

A MASS-SPECTROMETRIC STUDY OF THE HYDROXY ACIDS OF COTTONSEED OIL

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Eight new components of the hydroxy acids have been found in the seed oil of the cotton plant of variety Tashkent 1: six monohydroxy acids, of which three are isomeric hydroxyoctadecenoic, two are hydroxyheptadecenoic, and one is a hydroxyoctadecanoic acid, and two polyhydroxy acids — trihydroxystearic and dihydroxyoctadecenoic acids. The main acids in the combined material were 12-hydroxyoctadec-9-enoic acid and a trihydroxydecanoic acid.

As investigations of recent years have shown, many seed oils of plants of the family Malvaceae contain unusual fatty acids. These include unbranched acids with cyclopropene, cyclopropane, epoxy, and hydroxy groups in the chain.

The physiological activity of acids with cyclopropane groups [1] is responsible for interest in them, and at the present time the structure of these acids in cottonseed oil has been studied fairly well [2].

Less is known about the structure and properties of the epoxy and hydroxy acids of the seed oils of this family. This is due to their very small amount in many species of Malvaceae and their complex composition which is difficult to subject to analysis by classical methods of investigating lipids. Nevertheless, as early as 1957 in an analysis of the seed oil of *Malva parviflora* 12,13-epoxyoctadec-cis-9-enoic acid was isolated in an amount of 9.5% [3]. Previously, in a study of the seed oil of the cotton plant of variety 108-F we have detected in the seeds about 1% of a combination of 12,13-epoxyoctadec-cis-9-enoic and 9,10-epoxyoctadec-cis-12-enoic acids with trace amounts of isomeric 9(13)-hydroxyoctadeca-10,11(9,10)-dienoic acids [4]. Hydroxydionoic acids were found later in another five species of Malvaceae [2].

In the present paper we described the isolation and analysis of the structure of the main components of the hydroxy acids of the oil of the cotton plant of variety Tashkent 1. Ripe seeds of the 1979-1980 harvest were investigated immediately after they had been gathered. The results of the analysis of the hydroxy acids for the two years were identical.

When the combined neutral lipids of the seeds were separated by column chromatography, a fraction enriched with hydroxy acids amounting to 2.3% of the total was isolated. The fractions freed from impurities appeared in TLC in the form of two indistinctly separated spots with the common R_f value of 0.5 in system 1, 0.35 in system 2, and 0.24 in system 3. The IR spectrum had the bands of the vibrations of a hydroxy group bound by a hydrogen bond with maxima in the $3200-3600 \text{ cm}^{-1}$ interval. The remainder of the spectrum corresponded to the spectrum of ordinary triacylglycerols.

The PMR spectrum of the fraction contained a multiplet in the τ 5.5-5.6 ppm region relating to the protons of an esterified glycerol $\text{CH}_2\text{-O}$ group and a multiplet signal of protons at 6.52 ppm characteristic for a secondary $\text{CH}\text{-O}$ alcoholic group present in an aliphatic chain. This fraction had a specific rotation of $[\alpha]_D^{25} +7.9^\circ$ (c 0.3) chloroform). From a comparison of the chromatographic mobility and spectral characteristics of the fraction with literature information [5], it was assigned to the triacylglycerols containing the acyl residues of one hydroxy fatty acid and two unsubstituted fatty acids. To isolate the hydroxy acids we made use of acid methanolysis. The methanolysis products consisted of the methyl esters (MEs) of unsubstituted fatty acids (R_f 0.95) and the MEs of the hydroxy acids with R_f 0.4 and 0.2 (system 1) in a ratio of 2:1. The MEs of the hydroxy acids were isolated from the reaction mixture by preparative TLC. The amount of MEs with R_f 0.4 was four times greater than that of

