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LOW-MOLECULAR-WEIGHT TRIACYLGLYCEROLS OF THE SEED OIL OF *Artemisia absinthium*

N. T. Ul'chenko, E. I. Gigienova, A. U. Umarov, and U. A. Abdullaev

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Low-molecular-weight triacylglycerols have been isolated from the seed oil of *Artemisia absinthown* in which one acyl radical is derived from ethanoic, propanoic, butanoic, pentanoic, hexanoic, heptanoic, octanoic, or nonanoic acid. Their main representatives are the ethanoyl and propanoyl derivatives, position 2 being occupied mainly by the ethanoyl radical. The isomers with a short acyl radical in position 2 make up 75% of the total of the low-molecular-weight triacylglycerides isolated.

Triacyl glycerols in which one acyl radical is derived from a short-chain fatty acid are unusual components of seed oils. The first information in the literature on the presence of these compounds in plants appeared in 1967. These were acetyldiacylglygerols (ADGs) of the seed oil of *Impatiens edgeworthii*, family Balsaminaceae [1]. Other sources of these compounds have also been found — the seed oils of *Celastrus orbiculatus* (59% of ADGs on the oil) and of *Euonymus verrucosus* (68-69%), family Celastraceae, in which the acetyl is present in position 3 of the triacyl-sn-glycerol, [2]. Quite recently, ADGs have been found in the seed oils of *Polygala virgata* (74%) and of *Securidaca longipedunculata*, family Polygalaceae [3, 4]. In the first of these, the acetyl is found exclusively in position 2 of the triacyl-sn-glycerol. And, finally, it has become known that triacylglycerols of this type are also produced in the animal world: the ADGs of the insect *Icerya purchasi* [5], the sorboyldiacylglycerols of the aphid [6], and the isovaleroyldiacylglycerol of *Sotalia fluviatilus* [7]. The appearance of the above-mentioned low-molecular-weight triacylglycerols in some plant and animal tissues may bear definite biological information.

The present paper reports the presence of a whole series of low-molecular-weight triacylglycerols in the seed oil of *Artemisia absinthium* (family Asteraceae) of the 1979 harvest which differ from those mentioned above by the fact that they contain propanoyl, butanoyl, pentanoyl, hexanoyl, heptanoyl, octonoyl, and nonanoyl radicals.

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TABLE 1. Molecular Ions (relative intensity, %) of the Main Components of the Low-Molecular-Weight Triacylglycerols

Main molecular types	Short-chain fatty acids (Sh)							
	$C_{2:0}$	$C_{3:0}$	$C_{4:0}$	$C_{5:0}$	$C_{6:0}$	$C_{7:0}$	$C_{8:0}$	$C_{9:0}$
Natural								
LLSh	658(100)*	672(6)	686(5)	700(2)	714(0,5)	728(0,5)	742(2)	756(0,5)
LOSh	660(75)	674(5)	688(6)	702(1)	716(0,5)	730(0,4)	744(2)	758(0,5)
LPSh	634(20)	648(7)	662(20)	676(1)	690(8)	704(1)	718(6)	782(0,5)
Hydrogenated: 2- Isomers								
StStSh	666(100)	680(30)	694(tr.)	708(tr.)	722(tr.)	—	—	—
StPSh	638(75)	652(15)	666	680	694	—	—	—
1- and/or 3-isomers								
StStSh	666(100)	690(100)	694(10)	708	722(2)	736(1)	750(5)	764(2)
StPSh	638(30)	652(80)	666	680	694	708	722(0,5)	736(0,5)

*The peak in the range of masses of the molecular ions was taken as 100%.

The oil was isolated by the cold extraction method from seeds of the 1979 harvest collected at the end of September on the slopes of the western Tien Shan. By column chromatography, the oil yielded about 9% of triacylglycerols the structure of which was confirmed by their mass spectra (Table 1) and IR and PMR spectra. This components of the oil had chromatographic mobilities differing slightly from those of the triacylglycerols containing acyl radicals with numbers of carbon atoms of from 16 to 20. The R_f values of these unusual components of the oil coincide with the R_f values of epoxyacyldiacylglycerols. However, in a study of this sample of oil the problem of separating the two types of glycerol esters mentioned did not arise since they contained practically no epoxyacyldiacylglycerols.

The presence of acetyl groups in the triacylglycerols isolated was confirmed by their IR (1230 cm^{-1}, s) and PMR (s, δ, 1.98 ppm) spectra. In the water-soluble hydrolysis products, acetic acid was detected from its strong specific odor.

