The following solvent systems were used: 1) hexane—diethyl ether (8:2); 2) benzene—chloroform—diethy ether (50:50:2) [7]; 3) ethanol—ammonia—water (20:3:2); and 4) tert-butanol—ammonia—water (25:3:3).

Oxidative degradation by periodate-permanganate was performed by von Rudloff's method [7] and with permanganate by Hilditch's method [8].

For the acetolysis of the oil and of the saturated acid we used acetic anhydride and freshly-fused sodium acetate, 1 N potassium hydroxide, and 10% sulfuric acid.

SUMMARY

1. The seed oil of the Central Asian common wormwood has yielded 1.48% of cis-12,13-epoxyoctadec-cis-9-enoic acid, 5.94% of cis-9,10-epoxyoctadec-cis-12-enoic acid, and traces of 9,10-epoxyoctadecanoic acid, and their structures have been confirmed.

2. It is proposed to used the neutralization number of fatty acids to determine the amounts of epoxy acids in their combinations with α-hydroxydienic acids.

3. The possibility has been shown of determining the amount of ratio of the isomeric epoxy acids using oxidative degradation of the total oxy acids.

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OXY ACIDS OF THE SEED OIL OF THE COTTON PLANT OF VARIETY TASHKENT-1

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UDC 547.915+665.31

The seed oils of many plants include a large amount of individual oxidized fatty acids (oxy acids). However, oils are frequently found which contain complex mixtures of these acids [1, 2]. The oils, the mixture of oxy acids in which have not yet been studied, include cottonseed oil. At the present time, one of the wilt-resistant varieties of the cotton plant widely cultivated in Uzbekistan is Tashkent-1, and therefore we selected this variety for an investigation of the structure of the oxy acids of cottonseed oil.

The acids mentioned were studied in the form of the methyl esters (MEs). The mixture of MEs of the fatty acids was isolated from the oil by transesterification in methanol in the presence of sodium methoxide. The mixture obtained was separated by ascending column

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chromatography into the total unsubstituted and total oxidized fatty acid MEs. The latter amounted to ~1% of the total mixture of MEs, and they were additionally separated by ascending column chromatography into six zones.

The extreme lability [3, 4] and low content of oxy acids in the oil limited the number of characteristics studied.

The migration of each zone of the substances in a thin layer of silica gel (TLC/KSK) was compared with the migration of standards which we isolated from the seed oil of *Artemisia absinthium* (common wormwood) where they have been found previously and the structures of which have been studied [1, 2].

All the fragments of the oxidative degradation of the MEs were investigated first in a thin layer of cellulose and then, after methylation with diazomethane, by gas–liquid chromatography in the form of MEs of monocarboxylic and dicarboxylic acids. In an investigation in a thin layer of cellulose of the fragments of the degradation of the MEs of each zone we found dimethyl malonate among the low molecular fragments.

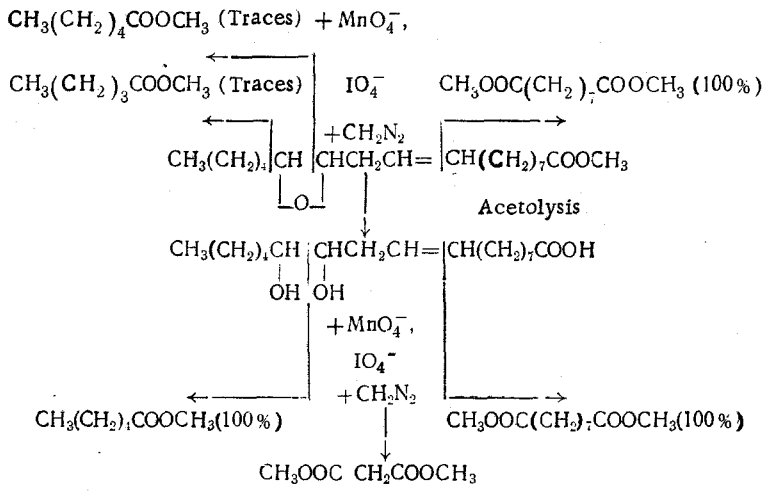
First Zone. The migration characteristics of the MEs of this zone on TLC/KSK (R_f 0.96) correspond to the migration of the MEs of unsubstituted fatty acids. Gas–liquid chromatography showed that these were the MEs of fatty acids commonly found in cottonseed oil that were present in the combined MEs of the oxy acids as impurities in the separation of the total mixture in a descending chromatographic column.