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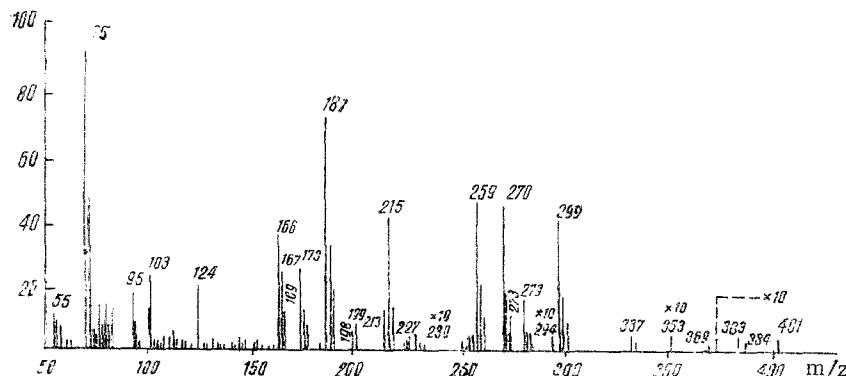


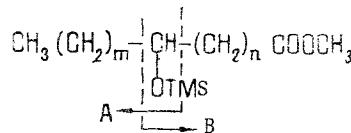
Fig. 1. Low-resolution mass spectrum of the TMS derivatives of the total MEs of the hydroxy acids of cotton seed oil.

the MEs with R_f 0.2. The IR spectrum of the MEs of the combined fatty acids contained, in addition to bands relating to the absorption of ester groups, bands of a hydroxy group at 3470, 1130, 1100, and 1050 cm^{-1} . In the PMR spectrum characteristic for the MEs of fatty acids there were signals at 6.3-6.8 ppm relating to the methine protons of a $\text{CH}-\text{O}$ group and a multiplet in the 4.55-4.9 ppm region of the protons of an isolated olefinic bond. The fraction was transparent in the UV region.

In order to establish the composition of the components of the total material of the two hydroxy acid fractions we used the mass spectrometry of their trimethylsilyl (TMS) derivatives, which were obtained and purified immediately before analysis.

The mass spectrum was studied in several steps. The low-resolution spectrum of the combined TMS derivatives (Fig. 1) revealed the presence of peaks which, according to the literature [7], are characteristic for the TMS derivatives of the MEs of hydroxy acids. The introduction of the same sample into a chromato-mass spectrometer confirmed the results of the first stage of the investigation, namely that the main component of the combined material was 12-hydroxyoctadec-9-enoic (ricinoleic) acid (more than 80% of the total). No accompanying components could be detected. In the later stages we used the advantages given by high-resolution instruments. When measuring the accurate masses of the characteristic fragmentary ions the fact that they were products of the decomposition of the TMS derivatives of hydroxy acids was confirmed. The masses of the mother ions, including the molecular ions, were determined by the method of metastable defocusing (MD) thereby re-establishing the scheme of fragmentation of the individual components of the mixture. The promising nature of the latter approach has been demonstrated, for example, by Batrakov et al. [6], who studied a complex mixture of lipid derivatives.

The main analytically important process in the fragmentation of the TMS derivatives of hydroxy acids is the cleavage of one of the α -C-C bonds with respect to the TMSO group [7]. This leads to the formation of two fragments including the methyl (A) or the carboxy (B) end of the chain.



The mass numbers and elementary compositions of these fragments determine the positions of the hydroxy groups and the degree of unsaturation of the aliphatic chain.

The peaks of the molecular ions of the TMSO derivatives of the hydroxy fatty acids have low intensities and their direct detection is unlikely even in the spectra of individual substances. More intensive are the peaks of the $(M - 15)^+$ ions arising by the detachment of CH_3 from the TMS group [7]. In addition to this, the M^+ ions of these compounds, like the B ions, are capable of losing a molecule of methanol or a hydroxymethyl radical. When more than one TMSO group is present in a molecule, the latter can be eliminated by the molecular ion or a fragmentary ion in the form of a TMSO radical or a TMSOH molecule.

