EPOXY ACIDS OF SEED OIL OF ZERAVSHAN SEA BUCKTHORN

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UDC 547.915+543.51

The composition of the total epoxy acids obtained by saponifying the fraction of epoxyacyldiacylglycerols of the seed oil of Zeravshan sea buckthorn has been studied with the aid of high-resolution mass spectrometry and MD spectra. The most complete structural information was obtained by analyzing the spectra of the TMS derivatives of the MEs of the di- and tetrahydroxy acids formed on the opening of the epoxide rings. On the basis of the results of the mass-spectrometric study, structures are proposed for 10 mono- and four diepoxy acids and one triepoxy acid.

A fraction has been isolated by column chromatography from the seed oil of Zeravshan sea buckthorn which, by the nature of its spectrum and a qualitative action has been assigned to the epoxyacyldiacylglycerols. The mass spectrum of the fraction contained the peaks of M^+ ions with m/z 890-898 corresponding to molecules containing one epoxide unit and from three to seven C=C bonds. The $(M - RCOO)^+$ fragment was represented mainly by ions with m/z 613 ($C_{39}H_{65}O_5$) and 615 ($C_{39}H_{67}O_5$), while epoxyacyl ions were presented by intense peaks with m/z 279 ($C_{18}H_{31}O_2$) and 277 ($C_{18}H_{29}O_2$).

The task of the investigation was to determine the structures of the epoxyacyl radicals by the use of high-resolution mass spectrometry and the metastable defocusing (MD) of characteristic ions. For this purpose, the epoxyacyldiacylglycerols were saponified, and the acids formed were methylated, and were separated by TLC into fractions of methyl esters (MEs) of unoxidized and epoxidized acids. For a number of reasons the mass-spectrometric characterization of the second fraction did not give sufficient information: the mass numbers of the molecular and fragmentary ions of the MEs of the epoxy acids coincided in many cases with those of the MEs of unoxidized compounds present as impurities in the fractions under investigation; the peaks of the key ions of the epoxy acids, with some exceptions, were not distinguished by a high intensity; and the above-mentioned coincidence of the mass numbers made the use of MD spectra pointless. The mixture under investigation was therefore converted into dihydroxy acids, the methyl esters of which were subjected to trimethylsilylation.

In this way, two samples were obtained, the first of which, judging from the general mass spectrum (Fig. 1, a), was enriched with the TMS derivatives of the MEs of dihydroxy acids, and the second with the TMS derivatives of the MEs of tetrahydroxy acids and epoxydihydroxy acids, i.e., with products in which one of the epoxy groups had not reacted (Fig. 1, b).

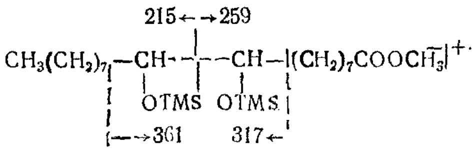
In establishing the structures of the main and auxiliary components of the mixtures, in addition to the mass-spectrometric characteristics we based ourselves on ideas about the genesis of epoxy compounds from unsaturated acids, the quantitative composition of which in the seed oils of the sea buckthorn is known. In accordance with this, the epoxy acids present in the mixture, which were analyzed in the form of TMS derivatives of the MEs of di- and tetrahydroxy acids (I-XIII), were grouped in accordance with the characteristics of their common biogenetic precursors; oleic, linoleic, linolenic, and hexadecanoic acids. Unsaturated acids with conjugated double bonds — precursors of compounds (XIV-XVI) — were not detected in the seed oil. In determining their structures, we were guided by their UV spectra, by the high concentration in the oil under investigation of hydroxy acids — coriolic and dimorphecolic [1] — close in structure to (XIV) and (XV), and by literature information on the mass spectrum of compound (XVI) [4].

Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 695-703, September-October, 1988. Original article submitted February 17, 1988.

0009-3130/88/2405-0592$12.50

Oleic acid

I.



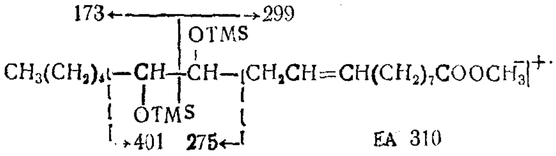
$CH_3(CH_2)_7-CH(OTMS)-CH(OTMS)-(CH_2)_7COOCH_3$

M^+ 474, ACI 443 $(M-OCH_3)^+$

RI 332, EA 312

Linoleic acid

II.



