Triacylglycerol Composition of *Pinus koraiensis* Seed Oil

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ABSTRACT: The triacylglycerol (TG) composition of *Pinus koraiensis* seed oil, which contains Δ5 nonmethylene-interrupted (NMI) fatty acids (FA) (the main acid is pinolenic, 18:3 Δ5,9,12), was determined. TG were preliminarily separated by argentation thin-layer chromatography (TLC), and the obtained fractions were analyzed by high-temperature gas chromatography (GC) on a capillary column with methyl phenyl silicone phase. Additionally, high-performance liquid chromatography (HPLC) of TG was applied. The FA composition of all TG fractions was identified. The identification of TG was carried out by combining TLC, GC, HPLC, and calculated equivalent carbon numbers of TG standards. The TG species identification was confirmed by comparison of the theoretical recalculated and directly analyzed FA compositions of all TLC fractions of TG. Species of TG with unsaturation degrees of 1 to 7 and trace amounts of saturated and octaenoic TG species were found. Except for minor compounds, 26 TG molecular species of 32 main components were quantitatively determined. The main species were oleoyl dilinoleoylglycerol (14.7%), dilinoleoyl pinolenoylglycerol (10.7%), palmitoyl oleoyl linoleoylglycerol (8.3%), triolein (7.6%), and dioleoyl, linoleoylglycerol (7.4%). Seven TG species contained Δ5 NMI acyl groups. Of these, the major were dilinoleoyl pinolenoylglycerol (10.7%), stearoyl linoleoyl pinolenoylglycerol (6.5%) dioleoyl, pinolenoylglycerol (5.4%), and palmitoyl linoleoyl pinolenoyl-glycerol (5.5%). TG species with two or three NMI acyl groups were not detected.
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KEY WORDS: High-temperature gas chromatography, molecular species, nonmethylene-interrupted fatty acids, pinolenic acid, *Pinus koraiensis*, silver ion thin-layer chromatography, triacylglycerols.

Natural mixtures of triacylglycerols (TG) from plant seed oils that contain common methylene-interrupted fatty acids (FA) have successfully been analyzed. The most complete results were obtained for several edible seed oils. A distinctive feature of Pinaceae plants (Gymnospermae) is the presence of a significant amount of unusual, nonmethylene-interrupted (NMI) polyenoic FA in lipids obtained from the leaves (needles) and seeds (1–6). The most interesting NMI acid found in the seeds of Pinaceae is pinolenic acid (18:3 Δ5,9,12). It takes part in eicosanoid biosynthesis, influences blood pressure, and is used in dietary nutrition (7,8). The regularity of the distribution of these NMI acids in the TG of Pinaceae seeds is of special interest. It was shown by chemical degradation (2) and ^{13}C nuclear magnetic resonance spectroscopy (9) that NMI acids are considerably enriched in α-chains as compared to the β-chains. The recent work reported that NMI acids of conifer seed oils are mainly esterified to the *sn*-3 position of TG (10).

While this paper was in preparation, Gresti *et al.* (11) published the results of the analysis of molecular species of TG from Pinaceae seed oils. They fractionated TG of *Pinus koraiensis* and *P. pinaster* by reversed-phase high-performance liquid chromatography (HPLC), examined the FA composition of each fraction by gas chromatography (GC), and showed that structure could be assigned to more than 92% of TG from both oils. As was expected, complete separation of the TG was not achieved. Theoretically, each HPLC fraction consists of up to five components, which were reconstructed on the basis of the FA composition of the fractions.

As a rule, a combination of two or more chromatography methods is used for complete separation of molecular species of TG. One of the main difficulties in the analysis of TG is the identification of chromatographic peaks, because of the small number of TG species eluted in a pure state. Traditionally, mixtures of TG are first separated according to the degree of unsaturation on silver-ion-loaded sorbents, and then the obtained fractions of TG are analyzed by reversed-phase HPLC with various types of detectors or by nonpolar-phase capillary GC (12,13). With the above chromatographic methods, the main problem is to separate TG according to degree of unsaturation and molecular weight simultaneously. Recently, high-temperature (up to 370°C) polar-phase capillary GC has been demonstrated as a powerful tool for separating acylglycerols according to the number of both double bonds and carbon atoms (14–16). In this work, a combination of silver-ion-loaded thin-layer chromatography (Ag-TLC) and high-temperature polar-phase capillary GC is used for the analysis of TG molecular species of *P. koraiensis* seed oil that contains about 17% of NMI fatty acids in the total lipids (2).