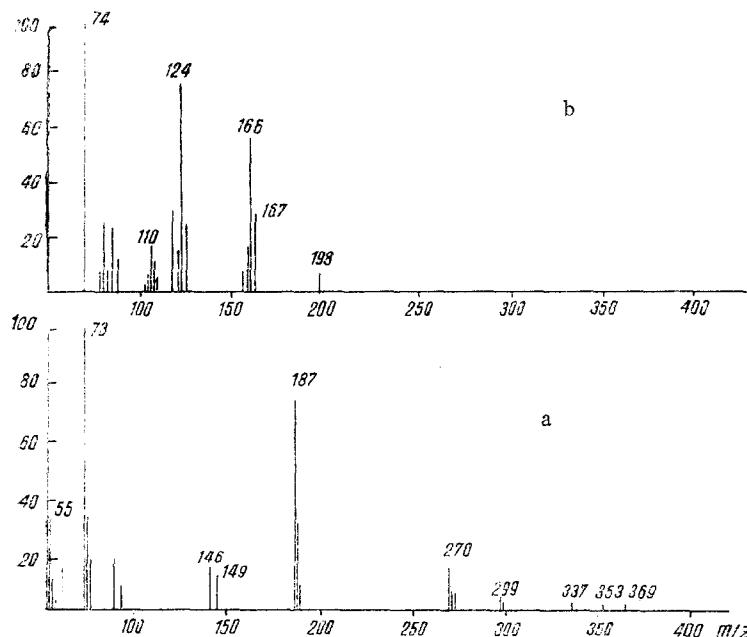
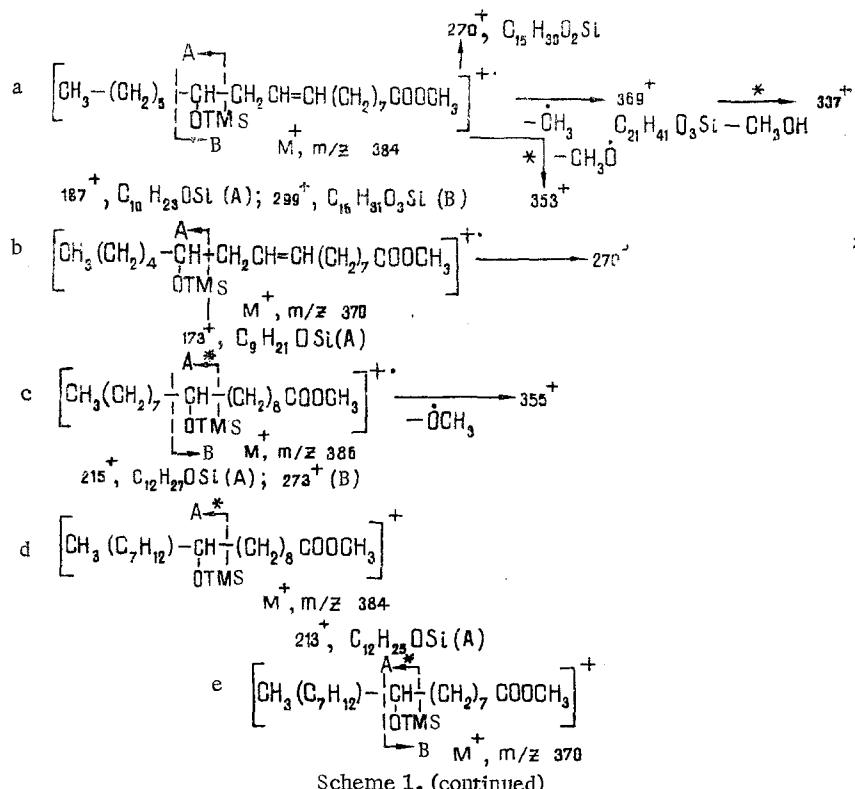


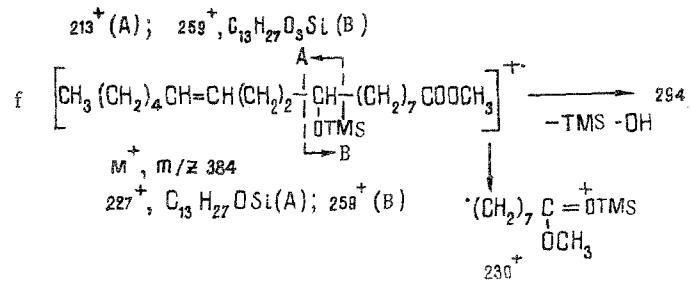
Fig. 2. Mass spectrum of the MEs of ricinoleic acid (TMS derivative, a) and of undecylenic acid (b) obtained in the investigation of the hydroxy acids of cottonseed oil.

These characteristics have been made the basis of the determination of the components of the combined hydroxy acids.

