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# OXYGENATED FATTY ACIDS OF THE SEEDS OF *Elaeagnus angustifolia*

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*Among the oxidized lipids in the seed oil of the olive* Elaeagnus angustifolia *we have detected six epoxy acids and five hydroxy acids, of these 12-hydroxyoctadeca-8,10-dienone being new.*

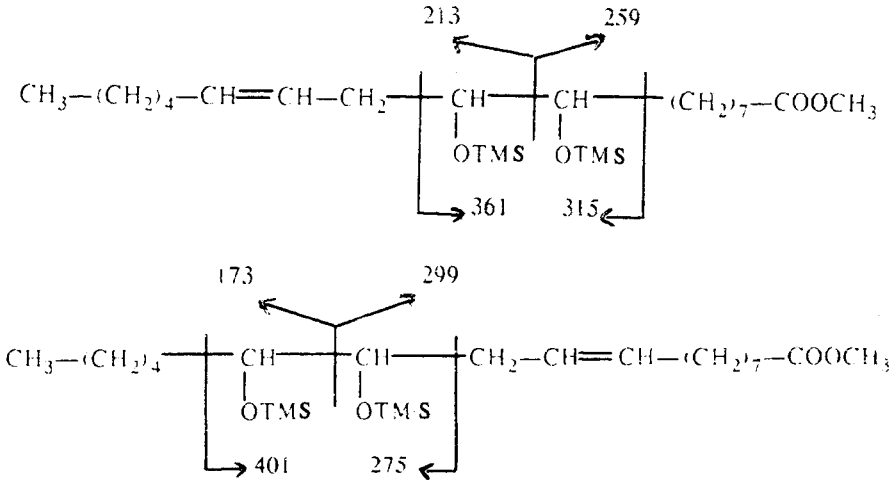
In the neutral lipids of the seeds of *Elaeagnus augustifolia* studied previously [1] we detected fractions of minor components which, on the basis of their chromatographic mobilities, qualitative reactions, and spectral characteristics, were assigned to the classes of epoxy- and hydroxytriacylglycerols (TAGs). The possibility of the GC-MS analysis of oxidized acids in the form of TMS derivatives of their methyl esters (MEs) has been shown in the case of the seed oil of sea buckthorn [2].

Continuing a systematic investigation of the lipids of plants of the family Elaeagnacae, we have analyzed the total epoxy and hydroxy acids isolated from the corresponding TAGs.

A chromatogram of the TMS derivatives of a purified fraction of the epoxy acids contained 6 chromatographic peaks (CPs), among which CP 3 stood out. The mass spectrum (MS) corresponding to it contained peaks of the molecular ion with *m/z* 470 and the peaks of characteristic fragmentary ions with *m/z* 455, 439, 412, 339, 310, 233, and 131 (100%). The combination of these facts showed that the main component of the total material was 15,16-epoxyoctadeca-9,12-dienoic acid, which is also characteristic for other materials [3, 4].

CP 1 corresponded to an artefactual contamination with palmitic acid ME ($M^+$ 270).

The spectrum of CP 2 contained the weak peak of the molecular ion with *m/z* 472 and also the peaks of $(M - 15)^+$, $(M - 31)^+$, and $(M - TMSOH)^+$ ions. A group of other intense peaks showed the presence of an unresolved mixture of two isomeric compounds — derivatives of 9,10-epoxyoctadec-12-enoic and 12,13-epoxyoctadec-9-enoic acids.

