MASS-SPECTROMETRIC BEHAVIOR OF OXIDIZED TRIACYLGLYCEROLS AND THEIR TMS DERIVATIVES

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The mass spectrometric fragmentation of oxidized triacylglycerols of castor oil and their TMS derivatives have been studied for the first time. Three types of fragmentary ions have been detected: 1) products of the successive elimination of TMSOH; 2) ions characteristic for unoxidized triacylglycerols; and 3) products of the cleavage of C—C bonds present in the α-positions to the TMSO groups, which are characteristic for these hydroxy acid derivatives. The origin of these ions has been confirmed by measurements of elementary composition and by the analysis of MD spectra. The specific cleavage of the C—C bonds in the α-positions to the OTMS (OH) groups permits the mass-spectrometric method to be proposed for the analysis of mixtures of oxidized triacylglycerols.

We have established previously the composition of the hydroxy acids of sea buckthorn seed oil by a study of the mass spectra of their TMS derivatives and a chromatographic analysis of the products of oxidative degradation [1]. However, a direct molecular-species analysis of the native triacylglycerols (TAGs) is most desirable. A knowledge of the general laws of fragmentation of this class of compounds and advances in the spectrometry of metastable ions [2] permit the omission of a preliminary chromatographic separation of the combined material into its individual components in the first stage of the investigations.

The behavior of oxidized TAGs under electron impact has not been studied and, therefore, before investigating a mixture of this type of substances we studied the fragmentation of model compounds using as examples the oxidized TAGs of castor oil — glycerol triricinoleate (I) and monooleoyldiricinoleoylglycerol (II) — and their TMS derivatives (III) and (IV). The choice of this material was due not only to the convenience of its production in the relatively pure state but also to the fact that ricinoleic acid residues are found in the majority of natural oxidized TAG compositions.

The mass spectra of (I-IV) are given in Figs. 1 and 2.

The spectra of the TMS ethers of (III) and (IV) present a complex but extremely characteristic picture. They contain the low-intensity peaks of the M^+ ions with m/z 1148 (III) and 1060 (IV). The $(M - 15)^+$ ions with m/z 1133 and 1045 corresponding to the TMS derivatives are represented by peaks of considerably greater height. The main fragmentary ions can be divided into three groups:

1) products of the successive elimination of TMSOH;

2) ions characteristic for the unoxidized triacylglycerols; and

3) products of the cleavage of the C—C bonds present in the α-position to the TMSO groups belonging to the given hydroxy acid derivatives.

From their appearance, the large number of ions is due to the superposition of all three or of two processes. The fragmentation of the TMS derivatives of (III) and (IV) are shown in schemes 1 and 2, respectively. The peaks of the ions of the first group in both spectra have low intensities, with the exception of $(M - 3\ TMSOH)^+$ with m/z 878 in the spectrum of (III) and of $(M - 2\ TMSOH)^+$ with m/z 880 in the spectrum of (IV) (Fig. 1, b, and 2, b).

