In the seeds of the hybrid L-477 × L-475, the level of the 18:2 acid had not changed in comparison with the parental lines, while the amount of the 16:0 acid was closer to that for L-477 and that of the 18:1 acid (14.3%) was lower than for L-475 (19.3%) but higher than for L-477 (11.3%).

Interesting changes were observed in another hybrid combination, L-476 × L-477. In the parents the amount of the 16:0 acid was 34.2 and 28.7%, respectively, and in the hybrid it was considerably lower (24.5%); the unsaturated acids occupied an intermediate position. The level of the 16:0 acid (24.3%) in the T-1 × L-454 hybrid was inherited from the L-454 (25.1%), and that of the 18:1 acid (19.1%) from the T-1, while the 18:2 acid occupied an intermediate position.

Thus, an analysis of the FAs of the seeds of cotton-plant lines has shown that the lines differ appreciably with respect to the amounts of the main components that they contain and also to the degree of variability of the FA composition under the influence of the conditions during the period of the formation and ripening of the seeds. At the same time, the clear differences in the amounts of individual FAs between $F_1$ hybrids permit certain lines to be recommended as donors for improving the food-value indices of cottonseed oil.

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# ANALYSIS OF THE HYDROXYACYLDIACYLGLYCERIDES OF SEA BUCKTHORN SEED OIL

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UDC 547.915+543.51

The TMS derivatives of the hydroxyacyldiacylglycerides of sea buckthorn seed oil have been analyzed by mass spectrometry. On the basis of charcteristic fragments it has been established that an acyl residue of one of the seven hydroxy acids found previously in hydrolysis products by the GC—MS method is attached in one of the positions of the hydroxyacyldiacylglycerides. The remaining positions are substituted by acyl radicals of the $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{16:0}$ acids. The 24 most probable components of the triglycerides include these acyl and hydroxyacyl substituents. Features of the fragmentation of the components due to presence of polyunsaturated acids have been revealed.

On the basis of the composition of the hydroxy acids of sea buckthorn seed oil obtained by the alkaline hydrolysis of the hydroxyacyldiacylglycerides and studied by mass spectrometry [1] and chromato-mass spectrometry [2], we have attempted to analyze the total hydroxyacyldiacylglycerides (o-TAGs) without preliminary hydrolysis. In this we used the laws of fragmentation under EI of glycerol trircinoleate and its TMS derivative [3].

The o-TAGs were isolated from sea buckthorn seed oil by column chromatography on silicagel. The fraction was repeatedly purified by TLC to eliminate accompanying substances.

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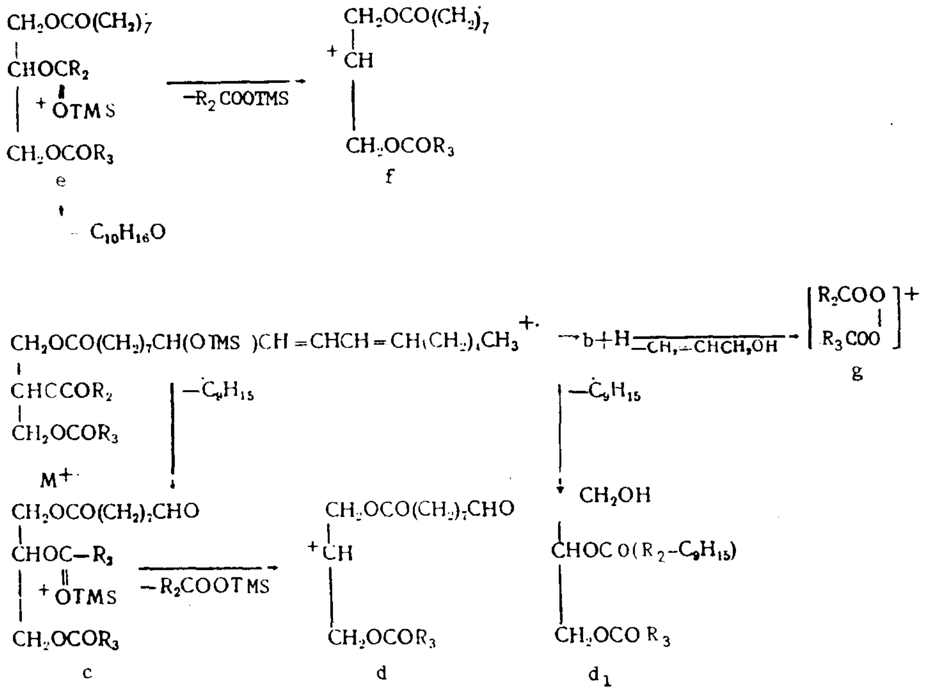
The mass spectrum of the native fraction of o-TAGs contained in the peaks of molecular ions, $M^+$, with m/z 890, 892, 894, 896, 898, and 900 and the peaks of the $(M - H_2O)^+$ ions corresponding to them. In addition, the spectrum contained the peaks of the $(acyl - H)^+$ ions that are chrcteristic for the unsaturated $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ acids (m/z 264, 262, and 260) and of the $(acyl)^+$ ions corresponding to the $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ hydroxy acids (m/z 281, 279, and 277, respectively). The most powerful peaks in the spectrum belonged to the ions of unoxidized TAGs and bore no structural information.