EXPERIMENTAL PROCEDURES

Materials. Plant seeds were received from the Research Department of the Botanical Garden, Vladivostok (Russia) in 1996. Seeds were stored at 4°C and constant humidity.

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1,2-Distearoyl-3-palmitoylglycerol, tripalmitin, tristearin, triolein, trilinolein, and trilinolenin were purchased from Sigma Chemical (St. Louis, MO). All reagents were of analytical grade.

Lipid preparation. Total lipids were extracted by the method of Bligh and Dyer (17). TG were separated by silica gel column chromatography with the use of hexane and benzene elution. The total lipids (100 mg in hexane) were introduced into a glass column with 5 g silica gel suspended in hexane. The column was eluted with 30 mL hexane, 30 mL of hexane/benzene (1:1, vol/vol), and 30 mL benzene. Fractions of 10 mL were collected, and their composition was checked by TLC. Fractions with pure TG were combined, and after evaporation, 55 mg of pure TG was obtained.

TLC of TG. The separation of TG according to degree of unsaturation was performed by preparative TLC on silica gel plates loaded with $AgNO_3$. The mixture of 20 g silica gel and 2 g $AgNO_3$ in 70 mL water was placed on a glass plate (20 × 38 cm). The plate was dried in air and activated 0.5 h at 110°C before use. A solution of TG in chloroform (16 mg/0.1 mL) was placed on a TLC plate and separated with hexane/diethyl ether (30:70, vol/vol) eluent. After development, spots were located by spraying with 0.2% solution of dichlorofluorescein in 95% ethanol and subsequently visualized under ultraviolet light. Bands were scraped from the plate, eluted with chloroform (30 mL), and stored in solution at −18°C.

GC of TG. An analysis of TG was performed on a Shimadzu (Kyoto, Japan) GC–9A gas chromatograph (flame-ionization detector), equipped with a high-temperature aluminum-clad capillary column (25 m × 0.25 mm i.d.), coated with bonded methyl 65% phenyl silicon (400-65HT-25-0.1F; Quadrex, New Haven, CT). The injector temperature was 370°C. The column and detector temperatures were 345°C; the carrier gas was helium. Chromatographic data were calculated with a Shimadzu Chromatopac C-R3A integrator. To identify TG peaks, the equivalent carbon number (ECN) was calculated according to the formula commonly used for identification of fatty acids (18):

$$\mathrm{ECN} = 52 + \frac{2(\log \mathrm{RT}_N - \log \mathrm{RT}_{\mathrm{PSS}})}{\log \mathrm{RT}_{\mathrm{SSS}} - \log \mathrm{RT}_{\mathrm{PSS}}}$$

where RT_N = retention time of unknown TG, RT_{PSS} = retention time of 1,2-distearoyl-3-palmitoyl-glycerol, and RT_{SSS} = retention time of tristearin.

Authentic TG standards were additionally used for identification.

FA analysis. Fatty acid methyl esters (FAME) were prepared by the consecutive treatment of TG fraction with 1% sodium in methanol and 5% HCl in methanol, according to Carreau and Dubacq (19), and purified by TLC in benzene. GC of FAME was performed on a fused-quartz capillary column (30 m × 0.25 mm i.d.), coated with Supelcowax 10 (Supelco Co., Bellefonte, PA). The column and detector temperatures were 210°C; the injector temperature was 240°C. Helium was used as the carrier gas, and the split ratio was 1:30. The identification of FAME was confirmed by chromatographic comparison with authentic standards and calculation of equivalent chainlengths (ECL) (18). Chromatographic data were calculated with a Shimadzu Chromatopac C-R3A integrator. To determine the quantity of TG, a known amount of tripentadecanoin was added to each TG fraction before preparation of FAME. The percentage of each fraction in total TG was calculated as the ratio between the peak area of pentadecanoic acid methyl ester and the total area of other FAME peaks.

HPLC of TG. Additional separation of the Ag-TLC fraction of TG was performed on a GPC liquid chromatograph with an RIDC-103 refractive index detector (Laboratorni Pristroje Co., Czechoslovakia) on a reversed-phase octadecyl-Si 100 (5 μm particles) column (9.5 mm × 25 cm; Supelco Co.). An isocratic elution with acetone at a flow rate of 4.5 mL/min was used for separation of TG. For each fractionation, about 0.6 mg of TG in 10 μL acetone was injected. Each detected peak was collected manually for further analysis. The TG and FA compositions of the fractions were analyzed by GC as described above.