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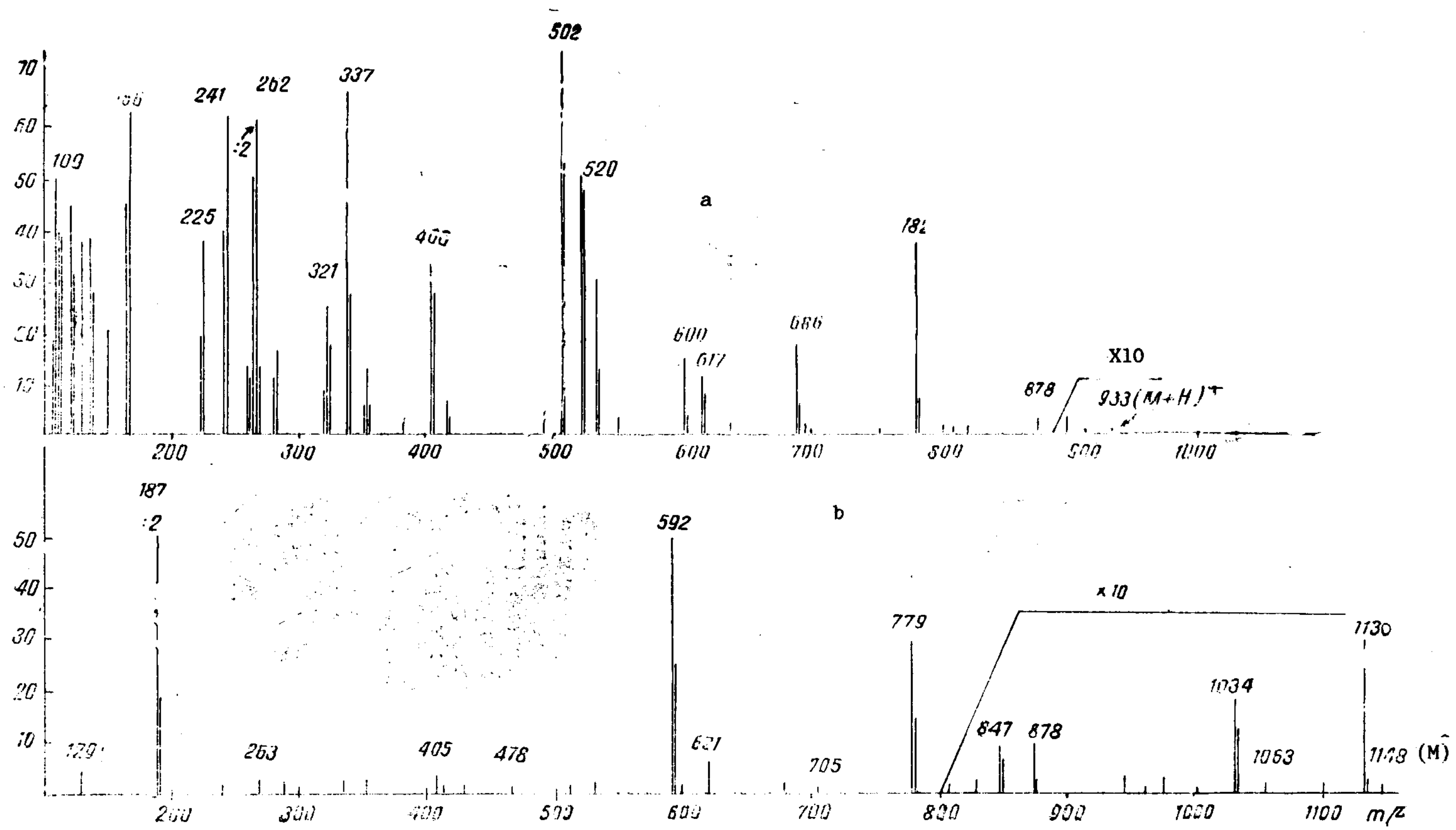


Fig. 1. Mass spectra of glycerol trircinoleate (I) (a) and of the TMS ether of glycerol triricinoleate (III) (b).

