OXYTRIGLYCERIDES OF THE SEED OIL OF THE TASHKENT-1 VARIETY OF COTTON PLANT

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It is natural to assume [1] that oxidized fatty acids (oxy-FAs) in oils containing them in high concentration will be present in the triglycerides (TGs) as acyl radicals. Nevertheless, the number of oxyacyl TGs (oxy-TGs) that has been studied is extremely small in comparison with the number of oxy-FAs that has been studied. (The prefix "oxy" denotes only the presence of an oxygen-containing functional group in the chain of the alkyl radical.)

We have previously [2] isolated from the seed oils of cotton plants of variety Tashkent-1 the methyl esters of α-hydroxyoctadecadienoic acids by transesterifying the oil with methanol, as for the majority of oxy-FAs described in the literature. The structure of the esters by the decomposition of which they were obtained has remained obscure. In the present paper we report the isolation from the above-mentioned oil of about 3% of triglycerides, one acyl radical of which is oxidized.

The presence of active oxygen in the molecule of an oxy-TG was detected qualitatively from the coloration of the thiocyanate reagent. Oxy-TGs instantaneously liberate free iodine from a solution of potassium iodide and readily form with formaldehyde a hydroxymethyl peroxide, and they instantaneously decolorize a solution of methylene blue.

To isolate the oxy-TGs from the oil we used column chromatography (CC) on silica gel and solvent systems 1 and 2. The results of IR and NMR spectroscopy showed the presence in the acyl radicals of hydroxyl and of secondary alcohol and hydroperoxy groups.

The IR spectra showed a region of absorption of the bonds of cis,trans-conjugated dienes together with those of isolated ethylene bonds.

The determination of the region of resonance of the hydroperoxy and hydroxylic protons (total 1 H) was difficult, since their chemical shifts vary from 2 to 7 ppm. Nevertheless, in some cases it was possible to detect them with the aid of trifluoroacetic acid at 3.25 and 5.35 ppm.

The NMR spectrum of the hydrogenated derivatives of the oxy-TGs differed from that of the initial oxy-TGs only by the absence of signals due to ethylenic bonds and revealed the one-proton signal of the esterified secondary alcohol group of glycerol —CHOCOR.
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When the oxy-TGs were hydrogenated, together with the saturated oxy-TGs, saturated unsubstituted TGs (n-TGs) were formed (~ 30% of the total hydrogenation products) as was shown by TLC in system 1 and a comparison of the R_f values with the mobilities of the initial oxy-TGs and the n-TGs isolation from the oil by the CC method. The mixture of these hydrogenation products was separated by preparative TLC on glass plates.

The IR spectrum of the n-TGs lacked the region of absorption of hydroxyl and hydroperoxy groups, and ethylenic bonds. The NMR spectrum of the n-TGs showed the complete absence of —CH(O⁻)— protons in the 3.5-ppm region.

The formation of n-TGs is connected with the fact that in the process of catalytic hydrogenation intermolecular transesterification of the triglycerides takes place.

The oxy-TGs were subjected to transesterification with methanol in the presence of a catalytic amount os sodium methanolate, to mild alkaline hydrolysis (Scheme 1).

From the water-soluble products of transesterification and alkaline hydrolysis a polyol was isolated which was detected as the only organic component in a thin layer of silica gel