Second Zone. The migration of the MEs of the second zone on TLC/KSK (R_f 0.60) was identical with that of a standard — methyl vernolate. The reaction of the MEs with picric acid for the presence of an epoxy group was positive. IR spectrum (ν_{max}, cm^{-1}): 2940, 1410, 1380 (methyl group), 2870, 1460, 725 (aliphatic chain), 1740 (ester group), 1440, 1250, 1135, 1080 (methylene of a $-CH_2COO-$ group), 3050, 1650, 765 (isolated cis-ethylenic bond), and 850, 840 (epoxy group). NMR spectrum: resolved triplet of the protons of a methyl group at δ 0.9 ppm (3 H); "methylene exaltation" at 1.2 ppm ($\approx$16 H); the signals of nonequivalent methylene protons of allyl $-CH_2CH=$ and $-CH_2COO-$ groups and of methylenes adjacent to an epoxy group in the 1.5-2.3 ppm region; symmetrical multiplet of the protons of an epoxy group at 2.69 ppm (2 H) which shifted downfield to the region of 2.87 ppm (2 H), when the spectrum was taken in deuterochloroform, showing the cis configuration of the epoxy group [5]; the singlet of the protons of a methoxy group at 3.5 ppm (3 H); and a multiplet of the protons of an isolated ethylene bond at 5.28 ppm (2 H), J = 30 Hz.

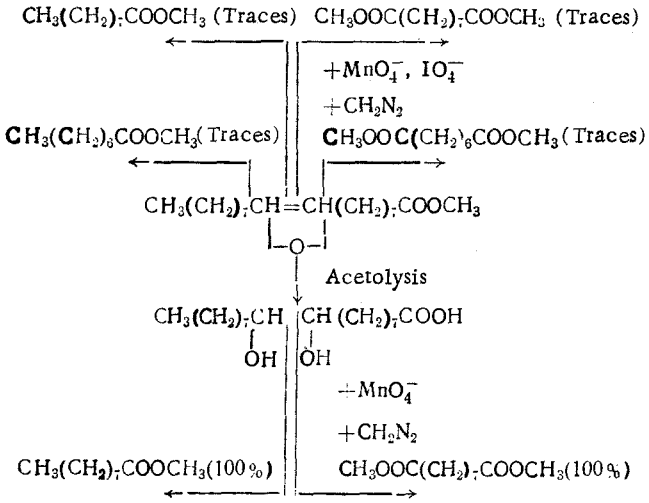
The fragments of the degradation of the MEs of the second zone by the periodate-permanganate reagent consisted of dimethyl azelate and traces of methyl valerate and caproate (Scheme 1). This shows the position of the ethylenic bond on the side of the ester group. At the same time, it is known that under mild conditions of periodate-permanganate oxidation the epoxide ring undergoes practically no cleavage. In view of this it may be assumed that the traces of esters of monocarboxylic acids are the result of the partial cleavage of the epoxy group. This hypothesis is confirmed by the following facts. We subjected the MEs of the second zone to acetolysis, as a result of which the epoxide ring should have opened with the formation of an α-dihydroxy group. In actual fact, the fragments of oxidative degradation of the product obtained by acetolysis — methyl caproate (C_6), dimethyl azelate (C_9), and dimethyl malonate (see Scheme 1) — showed that the epoxide ring did undergo cleavage. The results obtained show the position of the epoxy group on the side of the methyl end of the molecule. Since (according to spectroscopy), the ethylenic bond is isolated, the results obtained correspond to the structure of the ME of cis-12,13-epoxyoctadec-cis-9-enoic (vernolic) acid.

Third Zone. The migration of the MEs of this zone on TLC/KSK (R_f 0.55) was identical with the migration of methyl coronarate. A positive reaction with picric acid showed the presence of an epoxy group. The IR and NMR spectra were identical with the corresponding spectra of the MEs of the second zone. Among the fragments from periodate-permanganate degradation we found methyl caproate (C_6) and traces of methylcaprylate (C_8) and methyl pelargonate (C_9), and also traces of dimethyl azelate (C_9) and dimethyl suberate (C_8).