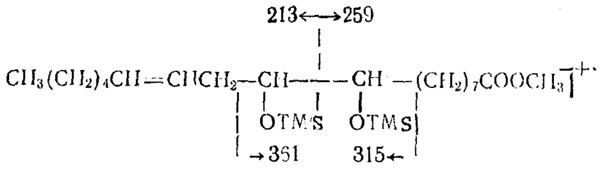
$CH_3(CH_2)_4-CH(OTMS)-CH(OTMS)-CH_2CH=CH(CH_2)_7COOCH_3$

M^+ 472, ACI , 441 $(M-OCH_3)^+$

RI 270, ACI 457 $(M-CH_3)^+$

EA 310

III.

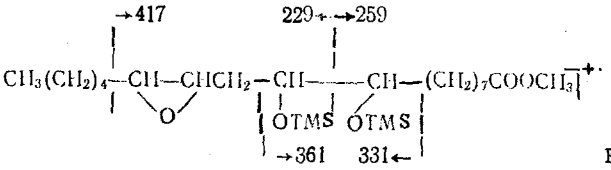


$CH_3(CH_2)_4CH=CHCH_2-CH(OTMS)-CH(OTMS)-(CH_2)_7COOCH_3$

M^+ 472, ACI 457, 441

EA 310, RI 332

IV.



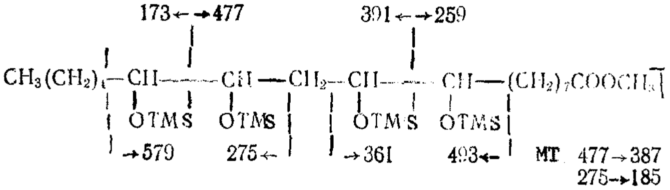
$CH_3(CH_2)_4-CH(-O-)CHCH_2-CH(OTMS)-CH(OTMS)-(CH_2)_7COOCH_3$

M^+ 488, MT 488→331

RI 332, 488→332

EA 326

V.



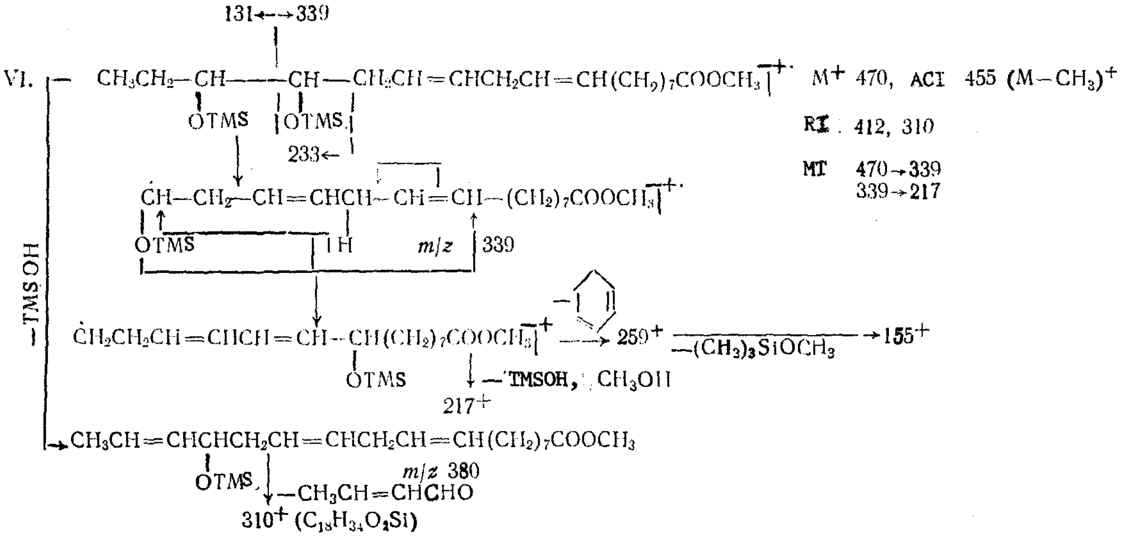
$CH_3(CH_2)_4-CH(OTMS)-CH(OTMS)-CH_2-CH(OTMS)-CH(OTMS)-(CH_2)_7COOCH_3$

$M^{+\cdot}$ 650, ACI 635, 619

RI 332, ACI 561, 560, 471, 191

Linolenic acid

VI.