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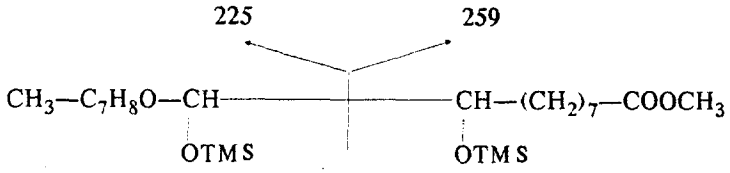
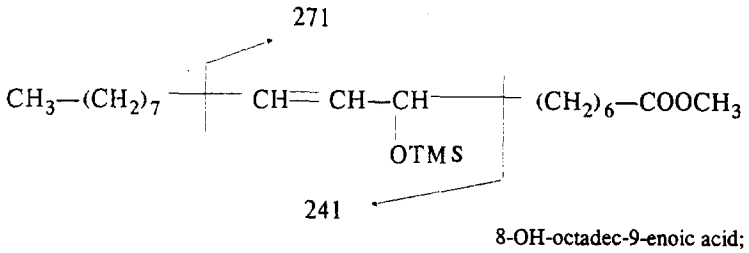
The two structures were confirmed by the presence in the spectrum of CP 2 of the peaks of rearranged fragments with *m/z* 332 and 270 formed, respectively, on the cleavage of the C-9-C-10 and C-11-C-12 bonds with the migration of the TMS radical from $C_{10}$ or $C_{12}$ to the methoxycarbonyl group [5].

The MSs of CPs 4-6 contained the weak peaks of molecular ions with *m/z* 488 (CP 4), 486 (CP 5), and 484 (CP 6), which correspond to the presence in the mixture of the $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ diepoxy acids, in each of which one of the groups had not taken part in the reaction. These compounds underwent partial thermal decomposition in the column, as the result of which products with the composition M − TMSOH were formed, giving in the spectra peaks of ions with *m/z* 398, 396, and 394, respectively. In view of this circumstance, it was impossible to determine their structures, all the more because in the spectra of CP 4 and CP 5 features characteristic of the main component of the mixture stood out clearly.

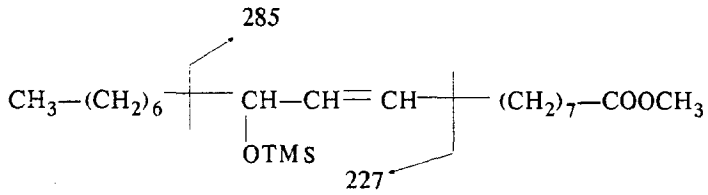
So far as concerns CP 6, thanks to the presence in its spectrum of the peaks of ions with *m/z* 361, 327, 259, and 225, it may be assumed that it contained a reduced epoxy group in the 9,10-position, two double bonds and an unchanged epoxy group in the $C_{11}$-$C_{18}$ chain.



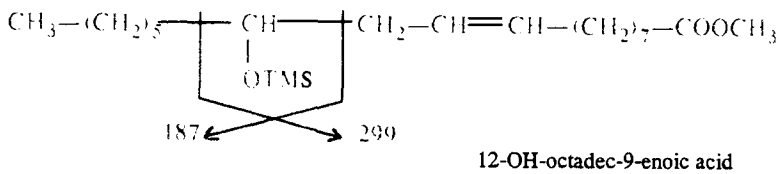

A chromatogram of the sum of the TMS derivatives of the MEs of the hydroxy acids under the same conditions revealed 9 CPs (CP7-CP15). Since the IR and UV spectra of this total material revealed the characteristic absorption of *cis-trans*-conjugated dienols, interpretation of the spectra was made by using information on the laws of the fragmentation of nonconjugated and conjugated dienic systems [5, 6]. The main component quantitatively was CP 12. Its MS proved to be the most complex in composition. The presence of the molecular ion $M^+$ 384, of characteristic ions with *m/z* 369, 353, and 337, and also of fragmentation ions with *m/z* 271 and 241, 285 and 227, and 187 and 299, showed a mixture of three $C_{18}$-hydroxy acids with double bonds at the 9-C atoms that were isomeric with respect to the position of the hydroxyl:




8-OH-octadec-9-enoic acid;



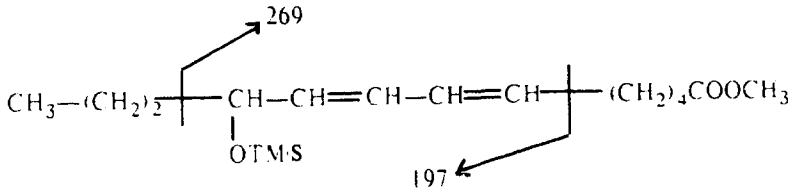

11-OH-octadec-9-enoic acid;



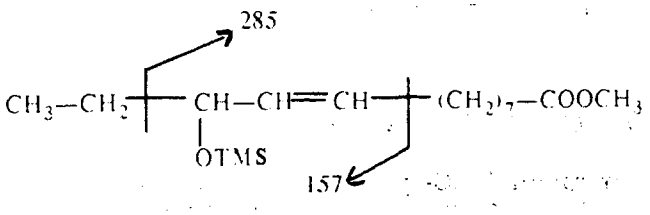

12-OH-octadec-9-enoic acid

No separation of the isomeric compounds took place, as had been observed previously [2].