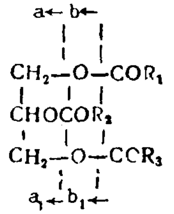
Scheme of the formation of fragments c-g

We subsequently studied the mass spectra of the TMS derivatives of the fractions mentioned. They contained $M^+$ peaks with m/z 962, 964, 966, 968, 970, and 972, and the peaks of the $(M - 15)^+$ ions that are characteristic for these derivatives. Also in the spectrum were intense $M^+$ peaks of o-TAGs containing a palmitic or a $C_{16}$ hydroxy acid residue (m/z 938, 940, 942, and 944) in one of the positions. The structures and compositions of the substituents in the molecules of the hydroxyacyldiacylglycerides and the mass numbers of the characteristic ions corresponding to them are given in Table 1 and the relative intensities of the peaks in Table 2.

The mass spectra described contain three types of ions characteristic of the TMS derivatives of o-TAGs – products of the splitting out of TMSOH, ions characteristic for unoxidized TAGs, and products of the cleavage of C—C bonds present in the α positions to TMSO groups – and also ions formed as the result of the successive occurrence of these processes. The presence of a TMSO group in one position, only, led to an appreciable decrease in the number of peaks of ions of the first type, but the ratio of the contributions of the ions of the second and third types was approximately the same as for the TMS ethers of the tri- and diricinoleates [3]. At the same time, because of the presence of compounds with oxidized acyl residues of different natures in the mixture, the relative roles of the various two-stage processes had changed. In view of the predominant presence of residues of dienic and trienic acids, alternative methods for the formation of cations of the second type appeared.

The nature of the oxidized substituents in the o-TAGs was determined mainly from the mass numbers of the peaks of the ions of type A [1, 4] with m/z 183, 187, 199, 225, and 227. The presence in the spectrum of two groups of $M^+$ ions and the detection of a number a number of peaks of ions, including a characteristic ion with m/z 227 in a mixture of the TMS ethers of methyl esters of the $C_{16}$ and $C_{18}$ hydroxy acids by the GC—MS method [2] permitted the assumption of the presence of the o-TAGs (1-4 and 16-20) in the mixture under investigation. We knew from the results of the previous investigations [1, 2] that one of the positions in each of compounds 11-15 could be occupied by isomeric coriolic and dimorphecolic acid residues.

