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GC–MS ANALYSIS OF HYDROXY AND EPOXY ACIDS FROM SEED OIL OF Hippophaë rhamnoides

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By the GC–MS method using a packed column, in the mixture of TMS derivatives of hydroxy acid methyl esters obtained from the seed oil of the sea buckthorn, in ten chromatographic peaks (CPs) 13 monohydroxy compounds have been characterized by their mass spectra, the main ones being derivatives of dimorphecolic and coriolic acids. Structures are proposed for four dihydroxy acids of the C_{17}–C_{20} series. The mixture of methyl esters of di- and tetra-TMS derivatives obtained from the mixture of epoxy acids from the same source has been analyzed by a similar method. In seven CPs, 11 compounds, reflecting the presence of nine epoxy acids in the initial mixture, have been characterized by their mass spectra. The main component of the mixture was 15,16-epoxyoctadeca-9,12-dienoic acid.

Having made use of the results of high-resolution mass spectrometry, metastable defocusing spectra, and known laws of the fragmentation of the TMS derivatives of oxidized fatty acids under electron impact (EI) [1], we predicted the presence of 20 hydroxy acids with chain lengths of C_{13}–C_{23} [2] and 14 C_{16}–C_{18} epoxy acids [3] in certain fractions of the seed oil of Zeravshan sea buckthorn. In the present work we have attempted to check the compositions of these fractions by chromato-mass spectrometry (GC–MS) using a packed chromatographic column.

The GC–MS analysis of hydroxy and dihydroxy acids has been performed previously in the study of the composition of a number of fractions of apple cutin [4], the products of the autooxidation of soybean oil [5], and other biological materials [6], but the authors of the papers referred to mentioned as the main disadvantage of the method used the incompleteness of the separation of the components.

Figure 1a shows a chromatogram of a fraction of TMS derivatives of the methyl esters of the hydroxy acids (I-XVII in Table 1) of sea buckthorn seed oil. Table 1 includes information on the mass numbers of the molecular and characteristic fragmentary ions and on the structures of the individual components of the mixture. The mass-spectral results are based on an analysis of the mass spectra recorded at the moment of reaching the apex of the corresponding chromatographic peak. On the basis of the mass spectra of ten chromatographic peaks (CPs) (Fig. 1) 17 compounds [13 derivatives of monohydroxy acids (I-XIII) and 4 derivatives of dihydroxy acids (XIV-XVII)] were detected, the presence of only 8 of them having been predicted in [2]: (I), (IV), (VI), (IX), (X), (XI), (XII), and (XVI), among which the main

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0009-3130/89/2505-0529$12.50

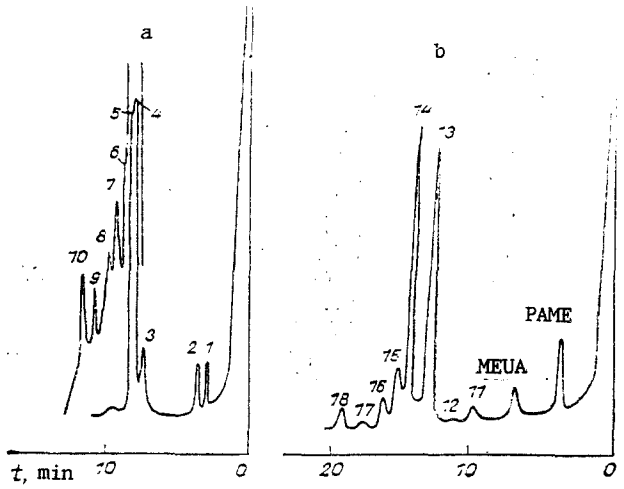


Fig. 1. Chromatogram of the TMS derivatives of the methyl esters of hydroxy acids (a) and of dihydroxy acids obtained from sea buckthorn oil (b).

components of the mixture were assumed to be dimorphecolic (X) and coriolic (XI) acids. Of the ten CPs, only 1, 2, 9, and 10 were separated satisfactorily from the other peaks. Nevertheless, the mass spectrum of CP 2 showed the presence in this peak of four compounds: a hydroxy acid of the C_{14} series (II) and three isomeric hydroxy acids of the C_{16} series (III-V). The presence of just these compounds was confirmed by the ratio found for the intensities of the peaks of the characteristic fragments with m/z 199 (A) and 257 (B), m/z 199 (A) and 285 (B), and m/z 227 (A) and 257 (B) corresponding to the mass spectra of the TMS ethers of hydroxy acids (II), (III), and (V) and the opposite ratio of the peaks of the ions with m/z 213 (A) and 271 (B) in the spectrum of compound (IV) that were found [1].

