HYDROXY ACIDS OF THE SEED OILS OF FIVE PLANTS OF THE FAMILY ASTERACEAE

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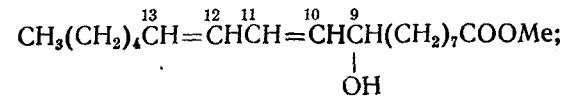
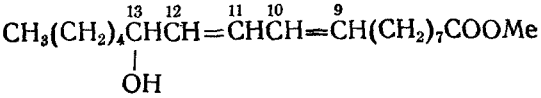
A comparative study has been made of the fatty hydroxy acids and epoxy acids of the seed oils of *Artemisia absinthium*, *Arctium tomentosum*, *Onopordum acanthium*, *Onopardum olgae*, and *Cousinia severzovii* by GLC, TLC, and IR and mass spectroscopy. It has been shown that the hydroxy acids present in largest amount are the products of the metabolism of 9-hydroperoxyoctadecadienoyl; the epoxy acids are represented mainly by 9,10-epoxy-cis-octadeca-12-enoic acid.

From five seed oils of plants of the family Asteraceae — *Artemisia absinthium* (common wormwood) — I; *Arctium tomentosum* (cotton burdock) — II; *Onopordum acanthium* (Scotch cotton-thistle) — III; *O. olgae* (Olga's cottonthistle) — IV; and *Cousinia severzovii* (Severtsov's cousinia) — V — we have isolated oxidized triacylglycerols containing a hydroxy acid or epoxy acid as one of the acyl radicals [1-3]. The main representatives of these acids in common wormwood have been identified from the results of chemical and spectral methods of analysis [4, 5]. In the present paper we give analyses of the corresponding acids of the seed oils of the above-mentioned plants in comparison with the hydroxy acids of common wormwood. As the main tests for comparison we used a combination of R_f values and qualitative reactions in thin-layer chromatography (TLC), relative retention times (RRTs) in gas–liquid chromatography (GLC), and the mass spectra of the methyl esters of the epoxy fatty acids and the trimethylsilyl (TMS) derivatives of the hydroxy acids.

To extract the hydroxy acids, all the oils were separated by column chromatography (CC) on silica gel [2]. In this way the hydroxyacyldiacylglycerols and epoxyacyldiacylglycerols were isolated. The total fatty acid methyl esters were isolated from these lipids by transesterification with methanol. Each of them was separated by preparative TLC in solvent system *a*. The combined hydroxy acid methyl esters and combined epoxy acid methyl esters were isolated from each of the five oils. The two combined groups were separated in thin layer of silica gel in solvent systems b and *a*, respectively, and by GLC on PEGS. The migration characteristics and RRTs of the main components of the hydroxy acids and epoxy acids from the five sources are given in Fig. 1 and in Table 1.

As can be seen from the facts given, three main components of the TMS derivatives of the hydroxy acid methyl esters were detected in all five samples. In addition, on gas–liquid chromatograms of derivatives of the acids of each of the samples a multiplicity of minor peaks was readily seen. As well as this, on TLC there were always two zones of hydroxy acid methyl esters corresponding to the migration of monohydroxy acid methyl esters. These may be not only α-hydroxydienic acids, but also di- and monoenic isomers of the type of ricinoleic acid. The lower of the two zones, the main one (Fig. 1b) corresponds to the migration of the 9-OH isomers with R_f 0.40, and the other zone to the migration of the 13-OH isomers [6] with R_f 0.45.





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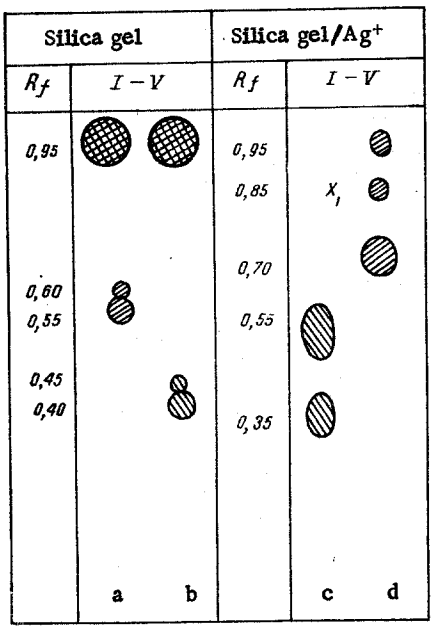


Fig. 1. TLC of the fatty acid methyl esters: a) from the epoxyacyldiacylglycerols; b) from the hydroxyacyldiacylglycerols; c) from the combined 9- and 13-OH isomers; d) from the combined 9, 10- and 12, 13-epoxy isomers.