TABLE 1. Compositions of the TMS Derivatives of the o-TAGs. Mass Numbers of Characteristic Fragments



| $R_1$ | Number | $R_2$ | $R_3$ | M+ | a | $M-R_1=C-O$ (b+H) | $a_1$ | $(b_1+H)^+-$TMSOH | $(a-R_2=C=O)^+$ | $(a_1-R_1=C=O)^+$ | $(a_1-R_2=C=O)^+$ −TMSOH | $(b+H-R_2C\dot{O})^+$ | $(b+H-R_2CO\dot{O})^+$ | $(a-R_2+CH=CH_2)^+$ | $(d_1)^+$ | f+ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $CH_3(CH_2)_6CH=CHCH(OTMS)$\|— 227← —$(CH)_5$— | 1 | $C_{17}H_{31}$ | $C_{17}H_{29}$ | 938 | 597 | — | — | — | 335 | 337 | 309 | 351 | 335 | 389 | 461 | 432 |
| | 2 | $C_{17}H_{31}$ | $C_{17}H_{31}$ | 940 | 599 | — | — | — | 337 | 337 | 309 | 353 | 337 | 391 | 463 | 434 |
| | 3 | $C_{17}H_{33}$ | $C_{17}H_{31}$ | 942 | 601 | — | — | — | 337 | 339 | 309 | 353 | 337 | 391 | 463 | 434 |
| | 4 | $C_{17}H_{33}$ | $C_{17}H_{33}$ | 944 | 603 | — | — | — | 339 | 339 | 309 | 355 | 339 | 393 | 465 | 436 |
| $CH_3(CH_2)_4CH=CHCH(OTMS)$\|— 199← —$(CH_2)_7$— 187← | 5 | $C_{17}H_{31}$ | $C_{17}H_{29}$ | 938 | 597 | — | — | 588 | 335 | 337 | 309 | 351 | 335 | 389 | 489 | 460 |
| | 6 | $C_{17}H_{31}$ | $C_{17}H_{31}$ | 940 | 599 | — | — | 588 | 337 | 337 | 309 | 353 | 337 | 391 | 491 | 462 |
| | 7 | $C_{17}H_{33}$ | $C_{17}H_{31}$ | 942 | 601 | — | — | 590 | 337 | 339 | 309 | 353 | 337 | 391 | 491 | 462 |
| | 8 | $C_{17}H_{33}$ | $C_{17}H_{33}$ | 944 | 603 | — | — | — | 339 | 339 | 309 | 355 | 339 | 393 | 493 | 464 |
| $CH_3(CH_2)_5CH(OTMS)CH_2$— —$CH=CH-(CH_2)_7$ | 9 | $C_{17}H_{33}$ | $C_{17}H_{31}$ | 970 | 601 | — | 691 | 618 | 337 | 339 | 337 | 353 | 337 | 391 | 531 | 502 |
| | 10 | $C_{17}H_{33}$ | $C_{17}H_{33}$ | 972 | 603 | — | 691 | — | 339 | 339 | 337 | 355 | 339 | 393 | 533 | 504 |
| $CH_3(CH_2)_4CH(OTMS)CH=CH$— —$CH=CH$\|$(CH_2)_7$— 225← | 11 | $C_{17}H_{29}$ | $C_{17}H_{29}$ | 962 | 595 | 612 | 685 | 612 | 335 | 335 | 335 | 351 | 335 | 389 | 489 | 460 |
| | 12 | $C_{17}H_{31}$ | $C_{17}H_{29}$ | 964 | 597 | 614 | 687 | 614 | 335 | 337 | 335 | 351 | 335 | 389 | 489 | 460 |
| | 13 | $C_{17}H_{31}$ | $C_{17}H_{31}$ | 966 | 599 | 616 | 687 | 614 | 337 | 337 | 335 | 353 | 337 | 391 | 491 | 462 |
| $CH_3(CH_2)_4CH=CHCH=CH$— —$CH-(OTMS)$\|$(CH_2)_7$— 225← | 14 | $C_{17}H_{33}$ | $C_{17}H_{31}$ | 968 | 601 | 618 | 689 | 616 | 337 | 339 | 335 | 353 | 337 | 391 | 491 | 462 |
| | 15 | $C_{17}H_{29}$ | $C_{15}H_{31}$ | 940 | 573 | 590 | 685 | — | 313 | 335 | 335 | 329 | 313 | 367 | 467 | 438 |
| $CH_3(CH_2)_4CH=CH(CH_2)_2$— —$CH(OTMS)$\|$(CH_2)_7$— 227← | 16 | $C_{17}H_{29}$ | $C_{17}H_{29}$ | 964 | 595 | — | 687 | 614 | 335 | 335 | 337 | 351 | 335 | 389 | 489 | 460 |
| | 17 | $C_{17}H_{31}$ | $C_{17}H_{29}$ | 966 | 697 | — | 689 | 616 | 335 | 337 | 337 | 351 | 335 | 389 | 489 | 460 |
| | 18 | $C_{17}H_{31}$ | $C_{17}H_{31}$ | 968 | 599 | — | 689 | 616 | 337 | 337 | 337 | 353 | 337 | 391 | 491 | 462 |
| | 19 | $C_{17}H_{33}$ | $C_{17}H_{31}$ | 970 | 601 | — | 691 | 618 | 337 | 339 | 337 | 353 | 337 | 391 | 491 | 462 |
| | 20 | $C_{17}H_{33}$ | $C_{17}H_{33}$ | 972 | 603 | — | 691 | — | 339 | 339 | 337 | 355 | 339 | 393 | 493 | 464 |
| $CH_3CH_2CH=CHCH=CH$— —$CH(OTMS)$\|— 183← —$C_{10}H_{18}$— | 21 | $C_{17}H_{31}$ | $C_{17}H_{29}$ | 962 | 597 | 614 | 685 | 612 | 335 | 337 | 333 | 351 | 335 | 389 | 529 | 500 |
| | 22 | $C_{17}H_{31}$ | $C_{17}H_{31}$ | 964 | 599 | 616 | 685 | 612 | 337 | 337 | 333 | 353 | 337 | 391 | 531 | 502 |
| | 23 | $C_{17}H_{33}$ | $C_{17}H_{31}$ | 966 | 601 | 618 | 687 | 614 | 337 | 339 | 333 | 353 | 337 | 391 | 531 | 502 |
| | 24 | $C_{17}H_{31}$ | $C_{15}H_{31}$ | 940 | 575 | 592 | 685 | — | 313 | 337 | 333 | 329 | 313 | 367 | 507 | 478 |