It can be seen from Fig. 1a that the acids of the C_{18} series (CPs 3-6) were partially separated according to their degrees of unsaturation, and under the chromatographic conditions used they issued in the following sequence $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$. It was impossible to detect hydroxy acids of the $C_{18:0}$ series. No separation of isomeric compounds took place, either. The mass spectrum of CP 3 was the most complex in composition, containing, in addition to the peaks of ions characteristic for the spectra of ricinoleic acid derivatives (VI), the peaks of the ions of three other isomers of the $C_{18:1}$ hydroxy acids (VII-IX). In this spectrum, the strongest peaks, of fragments with m/z 241 (A) and 285 (B), belonged to the spectra of two different isomers with M^+ 384. An ion of type B from compound (VIII) should have a mass number of 271, and an ion of type A from compound (VII) one of 227. In the mass spectrum the intensities of the peaks of these ions were several times lower than the intensities of the ions with m/z 241 and 285. According to the law given above, the two isomers should have structures (VIII) and (VII) (Table 1). The presence in the same spectrum of a rearrangement ion with m/z 230 and of ions with m/z 227 (A) and 259 (B) of medium intensity indicates the presence in the mixture of a fourth isomer, (IX) – 9-hydroxyoctadec-12-enoic acid, the mass spectrum of the methyl ester of the TMS derivative of which was given in [1]. To identify the isomeric hydroxyoctadecadienoic acids (X) and (XI), the spectra were recorded in the approach to the apex of the main peak of this group (CP 4) and at the beginning of the descent from it (CP 5). Both spectra were characterized by the peaks of M^+ ions with m/z 382 and the following ratios of the heights of the peaks of the ions with m/z 225 (A) and 311 (B) – 2:1 (CP 4) and 1:2 (CP 5), which are close to the mass spectra of the TMS derivatives at the methyl esters of dimorphecolic acid (X) and coriolic acid (XI) [1].

In the mass spectrum of CP 6, intense peaks of two A ions are observed with m/z 223 and, particularly, 183. The B ions corresponding to them have mass numbers of 259 and 351 and are characterized in the spectrum by peaks of medium intensity. According to the values of the mass numbers and the distribution of the intensities of these peaks, the first of the two isomers of the trienic hydroxy acids (XII) has a trienic system at the carbon end of the molecule separated from CH(OR) by a methylene group. The molecule of the second isomer (XIII) contains the increment $CH_3CH_2(CH{=}CH)_2CH(OTMS)$ (183 a.m.u.). The position of the third

TABLE 1. Structures of the TMS Derivatives of Methyl Esters of Hydroxy Acids Established in an Analysis of the Mass Spectra of the Chromatogram in Fig. 1a

