SUMMARY

The carbohydrate complex of the Chara alga *Chara aculeolata* Kütz includes an acid polysaccharide similar to the pectin substances of higher plants. It is based on a fragment constructed of α-1→4 bound residues of D-galacturonic acid in the pyranose form. The pectin is characterized by a high homogeneity, a considerable content of D-galacturonic acid, and a low degree of acidification of the carboxy groups.

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EPOXY ACIDS OF THE SEED OIL OF *Artemisia absinthium*

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The seed oil of *Artemisia absinthium* (common wormwood) has previously been found to contain about 15% of epoxy acids, on the total acids present in the form of acyl radicals in the triglycerides [1, 2]. The unusually high content of epoxy acids attracted our attention to this source of vernolic and coronaric acids. However, in the seed oil of plants of this species growing in Central Asia we detected no signals of the protons of an epoxide ring by nuclear magnetic resonance (NMR) while at the same time the IR spectrum of the oil showed a region of absorption of the vibrations of the bonds of an epoxide ring. The reason for this may be a content of epoxy acids considerably smaller than 15%.

To determine the concentration more accurately and for use as standards in the investigation of other plants we studied the structure of the epoxy acids of the seed oils of the Central Asian wormwood. The oil was subjected to transesterification with methanol in the presence of sodium methoxide and the mixture of methyl esters (MEs) of the fatty acids was isolated. By thin-layer chromatography (TLC) on Silufol plates (system 1) followed by treatment with picric acid, the mixture obtained was found to contain two sharp zones of the MEs of epoxy acids. Then the mixture of MEs (50 g) was transferred to a column 3 cm in diameter filled with silica gel (100 mesh) to a height of 10-15 cm. The MEs of the unsubstituted fatty acids were eluted with light petroleum ether (the process was monitored on Silufol plates in system 1).

The methyl esters of the oxy acids (acids in which an atom or atoms of hydrogen are the aliphatic chain are replaced by oxygen or a hydroxy group) were eluated from the column with diethyl ether. The resulting concentrate of oxy acids, containing MEs of unsubstituted fatty acids as impurities, was separated in an ascending chromatographic column (system 1). This gave two fractions of MEs of epoxy acids (Fig. 1a), each of which was subjected to TLC on silica gel (100 mesh) impregnated with 20% of silver nitrate in system 2. When the chromatograms were treated with iodine, sulfuric acid, and the picric acid reagent, a slowly moving zone was found to contain a mixture of MEs of unsaturated epoxy acids (II) and traces
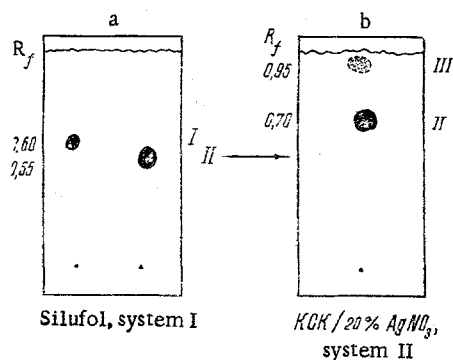
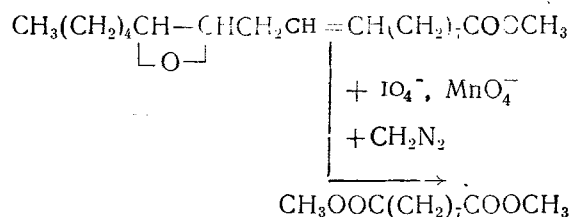Fig. 1. Thin-layer chromatography of the methyl esters of the epoxy acids.

of MEs of saturated epoxy acids (III) (Fig. 1b). The two components were isolated separately by preparative TLC with silver nitrate in the same system.