TABLE 2. Mass Numbers (m/z) and Relative Intensities (%) of the Ion Peaks in the Mass Spectra of TMS Derivatives of the o-TAGs

| m/z | % | m/z | % | m/z | % | m/z | % | m/z | % |
|---|---|---|---|---|---|---|---|---|---|
| 972 | 0.4 | 880 | 1,9 | 599 | 35 | 465 | 2,1 | 333 | 3,5 |
| 970 | 0,9 | 878 | 3, | 597 | 27 | 464 | 1,4 | 329 | 0,7 |
| 968 | 1,6 | 876 | 5,0 | 595 | 9,3 | 463 | 2,8 | 313 | 8,6 |
| 966 | 1,7 | 874 | 3,9 | 592 | 1,4 | 462 | 2,1 | 309 | 2,1 |
| 964 | 1,0 | 872 | 1,7 | 590 | 7 0 | 461 | 1 4 | 267 | 3,5 |
| 962 | 0,4 | 858 | 1,8 | 588 | 2,8 | 460 | 0,7 | 265 | 7,8 |
| 953 | 0,6 | 854 | 0,9 | 575 | 12 | 438 | 1.4 | 264 | 12 |
| 951 | 0.9 | 852 | 1,5 | 573 | 8.4 | 436 | 0,7 | 263 | 13 |
| 949 | 0,7 | 850 | 1,2 | 533 | 2.1 | 434 | 1,4 | 262 | 11 |
| 944 | 0,4 | 848 | 0,7 | 531 | 4,2 | 432 | 1 4 | 261 | 15 |
| 942 | 0,4 | 691 | 0.4 | 529 | 2,1 | 393 | 3.5 | 260 | 11 |
| 940 | 0,2 | 689 | 0,7 | 507 | 2,1 | 391 | 3,5 | 259 | 7,9 |
| 938 | 0,1 | 687 | 0,8 | 504 | 6,4 | 389 | 1,4 | 239 | 9,3 |
| 930 | 0,7 | 685 | 0,7 | 502 | 8,4 | 367 | 3,5 | 227 | 16 |
| 899 | 0,6 | 618 | 6,0 | 500 | 5,0 | 355 | 1,4 | 225 | 52 |
| 897 | 1,3 | 616 | 21 | 493 | 19 | 353 | 4,2 | 199 | 13 |
| 895 | 2,2 | 614 | 42 | 491 | 37 | 351 | 6 4 | 187 | 29 |
| 893 | 1,7 | 612 | 24 | 489 | 15 | 339 | 9,3 | 183 | 75 |
| 891 | 0,7 | 603 | 10 | 478 | 2,1 | 337 | 11 | 131 | 46 |
| 882 | 0,8 | 601 | 22 | 467 | 7,9 | 325 | 7,9 | 73 | 100 |