RESULTS AND DISCUSSION

Six main fractions of TG of *P. koraiensis* seeds were obtained by preparative Ag-TLC. The fractions were numbered from the starting point of the TLC plate to its front. These fractions corresponded to the substances that contained one to seven double bonds. However, each fraction might contain TG that differed by the number of double bonds. Only trace amounts of saturated and octanoic fractions were detected. The analytical variant of that chromatography showed the presence of 10 spots of TG, but some of them were placed closely and could not be separated under preparative conditions. Probably the appearance of the additional spots on the TLC plate was caused by the separation of isomeric TG with Δ5- and Δ9-octadecatrienoic acids.

As a rule, the presence of trienoic fatty acids in oils results in highly unsaturated TG [for example, 59.8% of linolenic acid results in 24% of trilinolenin in linseed oil (20)]. The oil under investigation contains approximately 19% triene acids (21). However, not even trace amounts of nonanoic TG were detected.

The FA compositions of the Ag-TLC fractions of TG are shown in Table 1. The content of polyunsaturated fatty acids regularly decreases from the first to the sixth fraction. Negligible amounts of saturated 16:0 and 18:0 acids in highly unsaturated TG fractions could be attributable to the type of silica gel, prewashing conditions of the TLC plate, and the polarity of the solvent system used for fraction elution. The data on FA were necessary for the check of TG identification in each TLC fraction and the reconstruction of the FA composition of the total TG. The FA composition of *P. koraiensis* seeds, described in detail by Takagi and Itabashi (2), is rather stable and may be used as a chemosystematic attribute (21). The total FA composition of *P. koraiensis* seeds, recalculated from fraction relative contents and composition, is close to

TABLE 1
Fatty Acid Compositions (wt%) of Triacylglycerol Fractions Obtained by Ag-TLC Separation of Seed Oil of *Pinus koraiensis*

	Fraction number (fraction content, %)						Total fatty acid composition[a]	
Fatty acid	1 (19.5)	2 (25.7)	3 (32.4)	4 (12.9)	5 (6.8)	6 (2.7)	Recalculated	Analyzed
16:0	2.1	5.5	5.4	12.4	6.5	19.4	6.2	5.0
16:1n-7	0.2	—	0.1	0.2	0.2	0.7	0.1	0.1
18:0	1.1	3.2	3.4	6.8	3.7	12.5	3.6	2.7
18:1n-9	10.5	24.0	33.1	45.9	82.3	59.0	32.1	28.1
18:1n-7	0.5	0.4	0.9	1.3	1.2	1.2	0.8	0.5
18:2 Δ5,9	0.5	4.1	3.4	—	—	—	2.3	2.2
18:2n-6	53.4	42.4	45.2	28.4	1.5	0.8	39.7	44.1
18:3 Δ5,9,12	28.9	17.2	4.4	—	—	—	11.5	13.9
18:3n-3	0.2	—	—	—	—	—	—	0.1
20:0	—	0.2	0.7	1.2	0.8	3.4	0.6	0.5
20:1n-9	0.6	0.7	2.1	2.7	3.5	1.9	1.6	1.3
20:2 Δ5,11	—	0.2	—	—	—	—	—	0.1
20:2n-6	—	—	—	—	—	—	—	0.5
20:3 Δ5,11,14	0.8	0.8	0.7	0.5	—	—	0.7	0.4
20:3 Δ7,11,14	1.4	1.1	0.3	—	—	—	0.7	0.4

[a]Total fatty acid composition of the triacylglycerols was recalculated on the basis of percentage fraction contents with comparison to original fatty acid composition. Ag-TLC, silver-ion-loaded thin-layer chromatography.

the FA composition of unfractionated TG analyzed previously (Table 1). The concentration of saturated and monoenoic FA in the reconstructed composition is slightly higher than that of the initial TG, as a consequence of the decrease in polyunsaturated FA content. Obviously, the partial loss of polyunsaturated FA is connected with the incomplete extraction of unsaturated TG from the TLC plate, but the increase of the polarity of extraction solvents leads to the elution of nonlipid materials and $AgNO_3$ in TG fractions.