Methyl Ester of Epoxy Acid (I): mol. wt. 310 (mass spectrum). IR spectrum, ν_{max}, cm^{-1}: 2940 s, 1410 m, 1385 m (CH$_3$—); 2867 s, 1460 s, 730 m (—CH$_2$—); 1750 s, 1440 m, 1370 m, 1255 s, 1210 s, 1180 s, 1120 m, 1025 m (—CH$_2$COOCH$_3$); 3025 w, 1663 w, 767 s (cis —CH=CH—); 843 s, 837 s (epoxy group). NMR spectrum, δ scale, in deuterochloroform: triplet of the protons of a methyl group at 0.9 ppm (3 H); multiplet of equivalent protons of isolated methylene groups at 1.31 ppm (16 H); signals of nonequivalent methylene protons (—CH$_2$CH=, —CH$_2$COO— and —CH$_2$CH—CH—) in the 1.5-2.3 ppm region; multiplet of the protons of a cis
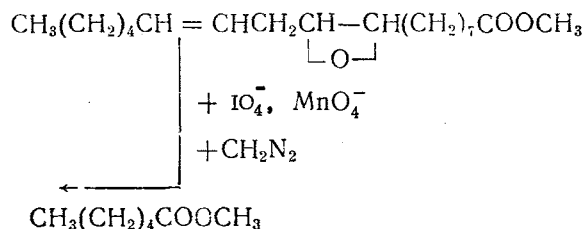$\quad\quad\quad\quad\quad\quad$ L$_O$—J
epoxy group at 2.87 ppm (2 H); singlet of the protons of a methoxy group at 3.54 ppm; broad multiplet of the protons of an isolated ethylenic bond at 5.4 ppm, J = 28 Hz.

The oxidative degradation of the (I) was performed by the periodate—permanganate reagent followed by the methylation of the degradation products with diazomethane.

Gas—liquid chromatography (GLC) at 204°C, monitored in thin layers of cellulose (system 3), showed that the only degradation fragment not containing an epoxide ring was dimethyl azelate. This shows the position of the ethylene bond on the side of the ester end, as in methyl vernolate:

$$CH_3(CH_2)_4CH—CHCH_2CH = CH(CH_2)_7COOCH_3$$
$$\quad\quad\quad\quad L_O—J$$
$$\quad\quad\quad\quad\quad\quad\quad | \quad + IO_4^-, \ MnO_4^-$$
$$\quad\quad\quad\quad\quad\quad\quad | \quad + CH_2N_2$$
$$\quad\quad\quad\quad\quad\quad\quad |———→$$
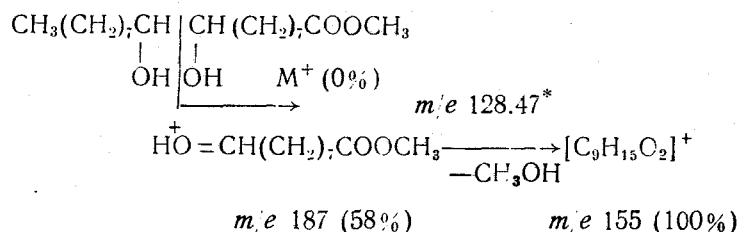$$\quad\quad\quad\quad CH_3OOC(CH_2)_7COOCH_3$$

Methyl Ester of Epoxy Acid (II): molecular weight 310 (mass spectrum). The IR and NMR spectra were identical with those given above. The only degradation fragment not containing an epoxy group detected by GLC at 123°C (monitoring in a thin layer of cellulose) was methyl caproate. Consequently, the ethylenic bond is on the side of the methyl end, as in methyl coronaroate:

$$CH_3(CH_2)_4CH = CHCH_2CH—CH(CH_2)_7COOCH_3$$
$$\quad\quad\quad\quad\quad\quad\quad\quad L_O—J$$
$$\quad\quad\quad | \quad + IO_4^-, \ MnO_4^-$$
$$\quad\quad\quad | \quad + CH_2N_2$$
$$←———|$$
$$CH_3(CH_2)_4COOCH_3$$

The observed positions of the epoxide groups in the position isomers (I) and (II) correspond to their migration characteristics (see Fig. 1a), since of the two unsaturated epoxy esters the least mobile is that in which the epoxide ring is located on the side of the ester end [3].

<u>Methyl Ester of Epoxy Acid (III)</u>: molecular weight 312 (mass spectrum). The acetolysis of (III) gave methyl 9,10-dihydroxyoctadecanoate with mp 64.0-64.5°C.