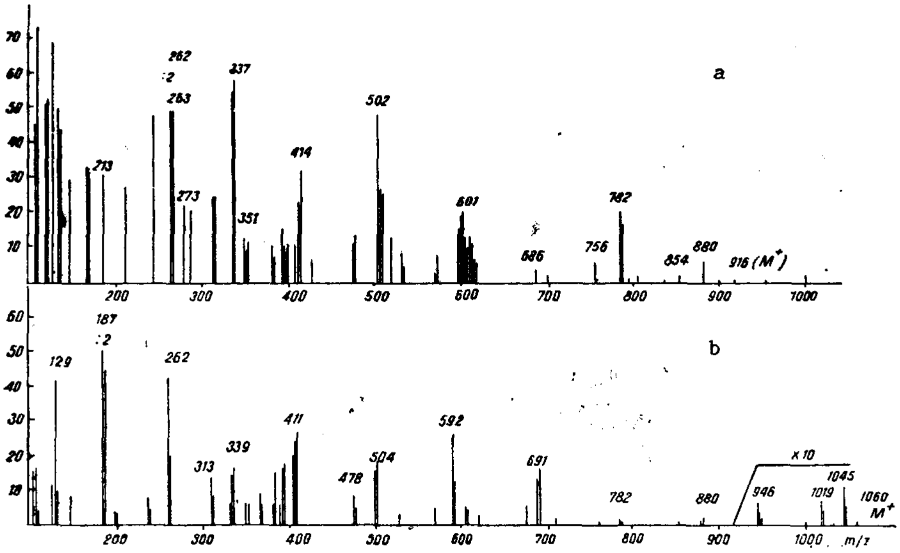


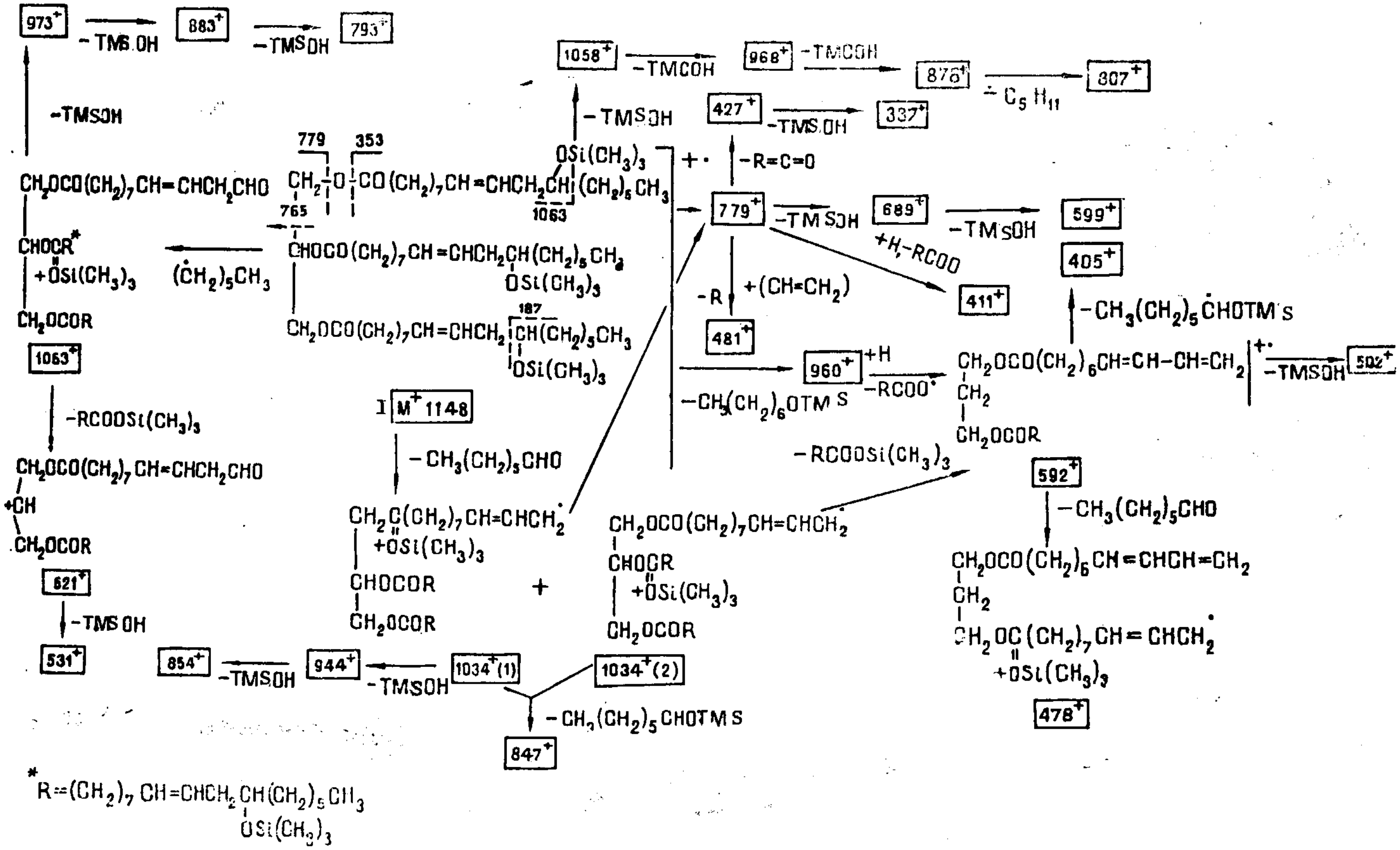
Fig. 2. Mass spectra of monooleoyldiricinoleoylglycerol (II) (a) and of its TMS derivative (IV) (b).