$CH_3CH_2-CH(OTMS)-CH(OTMS)-CH_2CH=CHCH_2CH=CH(CH_2)_7COOCH_3$

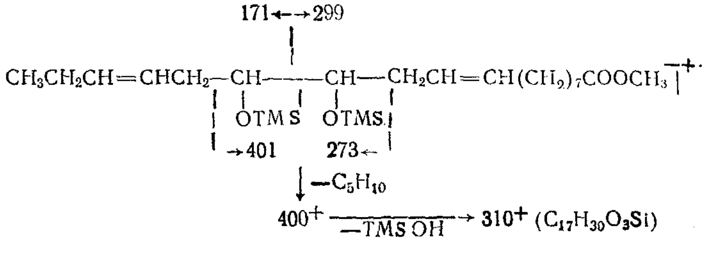
M^+ 470, ACI 455 $(M-CH_3)^+$

RI 412, 310

MT 470→339

339→217

VII.



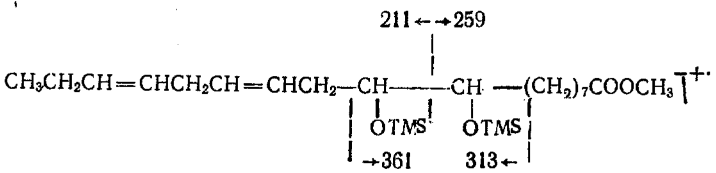
$CH_3CH_2CH=CHCH_2-CH(OTMS)-CH(OTMS)-CH_2CH=CH(CH_2)_7COOCH_3$

M^+ 470, ACI 455, 439

RI 270, ЭК 308

MT 470→299

VIII.



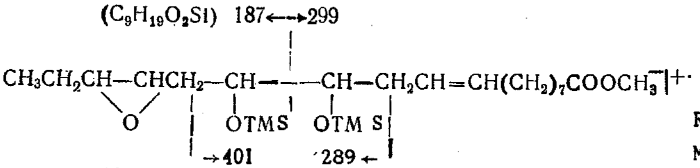
$$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}(CH_2)_7COOCH_3$$

M+ 470, ACI 455, 439

RI 332, EA 308

MT 470→259

IX.



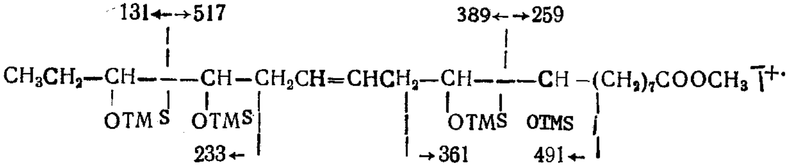
$$CH_3CH_2\overset{O}{CH{-}CH}CH_2{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}CH_2CH{=}CH(CH_2)_7COOCH_3$$

M+ 486, ACI 471, 455, 199 (289−90)

RI 270

MT 486→401

X.



$$CH_3CH_2{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}CH_2CH{=}CHCH_2{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}(CH_2)_7COOCH_3$$

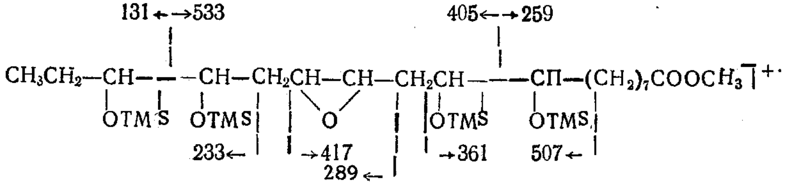
M+ 648, ACI 633

RI 590, 332

ACI 617, 559, 558, 469, 379

MT 648→558, 517→427

XI.



$$CH_3CH_2{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}CH_2\overset{O}{CH{-}CH}CH_2{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}(CH_2)_7COOCH_3$$