In the mass spectrum of this derivative, a daughter ion with m/e 155 is formed from the mother ion with m/e 187, as is confirmed by the formation of a metastable ion with m/e 128.47. This shows that the vicinal hydroxy groups are present on the 9th and 10th carbon atoms:

$$CH_3(CH_2)_7CH \mid CH (CH_2)_7COOCH_3$$
$$OH \mid OH \quad M^+ (0\%)$$
$$\xrightarrow{\hspace{1.5cm}} m/e \ 128.47^*$$

$$\overset{+}{H O} = CH(CH_2)_7COOCH_3 \xrightarrow[-CH_3OH]{\hspace{1.5cm}} [C_9H_{15}O_2]^+$$

$$m/e \ 187 \ (58\%) \qquad\qquad m/e \ 155 \ (100\%)$$

It follows from the results obtained that the position of the epoxide ring in the molecule of the ME must be the same as in methyl 9,10-epoxyoctadecanoate:

$$CH_3 (CH_2)_7CH-CH(CH_2)_7COOCH_3$$
$$\underset{\displaystyle O}{\rule{1cm}{0.4pt}}$$
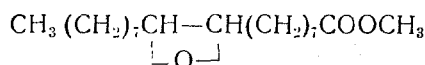
As is well known, the position of the epoxy group in the aliphatic chain of an acid has a predominant influence on the mobility of an ester of the epoxy acid in a thin layer of silica gel, while the presence of an isolated double bond has no effect whatever [3]. This is in harmony with the agreement of the migration characteristics of the MEs of the epoxy acids (II) and (III) (see Fig. 1a).

To determine the amount of oxy acids among the acyl radicals of the triglycerides, we used the neutralization number (N. No.) of the mixture of fatty acids. The oil was treated with 1 N potassium hydroxide at room temperature. The mixture of fatty acids was isolated from the solution of potassium salts by careful acidification [2] under a layer of diethyl ether, which prevented the cleavage of the epoxy group. The N. No. of the total fatty acids, according to a titrimetric determination [4], was 199.97 mg of KOH/g, which corresponds to a mean molecular weight of 280.54. On the other hand, the mean molecular weight of the total fatty acids is equal to the sum of the products of the molecular weight and the percentage content of each of the constituents. According to GLC, the amounts of unsubstituted acids were as follows (mole %): palmitic ($C_{16:0}$) 6.87; palmitoleic ($C_{16:1}$) 2.00; stearic ($C_{18:0}$) 2.44; oleic ($C_{18:1}$) 15.27; linoleic ($C_{18:2}$) 73.42. On the basis of these facts we calculated the mean molecular weight of the unsubstituted fatty acids as 278.67. The proportion of these acids in the total is (100 − x) % if the mixture of fatty acids contains x% of oxy acids. The molecular weight of the oxy acids is 296, since these are mainly isomeric epoxy acids — vernolic and coronaric — and isomers of α-hydroxydienic acids [5].
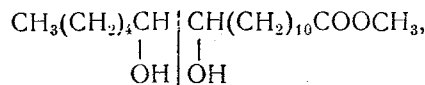
Thus, the mean molecular weight of the mixture of fatty acids can be described by the following equation: 278.67 (100 − x) + 296·x = 280.54·100. Solving this equation, we find that x = 10.8%.

To determine the proportion of epoxy acids among the 10.8% of oxy acids, the wormwood oil was subjected to acetolysis, which opens epoxide rings in acyl radicals of triglycerides. After this, we isolated from the oil a mixture of unsubstituted and hydroxy acids, hydrogenated the ethylenic bonds in the presence of palladium [5], and esterified the acids with diazomethane. This gave a mixture of the MEs of saturated fatty acids from which we isolated the hydroxy esters by column chromatography using light petroleum ether. The latter were subjected to destructive cleavage with permanganate (Scheme).
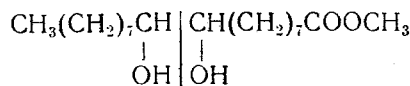
The composition of the monocarboxylic fragments, according to GLC at 123°C, was as follows (mole %): $C_{5:0}$ − 6.08; $C_{6:0}$ − 20.02; $C_{7:0}$ − 6.37; $C_{8:0}$ − 7.26; $C_{9:0}$ − 57.52; $C_{10:0}$ − 2.75.