After preliminary separation of TG according to the degree of unsaturation on the TLC plate, each fraction was analyzed by GC. High temperature is required for the GC analysis of TG; therefore, nonpolar temperature-stable chromatography phases are commonly used. Because such a GC phase type separates TG only by molecular weight, we applied recently developed high-temperature capillary columns of standard length (25–30 m) with the polar phase to separate TG according to both molecular weight and degree of unsaturation. The GC analysis yielded up to nine identifiable peaks in each TG fraction (Table 2). By analogy with ECL values for FAME, the ECN values were calculated for each peak. Saturated TG, designated as C_{50}, C_{52}, C_{54}, etc., had ECN values of 50, 52, 54, etc. GC of authentic TG standards showed that each double bond in unsaturated molecular species added 0.2–0.3 to the ECN values of saturated TG with the same number of carbon atoms. Thus, the preliminary identification of TG peaks was performed according to the general rules of TG elution order by Ag-TLC (13) and from the values of ECN (Table 2, left part). The reconstruction of the FA composition of TG fractions showed that several peaks consisted of two or three individual TG (Table 2, right part). For those, the percentages of TG molecular species were calculated from the percentages of the three component FA. It is, of course, possible to imagine a more complex mixture of TG, including numerous minor TG molecular species in each fraction, but in this work only basic authentic components were indicated. For all of them, the recalculated FA compositions of TG fractions agreed well with the experimentally obtained FA compositions (Table 2).

For the identification of TG, the majority of Ag-TLC fractions were additionally separated by HPLC. Each HPLC subfraction of TG was reanalyzed by GC, and its FA composition was determined and compared with the reconstructed FA composition, as described above. HPLC experimental data confirmed our preliminary identification of TG. Only four pure TG were isolated by HPLC (LLLn*, LLL, OLL, OOL; abbreviations given in Table 2), while other molecular species were eluted as mixture peaks. The GC analysis of the FA composition of pure TG subfraction gave the molar proportion of FAME peaks equal to that calculated from the formula of the corresponding TG.

Table 3 combines the TG composition of Ag-TLC fractions calculated in Table 2 and the list of total TG species of *P. koraiensis* seeds, based on the proportion and the composition of individual fractions. Thirty-one components with concentrations of more than 0.2% were detected in TG fractions, and 26 of them were identified. The main TG species were OLL (14.7%), LLLn* (10.7%), POL (8.3%), OOO (7.6%), and OOL (7.4%). This TG composition is similar to that of seed oils that contain linoleic acid as the major FA and a small percentage of linolenic acid. For example, soybean oil has 16% OLL, 7% LLLn, 2% OOO, and 8% OOL (20). Earlier we found that the distribution of TG of *P. koraiensis* seeds according to their molecular weight is similar to that of typical seed oils (21), and the presence of NMI FA does not appreciably influence this distribution. This investigation suggests

TABLE 2
Identified Triacylglycerol Molecular Species in Ag-TLC Fractions and Comparison of Recalculated and Directly Analyzed Fatty Acid Compositions of These Triacylglycerol Fractions[a]