M+ 664, RI 332

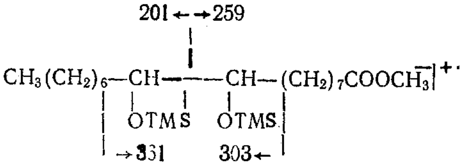
ACI 649, 633, 575, 574, 484, 443

MT 533→443, 405→315

EA 340

Pentadec-9-enoic acid

XII.



$$CH_3(CH_2)_6{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}(CH_2)_7COOCH_3$$

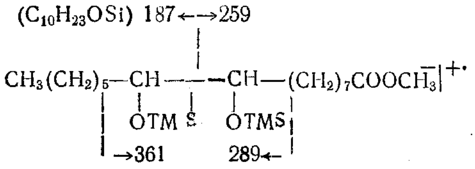
M+ 460, ACI 445, 429

RI 332, MT 460 → 259

EA 298

Hexadec-9-enoic acid

XIII.



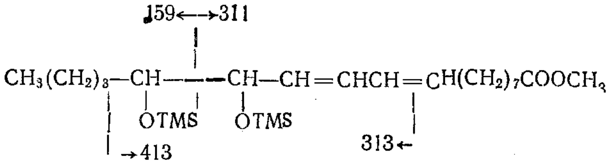
$$CH_3(CH_2)_5{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}(CH_2)_7COOCH_3$$

M+ 446, ACI 431

RI 332, MT. 446→259, 446→331

Octadeca-9,11,13-trienoic acid

XIV.

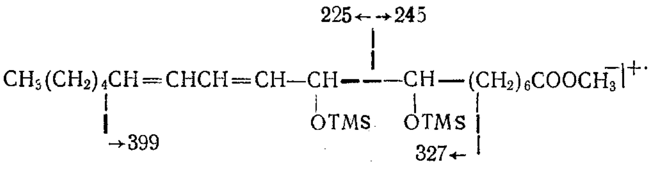


$$CH_3(CH_2)_3{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}CH{=}CHCH{=}CH(CH_2)_7COOCH_3$$

M+ 470, EA 308

Octadeca-8,10,12-trienoic acid

XV.

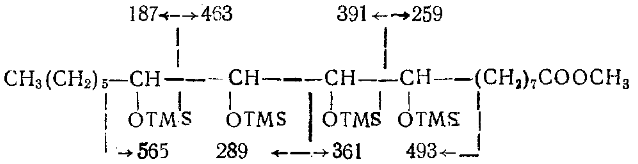


$$CH_3(CH_2)_4CH{=}CHCH{=}CH{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}(CH_2)_6COOCH_3$$

M+ 470, EA 308

Octadeca-9,11-dienoic acid

XVI.



$$CH_3(CH_2)_5{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}\underset{OTMS}{CH}{-}(CH_2)_7COOCH_3$$

M+ 650, ACI 635, 619, 560, 561, 471, 204

EA 326

Presumed biogenetic precursors (boldface) and mass spectrometric characteristics of the epoxy acids shown, which were isolated from sea buckthorn seed oil.

