abbreviations: COSY, correlation spectroscopy; 2D, two-dimensional; FTIR, Fourier transform infrared spectroscopy; FAME, fatty acid methyl esters; GC, gas chromatograpy; GC–MS, GC–mass spectrometry; ME, methyl ester; NMR, nuclear magnetic resonance; TLC, thin-layer chromatography.

EXPERIMENTAL PROCEDURES

Plant material. Fruits of *T. garganica* were collected, during 1999, from wild-growing plants at Sammichele (Bari, Italy). Fruits of *T. villosa* (two samples, 88-16, 2n = 44, and 88-9, 2n = 66), *T. laciniata*, *T. minor*, *T. maxima* I and *T. maxima* II were obtained from wild-growing plants as described before (12,16,17). Fruit vouchers are deposited at Dipartimento Farmaco-Chimico, Universitá di Bari (Italy).

Oil extraction and purification. Finely pulverized fruits (10 g) of *Thapsia* species were extracted in a Soxhlet apparatus with refluxing petroleum ether (bp 35–60°C) for 3 h. Evaporation of the solvent under vacuum gave an oily product, which was further investigated.

Each oil sample was first characterized by precoated silica gel 60 F254 TLC aluminum sheets (10×20 and 5×7.5 cm; Merck, Darmstadt, Germany) developed in $CHCl_3$. Lipid visualization was obtained with phosphomolybdic acid reagent (10% in EtOH; Sigma, Milan, Italy) and by charring the plates at 110°C.

Oils extracted from *T. garganica* consisted of a nearly pure fraction of acylglycerols; fruit extracts from the other species of *Thapsia* resulted instead in a mixture of acylglycerols plus other compounds (identification not reported here) mainly originating from the terpene pool of metabolites. All extracts were, however, purified by column chromatography (silica gel 60H, Merck) using $CHCl_3$ as the eluent to recover pure triacylglycerols.

Saponification and methylation. Triacylglycerols (15 mg) from *Thapsia* species were treated with 5% NaOH in MeOH (3 mL) for 20 min at room temperature. Methanolic 6 N HCl was then added to stop the reaction. After evaporation of the solvent, the residue was further reacted with CH_2N_2 according to Reference 18. The methylated fatty acid fractions were then analyzed by gas chromatography (GC) and GC–mass spectrometry (MS). For comparison, commercial tripetroselinin (Sigma) was also subjected to the above reactions. Free fatty acids (stearic, palmitic, oleic, linoleic, petroselinic, and linolenic; Sigma) were also methylated with CH_2N_2 and used as reference compounds for GC and GC–MS analyses.

Preparation of fatty acid butyl esters. Triacylglycerols (5 mg) from *Thapsia* species and commercial tripetroselinin were reacted with 1 mL 0.1 M Na *tert*-butylate in *n*-butanol

(Fluka, Milan, Italy). The reaction was performed for 1 h at room temperature, and then 5% $NaHSO_4$ in H_2O was added as described (19). The organic layer was used for GC and GC–MS analyses. Standard butyl esters of stearic, palmitic, petroselinic, oleic, and linoleic acids were also prepared and submitted to GC and GC–MS analysis.

GC analysis. A Carlo Erba HRGC 5160 gas chromatograph equipped with a flame-ionization detector and an on-column injector was used. Hydrogen was the carrier gas; air and H_2 were adjusted to yield optimal separation. Data were processed by a Spectra Physics SP 4290 computing integrator. All the samples were dissolved in $CHCl_3$.

Triacylglycerols from *Thapsia* oil samples, commercial tripetroselinin, triolein, trilinolein, tristearin, and tripalmitin were analyzed with an Easy 1 (Analitica, Milan, Italy) bonded phase fused-silica capillary column (25 m × 0.32 mm i.d.; 0.1–0.15 μm film thickness) either in isothermal conditions at 280°C or under programmed conditions, from 260°C, 7°C/min, up to 360°C. The detector port was kept at 380°C.