ECN	Peak area of TG (%)	TG name	Amount of fatty acids (%) P	S	O	L*	L	Ln*	Em	Amount of TG (%)
Ag-TLC fraction 1										
53.36	6.6	PLLn*	2.2				2.2	2.2		6.6
55.63	37.9	LLL					5.0			5.0
		+OLLn*			11.0		11.0	11.0		33.0
56.06	54.1	LLLn*					36.0	18.0		54.0
57.49	0.4	Unkn.[b]								0.4
57.76	1.0	Unkn.								1.0
FA composition of fraction 1										
Recalculated			2.2	0.0	11.0	0.0	54.2	31.2	0.0	
Analyzed[c]			2.1	1.1	11.0	0.5	53.4	28.9	0.6	
Ag-TLC fraction 2										
52.88	2.8	POL	0.9		0.9		0.9			2.7
53.15	0.2	Unkn.								0.2
53.28	16.4	PLLn*	5.4				5.4	5.4		16.2
55.03	29.6	OOLn*			13.8			6.9		20.7
		+SLLn*		3.0			3.0	3.0		9.0
55.20	6.1	Unkn.								6.1
55.40	29.7	OLL			5.9		11.8			17.7
		+LLL*				4.0	8.0			12.0
55.73	15.2	LLL					15.2			15.2
FA composition of fraction 2										
Recalculated			6.3	3.0	20.6	4.0	44.3	15.3	0.0	
Analyzed			5.5	3.2	24.4	4.1	42.4	17.2	0.7	
Ag-TLC fraction 3										
52.97	8.2	POL	2.7		2.7		2.7			8.1
53.32	11.5	PLL	3.8				7.6			11.4
54.74	3.9	SOL		1.3	1.3		1.3			3.9
54.93	3.1	OOO			3.1					3.1
55.16	38.2	OOL			10.0		5.0			15.0
		+OLL*			3.4	3.4	3.4			10.2
		+SLLn		4.4			4.4	4.4		13.2
55.59	30.4	OLL			10.2		20.4			30.6
57.22	0.7	Unkn.								0.7
57.40	1.2	Unkn.								1.2
57.67	2.7	LLEm					1.8		0.9	2.7
FA composition of fraction 3										
Recalculated			6.5	5.7	30.7	3.4	46.6	4.4	0.9	
Analyzed			5.4	3.4	34.0	3.4	45.2	4.4	2.1	
Ag-TLC fraction 4										
50.68	3.8	PPL	2.6				1.3			3.9
52.65	3.5	PSL	1.0	1.0			1.0			3.0
53.00	37.5	POL	12.5		12.5		12.5			37.5
54.75	15.9	SOL		5.3	5.3		5.3			15.9
54.92	16.3	OOO			16.3					16.3
55.19	19.2	OOL			12.8		6.4			19.2
56.75	1.8	SLEm		0.6			0.6		0.6	1.8
57.01	1.9	OLEm			0.6		0.6		0.6	1.8
FA composition of fraction 4										
Recalculated			16.1	6.9	47.5	0.0	27.7	09.0	1.2	
Analyzed			12.4	6.8	47.2	0.0	28.4	0.0	2.7	
Ag-TLC fraction 5										
50.65	0.8	PPL	0.6				0.3			0.9
52.54	16.0	POO	5.3		10.6					15.9
52.62	1.1	PSL	0.4	0.4			0.4			1.2
54.87	74.4	OOO			64.5					64.5
		+SOL		0.8	0.8		0.8			2.4
		+SOO		2.5	5.0					7.5
56.31	1.0	SOEm		0.3	0.3				0.3	0.9
56.62	6.6	OOEm			4.4				2.2	6.6
FA composition of fraction 5										
Recalculated			6.3	4.0	85.6	0.0	1.5	0.0	2.5	
Analyzed			6.5	3.7	83.5	0.0	1.5	0.0	3.5	
Ag-TLC fraction 6										
50.26	4.7	PPO	3.2		1.6					4.8
52.22	3.0	PSO	1.0	1.0	1.0					3.0
52.52	55.5	POO	18.5		37.0					55.5
54.17	1.3	SSO		0.8	0.4					1.2
54.43	29.4	SOO		9.8	19.6					29.4
56.30	6.0	SOEm		2.0	2.0				2.0	6.0
FA composition of fraction 6										
Recalculated			22.7	13.6	61.6	0.0	0.0	0.0	2.0	
Analyzed			19.4	12.5	60.2	0.0	0.8	0.0	1.9	

[a]P: palmitic; S: stearic; O: oleic; L*: 18:2 Δ5,9; L: linoleic; Ln*: 18:2 Δ5,9,12; Em: eicosanoic; ECN: equivalent carbon number; TG: triacylglycerol; FA: fatty acid. See Table 1 for other abbreviation.
[b]Unidentified component.
[c]Data from Table 1.

TABLE 3
Triacylglycerol Composition (wt%) of Ag-TLC Fractions and the Whole Oil of *Pinus koraiensis* Seeds[a]

		Fraction number						Amount of TG
ECN	TG	1	2	3	4	5	6	(%)
50.26	PPO						4.8	0.1
50.68	PPL				3.9	0.9		0.6
52.22	PSO						3.0	0.1
52.54	POO					15.9	55.5	2.6
52.62	PSL				3.0	1.2		0.5
52.97	POL		2.7	8.1	37.5			8.3
53.15	Unkn.		0.2					0.1
53.32	PLL			11.4				3.8
53.36	PLLn*	6.6	16.2					5.5
54.17	SSO						1.2	0.0
54.43	SOO					7.5	29.4	1.3
54.74	SOL			3.9	15.9	2.4		3.5
54.87	OOO			3.1	16.3	64.5		7.6
55.03	OOLn*		20.7					5.4
—	SLLn*		9.0	13.2				6.7
55.16	OOL			15.0	19.2			7.4
55.20	OLL*			10.2				3.4
55.40	OLL			17.7	30.6			14.7
—	LLL*		12.0					3.1
55.63	OLLn*	33.0						6.5
55.73	LLL	5.0	15.2					5.0
56.06	LLLn*	54.0						10.7
56.30	SOEm					0.9	6.0	0.2
56.62	OOEm					6.6		0.5
56.75	SLEm				1.8			0.2
57.01	OLEm				1.8			0.2
57.22	Unkn.			0.7				0.2
57.40	Unkn.			1.2				0.4
57.49	Unkn.	0.4						0.1
57.67	LLEm			2.7				0.9
57.76	Unkn.	1.0						0.2
Fraction content (%)		19.5	25.7	32.4	12.9	6.8	2.7	