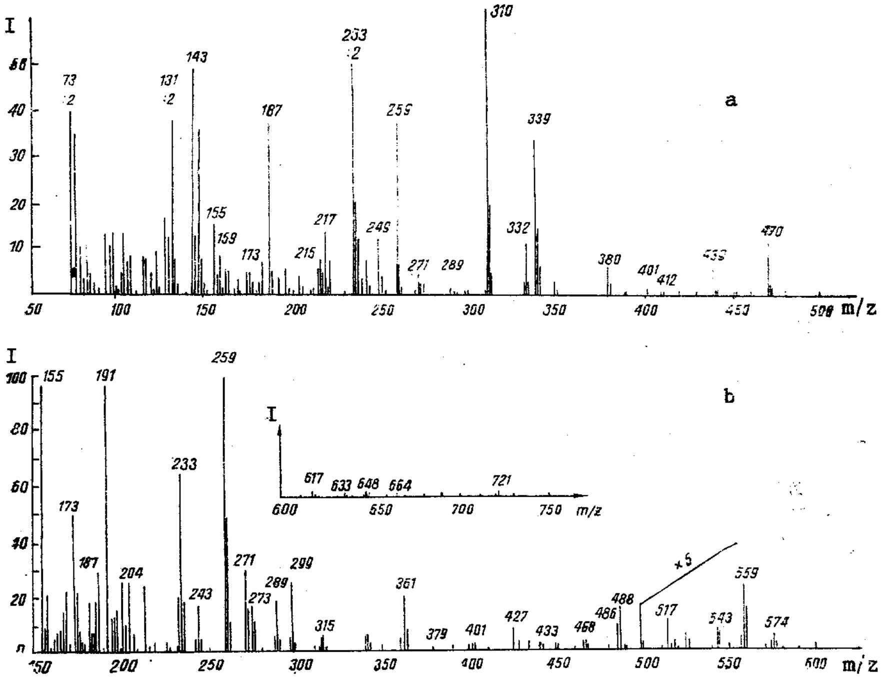


Fig. 1. Mass spectra of the TMS derivatives of methyl esters of dihydroxy acids: a) sample 1; b) sample 2.

In the formulas of all the compounds (scheme), cleavages leading to the formation of characteristics fragments are shown by dashed lines. In all cases, the comositions of the corresponding ions were confirmed by accurate mass measurements. In a number of experiments it was possible to show – and this is important from the mass-spectrometric point of view – that the processes leading to the appearance of the key fragments were multiple and that these fragments have a whole series of precursor ions. Under the formulas are given the mass numbers of the molecular ions (M^+), rearrangement ions (RIs), and additional characteristic ions (ACIs) and of metastable transitions (MTs) in the spectra of the TMS derivatives and the molecular masses of the corresponding epoxy acids (EAs).

Among the comopnents of the sample the spectrum of which is shown in Fig. 1, a, the highest concentration was that of the TMS derivative of the ME (VI) which corresponds to an initial 15,16-epoxyoctadeca-9,12-dienoic acid. The M^+ ions with m/z 470 and characteristic fragments with m/z 339, 310, 233, and 131 [2], the peaks of which were the strongest, therefore belonged to this derivative. The fragmentation pathways of compound (VI) are discussed below.

The existence of the metastable transition $470^+ \rightarrow 339^+$ and the composition of the latter ion confirmed the presence of OH groups at C-15 and C-16. In its turn, the ion with m/z 339 was one of the precursors of the ion with m/z 259 ($C_{13}H_{22}O_3Si$). In this transition, a C_5H_8 particle was lost, which can be explained by the migration of TMSO to the C-9 atom and the subsequent splitting out of a cyclohexadiene molecule (scheme). Then the ion with m/z 259 can rearrange into the structure suggested in [3] and, losing a $CH_3OSi(CH_3)_3$ molecule, be converted into an ion with m/z 155. The corresponding MT was also recorded. According to the MD spectrum another fragmentation pathway of the ion with m/z 339 consisted in the successive elimination of TMSOH and CH_3OH, which led to an ion with m/z 217 having the composition $C_{15}H_{21}O$.

The process of forming the ion with m/z 310 cannot amount to the migration of TMS to the carboxy group and cleavage of the C_{14}-C_{15} bond [2]. The MD spectrum of the ion with m/z 310 showed that its precursors were, in addition to the ion with m/z 470, ions with m/z 380 and 400. The ion with m/z 310 could have been formed as the result of the successive elimination of a TMSOH and a crotonaldehyde molecule.

The ions with m/z 400 and 401, the peaks of which had low intensities, corresponded to the compositions $C_{20}H_{40}O_4Si_2$ and $CO_{20}H_{41}O_4Si_2$, i.e., they were formed from the M^+ with m/z 470 after the ejection of C_5H_{10} and C_5H_9 fragments. The elimination of particles with such a comosition from the M^+ of compound (VI) is unlikely, and we therefore consider that the ions under consideration arose on the breakdown of the isomeric compound (VII), which was present in the mixture. The loss of a TMSOH molecule by the ion with m/z 400 led to an ion with m/z 310 having the composition $C_{17}H_{30}O_3Si$. We have detected an ion with such a composition under high resolution conditions. On electron impact, compound (VI) eliminated a TMS radical from the C-16 atom followed by the splitting out of crotonaldehyde and the formation of an ion with m/z 412.