Monohydroxy Acids. In the spectrum of the combined acids, the peaks of ions with m/z 187 (A) and 299 (B) correspond to the ricinoleic acid found by an independent method. It has been established by the MD method that the precursor of ion B is an ion with m/z 384, corresponding to the molecular ion. In addition to this, the transitions $384^+ \xrightarrow[-\text{CH}_2\text{OH}]{\longrightarrow} 353^+$ and $369^+ \xrightarrow[-\text{CH}_2\text{OH}]{\longrightarrow} 337^+$, which are characteristic for the TMS ether of methyl ricinoleate have been found [7] (Scheme 1a).



Scheme 1. (continued)



Scheme 1. Fragmentation of the TMS derivatives of the MEs of the monohydroxy acids: a) ricinoleic; b) 12-hydroxyheptadec-9-enoic; c) 10-hydroxyoctadecenoic; d) 10-hydroxyoctadecanoic; e) 10-hydroxyheptadecenoic; f) 9-hydroxyoctadec-12-enoic (the asterisks denote the transitions recorded by the MD method; together with the *m/z* values of the ions their elementary compositions are given in those cases where they were measured).

In addition to ricinoleic acid, the mixture contained a homolog - 12-hydroxyheptadec-9-enoic acid (b) - as was shown by the presence of peak with m/z 173 and the transition $370^+ \rightarrow 270^+$.

The appearance in the spectrum of the mixture of an intensive fragment with m/z 215 (A) having the composition $C_{12}H_{27}OSi$ and the simultaneous presence of the $386^+ \rightarrow 215^+$ transition shows the presence of 10-hydroxystearic acid (c). An additional confirmation of this is given by peaks of ions with m/z 273 (B) and 169 [8].

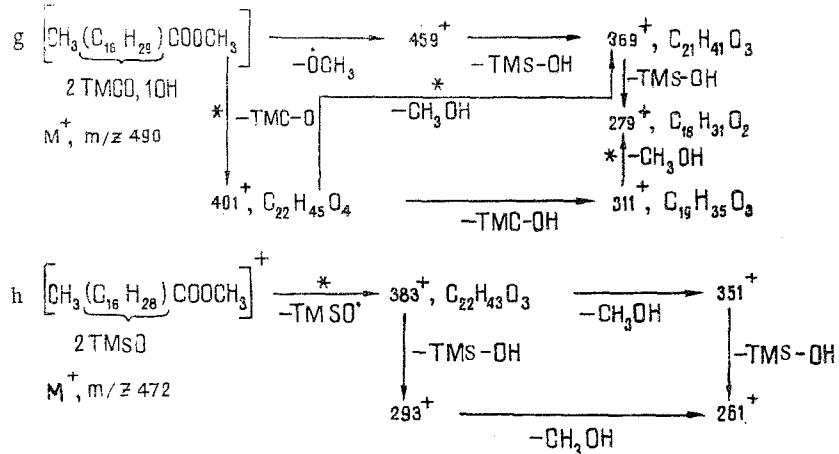
An unsaturated acid with a hydroxy group in the same position and a π -bond in the C₁₁-C₁₈ chain (d) is characterized by an ion with m/z 213 and the 384⁺ \rightarrow 213⁺ transition.

In addition to this transition, a $370^+ \rightarrow 213^+$ transition was recorded, showing the presence of the homologous 9-hydroxyheptadecenoic acid (e). A no less characteristic indication of 9-hydroxy acids is the presence of a strong peak of an ion with m/z 259 (B) having the composition $C_{13}H_{27}O_3Si$. Although we were unable to record the transitions of the maternal ions of this fragment, the presence in the spectrum of the combined material (see Fig. 1) of peaks with m/z 227, 230, 259, 294, 353, and 369 may indicate that they contained the ME of 9-hydroxydec-12-enoic acid [7]. The mass number of the type A ion for this acid is 227. However, in the high-resolution spectrum of the combined material, a triplet of peaks of ions with m/z 227 was observed. Only about 10% of the ions with this value of m/z is due to the component corresponding to composition $C_{13}H_{27}O_3Si$. Nevertheless, it was possible to record several weak transitions from parental ions in the m/z interval of 370-386 to an ion with m/z 227.