Determination of fatty acid methyl esters (FAME) and fatty acid butyl esters was carried out on a DB-5 (Superchrom, Milan, Italy) fused-silica capillary column, 30 m × 0.32 mm i.d.; 0.25 μm film thickness. The chromatographic conditions were as follows: detector temperature 300°C; column temperature was programmed from 70°C, 20°C/min to 170°C and then up to 280°C (5 min isothermal) at 5°C/min. Alternatively, FAME and fatty acid butyl esters from *Thapsia* acylglycerols were analyzed on a DB-23 (Superchrom) fused-silica capillary column (30 m × 0.32 mm i.d.; 0.25 μm film thickness) at the following conditions: 70°C, 15°C/min to 110°C and then up to 230°C (20 min isothermal) at 3°C/min. Detector port was maintained at 300°C.

GC–MS analysis. GC–MS analyses of FAME and fatty acid butyl ester derivatives were performed with a Hewlett-Packard 6890-5973 mass spectrometer interfaced with an HP 59970 Chemstation. The chromatographic conditions were as follows: column oven program from 70 (4 min isothermal) to 280°C (20 min isothermal) at 20°C/min; injector, 250°C. Helium was the carrier gas (flow rate, 1 mL/min). An HP-5-MS capillary column (30 m × 0.25 mm; 0.25 μm film thickness) was utilized. MS operating parameters were: ion source, 70 eV; ion source temperature, 230°C; electron current, 34.6 μA; vacuum 10^{-5} torr. Mass spectra were acquired over 40–800 amu range at 1 scan/s. The ion source was operated in the electron impact mode. The samples (1 μL) were injected using the splitless sampling technique.

Spectroscopic methods. Fourier transform infrared (FTIR) spectra were recorded using NaCl cells on a PerkinElmer Spectrum One.

Proton (^{1}H NMR) and carbon nuclear magnetic resonances (^{13}C NMR) were recorded on a DRX500 Avance Bruker instrument equipped with probes for inverse detection and with *z* gradient for gradient-accelerated spectroscopy. Standard Bruker automation programs were used for two-dimensional (2D) NMR experiments. 2D correlation spectroscopy (COSY) experiments were performed using COSYDFTP (double-quantum-filtered phase-sensitive COSY) and COSYG (gradient-accelerated COSY) sequences. Inverse detected normal and long-range ^{1}H-^{13}C heterocorrelated 2D NMR spectra were obtained by using the gradient-sensitivity enhanced pulse sequences INVIEAGSSI and INV4GPLRND, respectively. $CDCl_3$ was used as the solvent in all the NMR experiments. Residual ^{1}H and ^{13}C peaks of the solvent were used as internal standards to calculate chemical shifts referred to tetramethylsilane.

RESULTS AND DISCUSSION

Acylglycerols purified by chromatographic techniques from the total lipid extracts of the various species of *Thapsia* were characterized by chromatographic, spectroscopic, and chemical means. Homogeneous results were obtained with all the analyzed plant species, therefore reported spectroscopic assignments from spectra related to a single acylglycerol sample from *Thapsia* apply to all of them as well.

Infrared (IR) spectra of *Thapsia* lipids showed typical absorptions at 3003 (C–H olefins), 2949, 2921, 2852 (C–H), 1744 (C=O), 1464 (C–H), 1179 (C–O) and 721 cm^{-1}.

More structural information came from their ^{1}H and ^{13}C spectral data (Fig. 1). 2D proton-proton and proton-carbon correlations also facilitated the assigning of relative signals (Fig. 2).

The ^{1}H NMR signals at δ 4.11 (*dd*; glycerol α CH_2), 4.28 (*dd*; glycerol α CH_2), and 5.29 (*m*; glycerol β C*H*) indicated that the isolated lipids were triglycerides (Table 1). The observed chemical shifts in the ^{13}C NMR spectra at δ 62.52 (glycerol α-carbon atoms) and 69.32 (glycerol β-carbon atom) and their relative size also confirmed that the isolated compounds were triglycerides (Table 1). This was further supported by the highest chemical shift signals at about δ