Chromatographic peak	Compound	Characteristic ions, m/z				Structures of TMS derivatives of hydroxy acid methyl esters
		M+	A	B	Additional	
1	I	314	157	285	299, 283	$CH_3CH_2CH(OTMS)\ CH=CH(CH_2)_7COOCH_3$
2	II	328	199	257	313, 297	$CH_3(CH_2)_4CH=CHCH(OTMS)(CH_2)_5COOCH_3$
	III	356	199	285	341, 325	$CH_3(CH_2)_4CH=CHCH(OTMS)(CH_2)_7COOCH_3$
	IV	356	213	271	341, 325	$CH_3(CH_2)_5CH(OTMS)CH=CH(CH_2)_6COOCH_3$
	V	356	227	257	341, 325	$CH_3(CH_2)_6CH=CHCH(OTMS)(CH_2)_5COOCH_3$
3	VI	384	187	299	369, 353, 337, 270	$CH_3(CH_2)_5CH(OTMS)CH_2CH=CH(CH_2)_7COOCH_3$
	VII	384	227	285	369, 353, 337	$CH_3(CH_2)_6CH(OTMS)CH=CH(CH_2)_7COOCH_3$
	VIII	384	241	271	369, 353, 337	$CH_3(CH_2)_7CH=CHCH(OTMS)(CH_2)_6COOCH_3$
	IX	384	227	259	369, 353, 337, 230	$CH_3(CH_2)_4CH=CH(CH_2)_2CH(OTMS)(CH_2)_7COOCH_3$
4	X	382	225	311	367, 351, 335, 292, 130	$CH_3(CH_2)_4CH=CHCH=CHCH(OTMS)(CH_2)_7COOCH_3$
5	XI	382	225	311		$CH_3(CH_2)_4CH(OTMS)CH=CHCH=CH(CH_2)_7COOCH_3$
6	XII	380	223	259	365, 349, 333	$CH_3CH_2CH=CHCH=CHCH=CHCH_2CH(OTMS)(CH_2)_7COOCH_3$
	XIII	380	183	351		$CH_3CH_2CH=CHCH=CHCH(OTMS)C_{10}H_{18}COOCH_3$
7	XIV	456	157, 299	285, 427	441, 398	$CH_3CH_2CH(OTMS)CH=CHCH_2CH(OTMS)CH=CH(CH_2)_7COOCH_3$
8	XV	470	131, 313	259	455, 439, 412	$CH_3CH_2CH(OTMS)CH_2CH=CHCH=CHCH_2CH(OTMS)(CH_2)_7COOCH_3$
9	XVI	486	199	259, 299	455, 439, 396	$CH_3(CH_2)_2CH=CH(CH_2)_2CH(OTMS)(CH_2)_2CH(OTMS)(CH_2)_7-COOCH_3$
10	XVII	500	173, 343	429, 259	469, 453, 400, 230	$CH_3(CH_2)_4CH(OTMS)CH_2CH=CH(CH_2)_2CH(OTMS)(CH_2)_7-COOCH_3$

π-bond has not been established, although according to biogenetic analogies it is most probably located at C-9.

The last four peaks in the chromatogram shown in Fig. 1a (CPs 7-10) correspond to derivatives of C_{17}–C_{20} dihydroxy acids, the first two (7 and 8) being the esters of dienic acids (XIV and XV) and the last two (9 and 10) esters of monoenic acids (XVI and XVII). The structures of these compounds have been suggested on the basis of the following considerations. In the central part of spectrum of CP 7 the strongest peak is that of the ion with m/z 285, which corresponds to the fragment $TMSOCHCH{=}CH(CH_2)_7COOCH_3$. The presence in this spectrum of the peaks of $(M - C_2H_5)^+$ ions and of a rearrangement ion with m/z 398 indicates that the second TMSO group is located at C-15. An ion of type A with m/z 157 corresponds to the fragment $CH_3CH_2CH(OTMS)CH{=}CH$. Thus, the derivative under consideration has structure (XIV). Judging from the mass number of the molecular ion, in the spectrum of CP 8 (m/z 470) it corresponds to a derivative of a dihydroxy acid of the $C_{18:2}$ series.

A rearrangement ion with m/z 412 indicates that in this compound one of the TMSO groups is located at C-16, and the presence of an intense peak of a fragment with m/z 131 indicates the presence of a $CH_3CH_2CH(OTMS)$ group separated from the unsaturated chain by at least one methylene group. The maximum ion in the central part of the spectrum with m/z 259 indicates the presence of the increment $-CH_2CH(OTMS)(CH_2)_7COOCH_3$. Consequently, this dihydroxy acid must have structure (XV).

The spectrum of CP 9 corresponds to the di-OTMS derivative of the methyl ester of a $C_{19:1}$ acid with M^+ 486. The structure of this acid was substantiated in detail in [2]. The spectrum includes intense peaks of ions with m/z 199, 259, 299, 455, 439, and 396 (Table 1).