The intensities of the peaks of the ions of the second group in the spectrum of (III) (Fig. 1, b) are distributed in approximately the same way as in the spectra of the unoxidized compounds, namely: the product of the ejection of an acyloxy radical from one of the ester groups $(M - RCOO)^+$ with m/z 779 has the highest intensity, and the intensities of the peaks of the ions RCO^+ with m/z 353 and $(779 - R{=}C{=}O)^+$ with m/z 427 are considerably inferior to it. The peak of the ion $(M - RCOOCH_2)^+$ with m/z 765 is the smallest in this group. So far as concerns the TMS derivative of the monoacyldihydroxyacylglycerol (IV) (Fig. 2, b), although in this spectrum the total contribution of the ions of this type is increased, the peak of the $(M - RCOO)^+$ ion with m/z 691 does not so considerably exceed the peaks of the ions of this group (m/z 677, 353, 337).

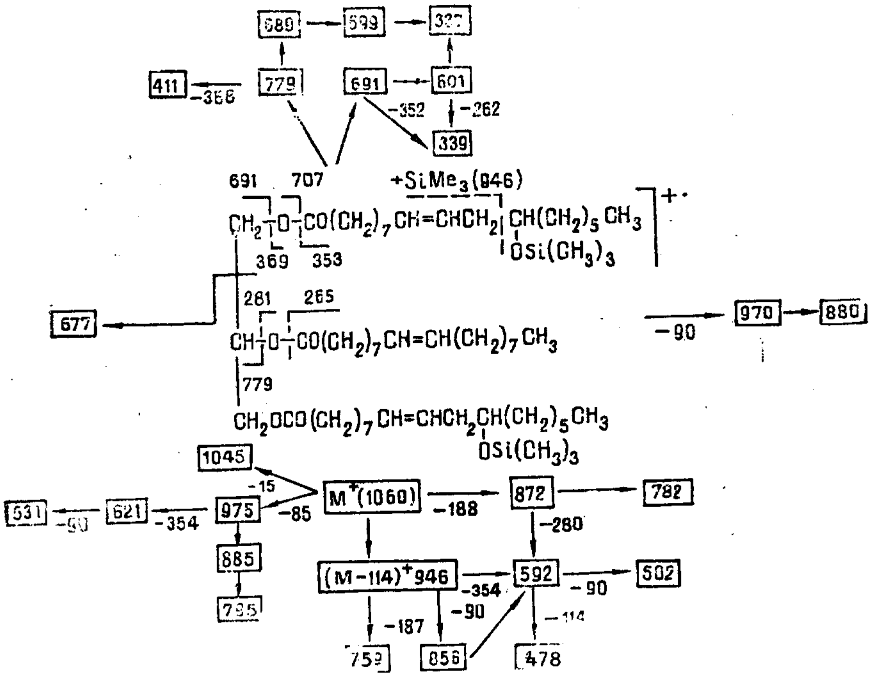
In connection with the presence in the (IV) of different acid residues, this specimen could have been a mixture of two position isomers, and the ions of the group under consideration could have two mass numbers. Thus, the ions $(M - RCOO)^+$ may be represented not only by the ion with m/z 691 but also by the ion with m/z 779, as in the spectrum of (III). The relative intensity of the latter (0.68%) is far less than that of the ion with m/z 691 (16%). If we assume the presence of a mixture of position isomers, this fact can be explained by a decreased amount of the isomer with the oleic acid residue in the sn-1 position or by a smaller tendency of the radical RCOO to split out from M^+ if the same residue is present in the sn-2 position. At the same time, the ratio of the intensities of the peaks of the ions $(M - RCOOCH_2)^+$ with m/z 765 and 677 (0.2 and 6.0%) in this spectrum is evidence in favor of the assumption that the oleic acid is attached mainly in the sn-2 position.