The combined fatty acids were isolated from the ether-soluble products of alkaline hydrolysis of the low-molecular-weight triacylglycerols and according to GLC at 198°C they had the following composition (mole % of the total) C_{12}—1.1; $C_{14:0}$—0.3; $C_{15:0}$—0.3; $C_{16:0}$—13.1; $C_{17:0}$—0.5; $C_{18:0}$—1.3; $C_{20:0}$—tr.; $C_{22:0}$— tr.; $C_{16:1}$—0.8; $C_{18:1}$—11.1; $C_{18:2}$—69.6; $C_{18:3}$—1.9. As can be seen, the main long-chain fatty acid components of the triacylglycerols isolated were linoleic (L in the Table), oleic (O), and palmitic (P) acids. Consequently in the mass spectra of the initial and of the hydrogenated low-molecular-weight triacylglycerols (Table 1) the main peaks are those of the molecular ions of the species corresponding to combinations of these acids and the hydrogenated derivatives of unsaturated acids (St) and short-chain acids (Sh).

The combined homologs of the short-chain fatty acids were identified by TLC and GLC at 132°C in comparison with model specimens of the acids. The following composition was found, mole % on the total: $C_{2:0}$—30.1; $C_{3:0}$—14.9; $C_{4:0}$—10.3; $C_{5:0}$—13.1; $C_{6:0}$—14.0; $C_{7:0}$—5.6; $C_{8:0}$—8.8; $C_{9:0}$—3.2. To determine the positions of the short-chain acyl radicals in the low-molecular-weight triacylglycerol molecules, the latter were subjected to enzymatic hydrolysis with pancreatic lipase. From the hydrolysis products by the TLC method in system A we isolated 2-monoacylglycerols of long-chain fatty acids (LFAs) with the following composition according to GLC (mole % on the total) $C_{12:0}$—1.3; $C_{14:0}$—0.8; $C_{16:0}$—2.5; $C_{17:0}$—1.2; $C_{18:0}$—0.9; $C_{16:1}$—0.5; $C_{18:1}$—16.6; $C_{18:2}$—74.1; $C_{18:3}$—2.1.

The yield of 2-monoacylglycerols of LFAs was about 25%. Consequently the remaining 75% was due to 2-monoacylglycerols of short-chain fatty acids, which were soluble in the aqueous layer.

The saturated derivatives of the low-molecular-weight triacylglycerols were separated by preparative TLC in solvent system b [3], into the 2- and 1(3)-isomers with respect to the

position of the short-chain acyl radicals. It was found that the amount of 2-isomers was almost three times that of 1(3)-isomers. These facts were confirmed by the results of enzymatic hydrolysis and it was shown that the short-chain fatty acids are present predominantly in position 2 of the triacyl-sn-glycerols isolated.

By studying the NMR spectrum of the hydrogenated 2-isomers and comparing the integral intensities of the α-methylene (in relation to the ester carbonyls) and acetyl protons, it was found that the integral intensity of the RCH_2CO protons amounted to 4-5 H and not to 6 H. Thus, of the three acyl radicals only one, on the whole, has no α-methylene protons. Consequently, position 2 of the triacyl-sn-glycerols is occupied predominantly by acetic acid. The other acyl radicals revealed from the results of mass spectroscopy (Table 1) are propanoyl ($C_{3:0}$), butanoyl ($C_{4:0}$), and traces of pentanoyl ($C_{5:0}$) and of hexanoyl ($C_{6:0}$). According to their mass spectrum, the hydrogenated 1(3)-isomers (Table 1) contained acyl radicals of all the acids mentioned above, and also heptanoyl ($C_{7:0}$), octanoyl ($C_{8:0}$), and nonanoyl ($C_{9:0}$).

Thus, about 75% of the low-molecular-weight triacylglycerols have in their second position mainly acetic acid with very small amounts of propanoic and butanoic and traces of pentanoic and hexanoic acids. About 25% of the low-molecular-weight triacylglycerols have mainly ethanoic and propanoic acids in positions 1 and/or 3 with small amounts of butanoic, pentanoic, hexanoic, heptanoic, octanoic, and nonanoic acids.

EXPERIMENTAL

The spectral characteristics of the substances were obtained on MKh-1310, UR-10, and JNM-4H-100/100 MHz instruments.

Gas–liquid chromatography was performed on a Khrom-4 instrument with 15% of Reoplex-400 on Chromaton N-AW-HMDS at 132°C for the methyl esters of the short-chain fatty acids, and ethylene succinate on Chromaton N-AW-HMDS at 198°C for the methyl esters of the high-molecular-weight fatty acids. In both cases, columns 2.5 m long with an internal diameter of 0.3 cm were used.