The structure of derivative (XVII) with a molecular mass of 500 (CP 10) was proposed on the basis of the following considerations. In the spectrum, the peaks of ions A with m/z 173 and B with m/z 259 stand out by their intensity, which most probably indicates the presence of TMSO groups in positions 15 and 9. This fact was reliably confirmed by the presence in the spectrum of an ion B with m/z 429 $(M - C_5H_{11})^+$ and an ion A with m/z 343 $[M - (CH_2)_7COOCH_3]^+$. We have previously established that the TMS ethers of dihydroxy acids can give two types of rearrangement ions with even mass numbers, depending on which of the TMS groups migrates to the methoxycarbonyl group [3]. In this case we observed ions of m/z 400 and 230. The first of them, $[M - CH_3 - (CH_2)_4CHO]^+$ unambiguously confirmed the presence of TMSO at C-15. The second is characteristic for the spectra of substances having a TMSO group at C-9 and a double bond at C-12 [1, 2].

Thus, the presence of many of the monohydroxy acids predicted in [2] could not be detected under the GC–MS conditions used. These were, in the first place the $C_{18:0}$ saturated acids and also the $C_{18:2}$ dienic acids. On the other hand, 10 new compounds were found, including five substances isomeric with those predicted previously (III, V, VII, VIII, XIII) and a number of new long-chain acids (see Table 1). In spite of the imperfection of both experiments [absence of the results of a study of the breakdown of metastable ions by the direct analysis of daughter ions (DADI) method or the method of linked (B/E = const) scanning, the unsatisfactory separation of the mixture in the packed column], we must nevertheless note as a positive fact that the GC–MS method has confirmed the structures of the main components of the mixture and has permitted the detection of 5 new compounds.

Figure 1b gives a chromatogram of the fraction of epoxy acids which were analyzed in the form of the TMS derivatives of the methyl esters of the dihydroxy acids, and Table 2 gives information on the structures and m/z values of the characteristic ions in the mass spectra of these derivatives (XVIII-XXVIII). According to the spectra of three of the ten CPs in Fig. 1b, three peaks contained artefactual compounds. This section of the first peak with respect to retention time corresponded to that of methyl palmitate (PAME), and the second to an unresolved mixture of the methyl esters of unsaturated acids (MEUA): $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$.

The spectrum of CP 12 shows the presence of dimorphecolic acid, which is probably formed as a product of the decomposition of poly-TMS derivatives during chromatography.

Of the 15 epoxy acid derivatives predicted in [3], in the spectra of the other chromatographic peaks (CPs 11 and 13-18) nine compounds were detected (XVIII-XXIV, XXVI, and XXVIII). The presence of a compound isomeric with derivative (XXV) was shown in the spectrum of CP 16. The possible presence of this compound in the mixture was mentioned in [3].

TABLE 2. Derivatives of Methyl Esters of Epoxy Acids Established in an Analysis of the Mass Spectra of the Chromatogram in Fig. 1b

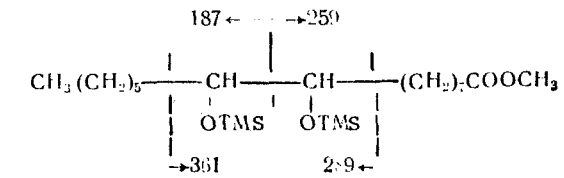
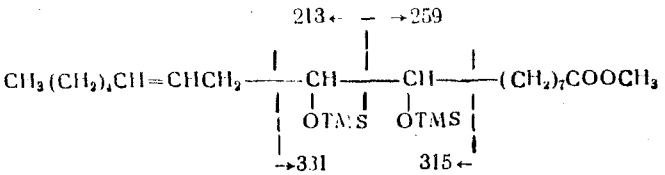
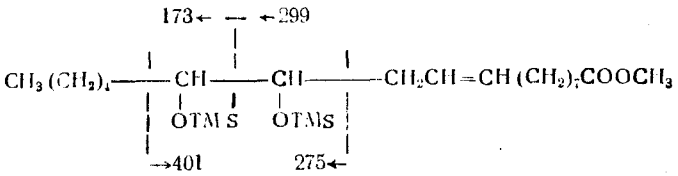
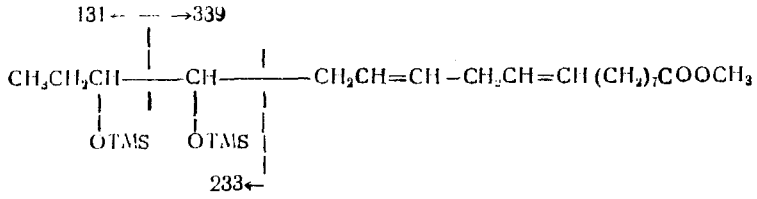
Chromatographic peak	Compound, M^+	Structure of the TMS derivatives of methyl esters of di- and tetrahydroxy acids and m/z values of characteristic fragments	m/z values of additional ions
11	XVIII 446		431, 415, 332
12	X 382	See Table 1	
13	XIX 472		457, 441, 332
	XX 472		
14	XXI 470		455, 439, 412, 310