The monomethyl fragments mentioned cannot be assigned simultaneously to one molecule of an unbranched aliphatic (according to IR spectroscopy) acid. Consequently the third zone contains a mixture of MEs of different epoxy acids. It is known that the migration in a thin layer of silica gel [6] of the ME of an unsaturated epoxy acid may coincide with the



Scheme 1



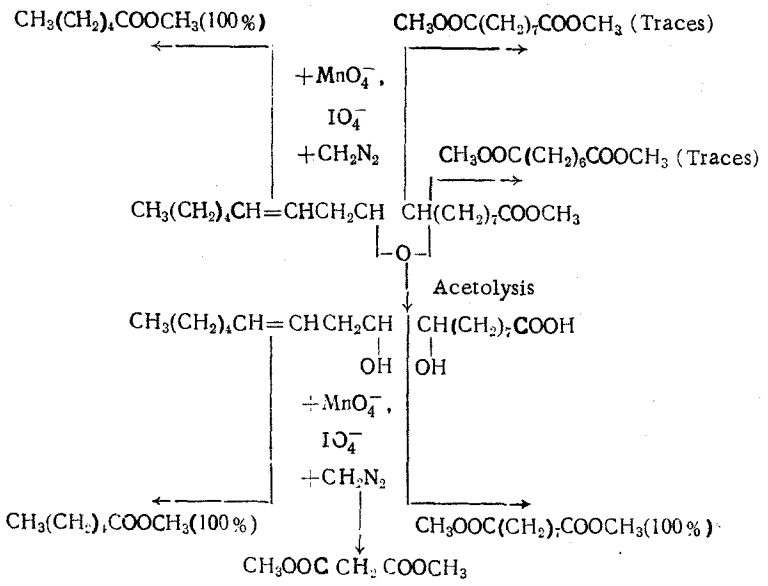
Scheme 2

migration of an ester of a saturated epoxy acid if the epoxy groups in the molecules of these compounds occupy the same position in the carbon chain. Assuming that this phenomenon may exist in our case, we subjected the MEs of the third zone to preparative chromatographic separation in a thin layer of silica gel containing silver nitrate (TLC/Ag^+). This led to the separation of the MEs of the saturated (R_f 0.95) and unsaturated (R_f 0.70) acids, each of which gave a positive reaction with picric acid.

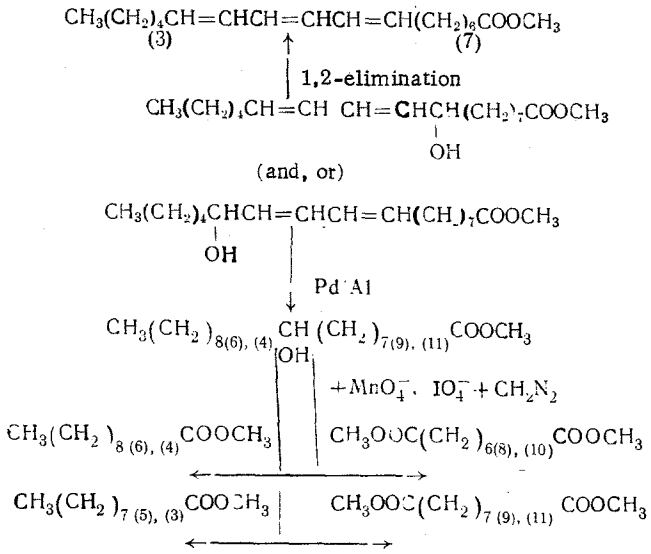
The epoxide ring of the MEs of the saturated epoxy acid opened on acetolysis, since the reaction with picric acid of the derivative obtained was negative, and the destructive cleavage of this derivative with permanganate was accompanied by the formation of only two fragments (Scheme 2) — methyl pelargonate (C_9) and dimethyl azelate (C_9) which corresponds to the structure of methyl cis-9,10-epoxyoctadecanoate.

After the acetolysis of the ME of the unsaturated epoxy acid and periodate-permanganate degradation of the derivatives so obtained (Scheme 3) we found three fragments — methyl caproate (C_6), dimethyl azelate (C_9), and dimethyl malonate (C_3). All this shows the cleavage of the epoxide ring located on the side of the ester group. The results obtained correspond to the structure of methyl cis-9,10-epoxyoctadec-cis-12-enoic (coronaric) acid.