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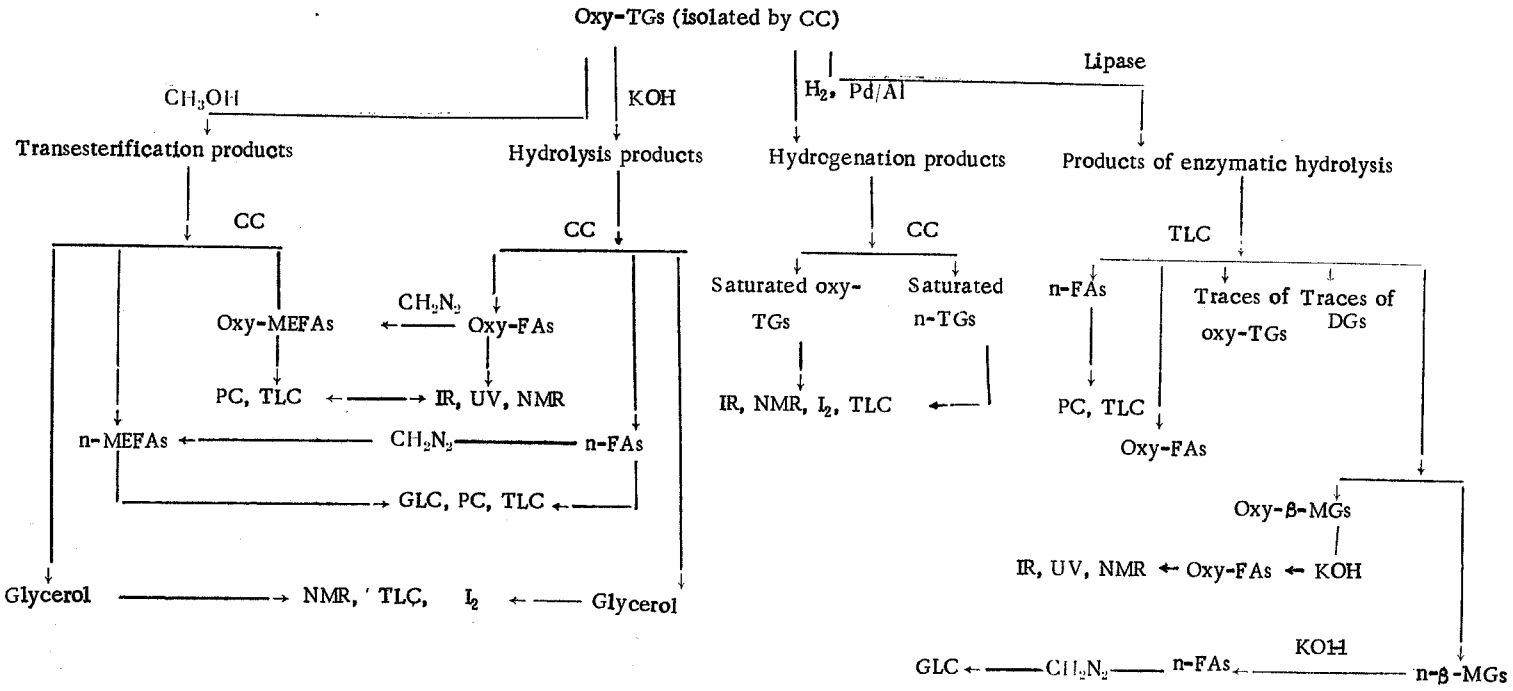
Oxy-TGs (isolated by CC)
CH3OH
KOH
H2, Pd/Al
Lipase
Transesterification products
Hydrolysis products
Hydrogenation products
Products of enzymatic hydrolysis
CC
CC
CC
TLC
CH2N2
Oxy-MEFAs
Oxy-FAs
Saturated oxy-TGs
Saturated n-TGs
n-FAs
Traces of oxy-TGs
Traces of DGs
PC, TLC
IR, UV, NMR
IR, NMR, I2, TLC
PC, TLC
Oxy-FAs
n-MEFAs
CH2N2
n-FAs
GLC, PC, TLC
Oxy-β-MGs
IR, UV, NMR
Oxy-FAs
KOH
Glycerol
NMR, TLC, I2
Glycerol
GLC
CH2N2
n-FAs
KOH
n-β-MGs

Scheme 1

using solvent systems 3, 4, and 5. The chromatographic mobility of the polyol corresponds to the mobility of glycerol. The polyol isolated and glycerol showed identical NMR spectra in deuterated methanol — broadened singlets of the HO—CH_2—CH(OH)—CH_2—OH protons at 3.51 ppm and of —OH at 4.79 ppm. When trifluoroacetic acid was added, the signal of the —OH protons (4.79 ppm) shifted into the 5.33-ppm region.