TABLE 2 (continued)

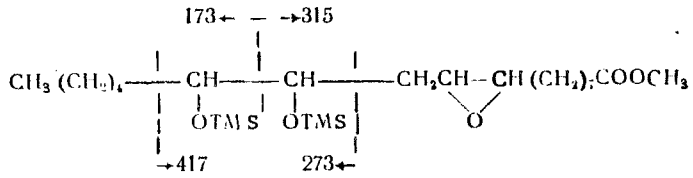
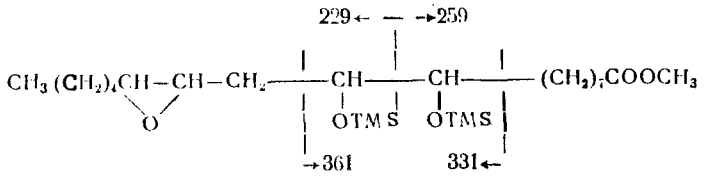
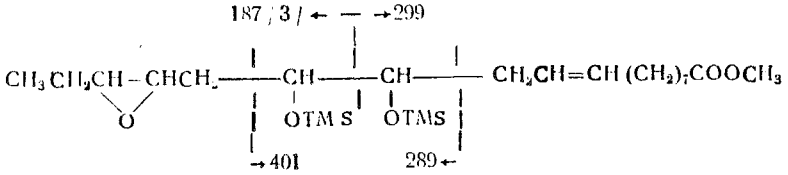
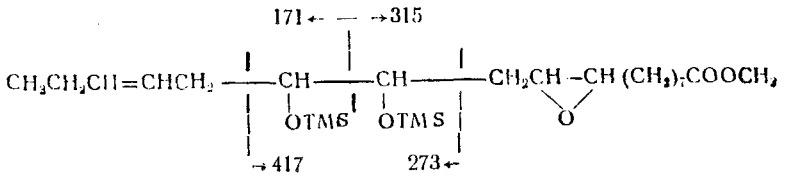
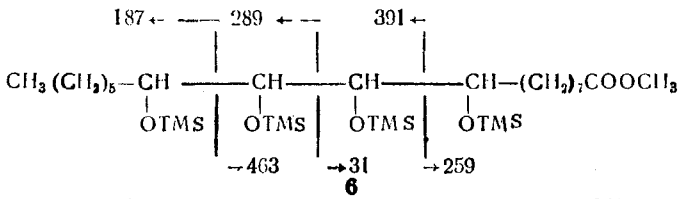
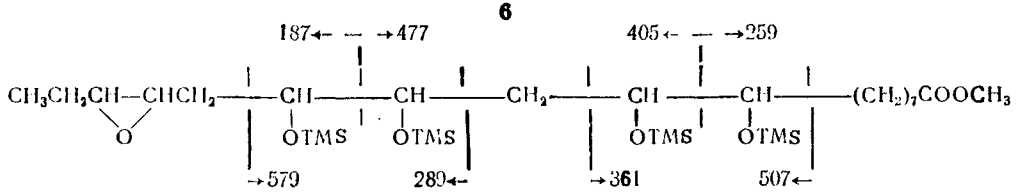
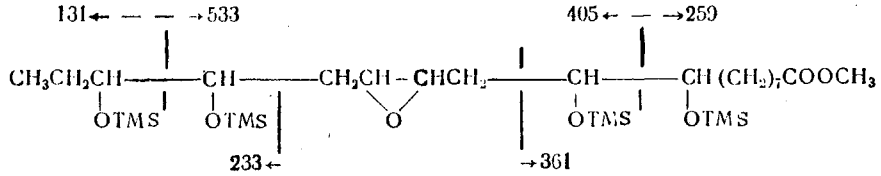
Chromatographic peak	Compound, M^+	Structure of the TMS derivatives of methyl esters of di- and tetrahydroxy acids and m/z values of characteristic fragments	m/z values of additional ions
15	XXII 488		473, 457
	XXIII 488		473, 457, 332
16	XXIV 486		471, 455, 270, 199
	XXV 486		471, 455, 327