Thin-layer chromatography was performed with the use of silica gel L 5/40 and the following solvent systems: a) hexane–ether (1:9), b) hexane–ether (3:2, by volume).

The oil was extracted from the previously comminuted seeds by steeping with petroleum ether at room temperature.

The low-molecular-weight triacylglycerols were isolated from a column containing silica gel L 100/250 μ with hexane–diethyl ether (8:2, by volume). On Silufol in the same system the low-molecular-weight triacylglycerols had R_f 0.35.

The IR spectra of the low-molecular-weight triacylglycerols, ν_{max}^{film}, cm^{-1}: 3010 m, 1660, w (–CH=CH–); 2960 s, 2870 s, 1370 m ($-CH_3$); 2930 s, 2860 s, 1465 m, 730 m ($-CH_2-$); 1750 s, 1420 m, 1230, 1170 s, 1105 m, 1055 m (–OCOR, $-OCOCH_3$).

PMR spectrum of the low-molecular-weight triacylglycerols, HMDS, δ, ppm: t 0.8 ($-CH_3$), m 1.22 ($-CH_2-$), m 1.62 ($-CH_2CH_2CO-$), s 1.98 (CH_3CO-), t 2.23 ($-RCH_2CO-$), m 2.68 ($=CHCH_2CH=$), m 4.06 ($-CH_2OCOR$), m 5.1 (–CHOCOR), m 5.2 (–CH=CH –).

PMR spectrum of the hydrogenated low-molecular-weight triacylglycerols (2-isomers with respect to the position of the short-chain acyl radical), HMDS, δ, ppm: t 0.8 ($-CH_3$, 6 H), s 1.24 ($-CH_2-$), s 1.97 (CH_3CO-, 3 H), m 2.22 (RCH_2CO-, 4-5 H), m 4.09 ($-CH_2OCOR$, 4 H), m 5.2 (–CHOCOR, 1 H).

The hydrogenation of the low-molecular-weight triacylglycerols was carried out in the presence of 5% (on the sample) of palladium on alumina (1:1) in a mixture of ethanol and diethyl ether at 60°C with the bubbling of an excess of hydrogen.

Alkaline hydrolysis was carried out with 1 M KOH in methanol at 40°C with a magnetic stirrer for 2 h.

The short-chain fatty acids were isolated by the method proposed by Yunusova et al. [8].

SUMMARY

In a study of one of the samples of a seed oil of *Artemisia absinthium* low-molecular-weight triacylglycerides, one acyl radical of which is formed by one of the eight homologs of normal saturated fatty acids with even and odd numbers of carbon atoms from $C_{2:0}$ to $C_{9:0}$, were detected and their presence was proved.

Of the components isolated, 75% contained the short-chain fatty acids. Mainly acetic, in position 2, and 25% contained them in positions 1 and/or 3 with a predominance of acetic and propionic acids.

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SEED OIL OF *Cousinia severzovii*

N. T. Ul'chenko, E. I. Gigienova, U. A. Abdullaev, K. L. Seitanidi, and A. U. Umarov

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The composition of the seed oil of *Cousinia severzovii* has been studied; about 20 types and classes of compounds were isolated from the oil, including 6% of triterpenes with a molecular weight of 426 in the free state, as the acetylated derivatives, and as derivatives acylated with higher fatty acids of the 34:0-13:0 group in a ratio of 1:1:2, respectively; and 3.8% of oxidized acylglycerols (seven types). The classes of lipids were represented by no individual compound but only by a series of homologs or isomers.

Lipids of complex composition have been detected in the seed oil of *Cousinia severzoii*, family Asteraceae growing on the western slopes of the Chatkal range. While with the aid of analytical thin-layer chromatography in systems a, c, e, and j only 6-8 zones of substances were detected in the seed oil, by means of column chromatography (100 g of oil) and preparative thin-layer chromatography in systems a-k 20 classes and types and lipids were isolated from it. The assignment of each of these components was based on a comparison of their chromatographic mobilities with those of model substances isolated from oils investigated by us previously, and it was confirmed by qualitative reactions 1-4 in a thin layer of silica gel and by spectral characteristics. The esters were subjected to alkaline and enzymatic hydrolysis. The ratio of the numbers of oxidized and unoxidized acyl radicals isolated from the products of the alkaline hydrolysis of the acylglycerols was determined gravimetrically after their separation in a thin layer of silica gel in system e and *l*.

The separation and identification of the fatty acids isolated was carried out by preparative chromatography in a thin layer of silica gel (systems e and j) by qualitative reac-

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