The ions of the third group in the spectra of (III) and (IV) either coincide in structure with the products of the fragmentation of the TMS derivative of methyl ricinoleate [4], such as, for example, the ion with m/z 187, $CH_3(CH_2)_5CH{=}\overset{+}{O}TMS$, or are analogous to them in their method of origin. Thus, the $(M - 85)^+$ ions with m/z 1063 (III) and 975 (IV) are analogous to the ion with m/z 299 of the hydroxy acid ester, and the ions $(M - 114)^+$ with m/z 1034 ($C_{59}H_{114}O_8Si_3$) (III) and 946 (IV) coincide in the composition of the fragment eliminated from M^+ with the rearrangement ions having m/z 270 [4]. The structure of the latter presupposes, as is well known, the migration of the TMS radical to the oxygen of the carbonyl group. However, on the basis of the similar form of the ion with m/z 1034 (1), where the transfer of the radical is realized within a single acid residue, it is difficult to imagine the formation of the ion with m/z 592 ($C_{35}H_{64}O_5Si$) that is the second in intensity in the spectrum of (III).

Judging from the metastable defocussing (MD) spectrum of the ion with m/z 592, its main precursor is an ion with m/z 1034, and the $779^+ \rightarrow 592^+$ transition is not realized because of the energetic unfavorability of the successive elimination of two radicals. Since the



Scheme 1. Fragmentation of the TMS derivative of glycerol triricinoleate.



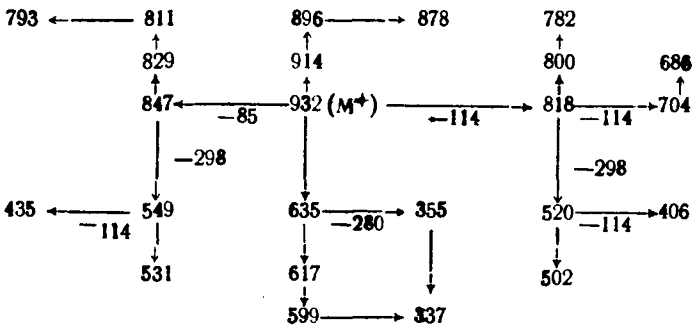
Scheme 2. Fragmentation of the TMS derivative of monooleoyldiricinoleoylglycerol (IV).

composition of the fragment eliminated in the $1034^+ \rightarrow 592^+$ transition corresponds to RCOOTMS, i.e., two TMS groups are eliminated in one stage (see scheme 1), it may be assumed that the parental ion has the 1034 (2) structure (scheme 1), being formed by the migration of a TMS radical from the sn-1 position to the oxygen of the sn-2 carboxyl. As can be seen from scheme 1 and Fig. 1, b, the precursor of the ion with m/z 592 may be the $(M - 188)^+$ ion with m/z 960. In its turn, the m/z 592 ion breaks down by the loss of a TMSOH molecule and also by the loss of 114 (m/z 478) and 187 (m/z 405) amu, which is confirmed by the MD spectra of the corresponding daughter ions.