The other components of the m/z 227 ions, with the compositions $C_{14}H_{27}O_2$ and $C_{13}H_{23}O_2$, show the presence of unoxidized acids and of keto acids in the combined material.

Polyhydroxy Acids. The search for polyhydroxy acids in the combined materials studied was based on the appearance in the spectrum of peaks of ions of medium intensity with m/z 441 and 383 having the compositions $C_{22}H_{45}O_4Si$ and $C_{22}H_{43}O_3Si$, respectively. The elementary transformation of the formulas gives: 401 [$(C_{17}H_{33}O)(OTMS)(COOCH_3)$] and 383 [$C_{17}H_{31}(OTMS)(COOCH_3)$]. The retention of the octadecanoic (octadecenoic) chain shows that these fragments could most probably be formed as the result of the splitting out of certain substituents. Using the MD method, $490^{+} \rightarrow 401^{+}$ and $472^{+} \rightarrow 383^{+}$ transitions showing the loss of TMSO groups were recorded. The subsequent decomposition of the ions with m/z 401 and 383, which is shown in Scheme 2, confirms the developed formulas of these ions. Thus, in the mixture under investigation there are trihydroxystearic (g) and dihydroxyoctadecenoic (h) acids. We have been unable to determine the concrete positions of the OH groups and double bonds in these acids.

Attention must be directed to a series of difficulties accompanying the performance of the experiments. In the first place, the contamination of the mixtures investigated by artifactual compounds such as, for example, dioctyl phthalate [9], giving strong peaks with m/z 149, 167, and 279. Only with the aid of the high-resolution method was it possible to establish that in addition to the ion with m/z 279 of dioctyl phthalate with the composition $C_{16}H_{23}O_4$ there was a $C_{18}H_{31}O_2$ ion arising in the decomposition of the ester of trihydroxy-stearic acid.



Scheme 2. Fragmentation of the TMS derivatives of the MEs of polyhydroxy acids; g) trihydroxystearic; h) dihydroxyoctadecenoic.

Another type of artifactual impurities was represented by the polymethylsiloxanes which constantly accompany silylation products in spite of their previous purification. The pyrolytic decomposition of siloxanes begins under milder conditions than is required for the evaporation of the TMS ethers of hydroxy acids and continues after the volatilization of the latter. This makes it necessary to select the temperature conditions of the ion source and of the inlet system carefully and also to recharge the sample repeatedly in the course of an experiment. Furthermore, the TMS ethers of the hydroxy acids themselves apparently undergo pyrolysis under the conditions of the mass spectrometry with the formation of low-molecular-weight products. Thus, a purified sample of the TMS derivative of the ME of ricinoleic acid on introduction into a chromato-mass spectrometer gave a spectrum of an accompanying compound with a molecular weight of 198, which was identified as undecenoate (Fig. 2). At the same time, the chromatography of a sample of the ME of ricinoleic acid in a thin layer with a marker consisting of the ME of undecylenic acid showed no indications of the presence of the latter in admixture with the ricinoleic acid. We also directed our attention to the presence of the doublet of peaks of ions with m/z 167 ($M - OCH_3$)⁺ and 166 ($M - CH_3OH$)⁺ that is characteristic for methyl undecenoate in the spectrum of the combined material (Fig. 1) and to the sharp rise in the heights of these peaks in the spectrum of methyl ricinoleate taken at an elevated temperature (140°), which shows the possibility of the pyrolysis of the TMS ether of ricinoleic acid.

Thus, the main hydroxy acid of cottonseed oil is 12-hydroxyoctadec-9-enoic (ricinoleic), which is accompanied by its isomers 10-hydroxyoctadecenoic and 9-hydroxyoctadec-12-enoic acids and its homologs 12-hydroxyhepta-9-enoic and 9-hydroxyheptadecenoic acids, and also by 10-hydroxystearic acid. Polyhydroxy acids — a dihydroxyoctadecenoic and trihydroxystearic acid, with the predominance of the latter — are present in considerably smaller amount.

Until recently, the sole known source of ricinoleic acid was castor oil (the oil of *Ricinus communis*), which contains 90% of it. Recently, with the aid of modern methods of analysis, ricinoleic acid and its isomers have been detected in the seeds of some other species [10-12]. 10-Hydroxystearic acid together with ricinoleic acid has been found in the surface waxes of the leaves of *Rosmarinus officinalis* [8]. Polyhydroxy acids are very rare in seed oils, but some trihydroxyoctadecanoic acids are present in the cuticular lipids of leaves [8].