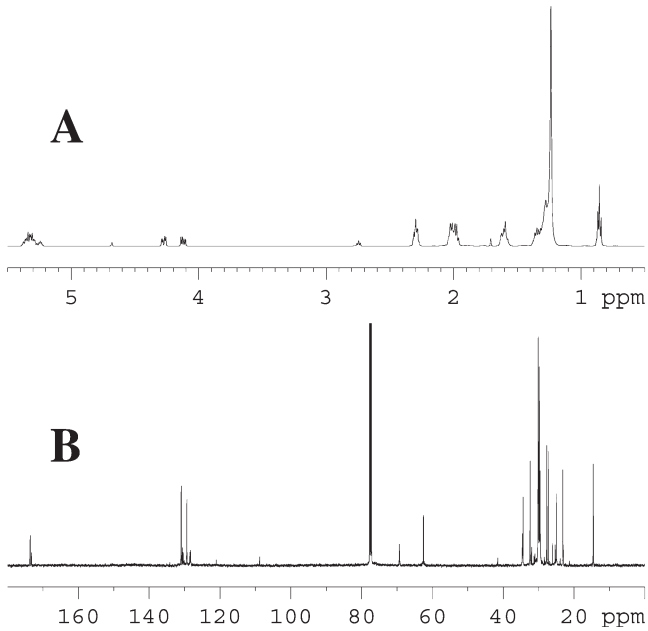

FIG. 1. ^{1}H (A) and ^{13}C (B) 500 MHz nuclear magnetic resonance (NMR) spectra of acylglycerol constituents from the fruits of *Thapsia* sp.

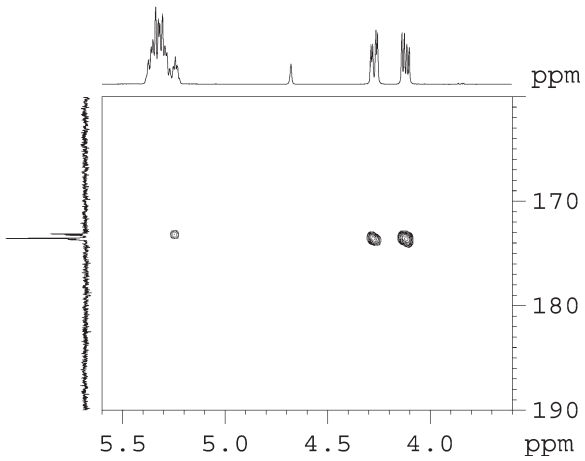

FIG. 2. Long-range two-dimensional heteronuclear NMR spectrum of acyglycerols from *Thapsia* sp. Selected region shows cross peaks between carbonyl resonances of C1 α and β chains and glycerol α and β hydrogens. For abbreviation see Figure 1.

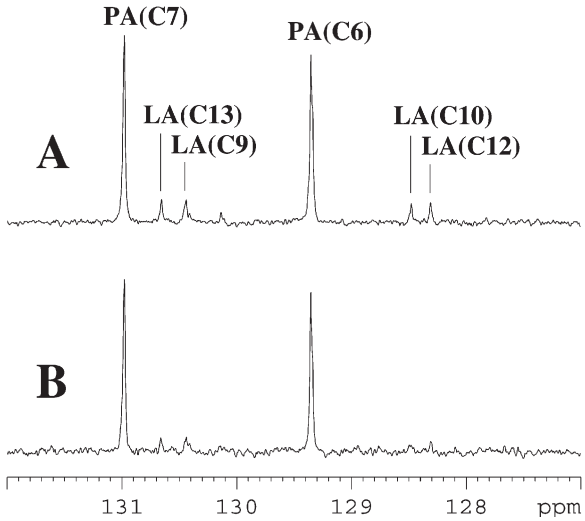

FIG. 3. Expansion of the olefin region of ^{13}C NMR spectra of the acylglycerols from (A) *T. garganica* and (B) *T. villosa* (88-16). PA, petroselinic acid; LA, linoleic acid. For abbreviations see Figure 1.

173.11 (C1 β chains) and δ 173.52 (C1 α chains) in all the lipid samples from *Thapsia* species. The 2D NMR experiments were also useful to corroborate the 1,2,3 substitution pattern of the isolated esters. Cross peaks (Fig. 2) were in fact observed in the long-range ^{1}H-^{13}C heteronuclear correlated spectra between the carbon resonance at δ 173.52 and 173.11 (C1 α and β chains) and proton resonances at δ 2.30 (C2) and δ 4.28, 4.11 and 5.29 (glycerol α and β hydrogens).