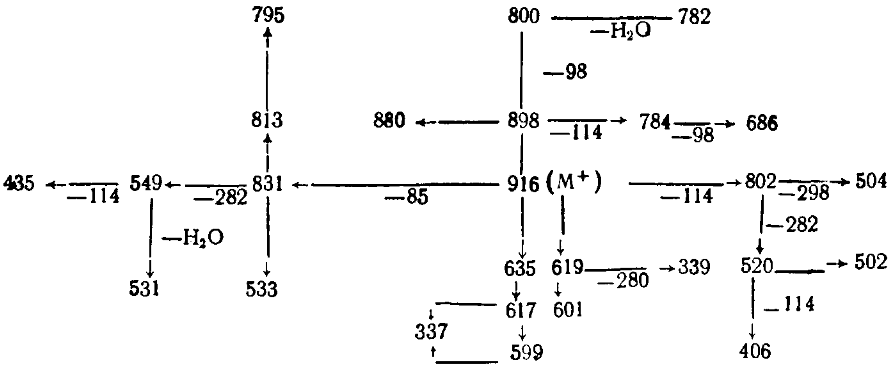
The $(M - 85)^+$ ion with m/z 1063, just like the m/z 1034 ions, loses a RCOOTMS molecule in one stage, forming an ion with m/z 621 ($C_{36}H_{65}O_6Si$). This permits the assumption that the stabilization of this parental ion also takes place through an interchange transfer of a TMS radical. In favor of this hypothesis is also the low intensity of the peaks of the $(1034 - RCOOH)^+$ and $(1063 - RCOOH)^+$ ions, which, it would appear, should compare with the 592 and 621 ions if the TMS group did not migrate to the neighboring substituent. Scheme 2 describes in generalized form the breakdown of the TMS derivative (IV), largely repeating the scheme for (III).

Let us now consider the mass-spectrometric behavior of the native triacylglycerols (I) and (II) (schemes 3 and 4). In both spectra, low-intensity peaks of the protonated (I) or unprotonated molecular ion with m/z 933 (I) and 916 (II) are observed (see Fig. 1, a, and 2, a). The peaks of the $(M - H_2O)^+$ ions are weak, and the peaks of the $(M - 3\,H_2O)^+$ ions in the spectrum of (I) and of the $(M - 2\,H_2O)^+$ ions in that of (II) achieve a greater intensity. This fact can obviously complicate the analysis of mixtures of triacylglycerols where, together with oxidized acid residues, there may be radicals of unoxidized acids with a high degree of unsaturation. In following the scheme of the separation of ions into groups proposed above, we may note that the ions characteristic of the unoxidized triacylglycerols [3] make a smaller contribution to the spectra of (I) and (II) than to the spectra of the TMS derivatives (III) and (IV). Only the peaks of the RCO^+ and, particularly, the $(RCO - H)^+$ ions with m/z 263 and 262 possess a high intensity. The head ions of the $(M - RCOO)^+$ series with m/z 635 (I) and 619 (II) are also stabilized by the loss of one or two molecules of water.

So far as concerns the breakdown of the M^+ ions of (I) and (II) at bonds present in the α-positions relative to OH groups, by analogy with the spectra of the TMS derivatives (III) and (IV), here the peaks of the $(M - 85)^+$ and $(M - 114)^+$ ions are observed, the peaks of the ions of the second type also being stronger. A feature of the spectra of glycerol



Scheme 3. Fragmentation of glycerol triricineolate.



Scheme 4. Fragmentation of monooleoyldiricineoleoylglycerol.

triricinoleate (I) consists in the successive elimination of the two 114 amu fragments and the formation of a stable fragment with m/z 686 after the additional ejection of a water molecule. No similar process is observed in the corresponding TMS derivative (III). In contrast to (I), in the spectrum of the monoacyldihydroxyacylglycerol (II) the ejection from M^+ of two fragments of 114 amu each is uncharacterisic, but the peak of an ion with m/z 686 stands out by its intensity and, together, with this, there is a peak with m/z 800 which can be regarded as $(M - H_2O - 98)^+$ and be taken as the consequence of the splitting out from $(M - H_2O)^+$ of a C_7H_{14} fragment as the result of the cleavage of the C_{11}–C_{12} bond in an unoxidized acid residue. The combination of these facts confirms that this residue is located in the sn-2 position. The ejection of RCOOH from the $(M - 114)^+$ and $(M - 85)^+$ ions forms analogs of the ions with m/z 592 and 621 – ions with mass numbers of 520 and 549, respectively. Both fragments are capable of losing 114-amu fragments, forming ions with m/z 406 and 435. The first of them is stabilized after the loss of an H_2O molecule.