There is no doubt that the ion with m/z 259 with the same elementary composition could also be formed in the fragmentation of compounds with TMSO groups at C-9 and C-10. Such substances were present in the sample in the form of impurities. This hypothesis has been shown by the detection of the metastable transitions $M^+ \rightarrow 259^+$ [M^+ –474(I), 472(III), 460(XII), 446(XIII)]. Furthermore, the occurrence of the MT.$470^+ \rightarrow 259^+$ may indicate the presence in the mixture of a third isomer of an epoxydienoic acid formed from linolenic acid (the TMS ether (VIII).

The molecular ions of the MEs of the TMS derivatives of the monoenoic (II, III) and saturated (I, XII, XIII) dihydroxy acids were less stable, and the presence of these compounds was judged mainly from the fragments $(M - 15)^+$ and $(M - 31)^+$, which are characteristic for ions with m/z 215 (I), 173 and 275 (II), 213 (III), 201 (XII) and 187 (XIII), and from the occurrence of the metastable transitions $460^+ \rightarrow 259^+$ (XII) and $446^+ \rightarrow 259^+$ (XIII). Without the complete separation of the components it was impossible to judge the amounts of these compounds relative to the main compound (VI). It is sufficient to note that in the spectrum of sample (I) in Fig. 1, a, the peak of the ion with m/z 187 ($C_{10}H_{23}OSi$) that was characteristic for derivative (XIII) had a high intensity.

In the UV spectrum of the mixture of epoxy acids, a sharp maximum was observed with its center at 233 nm, which corresponded to the presence of conjugated dienic compounds. The most probable explanation of this fact is the presence among the epoxy acids of compounds biogenetically close to the main hydroxy acids of the sea buckthorn oil, coriolic and dimorphic acids. In the spectrum of the sample there were medium-intensity peaks characteristic for the corresponding M^+ ions of the TMS derivatives of the MEs of the dihydroxy acids (XIV) and (XV).

Of the diepoxy acids in the mixture, the most representative was the 9,12-diepoxy-$C_{18:0}$ acid, which is biogenetically linked with linolenic acid. As shown above, when the combined material was treated with acetic acid, one of the epoxy groups reacted preferentially. Analysis of the spectra of the TMS derivatives of the MEs (Fig. 1b) showed that the compound with M^+ 488 most probably corresponded to structure (IV) and that, consequently, on treatment with AcOH, the epoxide ring at C-9, C-10 opened first. The choice of structure (IV) is also justified by the fact that the MD spectrum showed the metastable transitions $488^+ \rightarrow 332^+$ and $488^+ \rightarrow 361^+$; however, the presence of ions with m/z 332 and 361 in the spectrum may indicate that the mixture contained compounds (I), (III), (V), (VIII), (X), (XII), (XIII), and (XVI).

The mixture contained a smaller amount of the $C_{18:1}$ diepoxy acid. The molecular mass of the TMS derivative of the ME obtained on the opening of one epoxide ring was 486. The number of possible isomers of this substance, based only on the most probable precursor — linolenic acid — is six (three epoxy acids isomeric with respect to the positions of the rings). We have dwelt on the structure of (IX) as the most abundant in the mixture for the following reasons. The bulk of the ions with m/z 187 in sample 2 corresponded to the composition $C_9H_{19}O_2Si$. This structure was satisfactorily confirmed by an ion with m/z 289 having the composition $C_{13}H_{29}O_3Si_2$, the peak of which was of high intensity in the spectrum (Fig. 1, b). This ion, losing TMSOH, formed an ion with m/z 199 ($C_{10}H_{19}O_2Si$). Finally, the MD spectrum showed the occurrence of the metastable transition $486^+ \rightarrow 401^+$.