TABLE 1. Compositions of the Main Components of the Hydroxy Acids of Five Seed Oils of Plants of the Family Asteraceae (GLC on PEGS)

Sample	Content, % on the total							
	hydroxy acids			epoxy acids				
	relative retention time with respect to $C_{16:0}$							
	3,9	4,6	5,6	6,0	7,7	8,8	10,7	18,8
I	6,4	78,2	15,4	5,3	23,6	71,1	—	+
II	38,4	40,5	21,1	7,8	20,3	63,5	8,4	+
III	43,7	33,5	22,8	5,2	26,7	68,1	—	+
IV	47,7	38,6	13,7	2,6	34,7	62,7	—	+
V	32,4	21,6	46,0	7,7	21,0	71,3	—	+
Va	41,7	58,3	—	"	"	"	—	"
Vb	—	—	100,0	"	"	"	—	"

To identify the main components of the hydroxy acids, the TMS derivatives of the hydroxy acid methyl esters of common wormwood were separated with aid of preparative GLC on SE-30. This gave three main fractions of esters in amounts sufficient for mass-spectrometric analysis. The molecular ions of each of them corresponded to a mass number of 382. Such a molecular weight is possessed by derivatives of α-hydroxyoctadecadienoic acids with the general formula $C_{18}H_{32}O_2$, the structure of which has been studied in a sample of common wormwood [5].

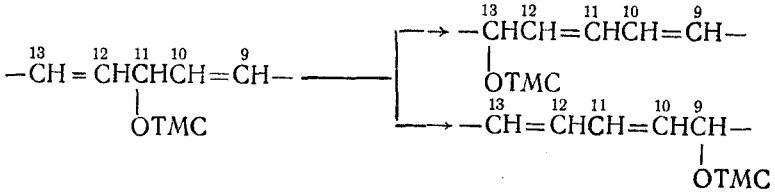
The main trimethylsilyl fragments in the mass spectra of the TMS derivatives of the hydroxy acids of common wormwood and of Scotch cottonthistle, with m/e 255 and 311 [7], correspond to the structure of the two isomers, 9-OH and 13-OH. No specific fragments of other isomers were detected in the mass spectra.

The isomers of the methyl esters of the acids of each of the TLC zones (Fig. 1b) were isolated preparatively. The IR spectrum shows that in the esters of the lower and upper zones there were simultaneously cis,trans- and trans,trans-conjugated dienic bonds (955 and 980 cm^{-1}, the second band being stronger than the first). These facts were confirmed with the aid of TLC/$AgNO_3$ in solvent system c: The mixture of hydroxy acid methyl esters formed two zones corresponding to the separation of the cis,trans- and trans,trans-dienes (Fig. 1, c, R_f 0.55 and 0.35, respectively). The TMS derivatives of the methyl esters of the α-hydroxyoctadienoic acids were separated similarly by GLC on OV-101 [7].

Summarizing the results obtained, it may be concluded that the first peak with an RRT of 3.9 corresponds to the 9-OH and 13-OH isomers with the cis,trans configuration of the

ethylenic bonds, and the second peak with RRT 4.6 corresponds to the 9-OH and 13-OH isomers with the trans,trans configuration of the dienic system. More convincing arguments in favor of this conclusion were obtained on chromatography by the GLC method on PEGS of the components enriched only in the α-hydroxy-cis,trans- or only in the α-hydroxy-trans,trans-dienic isomers. The first were obtained as the result of the transesterification of the hydroxyacyldiacylglycerols, and the second as the result of severe alkaline hydrolysis. In the first case, the main peak in the GLC of the acid derivatives was the peak with the RRT of 3.9 and the second that with the RRT of 4.6.

The isomers concealed under the peak with the RRT of 5.6 gave no specific trimethylsilyl fragments. According to the value of M^+ (mass spectrum) they were TMS derivatives of methyl esters of acids with a molecular weight of 382. Consequently a possible variant in this case may be 11-hydroxydienic isomers which have been detected previously in the form of bound acyl radicals [8]. Under the conditions of mass spectrometry one may expect an allyl rearrangement of the TMS derivatives of the methylesters here, as a consequence of which they do not give specific fragments:



The interdependence between the appearance of the peaks with RRT 3.9 and 4.6, on the one hand, and the peak with RRT 5.6, on the other hand, shows the following facts. We obtained the gas–liquid chromatograms in a single run connected each time with a new isolation of hydroxy acids from the hydroxyacyldiacylglycerols. This enabled us to detect changes in the gas–liquid chromatograms only in the case of samples of V. In one case, these chromatograms corresponded to those of the samples of all the other plants (Table 1, variant V from the 1978 harvest). In another case the peak with RRT 5.6 was absent (variant Va, 1979). In a third case, of the three main peaks on GLC common to all the samples only one appeared — with RRT 5.6 (variant Vb, 1978). In the last case, in addition to this peak new peaks appeared with higher RRT values. Under these conditions the TLC results remained the same as shown in Fig. 1b, and the IR spectrum corresponded to the spectrum of compounds from sample III — 955, 980, and 3050 cm^{-1}. If the isomer with the RRT of 5.6 arose after, and not before, isolation from the oil, the GLC analysis of the TMS derivatives of the hydroxy acid methyl esters from all the samples would have given similar anomalies.