Compound (I) possesses an unusual property among the series of compounds studied: its spectrum shows the presence of a stable fragment with m/z 697 formed by the elimination of (85 + 114) amu and two water molecules.

In each of the spectra of the two native triacylglycerols (Fig. 1a and 2a) one of the strongest peaks is that of a rearrangement ion with m/z 166 corresponding to a ricinoleic acid derivative. It is formed by the elimination from the $(\text{acyl} - H)^+$ ion with m/z 280 of a fragment with 114 amu.

An analysis of the spectra of (I)-(IV) with the aim of finding the impurities characteristic for castor oil [5] permitted the detection in the region of high mass numbers of the peaks of ions with m/z 854 (I and II) and 1019 (III and IV) not belonging to the products of the breakdown of the M^+ ions of the main compounds. In combination with results from the measurement of the accurate masses and the MD spectra of some ions in the central part of the spectrum, these peaks can be assigned to a minor component of the mixture – a monoacyldihydroxyacylglycerol substituted in one of the positions by a palmitic acid residue (M^+ 890). Thus, in the spectrum of the TMS derivative (III) the ion with m/z 478 is a doublet (1:1), one of the components of which corresponds to the composition $C_{28}H_{50}O_4Si$, and the other to $C_{30}H_{54}O_4$. As shown in Scheme 1, the former arises from an ion with m/z 592 by the loss of 114 amu. This transition is recorded in the MD spectrum of the ion with m/z 478.

In the same spectrum a $920^+ \rightarrow 478^+$ transition is observed which is due to the loss of RCOOTMS and is analogous to the above-mentioned $1034^+ \rightarrow 592^+$ transition of the main compounds (III). The existence of the $920^+ \rightarrow 478^+$ transition explains the composition of the second component of this daughter ion. Thus, the molecular weight of the TMS derivative of the impurity is 1034, and the 1019 ion corresponds to $(M - 15)^+$, which is confirmed by its elementary composition ($C_{60}H_{115}O_8Si_2$). In the spectrum of the TMS derivative (IV) the peak of an ion with m/z 1034 does not overlap the peak of the main compound and can be seen separately.

On the basis of the facts given above, the ion with m/z 854 in the spectra of the native triacylglycerols is the $(M - 2\ H_2O)^+$ ion of an impurity.

EXPERIMENTAL

MKh 1310 mass spectrometer, SVP5 system for the direct introduction of the sample, temperature of the ionization chamber and of the evaporation bulb 220-240°C, ionizing voltage 50 V, collector current 40 μA. The accurate mass measurements and the recording of the MD spectra were performed as in [1].

The lipids were extracted from the seeds with hexane at room temperature. The individual classes of lipids were isolated by repeated thin-layer chromatography in the hexane—diethyl ether (6.5:3.5) system. The oxidized triacylglycerols were silylated as described by Gunstone and Schuler [6]. The silylation products were purified by preparative TLC on silica gel in the solvent system hexane—diethyl ether (9:1).

SUMMARY

The mass-spectrometric fragmentation of the oxidized triacylglycerols of castor oil and their TMS derivatives has been studied for the first time. Three types of fragmentary ions have been detected the origin of which has been confirmed by measurements of elementary composition and by analysis of MD spectra. The specific cleavage of C—C bonds in the α-position to OMTS (OH) groups permits a mass-spectrometric method of analyzing mixtures of oxidized triacylglycerols to be proposed.

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