To identify the ether-soluble reaction products we used paper chromatography, thin-layer chromatography, and gas—liquid chromatography. Under these conditions we detected: in the transesterification products, the methyl esters of unsubstituted FAs (n-MEFAs) and the methyl esters of oxy-FAs (oxy-MEFAs); in the products of alkaline hydrolysis, unsubstituted FAs (n-FAs) and oxy-FAs; and in the products of enzymatic hydrolysis, acyl monoglycerides (MGs) and oxyacyl MGs, traces of acyl diglycerides and oxyacyl diglycerides, n-FAs and oxy-FAs, and traces of TGs.

The reaction products were separated into their individual components by the CC method in solvent systems 2 and 6.

According to UV, IR, NMR, and mass spectroscopy, the oxy-MEFAs were identical with the methyl esters of α-hydroxyoctadecadienoic acids that we had identified previously.

We are the first to have isolated free oxy-FAs from the oil under investigation. According to UV, IR, and NMR spectroscopy they correspond to the acyl fraction of the methyl esters of the α-hydroxyoctadecadienoic acids that we had isolated previously (hydroxy-$C_{18:2}$).

The results obtained show that in the native form the oxyacyl radicals of the oxy-TGs are α-hydroperoxyoctadecadienoic radicals. On isolation from the oils of the methyl esters of the acids and their separation, as is well known, hydroperoxy groups, which have a low reduction potential, readily lose their active oxygen, being reduced to hydroxy groups. It is just for this reason that the hydroperoxy group has not been detected in the methyl esters of the oxyoctadecadienoic acids.

According to gas—liquid chromatography (GLC) the n-MEFAs had the following composition (%): $C_{14:0}$, 0.7; $C_{16:0}$, 30.9; $C_{16:1}$, 2.2; $C_{18:0}$, 3.0; $C_{18:1}$, 25.9; $C_{18:2}$, 37.3. The composition of the n-FAs detected by paper chromatography confirmed the qualitative results of thin-layer chromatography and gas—liquid chromatography given above for the methyl esters.

The weight ratio between the sum of the n-FAs and the sum of the oxy-FAs (as also between the sum of the methyl esters of the two species isolated from the hydroxy-TGs) was 2:1. This means that among the acyl radicals of the oxy-TGs the oxy-FAs amounted to one third (33.3% of the sum of the FAs). Consequently, the composition of the remaining 66.7% of fatty acids corresponds to the GLC results given above. From this it is easy to calculate the fatty-acid composition of the oxy-TGs taking the ocyacyl radical into account (%): $C_{14:0}$, 0.5; $C_{16:0}$, 20.6; $C_{16:1}$, 1.5; $C_{18:0}$, 2.0; $C_{18:1}$, 17.2; $C_{18:2}$, 24.9; oxy-$C_{18:2}$, 33.3.

By TLC on glass plates in system 6 we isolated two types of MGs from the products of the enzymatic hydrolysis of the oxy-TGs: α-oxyoctadecadienoyl and ordinary acy types. The weight ratio between them, found by the gravimetric method, was 1:1.7.

The acyl MGs were obtained by mild alkaline hydrolysis. From the hydrolysis products we isolated the n-FAs, which were methylated with diazomethane. The resulting mixture of methyl esters was identified by GLC. The fatty-acid composition of the acyl monoglycerides was, according to GLC, as follows (%): $C_{16:0}$, 3.7; $C_{18:1}$, 20.3; $C_{18:2}$, 76.00.

Knowing the quantitative ratio and the fatty-acid composition of the two types of monoglycerides, we calculated the total fatty-acid composition of the total MGs (%): $C_{16:0}$, 2.3; $C_{18:1}$, 12.8; $C_{18:2}$, 47.9; oxy-$C_{18:2}$, 37.0.