In all cases the main components of the hydroxyacyl radicals corresponded to the reduction products of linoleoyl hydroperoxides formed under the action of lipoxygenase in aerobic or anaerobic conditions [8]. Thus, on the basis of the results obtained it may be assumed that a characteristic feature of plant V is the ease of switching the mechanism of the peroxide oxidation of linoleoyl by lipoxygenase from aerobic conditions to anaerobic conditions, and conversely.

As can be seen from the TLC results (Fig. 1a), the main component of the epoxy acids from all five sources is 9,10-epoxy-cis-octadec-12-enoic (coronaric) acid with R_f 0.55. The IR spectra of the epoxy acid methyl esters showed no trans-ethylenic bonds. A small amount of the 12, 13-epoxy isomers (Vernolic acid) with R_f 0.60, detectable on TLC in solvent system *a*, gave no separate peak in GLC on PEGS of the mixtures of epoxy acid methyl esters. It follows from information in the literature [9, 10] that the two isomers migrate in one peak. To investigate the behavior of these isomers under the conditions of GLC on PEGS that we used, pure vernolic and coronaric acids were isolated from samples I, III, and IV. It was found that methyl coronarate had an RRT of 8.8 and methyl vernolate one of 18.8.

The combined 9,12- and 12,13-epoxy isomers (Fig. 1a, R_f 0.55 and 0.60) were isolated by preparative TLC in system *a* and were analyzed by the TLC/$AgNO_3$ method. It can be seen in Fig. 1d that together with the 9,10- and 12,13-epoxy monoenic isomers migrating as one zone with R_f 0.70 there were two other components — with R_f 0.85 (X_1) and 0.95. Each of them was isolated by preparative TLC/$AgNO_3$ in system d. They both had a molecular weight of

312 (mass spectrum) and the same position of the epoxide ring at the C_9 and C_{10} atoms of the octadecane chain, since the main fragments in the corresponding mass spectra were those with m/e 199 and 155 [11].

In GLC on PEGS, the component with R_f 0.05 had RRT 7.7, and that with R_f 0.85 had RRT 6.0. Consequently, they are methyl esters of 9,10-epoxysteric acids differing only in the configuration of the epoxide ring. In actual fact, X_1 (RRT 6.0) issued in GLC earlier than the component with R_f 0.95 (RRT 7.7), i.e., the trans-epoxysterate outstripped the cis-epoxysterate [12, 13].

In the GLC on PEGS of mixtures the epoxy acid methyl esters of sample II, component X_2 with RRT 10.7 was detected, although it gave no separate zone on TLC. It cannot be an artifact, either, since it was detected in only one of the sources.

Thus, among the epoxy acids in all five samples, that present in the largest amount is coronaric acid, while vernolic and cis-9,10- and trans-9,10-epoxyoctadecanoic acids were detected in small amounts.

It is known that epoxy acids are unstable compounds [14]. However, we, like other authors [9, 13], did not detect any peaks in GLC which could have been ascribed to artifacts.

EXPERIMENTAL

The spectra were taken on UR-10 and MKh-1303 instruments. Gas-liquid chromatography was performed preparatively on a Varian-2800 instrument, and analytically on a Khrom-41 instrument.

The oils were isolated from previously comminuted seeds by cold extraction with petroleum ether.

The methyl esters of the oxidized fatty acids (oxyacids) were obtained either by the transesterification of the oxidized triacylglycerols with methanol in the presence of catalytic amounts of sodium methanolate or by alkaline hydrolysis followed by methylation with diazomethane.

TLC was performed on glass plates, 18 × 24 cm, with a layer of type L 5/40 silica gel fixed with 5% of gypsum, TLC/$AgNO_3$ – on the same silica gel containing gypsum but with the addition of 20% of silver nitrate.

Qualitative reactions: 50% sulfuric acid followed by carbonization, picric acid.

Solvent Systems. Hexane–diethyl ether: a – (8:2); b – (1:1); benzene–chloroform–diethyl ether: c – (50:50:15) [15]; d – (50:50:2).

The TMS derivatives of the hydroxy acids were obtained with the aid of hexamethyldisilazane (HMDS) and chlorotrimethylsilane [16]. GLC on SE-30: column 6.0 m long with an internal diameter of 9.5 mm filled with 3% of siliconized SE-30 on Chromosorb G; programming of the temperature from 200 to 290°C at 6°/min. TLC on PEGS: column 2.25 m long with an internal diameter of 3 mm filled with 17% of poly(ethylene succinate) on Celite 545, 60-80 mesh; temperature 203 ± 2°C.