Moreover, the multiplet at δ 5.33 in the proton spectrum, together with the signals in the range between δ 128 and 131 in the ^{13}C NMR spectra, suggested the presence of olefinic structural units. In addition, resonances of allylic and diallylic methylenes can be identified in the spectra (Table 1). The olefin region in the carbon NMR spectrum appears particularly important for the characterization of the oil composition (20–22). A closer inspection of this peak area, in fact, allowed us to distinguish in all the samples, on the basis of their chemical shifts, the following two unsaturated fatty acids esterified to the unit of glycerol: petroselinic acid, δ 130.95 (C7) and δ 129.33 (C6); linoleic acid (*cis*-9,12-octadecadienoic), δ 130.61 (C13), δ 130.37 (C9), δ 128.48 (C10), and δ 128.29 (C12) (Fig. 3).

Identity of the two fatty acids was further confirmed by comparison with NMR spectra of commercial tripetroselinin and trilinolein and with spectra obtained from constructed mixtures of saturated (tripalmitin) and unsaturated (tripetroselinin, trilinolein, and triolein) triacylglycerols. Literature data have also been evaluated for their identification (20–25).

Quantification of the relative proportions of petroselinic and linoleic acids in the NMR spectra according to Mallet *et al.* (22) indicated that petroselinic acid was by far the most abundant (≅90%) unsaturated fatty acid esterified to glycerol

TABLE 1
Nuclear Magnetic Resonance Spectral Data of Fruit Triacylglycerols from *Thapsia* Species

^{1}H	δ (*J*)	^{13}C	δ
2,2′	2.30 (*m*)	CH_3, ω1	14.51
3,4	1.61 (*m*)	CH_2, ω2[a]	23.09
Allylic CH_2 *(cis)*	2.00(*m*)	CH_2, C–3	24.88
Diallylic CH_2 *(cis)*	2.75 (*m*)	–C=C–CH_2–C=C–	26.04 (C11 LA)
Olefinic *(cis)*	5.33 (*m*)	–CH_2–C=C–CH_2 *(cis)*	27.21 (C5 PA)
			27.66 (C8 PA)
Other (*n*-CH_2)	1.34 (*m*)	*n*-CH_2	29.56–30.14
CH_3, ω1	0.85 (*t, J* = 7 Hz)	CH_2, ω3[a]	32.33
Glycerol α CH_2 (1″ a, 3″ a)	4.11 (*dd, J* = 18.6 Hz)	Glycerol CH_2 (α carbons)	62.52
Glycerol α CH_2 (1″ b, 3″ b)	4.28 (*dd, J* = 17.5 Hz)	Glycerol CH (β carbons)	69.32
Glycerol β C*H*	5.29 (*m*)	–C=CH	128.29 (C12 LA)
			128.48 (C10 LA)
			129.33 (C6 PA)
			130.37 (C9 LA)
			130.61 (C13 LA)
			130.95 (C7 PA)
		C-1, C-1′	173.52, 173.11
		C-2, C-2′	33.90, 34.07

[a]Weak signals at 22.98 δ [ω2 linoleic acid (LA)] and at 31.93 δ (ω3 LA) were also observed. PA, petroselinic acid.

TABLE 2
Relative Amount (%) of Fatty Acids Esterified to Glycerol in Fruit Oils from *Thapsia* Species

Species	Palmitic	Stearic	Petroselinic	Oleic	*cis*-Vaccenic	Linoleic
T. garganica	4.9	Trace	82.8	4.4	Trace	7.9
T. villosa (88-16)	8.2	4.9	73.0	7.0	1.9	5.0
T. villosa (88-9)	4.7	Trace	72.3	6.1	0.6	16.3
T. laciniata	7.2	1.8	73.3	6.2	0.7	10.8
T. minor	8.2	1.7	69.7	4.0	0.7	15.7
T. maxima I	6.8	1.9	73.5	4.2	1.1	12.5
T. maxima II	5.0	0.8	74.8	4.6	0.4	14.4

in *Thapsia* fruit lipids. Petroselinic acid represents a positional isomer of oleic acid (18:1n-9*cis*). The two fatty acids often occur in combination as constituents of Apiaceae oils, and they are generally difficult to separate. According to our NMR results, *Thapsia* fruit oils should not contain oleic acid, or only in low amounts (Fig. 3). Oleic acid, as well as petroselinic and linoleic acids, shows well-defined signals related to the olefin region in the ^{13}C NMR spectra, namely, at around δ 129.7 (C9) and 130.0 (C10), the latter often overlapping with the C9 peak of linoleic acid (20–22). A weak signal at δ 130.1 attributable to oleic acid was sometimes detectable in our oil samples suggesting a trace quantity of oleic acid in *Thapsia* oils.

