Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1146 (2007) 67–77
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

High-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry and gas chromatography–flame ionization detection characterization of Δ5-polyenoic fatty acids in triacylglycerols from conifer seed oils

Miroslav Lísa[a], Michal Holčapek[a,*], Tomáš Řezanka[b], Naděžda Kabátová[c]

[a] *Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Nám. Čs. Legií 565, 53210 Pardubice, Czech Republic*
[b] *Institute of Microbiology, Vídeňská 1083, 14220 Prague, Czech Republic*
[c] *Central Institute for Supervising and Testing in Agriculture, Hroznová 2, 656 06 Brno, Czech Republic*

Received 4 September 2006; received in revised form 23 January 2007; accepted 25 January 2007
Available online 6 February 2007

Abstract

Edible conifer seeds can serve as a source of triacylglycerols (TGs) with unusual Δ5 unsaturated polymethylene interrupted fatty acids (UPIFAs), such as *cis*-5,9-octadecadienoic (taxoleic), *cis*-5,9,12-octadecatrienoic (pinolenic), *cis*-5,11-eicosadienoic (keteleeronic) and *cis*-5,11,14-eicosatrienoic acids (sciadonic). Conifer seed oils from European Larch (*Larix decidua*), Norway Spruce (*Picea abies*) and European Silver Fir (*Abies alba*) have been analyzed by non-aqueous reversed-phase high-performance liquid chromatography (NARP-HPLC) with atmospheric pressure chemical ionisation (APCI)-MS detection. The influence of different positions of double bonds in Δ5-UPIFAs on the retention and fragmentation behavior is described and used for the successful identification of TGs in each oil. TGs containing Δ5-UPIFAs have a higher retention in comparison with common TGs found in plant oils with single methylene interrupted Δ6(9)-FAs and also significantly changed relative abundances of fragment ions in APCI mass spectra. Results obtained from HPLC/MS analyses are supported by validated GC/FID analyses of fatty acid methyl esters after the transesterification. The total content of Δ5-UPIFAs is about 32% for European Larch, 27% for Norway Spruce and 20% for European Silver Fir. In total, 20 FAs with acyl chain lengths from 16 to 24 carbon atoms and from 0 to 3 double bonds have been identified in 64 triacylglycerols from 3 conifer seed oils.

Keywords: Conifer seed oil; Triacylglycerol; Triglyceride; Delta 5-Olefinic; Polyenoic fatty acid; HPLC/MS

1. Introduction

Triacylglycerols (TGs) are natural compounds consisting of saturated and unsaturated fatty acids (FAs) differing in their acyl chain lengths, in the number and positions of double bonds (DBs), in the *cis*/*trans* configuration of DBs, in positional isomerism and *R*/*S* optical isomerism of TGs containing three different FAs. The standard notation of TGs is based on the initials of FA trivial names (see Table 1), arranged in the order of their stereochemical positions on the glycerol skeleton. In addition to saturated and monounsaturated FAs, common plant oils contain polyunsaturated FAs most frequently with 18 carbon atoms and single methylene interrupted DBs [1–5], i.e. *cis*-9,12-octadecadienoic (linoleic acid) and *cis*-9,12,15-octadecatrienoic (linolenic acid). TGs with unusual FAs, such as those containing conjugated DBs (e.g. conjugated linolenic acids) [6] or unsaturated polymethylene interrupted fatty acids (UPIFAs) [7–14], can be found in some taxonomical groups.

Gymnosperms (*Gymnospermae*) includes trees and shrubs and they are commonly known as conifers. This large taxonomical group is known for the presence of unusual FAs with the first site of unsaturation at the fifth carbon atom (Δ5-UPIFAs) and *cis* (Z) configuration, such as *cis*-5,9-octadecadienoic (taxoleic), *cis*-5,9,12-octadecatrienoic (pinolenic),

---

[*] Corresponding author. Tel.: +420 46 603 7087; fax: +420 46 603 7068.
E-mail address: michal.holcapek@upce.cz (M. Holčapek).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.01.122

Table 1
Systematic and trivial names of fatty acids found in triacylglycerols of studied conifer seed oils listed with their abbreviations, carbon numbers (CN), double bond (DB) numbers and equivalent carbon numbers (ECN)

Systematic name	Trivial name	Abbreviation	CN:DB	ECN
Hexadecanoic	Palmitic	P	C16:0	16
cis-9-Hexadecenoic	Palmitoleic	Po	C16:1	14
Heptadecanoic	Margaric	Ma	C17:0	17
Octadecanoic	Stearic	S	C18:0	18
cis-9-Octadecenoic	Oleic	O	C18:1	16
cis-11-Octadecenoic	Vaccenic	Va[a]	C18:1	16
cis-9,12-Octadecadienoic	Linoleic	L	C18:2	14
cis-5,9-Octadecadienoic	Taxoleic	Ta[a]	C18:2	14
cis-9,12,15-Octadecatrienoic	Linolenic	Ln	C18:3	12
cis-5,9,12-Octadecatrienoic	Pinolenic	Pi[a]	C18:3	12
Nonadecanoic	–	–	C19:0	19
Eicosanoic	Arachidic	A	C20:0	20
cis-9-Eicosenoic	Gadoleic	G	C20:1	18
cis-11,14-Eicosadienoic	–	–	C20:2	16
cis-5,11-Eicosadienoic	Keteleeronic	Ke[a]	C20:2	16
cis-8,11,14-Eicosatrienoic	–	–	C20:3	14
cis-5,11,14-Eicosatrienoic	Sciadonic	Sc[a]	C20:3	14
Docosanoic	Behenic	B	C22:0	22
Tricosanoic	–	–	C23:0	23
Tetracosanoic	Lignoceric	Lg	C24:0	24