[a]Abbreviations as in Tables 1 and 2.

that the general distinction of TG species that include NMI FA consists of a different distribution of linoleic (18:2n-6) and pinolenic (18:3 Δ5,9,12) acids among the TG molecular species. TG with two or three acyl chains of pinolenic acid, the NMI structural analog to linolenic acid, were practically absent. Also, the percentage of LLL (5.0%) was reduced, and TG species with two linoleoyl groups prevailed in the total TG of the seeds. This result confirms the data about the location of linoleic acid in *P. koraiensis* seed oil at the *sn*-1 (50.1 mol%) and *sn*-2 (66.4 mol%) positions of TG (11). Using the methods of chemical cleavage with subsequent HPLC analysis, Wolff *et al.* (10) showed that Δ5-NMI FA of *P. koraiensis* seed oil were mainly esterified to the *sn*-3 position of TG (96.1%). Formerly, they suggested that NMI FA was enriched in only one position of TG (22). Such TG species are LLLn* (10.7%), OLLn* (6.5%), SLLn* (6.7%), OOLn* (5.4%), and PLLn* (5.5%).

The minor NMI FA 18:2 Δ5,9 was found in two TG species, OLL* (3.4%) and LLL* (3.1%). Like pinolenic acid, TG species did not esterify more than one 18:2 Δ5,9 acid and did not register in appreciable amounts. The distribution of C_{20} NMI FA in TG species of *P. koraiensis* is not quite clear, but we propose that two components in the first Ag-TLC fraction, with ECN of 57.49 (0.1%) and 57.76 (0.2%), are TG species esterified with two linoleic acids and one of either 20:2 or 20:3 acid. To our observation, at least 41.3% of the total TG species contained one NMI FA. This value is in good agreement with the same parameter (45.6%) calculated earlier (11).

From an analytical point of view, we have found that high-temperature GC of TG on a capillary column of medium polarity is suitable, in general, for the complete separation of TG mixtures according to degree of unsaturation and molecular weight after preliminary separation by another chromatographic method. Attempts to perform the direct GC analysis of total TG of *P. koraiensis* were unsuccessful. We had some problems in the identification and calculation of small peaks of high-molecular-weight TG species because these peaks were wide and their RT was not stable. Long-length capillary columns for TG analysis have a very thin film of liquid phase to reduce the retention time of high-boiling substances. Therefore, even an insignificant overload of the column leads

to the appearance of wide, "triangular" peaks. Thus, two components that were successfully divided at small concentrations, divided poorly at a large column loading. Also, the aluminum-clad capillary column (made by Quadrex Co.) has low mechanical durability and can be broken during its storage in the chromatograph oven.

Gresti *et al.* (11) have reported that the elution volume of the Δ5 isomer of octadecatrienoic acid methyl ester was higher than that for the Δ9 isomer with reversed-phase HPLC and that the elution volume of the TG isomer with Δ5 acyl group was higher as well (11). The reversed elution order of the above FAME isomers was observed in GC analysis, and we found the same order of TG isomers with GC analysis. For example, OOL* eluted earlier than OOL, and PLL and PLLn* coeluted together on the polar GC column (Table 3).

At the present time, there is no universal method for the analysis of TG molecular species, and the choice of methods depends on the nature of the object to be analyzed and on the equipment that is at the disposal of the researcher. Preparative Ag-TLC plates separated TG of different degrees of unsaturation quite well, but some TG bands on the plate were wide and cross-covered, as was shown by GC (Table 3). Also, the loss of highly-unsaturated components was observed. Compared with the HPLC separation of TG of conifer seed oils, the best methods for direct analysis of these TG would, in our opinion, be the combination of HPLC, followed by high-temperature GC of TG.

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