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TRIGLYCERIDE COMPOSITION OF *Pinus sibirica* OIL

V. I. Deineka[1] and L. A. Deineka[2]

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The triglyceride composition of pine oil was investigated by reversed-phase HPLC. The fatty-acid composition of the oil was calculated (mol %): 18:3, 17.1 ± 2.0; 18:2, 49.0 ± 2.3; 18:1, 23.8 ± 2.1; 18:0, 2.5 ± 0.1; 16:0, 6.3 ± 2.2. The retention times of the triglycerides containing octadecatrienoic acid were consistent with the 18:3(5,9,12) structure.

Key words: HPLC, triglycerides, fatty acids, pine oil.

Oil of pine nuts (*Pinus sibirica* Du Tour) is popular as a medicinal and biologically active additive. Information on the fatty-acid composition of this preparation, especially the octadecatrienoic acid, is contradictory. Comparison of the results of two investigations [1] favors the one [2] in which the positions of the double bonds in octadecatrienoic acid are determined as 18:3(5,9,12). This acid makes up 30.17% of the total. The presence of 18:3(5,9,12) acid in pine-nut oil was confirmed [3]. This acid was used for taxonomic classification of plants in the Gymnospermae class [4]. However, information from OOO "Cybervision" on the internet reports the inclusion of γ-linolenic triglycerides (19.09%) in the composition. Some investigators identify the octadecatrienic acid in the oil of this plant as linolenic, although its fraction was estimated as 3.5% [5] and 25.6% [6].

HPLC is a method that enables the analysis of triglycerides and not their conversion products. It has advantages over traditional methods [7, 8]. We used reversed-phase HPLC with refractive-index detection to determine the composition of pine-nut oil.

Figure 1a shows the chromatrogram of the oil. Comparison with the chromatogram of apricot or peach oils [7] identifies the signals of **3** (trilinoleate, L_3), **6** (dilinoleate—oleate, L_2O), **10** (linoleate—dioleate, LO_2), and **13** (trioleate, O_3). The signal of another triglyceride is superimposed on that of **7**, which corresponds to dilinoleate—palmitate (L_2P). This is evident in the broadening of this peak. The doubling of the signals for **11** and the pair **14** + **15** is typical for co-eluting problematic triglycerides of composition L_2S + LOP and LOS + O_2P (S represents steric acid) [8]. Thus, the distinguishing triglycerides of pine-nut oil relative to linoleic—oleic oils are triglycerides **1**, **2**, **4**, and **5** (Table 1).

The equality of the two successive increments for the more weakly retained triglycerides **1** and **2** on going from 1→2 and 2→3 (0.064 and 0.064) is consistent with a uniform exchange of acid moieties. Designating the 18:3(5,9,12) acid as A, it can be confirmed that triglycerides **1** and **2** have the compositions A_2L and AL_2, respectively. The incremental approach easily identifies the signals of **4** and **5** as ALO and ALP. This is also typical of oils containing octadecatrienoic acid. Triglycerides ALO and L_3 are separated well enough. However, the analogous pair for α-linolenic acid under these conditions is practically not separated [8]. Therefore, the A moiety does not correspond to α-linolenic acid.

The studied oil was compared with the extract of *Borago officinalis* seeds, a known source of γ-linolenic acid (γL), to check for the presence of this acid [10]. However, the retention times of the pine-oil triglycerides are longer, as before, than those for their γ-linolenic analogs. This was confirmed by the doubling of the signals corresponding to the pairs A_2L + γL_2L and AL_2 + γLL_2 upon elution of a mixture of these oils.

Acid radicals with the 18:3(5,9,12) arrangement of double bonds were observed by Uzbek scientists in oil of plants of the Ranunculaceae family [11]. The configuration of the double bonds was established. This 18:3(*trans*-5,*cis*-9,*cis*-12) acid was called ranunculenic.

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1) Belgorod State University, 308015, Belgorod, ul. Pobedy, 85; 2) Belgorod State Agricultural Academy, 309503, Belgorod Dist., Maiskii, ul. Vavilova, 1. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 126-128, March-April, 2003. Original article submitted April 8, 2003.