From the results of the fatty-acid composition of the oxy-TGs and of the MGs isolated from them, we calculated the typical triglyceride composition based on the following types of acids — palmitic (P), oleic (O), linoleic (L), and hydroxyperoxy (H) (%):

α-oxy TGs (61.9%)			β-oxy-TGs (38.1%)	
PPH, 1.1	POH, 5.9	LPH, 22.2	PHP, 9.1	OHO, 3.8
OPH, 0.7	OOH, 6.1	OLH, 14.3	PHO, 11.8	OHL, 4.7
LPH, 0.4	LOH, 2.4	LLH, 8.8	PHL, 7.3	LHL, 1.4

The results obtained showed that the α-oxyoctadecadienoyl radical is present in the β position of only 38% of the triglyceride molecules; in the remaining 62% of oxy-TGs the oxyacyl radicals are localized in the α position.

EXPERIMENTAL

The UV spectra were taken on a Hitachi instrument, the IR spectra on a UR-10, and the NMR spectra on a JNM-4H-100/100 MHz instrument using concentrations of 10-12% in carbon tetra chloride with HMDS as internal standard. Gas–liquid chromatography was carried out on a UKh-instrument under the following conditions: 15% of Reoplex-400 on Chromaton N-AW-HMDS at 203°C, copper column with an internal diameter of 4 mm and a length of 2.5 m.

Isolation of the Oil. Immediately after their comminution, the oil was extracted from the seeds with hexane at room temperature by the steeping method.

The oxy-TGs were isolated by column chromatography using silica gel L 100/250 μ as adsorbent.

The isolated oxy-TGs were soluble in hexane, diethyl ether, carbon tetrachloride, chloroform, acetone, etc. Their optical activity was $[\alpha]_D^{24}$ + 17.4° (21 mg/ml, in chloroform).

IR spectrum of the oxy-TGs, ν_{max}^{film}, cm^{-1}: 3550-3480 m (–OOH or –OH); 3010 m, 1635 w, 990 and 950 m (–CH=CH–); 2965 s, 2870 s, 1380 m ($-CH_3$); 2930 s, 2860 s, 1465 m, 730 m ($-CH_2-$) 1745 s, 1420 m, 1245 s, 1170 s (–OCOR); 1110 and 1120 s [–CH(O⁻)–]; 1070 and 855 w m (–OOH).

The IR spectrum of the hydrogenated derivatives of the oxy-TGs, ν_{max}^{film}, cm^{-1}: 3500-3400 (–OOH and –OH); 2965 s, 2880 s, 1380 m ($-CH_3$); 2930 s, 2860 s, 1475 m, 1300-1200 m, 730 m ($-CH_2-$); 1745 s, 1420 m, 1260 s, 1180 s (–OCOR); 1110 s [–CH(O⁻)–]; 1070-800 w.

NMR spectrum of the oxy-TGs, δ scale, ppm: unsymmetrical to 0.86 ($-CH_3$, 9 H), m 1.26 ($-CH_2-$), m 1.55 ($-\underline{CH_2}CH_2COO^-$), superimposed m 2.0 ($-\underline{CH_2}CH=$ and $-\underline{CH_2}CH_2CH=$), t 2.22 ppm ($-CH_2COO^-$), m 2.68 ($=CH\underline{CH_2}CH=$), m 3.51 [–CH(O⁻)–, 1 H], m 4.06 ($-\underline{CH_2}OC-$, 4 H), m 5.1 (–CHOC–), m 5.23 (–CH=CH–).

Thin-layer chromatography was performed on "Silufol" plates or on glass plates (18 × 24 cm) with silica gel L 5/40 μ and 5% of gypsum. Solvent systems: 1) hexane–ether (8:2); 2) hexane–ether (1:1); 3) 2% ammonia–methanol (2:3); 4) chloroform–methanol (85:15); 5) isopropanol–25% ammonia–water (7:1:2); 6) ether–hexane (9:1).

The method of paper chromatography has been described previously [3].