To achieve a good quantification of the fatty acids in their fruit oils, lipids from *Thapsia* plants were submitted to transmethylation and transbutylation reactions, and the methyl and butyl esters formed were subjected to GC analyses. Separation on a DB-23 column allowed the best determination and quantification of the fatty acid content in the acylglycerols from *Thapsia* oils (Table 2; Fig. 4). The column produced a good separation of the three isomers—oleic methyl ester (ME), petroselinic ME, and *cis*-vaccenic ME—as well as for the separation of their corresponding butyl esters (Fig. 4), which were used for the quantitative analysis. Neverthless, GC further supported NMR findings, that is, *Thapsia* triacylglycerols were mainly made up of petroselinic (69–82%) and

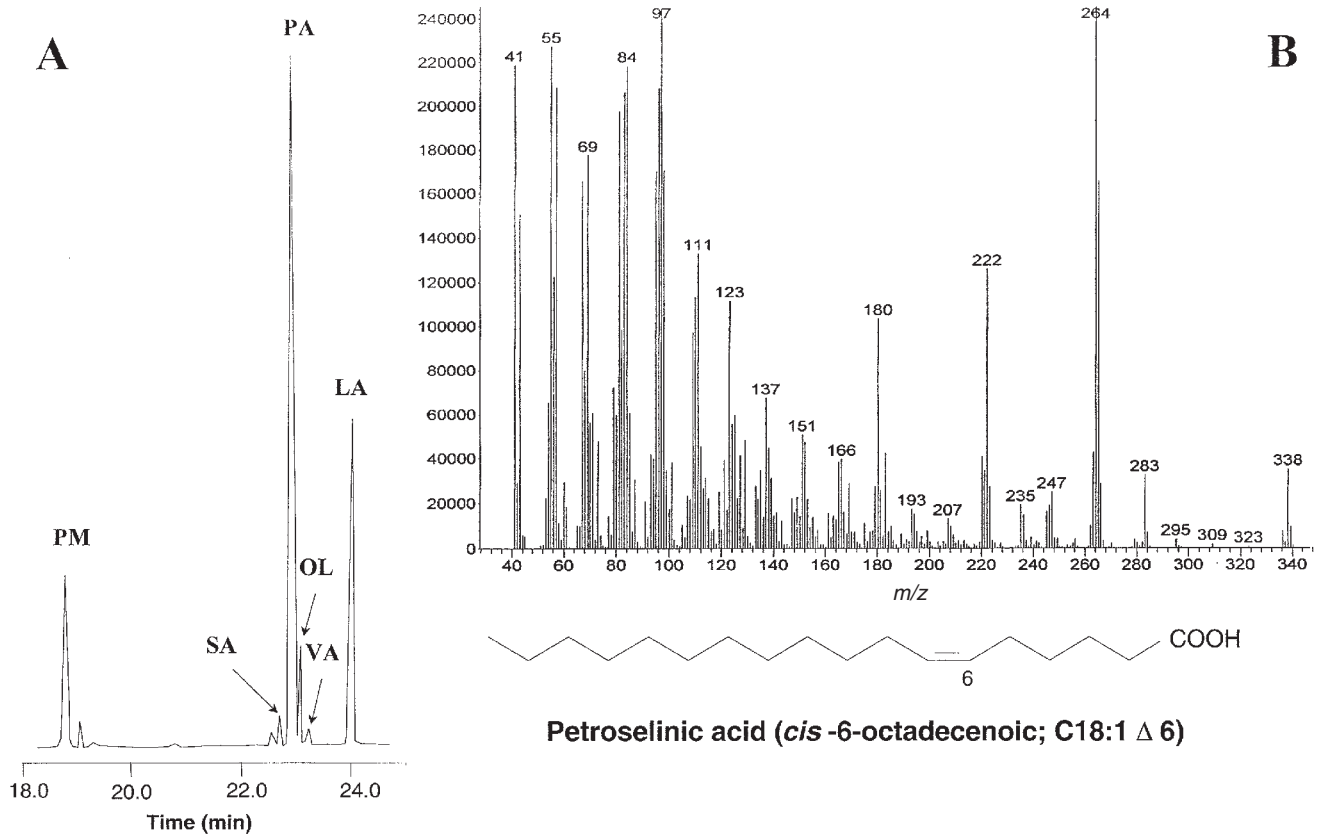

FIG. 4. Gas–liquid chromatographic analysis (A) of the butyl esters obtained from the acylglycerols of *T. minor,* and mass spectrum (B) of PA. PM, palmitic acid; SA, stearic acid; OL, oleic acid; VA, *cis*-vaccenic acid. For other abbreviations see Figure 3.

linoleic (trace–16%) acids. The amount of oleic acid generally ranged from 4 to 6%. GC analysis of the butyl ester derivatives from the oils also allowed the quantification of *cis*-vaccenic acid as minor component (from traces up to 1%). The two saturated palmitic (5–8%) and stearic (trace–8%) acids were also identified in the reaction mixtures (Table 2; Fig. 4). Their presence was not further investigated in the NMR spectra, however.

All the derivatized oil fractions were also analyzed by GC–MS; MS fragmentation of petroselinic acid butyl ester was as follows: *m/z* (relative intensity, r.i.), 338, [M^+, $C_{22}H_{42}O_2$ (14)], 264 (100) ($M^+ - 74$), 222 (51), 180 (42), 166 (16), 151 (21), 137 (25), 123 (45), 111 (54), 97 (99), 84 (89), 69 (72), 55 (92), 41 (89).