In the region of high mass numbers in the spectrum of the total o-TAGs there were the peaks of $(M - 71)^+$ ions with m/z 891, 893, 895, 897, and 899, which are characteristic for the corresponding acids. However, it must be borne in mind that similar ions are present in the spectrum of the trilinoleate and can also be formed as the result of the breakdown of o-TAGs having at sn-1 substituents with π-bonds in positions 12 (16-20).

Ideas about a possible set of substituents $R_2$ and $R_3$ for each of the $R_1$'s were based on the set of mass numbers of the $M^+$, a, b+H and $RCO^+$ ions.

IN the spectrum, the ions a, which were common for all TAGs, had odd numbers from 595 to 603 (when $R_2$ and $R_3$ were of the $C_{18}$ series) and 575 and 573 if one of the substituents belonged to the $C_{16}$ series. The ions $a_1$ analogous to them were far less stable. The ions analogous to them were far less stable. The ions b+H $(M—R_1=C=O)$ were characteristic for dienic and more unsaturated acid residues. As is known [5], after the formation of ions a, saturated and monoenic residues break down through cleavages in the sn-2 or sn-3 chains. The formation of ions of the b+H type is also characteristic for unoxidized TAGs. Thus, in the spectrum of the trilioleate with $M^+$ 878 there are strong peaks of ions with m/z 616 (b+H) and 262 (acyl −H) (616 + 262 = 878). The peaks of the ions $b_1$+H were not observed in the spectrum of the TMS ether of the o-TAG under consideration They were probably stabilized by the elimination of TMSOH from sn-1.

In the region of mean mass numbers of the spectrum in the 300-400 a.m.u. region there were several groups of peaks of ions of medium intensity that are charctersitic for TAGs of all types and are formed in their majority by alternative pathways: 309, 313, 333, 335, 337, 339, 329, 351, 353, 355, 367, 389, 391, and 393. Of these groups, the ions $(a-R_2=C=O)^+$ and $(b+H-R_2COO)^+$ (Table 1) have identical mass numbers but isomeric structures. The ions $(a-R_2=C=O)^+$ and $(a_1-R_1=C=O)^+$ are isomeric if the substituents $R_2$ and $R_3$ in the initial molecules have the same composition. The ions $(b+H-R_2CO)^+$ with m/z 351, 353, and 355 have the same mass numbers as $(R_1CO)^+$ ions with a different elementary composition. Thus a measurement of the accurate masses of the ion with m/z 351 showed that it consisted of particles with the compositions $C_{21}H_{35}O_4$ $(b+H-R_2CO)^+$ and $C_{29}H_{31}O_2Si$ $(R_1CO)^+$ in a ratio of 1:1.