TABLE 2 (continued)

Chromatographic peak	Compound, M^+	Structure of the TMS derivatives of methyl esters of di- and tetrahydroxy acids and m/z values of characteristic fragments	m/z values of additional ions
17	XXVI 650		560, 470, 373, 301, 204, 191
	XXVII 664		574, 484, 387, 417, 439, 315
18	XXVIII 664		574, 484, 391, 433, 315

In the spectrum of CP 17, peaks of ions corresponding to the mass spectrum of compound (XXVII), an isomeric derivative of the triepoxy acid (XXVIII), were found.

The high percentage of agreement with the structures of epoxy acids established on the basis of mass spectra of mixtures that had not undergone preliminary separation [3] and those determined by the GC–MS method is due, in our opinion, to the considerably greater set of characteristic fragments in the mass spectra of this group of compounds as compared with the spectra of derivatives of monohydroxy acids. No small role in this was also played by the prediction of the structures of epoxy acids based on their genesis from unsaturated acids the composition of which in buckthorn seed oil was studied in [7].

The GC–MS results confirmed the presence in the mixture of di- and triepoxy acids in which, on conversion into hydroxy derivatives, not all the epoxide units reacted completely (XXII-XXV, XXVII, XXVIII). Thus, in this group of substances two pairs of compounds (XXII and XXIII, and XXVII and XXVIII) were derivatives of identical di- and triepoxy acids (9,12-diep-$C_{18:0}$ and 9,12,15-triep-$C_{18:0}$).

The chromatogram in Fig. 1b shows that the maximum peak was CP 14 and, consequently, the main component of the mixture was 15,16-epoxyoctadeca-9,12-dienoic acid. In the spectrum of its derivative (XXI) we found, together with the M^+ peak having m/z 470, the peaks of all the characteristic fragments coinciding in mass numbers with those predicted previously [3, 6].

EXPERIMENTAL

GC–MS analysis was performed on a MS-25 RF instrument (United Kingdom) under the following conditions: glass column, ℓ = 1 m, d = 4 mm, phase 2% of OV-17 on Chrom W, carrier gas He. Programming of the temperature from 150 to 250°C at the rate of 5°C/min. Temperature of the jet separator 250°C.

Recording of the mass spectra: ionizing voltage 70 V, collector current 40 μA. Accelerating voltage 4 kV.

The isolation of the epoxy and hydroxy acids and the preparation of the TMS derivatives of methyl esters of these acids were performed as described in [3].

SUMMARY

By the GC–MS method using a packed column, in a mixture of the TMS derivatives of the methyl esters of the hydroxy acids of sea buckthorn seed oil, in ten chromatographic peaks (CPs) 13 monohydroxy acids have been characterized by their spectra, the main ones of them being derivatives of dimorphecolic and coriolic acids.

The structures of four dihydroxy acids of the C_{17}–C_{20} series have been suggested.

The mixture of methyl esters of di- and tetra-TMS derivatives obtained from the mixture of epoxy acids from the same source has been analyzed by a similar method. In seven CPs 11 compounds have been characterized by their mass spectra, reflecting the presence in the initial mixture of nine epoxy acids (three mono-, four di-, and two triepoxy acids). The main component of the mixture of epoxy acids was 15,16-epoxyoctadeca-9,12-dienoic acid.

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