The following MS fragments were obtained for petroselinic (i) and linoleic (ii) ME derivatives, respectively, *m/z* (r.i.): (i), 296, [M^+, $C_{19}H_{36}O_2$ (100)], 264 (83) [$M^+ - 32$], 222 (49) [$M^+ - 74$], 180 (33), 123 (40), 110 (46), 96 (92), 84 (93), 74 (100), 55 (99), 41 (75); (2), 294, [M^+, $C_{19}H_{34}O_2$ (100)], 263 (15) [$M^+ - 31$], 220 (6) [$M^+ - 74$], 178 (8), 164 (10), 150 (14), 136 (15), 123 (18), 109 (35), 95 (63), 81 (77), 67 (100), 55 (54), 41 (44). Data were in agreement with mass spectra obtained with reference compounds.

Similarly, GC of the intact triglycerides allowed the separation of one main component (80–90%) coeluting with commercial tripetroselinin. Trilinolein resulted in a partially overlapping shoulder to the GC peak of tripetroselinin.

To our knowledge, this is the first extensive study on the distribution of tripetroselinate in the genus *Thapsia*. Petroselinic acid is one of the most common fatty acids in Apiaceae seed oils, thus its isolation from the genus *Thapsia* can be anticipated. Moreover, a previous report (26) showed that petroselinic acid, a component of the oil from *T. villosa*, amounted to 75%. Other studies showed that the different species of *Thapsia* can be distinguished by their phytochemical characters; in particular, the presence/absence of the typical sesquiterpene lactones and the constituents of the essential oils were of chemotaxonomic value in differentiating them (8,9). The most pronounced heterogeneity was found among plants identified as *T. villosa* species, which were divided into five types, corresponding to two distinctly different chemical, cytological and morphological groups (16,17). We cannot know what type of *T. villosa* has been investigated previously (26). However, according to the present investigation, in all the analyzed species belonging to the genus *Thapsia*, the acylglycerols have almost the same composition and tripetroselinate is equally abundant in all of them. Thus, in contrast to other chemical classes synthesized by *Thapsia* plants, this metabolite cannot further contribute to the chemotaxonomy of the genus. Nevertheless, the genus *Thapsia* should be regarded as an important source of petroselinic acid, an oleochemical raw material that is used in cosmetics, pharmaceuticals, or food (6,7,27,28).

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