As can be seen from the scheme, in the case of an isolated secondary alcoholic group the cleavages of the carbon—carbon bonds are equiprobable, as is shown by the ratio of the amounts of the $C_{7:0}$ and $C_{8:0}$ fragments. This is due to a weakening of the influence of the induction effect of the carboxylic group, since the length of the chains separating it from the secondary alcohol group is more than seven carbon atoms. Hence it may be considered that the amount of each of the monocarboxylic fragments, $C_{5:0}$ and $C_{6:0}$, formed in the deg-
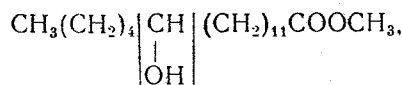
Scheme 1

Epoxy acid derivatives

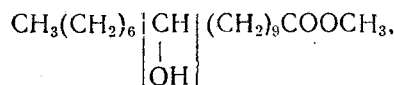$$CH_3(CH_2)_4CH \mid CH(CH_2)_{10}COOCH_3,$$
$$OH \mid OH$$

$$\longleftarrow$$

$$C_{6:0}\ (13.94\%)$$

$$CH_3(CH_2)_7CH \mid CH(CH_2)_7COOCH_3$$
$$OH \mid OH$$

$$\longleftarrow$$

$$C_{9:0}\ (54.77\%)$$

α-Hydroxydienic acid derivatives

$$CH_3(CH_2)_4 \mid CH \mid (CH_2)_{11}COOCH_3,$$
$$OH$$

$$\longleftarrow$$

$$C_{5:0}\ (6.08\%)$$

$$\longleftarrow$$

$$C_{6:0}\ (6,08\%)$$

$$CH_3(CH_2)_6 \mid CH \mid (CH_2)_9COOCH_3,$$
$$OH$$

$$\longleftarrow$$

$$C_{7:0}\ (6.37\%)$$

$$\longleftarrow$$

$$C_{8:0}\ (7.26\%)$$

$$CH_3(CH_2)_8 \mid CH \mid (CH_2)_7COOCH_3$$
$$OH$$

$$\longleftarrow$$
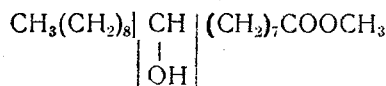
$$C_{9:0}\ (2.75\%)$$

$$\longleftarrow$$

$$C_{10:0}\ (2.75\%)$$

radation of 13-hydroxyoctadecanoate is 6.08% and the amount of each of the $C_{9:0}$ and $C_{10:0}$ fragments formed in the degradation of 9-hydroxyoctadecanoate is 2.75%. Then the amount of the $C_{6:0}$ fragment formed in the degradation of the 12,13-dihydroxyoctadecanoate is 20.02 — 6.08 = 13.94% and the amount of the $C_{9:0}$ fragment formed in the degradation of the 9,10-di-hydroxyoctadecanoate is 57.52 — 2.75 = 54.77%.

It can be seen from the scheme that the sum of the dihydroxy acids derived from the epoxy acids is 54.77 + 13.94 = 68.71%, and the sum of the saturated hydroxy acids derived from α-hydroxydienic acids is 2·6.08 + (6.37 + 7.26) + 2·2.75 = 31.29%.

Consequently, of the 10.8% of oxy acids the proportions of epoxy acids is ~7%, and of α-hydroxydienic acids ~3% (2.3% according to UV spectroscopy in the 230-233 nm region in hexane; the smallness of this value is natural because of the formation of a α-hydroxydienic tautomer with nonconjugated ethylenic bonds).

Thus, the amount of epoxy acid radicals in the triglycerides of the seed oil of the Central Asian common wormwood is only half that in the American plant of the same species, and the amount of epoxy acid radicals is almost twice as great than that of α-hydroxydienic radicals. The ratio of the amounts of coronaric and vernolic acyl radicals is 4:1.

EXPERIMENTAL

All the spectra were taken and GLC was performed in the instruments and under the conditions described previously [6]. The seeds were ground in an electric mill. The oil was extracted from the seeds with light petroleum ether by steeping at room temperature.

Type KSK silica gel washed with hydrochloric acid, water, and acetone and ground in a porcelain mill was used as the adsorbent for thin-layer and column chromatography.

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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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