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TABLE 1. Triglyceride Composition of *Pinus sibirica* Du Tour Nut Oil

No.	Retention time, t_R, min	Logarithm of capacity coefficient, log k′	Identification	Relative content, mol %
1	6.10	0.361	A_2L	1.3
2	6.77	0.425	AL_2	21.2
3	7.55	0.489	L_3	11.8
4	8.07	0.527	ALO	12.7
5	8.56	0.560	ALP	4.3
6	9.06	0.591	L_2O	12.0
7	9.7	0.628	$AO_2 + L_2P$	12.8
8	10.2	0.655	ALS	2.7
9	10.65	0.677	AP_2	1.4
10	11.0	0.694	LO_2	5.3
11	11.56	0.720	L_2S + LOP	3.2
12	12.52	0.761	LP_2	2.8
13	13.5	0.799	O_3	3.2
14	14.1	0.821	LOS	1.1
15	14.45	0.833	O_2P	1.2
16	15.15	0.857	LPS + OP_2	0.5

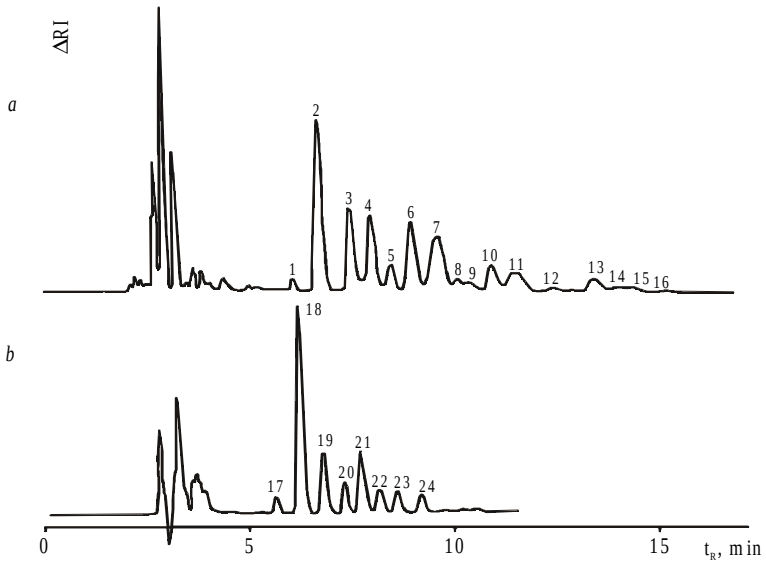

Fig. 1. Separation of pine-nut-oil (a) and aquilegia-seed (b) triglycerides. Column: Diasphere-110-C18, 6μm (250×4 mm). Eluent: 10 vol % CH_3CN in acetone, 1 mL/min. Peaks are assigned in the text and Table 1.

In fact, the chromatogram (Fig. 1b) of the acetone extract of aquilegia seeds (*Aquilegia hybrida hort*) shows basically triglycerides that contain ranunculenic acid. Using the incremental method to identify the composition of these triglycerides is not particularly difficult. R_3 (R is ranunculenic acid) is present in insignificant quantities (Fig. 1b, **17**); R_2L (**18**) is the main component. The content of triglyceride RL_2 (**19**) is much lower; of R_2O (**20**), slightly lower than that of the problematic pair $L_3 + R_2P$ (**21**). The quantitative ratio is close to unity for the pair RLO (**21**) + RLP (**22**). The last of the significant signals was identified as the triglyceride containing steric acid, R_2S (**24**).

Comparison of the chromatograms confirms the identical position of the double bonds in octadecatrienoic acids of pine-nut oil and aquilegia. Analogous combinations of triglycerides have similar retention times (a difference of 0.03-0.07 min is

typical of the reproducibility). However, slightly broadened peaks were found in the chromatograms of a mixture of these extracts. This indicates that the corresponding triglycerides do not completely coincide. This could be a consequence of different distributions of the *cis*- and *trans*-bonds in the octadecatrienoic acids or different positions for the radicals.

A recalculation of the triglyceride composition of fatty acids taking into account the uncertainty due to the presence of inseparable pairs of triglycerides and differences in the theoretical refractive indices of the triglycerides (using the ACDLabs program) gives the composition (mol %): 18:3, 17.1 ± 2.0; 18:2, 49.0 ± 2.3; 18:1, 23.8 ± 2.1; 18:0, 2.5 ± 0.1; 16:0, 6.3 ± 2.2. These are close to the literature values [5].

EXPERIMENTAL

We used pine-nut oil from OOO TPK "Aromas of Life" and oil obtained by acetone extraction of nuts acquired through retail sales. The compositions of the triglycerides of both oils do not differ in principle. Aquilegia seeds were taken from plants grown in Belgorod district (2002 season).

HPLC of the oils and the retention times were calculated as before [7-9].

Thus, the triglyceride composition of pine-nut oil was studied. It was found that it does not contain α- and γ-linolenic acids. The principal components of the oil are linoleic (49 mol %) and oleic (24 mol %) acids. The 18:3(5,9,12) structure of the octadecatrienoic acid of this oil (17 mol %) is consistent with the retention times of triglycerides under reversed-phase HPLC conditions. However, triglycerides of this oil are different from the corresponding triglycerides of aquilegia, which include ranunculenic acid with an analogous distribution of double bonds in the carbon chain.

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