[a] Suggested abbreviations used in this work.

cis-5,11-octadecadienoic (ephedrenic), *cis*-5,11-eicosadienoic (keteleeronic), *cis*-5,11,14-eicosatrienoic (sciadonic), *cis*-5,9,12,15-eicosatetraenoic (coniferonic) and *cis*-5,11,14,17-eicosatetraenoic (juniperonic) acids. Sciadonic acid has been identified in *Podocarpus nagi* seed oil as early as in 1962 [15]. The presence of the following Δ5-UPIFAs has been reported in Gymnosperm plants: keteleeronic and sciadonic acids in *Ginkgo biloba* [16,17], pinolenic acid in *Larix leptolepsis* [18] and *Pinus koraienesis* [19], taxoleic and sciadonic acids in *Taxus baccata* [20], sciadonic acid in *Toreya nucifera* [19,21], ephedrenic, keteleeronic, sciadonic and juniperonic acids in *Ephedra campylopoda* [22], ephedrenic and sciadonic acids in *Ginkgo biloba* [23], pinolenic acid in *Picea abies* [24], taxoleic, pinolenic, coniferonic, sciadonic and juniperonic acids in many species of conifers [25].

In contrast to several papers dealing with methyl esters of Δ5-UPIFAs (e.g. reviews [7,8,11]), much less attention has been paid to intact TGs that contain those Δ5-UPIFAs. ^{13}C nuclear magnetic resonance (NMR) spectroscopy of oils from three *Taxus* and one *Torreya* species has confirmed that Δ5-olefinic acids are apparently excluded from the *sn*-2 position which is characteristic for all *Gymnosperm* species analyzed so far [12]. ^{13}C NMR spectroscopy of the seed oil from two *Ephedra* species shows that Δ5-UPIFAs are excluded from the *sn*-2 position, a characteristic common to all analyzed *Conipherophytes* species (more than 30 species), with the possibility of an exclusive esterification at the *sn*-3 position [13]. The chemical degradation of conifer seed oils followed by gas chromatographic analysis of dibutyroyl derivatives of monoacylglycerols reveals that seed oils of 18 species from 5 conifer families contain Δ5-olefinic acids esterified mainly at primary positions (i.e. *sn*-1 and *sn*-3) of the glycerol backbone, whereas less than 8% of Δ5-olefinic acids are esterified in the secondary position (i.e. *sn*-2) [14].

Non-aqueous reversed-phase high-performance liquid chromatography (NARP-HPLC) has been widely used for the separation of complex natural lipid samples [1–5,26–41]. The retention in NARP-HPLC increases with increasing equivalent carbon number (ECN) defined as the total carbon number (CN) in all acyl chains minus two times the number of DBs, i.e. ECN = CN − 2DB. Under optimized separation conditions, the separation of most TGs within the same ECN group is also possible, for example the critical pair LLL/OLLn or the group of OOO, OOP, OPP and PPP can be well resolved [1,2,4,5,26,27]. The separation of TGs differing in the positions of DBs is also feasible [28]. On the other hand, NARP-HPLC is not suitable for the separation of three types of isomerism, i.e. regioisomers, *R*/*S* isomers and *cis*/*trans* isomers. Various mobile phase systems, mostly in gradient elution mode, are described in the literature, such as 2-propanol/acetonitrile [1–3,27,29], 2-propanol/acetonitrile/hexane [30,31], acetone/acetonitrile [26,31-34], 100% propionitrile [4], acetonitrile/chloroform [5], acetonitrile/dichloromethane [35–41], etc.

The coupling of HPLC and mass spectrometry (MS) is a powerful tool in lipid analysis, because it provides both structural information and usually also the highest sensitivity among all available chromatographic detectors [30]. Atmospheric pressure chemical ionization (APCI) is the most frequently used ionization technique for TG analysis because of easy coupling to non-aqueous mobile phase systems and high ionization efficiency for non-polar species. The presence of both protonated molecules $[M+H]^+$ and fragment ions $[M+H-R_iCOOH]^+$ is important for structure elucidation [1–5,26–42]. Electrospray ionization (ESI) mass spectra exhibit $[M+Na]^+$ and $[M+K]^+$ adduct ions instead of protonated molecules and also some fragment ions, such as $[M+Na-R_iCOOH]^+$ and $[M+Na-R_iCOONa]^+$, but with lower relative abundances