Fourth Zone. On TLC/KSK the MEs of the acids with R_f 0.30 absorb in the near ultraviolet as dienes with conjugated ethylenic bonds (233 nm). IR spectrum (ν_{max}, cm^{-1}): 3600-3000, 1180 and 1100 (secondary hydroxyl), 2945, 1410, 1380 (methyl group), 2875, 1460, 730 (aliphatic chain), 1735 (ester group), 1440, 1255, 1130, 1070 (methylene in a $-CH_2COO-$ group), and 985, 955 (cis,trans-conjugated ethylenic bonds). These facts show the presence



Scheme 3

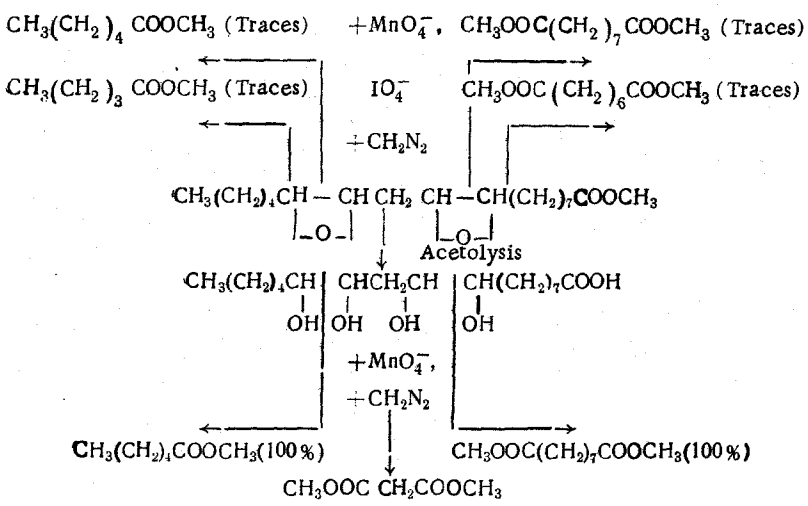


Scheme 4

of a free hydroxy group and of conjugated ethylenic bonds in the structure of the MEs of the fourth zone. NMR spectrum: triplet of the protons of a methyl group at δ 0.85 ppm (3 H); signal of the equivalent protons of methylene groups at 1.2 ppm (≈16 H); signals of non-equivalent methylene protons at 1.5-2.2 ppm; unsharp signals (≈1 H) of the protons of hydroxy groups at 2.65 [—CH(OH)CH=] and 3.15 ppm [=CHCH(OH)CH=] formed in tautomeric transitions; sharp singlet of methoxy protons at 3.55 ppm (3 H); broad multiplet of a methine group at 4 ppm (J = 20 Hz); broad multiplet of olefinic protons at 5.1-5.7 ppm (J = 60 Hz).

After being boiled in glacial acetic acid, the MEs absorbed in the UV region of the spectrum as trienes with conjugated ethylenic bonds — ν_{max}^{hexane} at 260, 270, and 280 nm. This indicates 1,2-elimination accompanied by the formation of a third conjugated ethylenic bond. Consequently, the hydroxyl is present in the α position to the system of conjugated ethylenic bonds (Scheme 4).

Oxidative degradation with periodate-permanganate gave only two fragments — dimethyl azelate (C_9) and methyl caproate (C_6). Consequently the α-hydroxydiene system is located between the 9th and 13th carbon atoms.



Scheme 5

The methyl esters of this zone were hydrogenated in the presence of palladium. The saturated derivatives were cleaved by permanganate with the formation of 12 degradation fragments (see Scheme 4) which indicates the hydrogenation of three positions (with respect to the hydroxy group) of the saturated isomers. This also confirms the fact that in the fourth zone we have either one or two isomers of α-hydroxydienic systems with conjugated ethylenic bonds. Even one of these tautomers present in the seed oil may be the source of the appearance of tautomers in the presence both of a static and of a dynamic conjugation effect (in this case, in the hydrogenation process). Thus, the results obtained cannot give an answer to the question of whether one or two tautomers with conjugated ethylenic bonds are native compounds: 9-hydroxyoctadeca-trans,cis-10,12-dienoic and 13-hydroxyoctadeca-cis, trans-9,11-dienoic acids.

Fifth Zone. The R_f value of 0.18 for the MEs of this zone on TLC/KSK corresponds to that for the ME of a diepoxyoctadecanoic acid [4]. IR spectrum (ν_{max}, cm^{-1}): 2940, 1410, 1380 (methyl group), 2875, 1460, 725 (aliphatic chain), 1735 (ester group), 1440, 1260, 1140, 1090 (methylene in $-CH_2COO-$), 890, 850 (epoxy group). No unsaturation was detected. It gave a positive reaction with picric acid.

The oxidative degradation with periodate-permanganate of the MEs of this zone gave traces of the fragments shown in Scheme 5. In addition, after acetolysis of the MEs and permanganate degradation of the derivative obtained we found three fragments — methyl caproate (C_6), dimethyl azelate (C_9), and dimethyl malonate (C_3). These facts correspond to the structure of 9,10:12,13-diepoxyoctadecanoic acid.