Hydrogenation was carried out under the conditions recommended for the hydrogenation of phospholipids [4]. To isolate the hydrogenation products from the reaction mixture the diethyl ether and ethanol were distilled off. Then the residue was treated with acetone, after which the catalyst was filtered off. The hydrogenate was crystallized from aqueous acetone in the vacuum of the water pump, after which crystallization from acetone was repeated several times. A white powdery substance (the sum of the saturated TGs and the oxy-TGs) was obtained. The filtrate was colored dark brown. The NMR spectrum of the residue after the evaporation of the filtrate showed no foreign impurities and corresponded to saturated TGs.

Enzymatic hydrolysis followed by a Coleman species calculation was performed by a published method [5].

Mild alkaline hydrolysis was carried out at 37°C in a 0.1 M solution of caustic potash in methanol for 1 h.

Isolation of the Polyol from the Hydrolysis Products. The aqueous solution after decomposition of the potassium salts was treated four times with diethyl ether to eliminate residues of ether-soluble hydrolysis products. The excess of sulfuric acid was neutralized with potassium hydroxide. The solution was evaporated to dryness. From the dry residue acetone extracted the organic part. The extract after the elimination of the acetone yielded a polyol. In a thin layer, the polyol was revealed with sulfuric acid followed by heating to 140°C, and also with an ammoniacal solution of silver nitrate and an iodine vapor.

SUMMARY

Mono(hydroxyperoxy)octadecadienoyldiacyl triglycerides have been isolated from the seed oil of the cotton plant Tashkent-1, their amount in the oil being about 3%. It has been

established that the hydroperoxyoctadecadienoyl radical is localized mainly in the α position of the oxy-TGs. On the basis of the fatty-acid composition and the results of enzymatic hydrolysis, the proportions of the 15 possible types of oxytriglycerides have been calculated.

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QUINOID PIGMENTS OF ECHINODERMATA

V. PIGMENTS OF THE SEA URCHIN *Strongylocentrotus drŏebachiensis*

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The sea urchin *Strongylocentrotus drŏebachiensis* (O. F. Müll) is a species that is widely distributed in the boreal regions of the world ocean. The presence of spinochromes A, B. C, D, and E [1] and also of a substance which has been identified as anhydroethylidene-3,3'-bis-(2,6,7-trihydroxynaphthazarin) [2] has been established among the quinoid pigments of its shell and needles.

In an investigation of the pigments of animals of the same species caught in the Bering Sea, we also isolated spinochromes A, C, D, and E, and two other substances — X and Y. Substance X predominated in the total pigment extract. The chromatographic mobility and the absorption, mass, and PMR spectra of the pigment and its hexamethyl ether were similar to those of the binaphthoquinone isolated previously from the sea urchin *Strongylocentrotus intermedius* [3], for which the structure of ethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin) has been proposed.

These indices also do not differ greatly from those found by Mathieson and Thomson for a binaphthoquinone (and its hexamethyl ether, respectively) isolated for the first time from the sea urchin *Spatangus purpureus* [2]. However, these authors considered that a more probable structure of this substance was that of ethylidene-3,3'-bis(2,6,7-naphthazarin).

A comparison of the chromatographic mobilities (in two chromatographic systems) and absorption spectra of substance X and the binaphthoquinones from *Strongylocentrotus* and *Spatangus purpureus*,* and also their hexamethyl ethers, showed no differences whatever between them. Practically the only difference between the binaphthoquinones mentioned is found in their melting points. However, it is possible that this difference could be caused by the presence of impurities and by the tendency of these compounds to form extremely stable solvated crystals. Thus, we previously gave for the hexamethyl ether of pigment X mp 71-73°C [4], while on extremely prolonged drying in a pistol over P_2O_5 it was found to increase and finally reached 134-136°C, i.e., it coincided with the figure given previously [3].

Mathieson and Thomson gave as one of the indications of the proposed structure of the binaphthoquinone the coincidence of its properties with the properties of the binaphthoquinone obtained by the action of acetaldehyde on spinochrome D [2]. We have repeated this synthesis, using spinochrome D obtained from the shell of the sea urchin under investigation. The reac-

*A sample of the binaphthoquinone was kindly given to us by Prof. Thomson.

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