The hydroxyheptadecenoic acids are new, and this is the first time they have been found in seed oils. Since 9-hydroxyheptadecenoic acid could be formed by the α -oxidation of 10-hydroxyoctadecenoic acid, their combined presence in one oil may show a biosynthetic link between them.

EXPERIMENTAL

For the conditions of recording the IR, PMR, and UV spectra see [5]. The GLC-MS analysis was performed on an LKB-2091 instrument in the A. V. Topchiev Institute of Petrochemical Synthesis of the Academy of Sciences of the USSR. The temperature of the ion source and of the separator was 200°C, the 1 m \times 3 mm column was filled with 5% of SE 30 on Chromaton, the

rate of flow of the carrier gas, He, was 10 ml/min, and the temperature was programmed from 8 to 280°C at the rate of 10°/min.

The low-resolution spectra of the combined TMS ethers of the MEs of the hydroxy acids were obtained by using a direct inlet system on an MKh 1303 instrument (140°C, ionizing energy 50 eV) and an MKh 1310 instrument (ampul-evaporator 60–70°C, ionization chamber 100°C; 50 eV; collector current 60 μA). Metastable defocusing and the measurements of the elementary compositions of the ions were carried out on the MKh 1310 mass spectrometer.

Thin-layer chromatography was carried out on type L 5/40 silica gel (Chemapol) in system 1 (hexane–diethyl ether (6.5:3.5)) and on Silufol in system 2 (heptane–methyl ethyl ketone–acetic acid (47:7:1)), and in system 3 (hexane–diethyl ether (7:3)).

Acid methanolysis was carried out as described by Christie [13] and the silylation of the methyl esters of the hydroxy acids according to Gunstone and Schuler [14]. The silylation products were purified by preparative TLC on silica gel in the solvent system hexane–diethyl ether (9:1).

The lipids were extracted from the seeds by steeping in petroleum ether (40–60°C) at room temperature. The isolation of the hydroxyacylglycerols and of ricinoleic acid from castor oil has been described previously – [15] and [4], respectively.

Methyl esters of hydroxy acids: R_f 0.4 in system 1. IR spectrum, $\nu_{\text{max}}^{\text{film}} (\text{cm}^{-1})$; 3200–3600 s, 3010 m, 2930 s, 2860 s, 1740 s, 1730 s, 1470 s, 1440 s, 1380 m, 1320 w, 1250 m, 1200 m, 1180 m, 1120 m, 1100 m, 950 w, 850 w, 730 m.

PMR spectrum (τ , ppm): 9.14 t, 8.78 s, 7.9 m, 7.82 t, 6.1–6.9 m, 6.46 s, 4.5–5.0 m.

Methyl ester of undecylenic acid: mass spectrum. m/z : M^+ 198 (1.8%), 166 (17.6%), 167 (11.5%), 148 (10.4%), 137 (3.7%), 124 (23.7%), 110 (6.6%), 96 (36.6%), 74 (100%).

PMR spectrum (ppm): 8.73 s, 8.01 m, 7.84 t, 6.47 s, 5.22 m, 4.35 m.

IR spectrum $\nu_{\text{max}}^{\text{film}} (\text{cm}^{-1})$: 3090 s, 2940 s, 2870 s, 1750 br. s, 1650 s, 1440 s, 1370 m, 1200 m, 1170 m, 1120 m, 2000 s, 915 s, 730 w.

CONCLUSION

Eight components of the hydroxy acids have been detected in the seed oil of the cotton plant of the variety Tashkent 1: 12-hydroxyoctadec-9-enoic (ricinoleic), forming the bulk of the combined hydroxy acids- and isomers and homologs of it – 10-hydroxyoctadecenoic, 9-hydroxyoctadec-12-enoic, 12-hydroxyheptadec-9-enoic, 9-hydroxyheptadecenoic – and also 10-hydroxystearic trihydroxystearic, and dihydroxyoctadecenoic acids.

Two new hydroxyheptadecenoic acids have been found in a seed oil for the first time.

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