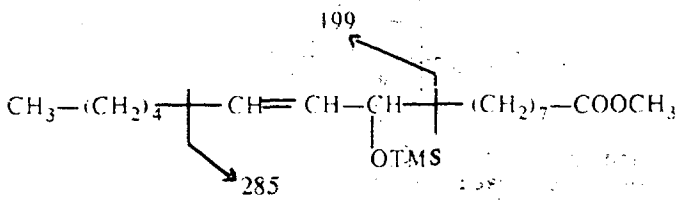
The components corresponding to the chromatogram of CP 7 and CP 9 also proved to be unresolvable mixtures. The MS of CP 7 contained two molecular ions, with $M^+$ 312 (*m/z* 297, 281, 265) and $M^+$ 314 (*m/z* 299, 283, and 267) and a set of fragmentary ions with *m/z* 269 and 197, and *m/z* 285 and 157, which permitted the identification of 10-OH-trideca-6,8-dienoic acid




and 11-OH-tridec-9-enoic acid

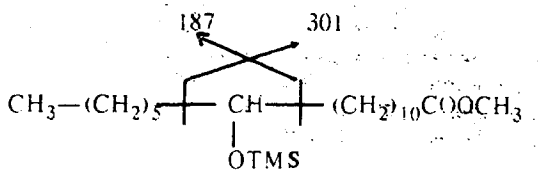



The MS of CP 9 was characterized by the molecular ion $M^+$ 372 and by peaks with *m/z* 357, 341, 325, 259, and 215, which corresponds to a saturated pentadecanoic acid with a hydroxyl in position 9. In addition to this, the spectrum contained a less intense molecular ion with $M^+$ 356 and the peaks of ions with *m/z* 341, 325, 309, 285, and 199, which are characteristic for 9-OH-hexadec-10-enoic acid:

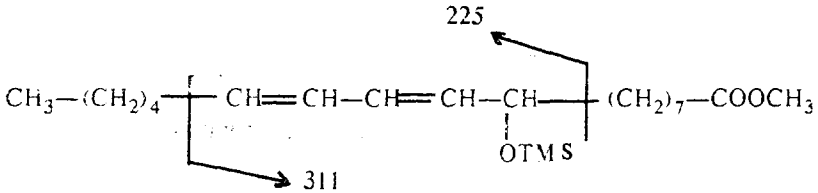



For CP 8 and CP 11, the ratio of the peaks of these substances to the background was so unfavorable that it was impossible to interpret these spectra.

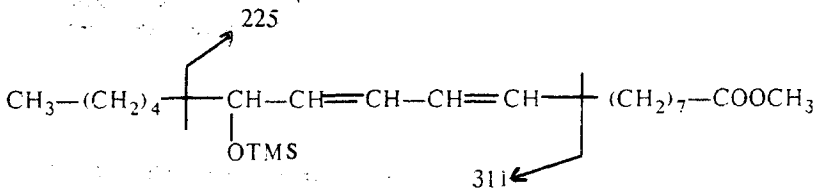
In the MS of CP 10 we detected the molecular ion $M^+$ 386 (peaks of characteristic ions with *m/z* 371, 355, and 339) and fragmentary ions with *m/z* 187, 301 corresponding to the structure of the saturated 12-OH-octadecanoic acid:



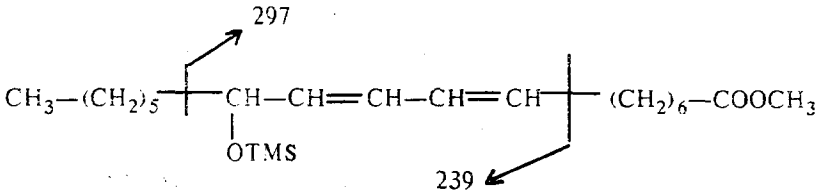

The further interpretation of the spectra showed the separation of three hydroxy acids of the $C_{18:2}$ series ($M^+$ 382) isomeric both with respect to the position of the hydroxy group and with respect to that of the double bond:




(m/z 367, 351, 335, 341, 292) 9-OH-octadec-10,12-dienoic (CP 13)

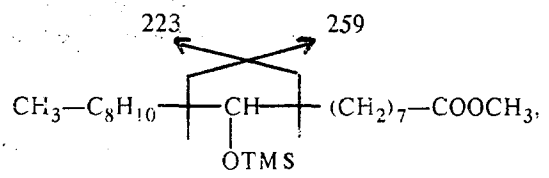



(m/z 367, 351, 335, 130) 13-OH-octadeca-9,11-dienoic (CP 14)




(m/z 367, 351, 335) 12-OH-octadeca-8,10-dienoic (CP 15)

On a chromatogram of the TMS derivatives of the MEs of the hydroxy acids, a weakly resolved minor peak was detected on the shoulder of the peak of CP 14, and this was reflected by the appearance in the corresponding MS of, in addition to the main molecular ion $M^+$ 382, an impurity ion with $M^+$ 380 and fragmentary ions corresponding to it with *m/z* 223 and 259:




which is characteristic for a $C_{18:3}$ acid with the preferential location of all three double bonds in the terminal chain of the molecule.

By its content of TAGs with unoxidized fatty acids, the seed oil of the plant under study is linoleic-containing, with a small amount of linolenic acid [1]. And although there are biogenetic precursors for the formation of the corresponding 18:3-oxidized products, some inconstancy of the quantitative proportions on repeated GL chromatography gives grounds for assuming that the given peak most probably appeared as the result of the thermal decomposition of the preceding acid and cannot be regarded as a structural component of the initial oxidized TAGs.

The separation of the isomeric 18:2-acids is interesting. In the best-known studies of the separation of the products of the oxidation of unsaturated fatty acids of the $C_{18}$ series [7-9], the HPLC method has been used and some law in the sequence of issuing of the isomers with time has been traced. Thus, Frankel [8] established that during HPLC the peaks of hydroxy acids with the most remote position of the hydroxyl in the chain issue first; in the case of acids analogous with respect to the position of the hydroxyl, *cis-trans*-conjugation of the dienols plays a role. Isomers with the *trans-trans*-arrangement of the double bonds have greater retention times than acids with the *cis-trans*-configuration.

For the packed columns used in our work there is practically no literature information on the separation of isomers. The separation of pairs of acids — 9-OH-10,12-18:2 and 13-OH-9,11-18:2 — was similar to that established previously for the case of sea buckthorn seed oil [2]. It is quite likely that the appearance of the 12-OH-octadeca-8,10-dienoic acid is also connected with the spatial configuration of the dienic system, and the finding of a definite law requires accurate determination of the structures, which was not part of the aim of the present investigation

Thus, among the oxidized acids of olive seeds we have detected by the GC/MS method six epoxy acids (three of which were of undetermined structure) and 12 hydroxy acids, one of which — 12-OH-octadeca-8,10-dienoic — is new.

## EXPERIMENTAL

The isolation of the epoxy- and hydroxy TAGs, their saponification, and the preparation of the TMS derivatives of the MEs of the epoxy- and hydroxy acids, and also the GC-MS analysis of the derivatives obtained were carried out as in [2].

UV spectrum of the total hydroxy acids: 234 nm (conjugated dienols). IR spectrum of the total hydroxy acids: 1730 and 1173 $cm^{-1}$ (ME carbonyl), 3620 and 3500 $cm^{-1}$ (hydroxy group), and 3010, 985, and 950 $cm^{-1}$ (*cis-trans*- and *trans-trans*-conjugated double bonds). The spectra were taken on a Hitachi spectrometer and on a UR-10 instrument with the specimens in the form of films.

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