The sequence of formation of all the fragments given in Table 1 and in the scheme was confirmed not only with the aid of literature analogies but also by an analysis of the linked-scanning (B/E = const) and metastable defocusing (MD) spectra. In this way it was possible to show, for example, that the groups of $a^+$ and $(b+H)^+$ ions were formed from the $(M - TMSOH)^+$ ions, while representatives of all the groups of ions in the 330-400 a.m.u. interval arose from ions with m/z 599 $(a)^+$ and 614 $(b+H)^+$. According to the literature, the ions with m/z 389, 391, and 393 have the structure $(a-R_2+CH=CH_2)^+$ [5]; however, the presence of the metastable transition $614^+ \rightarrow 393^+$ in the linked-scanning spectrum showed an alternative structure for the daughter ions of this group. The MD spectrum of the ion with m/z 614 gave as precursors the $(M - TMSO)^+$ ions and groups of $M^+$ ions with their centers at m/z values of from 968 to 940.

The peak of an ion of the type d(d$_1$) with m/z 491 ($C_{30}H_{51}O_5$) was one of the most intense in the spectrum of sample of o-TAGs under consideration. The MD spectra of this ion showed that there were at least two pathways for its appearance. These fragmentation pathways are illustrated in the scheme, taking as an example the breakdown of the o-TAG esterified at sn-1 with dimorphecolic acid. The first pathway consists in the splitting out of the radical $\dot{C}_9H_{15}$ with the simultaneous migration of the TMS group to the oxygen of the carbonyl at sn-2 (ion b), followed by the splitting out of $R_2COOTMS$, which gives ion d$_1$ The similar breakdown pathway for glycerol trilinoleate led to an ion with m/z 621 [3] giving a peak of medium intensity. The linked-scanning spectrum of the ion $(b+H)^+$ with m/z 614 showed the splitting out of $\dot{C}_9H_{15}$ from sn-2, giving rise to the isomeric ion d. Under these conditions the substituent at sn-1 and the palmitoyl residue at sn-3 formed the ions d(d$_1$) with m/z 467 (Table 1). When sn-1 is substituted by different hydroxy acids ions d(d$_1$) with different m/z values are formed.

In addition to the group of ions f with even mass numbers, which are formed from the ions e, we also detected the ions d, which were formed from $M^+$ with the transfer of TMS to sn-2, followed by the splitting out of $R_2OTMS$. From their method of formation they were similar to ions with m/z 592 in the spectrum of the trilinoleate [3]; however, they made a far smaller contribution to the total ion current.

It must be mentioned that the peaks of fragments of the d and f types were also observed in the spectra of unsilyated o-TAGs. However, only in the spectra of the TMS ethers were there the peaks of ions with m/z 522, 554, and 556. The elementary composition of the ion with m/z 554 was $C_{36}H_{58}O_4$, and, judging from the linked-scanning spectrum, this ion was formed from an ion with m/z 612 as the result of the loss of a $C_3H_6O$ group. Such a decomposition pathway could most probably arise through the splitting out of a molecule of allyl alcohol. The breakdown of the $(b+H)^+$ ion with the linkage of $R_2CO\dot{O}$ and $R_3CO\dot{O}$ radicals led to the ion g. In the region of high mass numbers there was a peak of an ion with m/z 930 corresponding to the presence of the residue of a $C_{15}$ acid in one of the positions. A single acid of this series (9-hydroxypentadeca-11,12-dienoic) has been detected among the TMS derivatives of methyl esters of the hydroxy acids [1]. The type A ion corresponding to it is characterized by a mass number of 183. On the basis of molecular masses, the sn-2 and sn-3 positions of such an o-TAG must be substituted by oleic and stearic acid residues. The peak of an ion $C_{17}C_{35}CO^+$ with m/z 267 was present in low intensity in the spectrum, so that the presence of this component may be assumed.

## EXPERIMENTAL

MKh 1310 mass spectrometer, temperature of the ionization chamber and evaporator bulb 180-200°C, ionizing voltage 60 V, collector current 80 μA. For the conditions of obtaining the MD and linked-scanning (B/E = const) spectra, see [6].

The isolation of the o-TAGs and their purification and silylation were carried out as described in [3].

Yu. M. Mil'grom took part in the performance of the mass-spectrometric experiment.

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