Sixth Zone. The migration of the MEs of this zone on TLC/KSK (R_f 0.00) corresponds to the migration of a methyl α-hydroxyoctadecadienoate [3]. The IR spectrum lacked the region of absorption of conjugated ethylenic bonds, which explains the transparency of these esters in the near ultraviolet.

The oxidative degradation of the MEs of the sixth zone gave the fragments that were formed in the degradation of the MEs of the fourth zone and, in addition, another four fragments corresponding to cleavage at the eighth carbon atom. Such fragments cannot be assigned similarly to a single ME molecule. Consequently, the sixth zone contains a mixture of MEs of different acids. We separated this mixture by the TLC/Ag^+ method. Part of the initial MEs was subjected to oxidative degradation with the periodate-permanganate reagent and another part to hydrogenation on palladium followed by degradation with permanganate.

The results of the degradation of the initial unhydrogenated MEs of the sixth and the fourth zones were identical. It follows from this that the sixth zone contains a methyl α-hydroxyoctadecadienoate with nonconjugated (according to spectroscopy) ethylenic bonds, namely methyl 11-hydroxyoctadeca-cis,trans-9,12-dienoate. This compound is extremely unstable, rapidly losing its cis-ethylenic bond with the formation of a trans-ethylenic bond as the result of an allyl rearrangement and geometrical isomerization.

EXPERIMENTAL

The UV spectra were taken on a Hitachi instrument, the IR spectra on a UR-10 instrument, and the NMR spectra on a JNM-4H-100/100 MHz spectrometer in carbon tetrachloride with hexamethyldisoloxane as internal standard. Gas–liquid chromatograms were obtained on a UKh-2 instrument under the following conditions: 15% of Reoplex 400 on Chromaton N-AW-HMDS at 125 and 203°C using a copper column with an internal diameter of 4 mm and a length of 2.5 m.

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

Oxidative degradation by the periodate-permanganate reagent was performed by von Rudloff's method [7] and with permanganate by Hilditch's method [8]. Acetolysis was performed in the following way: the MEs of the epoxy acids were acetylated in acetid anhydride in the presence of freshly fused sodium acetate, the resulting acetyl derivates were treated with 1 N caustic potash, and the sodium salts of the dihydroxy acids were decomposed with 50% sulfuric acid.

The methyl esters of the hydroxy acids were hydrogenated in ethyl acetate at 50°C in the presence of Pd/Al taken in an amount of 0.5% of the weight of the MEs.

As adsorbent for chromatography we used type KSK silica gel, which was washed with hydrochloric acid, water, and acetone, ground, and sieved.

Descending column chromatography: silica gel (100 mesh), petroleum ether (for eluting the combined MEs of unsubstituted fatty acids), and diethyl ether (for eluting the combined MEs of oxy acids).

Ascending column chromatography: silica gel (100 mesh), hexane–diethyl ether (8:2).

Thin-layer chromatography on silica gel: glass plates (18 × 24 cm), silica gel (150 mesh) with 5% of gypsum, hexane–diethyl ether (8:2).

Thin-layer chromatography on silica gel with silver nitrate: glass plates (18 × 24 cm), silica gel (150 mesh) with 20% of silver nitrate and 5% of gypsum, benzene–chloroform–diethyl ether (50:50:2) to separate the MEs of epoxy acids, and the same solvents (50:50:15) to separate the MEs of α-hydroxyoctadecadienoic acids.

Thin-layer chromatography on cellulose of carboxylic acids in the form of their ammonium salts: glass plates (6 × 12 cm), cellulose with 5% of gypsum, ethanol–ammonia–water (20:3:2) and tertiary butanol–ammonia–water (25:3:3).

SUMMARY

Seven oxy acids have been isolated from cottonseed oil of variety Tashkent-1 for the first time and their structures have been shown: cis-12,13-epoxyoctadec-cis-9-enoic, cis-9,10-epoxyoctadec-cis-12-enoic, cis-9,10-epoxyoctadecanoic, 9,10:12,13-diepoxyoctadecanoic, 9-hydroxyoctadeca-trans,cis-10,12-dienoic, 13-hydroxyoctadeca-cis,trans-9,11-dienoic, and 11-hydroxyoctadeca-cis,trans(trans,cis)-9,12-dienoic (unstable).

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