The spectra of samples of the TMS derivatives of the MEs of the epoxy acids showed the presence in the mixture of compounds with four TMSO groups (molecular masses 648, 650, and 664). The peaks of the following fragments of the first of the compounds were observed in the spectrum, m/z: 633 $(M - CH_3)^+$, 617 $(M - OCH_3)^+$, 559 $(M - TMSO)^+$, 469, and 379 $(M - TMSO - 2TMSOH)^+$. The composition of the last-mentioned ion was confirmed by accurate mass measurements. A consideration of the characteristic fragments formed on the fragmentation of the M^+ ions of the three possible isomers of the diepoxy derivatives of linolenic acid enabled the compound investigated to be characterized as 9,15-diepoxyoctadec-12-enoic acid. Its spectrum contained the peaks of all the ions corresponding to the fragmentation of derivative (X), including the peak of an ion with m/z 517 ($C_{25}H_{53}O_5Si_3$) of medium intensity which, on losing a TMSOH molecule, was converted into an ion with m/z 427 ($C_{22}H_{43}O_4Si_2$), the peak of which was one of the most intense in the region of high mass numbers.

The mixture of TMS derivatives of the MEs apparently contained not less than two compounds with a molecular mass of 650, the fragmentation of the M^+ ions of which was accompanied by the formation of the ions shown in the scheme. One of them consisted of the product of the opening of both epoxide rings of the $C_{18:0}$ 9,12-diepoxy acid (V). The occurrence of the transitions $477^+ \rightarrow 387^+$ and $275^+ \rightarrow 185^+$ in the MD spectrum and the determination of the elementary composition of the ions with m/z 387 and 185 confirmed this hypothesis. In the spectrum of sample 2 (Fig. 1, b), the intensity of the peak of the ion with m/z 191 $[(CH_3)_3SiO^+ = CH{-}OSi(CH_3)_3]$ had risen sharply and the peak of an ion with m/z 204 $[(CH_3)_3SiOCH = CHOSi(CH_3)_3]^+$ had appeared.

The first of them is characteristic for poly-TMS derivatives in general, and the second only for compounds with not less than four TMSO groups arranged in succession [4]. Thus, the second compound with M^+ 650 is most probably represented by structure (XVI).

The compound with M^+ 664 was a derivative of a triepoxy acid in which one epoxide ring had been retained. The fragmentary ions with m/z 649, 633, 575, 574, and 484 were formed on the elimination of $^\cdot CH_3$, $^\cdot OCH_3$, TMSO, and TMSOH. The occurrence of the metastable transitions $533^+ \rightarrow 433^+$ and $405^+ \rightarrow 315^+$ and a knowledge of the elementary comositions of the daughter ions mentioned permits the hypothesis that this TMS derivative had the structure (X), and the acid was, accordingly, the $C_{18:0}$ 9,12,5-triepoxyacid. The spectrum (Fig. 1, b) also showed an ion with m/z 721 which was obviously the product of the splitting out of CH_3 and TMSOH $[(M - 105)^+]$ from the M^+ with m/z 826 of the TMS derivative of the ME of the $C_{18:0}$ hexahydroxy (triepoxy) acid.

EXPERIMENTAL

A MKh 1310 mass spectrometer with a SVP5 attachment for the direct introduction of the sample was used. The temperatures of the evaporator bulb (ionization chamber) for the MEs of the acids and the triacylglycerols were 80/100 and 170/180°C, respectively. For the conditions of accurate mass measurements and the acquisition of the MD spectra, see [1].

The isolation of the epoxyacyldiacylglycerols, their saponification, and the preparation of methyl esters were carried out as described in [1]. Thin-layer chromatography was conducted on Chemopol silica gel, type 5-40, in the hexane—diethyl ether (8:2) system. Epoxy acids were converted into dihydroxy acids by the procedure described in [5]. The hydroxy acid derivatives wre silylated as in [1]. The silylation products were purified by preparative TLC on silica gel in the solvent system hexane—diethyl ether (9:1). In the isolation of the epoxy acids by the TLC method, the top part of the spot was removed and a fraction was obtained that was enriched with 15,16-epoxyoctadeca-9,12-dienoic acid.

SUMMARY

The composition of the mixture of epoxy acids obtained by the saponification of the epoxydiacylglycerol fraction of the seed oil of Zeravshan sea buckthorn has been studied with the aid of high-resolution mass spectrometry and MD spectra. The greatest amount of structural information was given by the spectra of the MEs of the TMS derivatives of the di- and tetrahydroxy acid formed on the opening of the epoxide rings. Structures have been proposed for ten monoepoxy and four diepoxy acids and for one triepoxy acid.

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