SUMMARY

A comparative evaluation of the results of TLC and GLC and the mass and IR spectra of methyl esters of the hydroxy and epoxy acids isolated from the oxidized triacylglycerols of the seed oils of five plants of the family Asteraceae hss been performed.

It has been found that the main components of the oxidized acids from all five sources are identical: the hydroxy acids correspond to the products of the metabolism of 9-hydroperoxyoctadecadienoyl appearing in the seeds as the result of the peroxide oxidation of linoleoyl by lipoxygenase; the epoxyacyl radicals are represented mainly by 9, 10-epoxy-cis-octadec-12-enoic acid radicals.

LITERATURE CITED

1. N. T. Ul'chenko, E. I. Gigienova, K. L. Seitanidi, M. R. Yagudaev, and A. U. Umarov, Khim. Prir. Soedin., 471 (1979).

2. N. T. Ul'chenko, E. I. Gigienova, K. L. Seitanidi, M. R. Yagudaev, and A. U. Umarova, Khim. Prir. Soedin., 699 (1978).
3. N. T. Ul'chenko, E. I. Gigienova, and A. U. Umarov, Khim. Prir. Soedin., 311 (1980).
4. N. T. Ul'chenko, E. I. Gigienova, and A. U. Umarov, Khim. Prir. Soedin., 701 (1974).
5. N. T. Ul'chenko, E. I. Gigienova, and A. U. Umarov, Khim. Prir. Soedin., 584 (1976).
6. M. Hamberg, Anal. Biochem., 43, 515 (1971); H. W. Gardner, Lipids, 10, 248 (1975).
7. E. N. Frankel, W. E. Neff, W. K. Rohwedder, B. P. S. Khambay, R. F. Harwood, and B. C. L. Weedon, Lipids, 12, 908 (1977).
8. G. A. Veldink, J. F. G. Vliegenthart, and J. Boldingh, Prog. Chem. Fats Other Lipids, 15, 131 (1977).
9. S. F. Herb, P. Magidman, and R. A. Barford, J. Am. Oil Chem. Soc., 41, 222 (1964).
10. R. A. Barford, S. F. Herb, F. E. Luddy, P. Magidman, and P. W. Reimenschneider, J. Am. Oil Chem. Soc., 40, 136 (1963).
11. A. K. Sengupta, Chem. Ind. (London), 257 (1972).
12. E. A. Emken, Lipids, 7, 459 (1972).
13. L. J. Morris, R. T. Holman, and K. Fontell, J. Lipid Res., 2, 68 (1961).
14. L. J. Morris, R. T. Holman, and K. Fontell, J. Lipid Res., 1, 412 (1960).
15. R. Kleiman, G. F. Spencer, L. W. Tjarks, and E. A. Earle, Lipids, 6, 617 (1971).
16. C. N. Joo, T. Shier, and M. Kates, J. Lipid Res., 9, 782 (1968).

LOW-MOLECULAR-WEIGHT TRIACYLGLYCEROLS OF THE SEED OIL OF *Artemisia absinthium*

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Low-molecular-weight triacylglycerols have been isolated from the seed oil of *Artemisia absinthown* in which one acyl radical is derived from ethanoic, propanoic, butanoic, pentanoic, hexanoic, heptanoic, octanoic, or nonanoic acid. Their main representatives are the ethanoyl and propanoyl derivatives, position 2 being occupied mainly by the ethanoyl radical. The isomers with a short acyl radical in position 2 make up 75% of the total of the low-molecular-weight triacylglycerides isolated.

Triacyl glycerols in which one acyl radical is derived from a short-chain fatty acid are unusual components of seed oils. The first information in the literature on the presence of these compounds in plants appeared in 1967. These were acetyldiacylglygerols (ADGs) of the seed oil of *Impatiens edgeworthii*, family Balsaminaceae [1]. Other sources of these compounds have also been found — the seed oils of *Celastrus orbiculatus* (59% of ADGs on the oil) and of *Euonymus verrucosus* (68-69%), family Celastraceae, in which the acetyl is present in position 3 of the triacyl-sn-glycerol, [2]. Quite recently, ADGs have been found in the seed oils of *Polygala virgata* (74%) and of *Securidaca longipedunculata*, family Polygalaceae [3, 4]. In the first of these, the acetyl is found exclusively in position 2 of the triacyl-sn-glycerol. And, finally, it has become known that triacylglycerols of this type are also produced in the animal world: the ADGs of the insect *Icerya purchasi* [5], the sorboyldiacylglycerols of the aphid [6], and the isovaleroyldiacylglycerol of *Sotalia fluviatilus* [7]. The appearance of the above-mentioned low-molecular-weight triacylglycerols in some plant and animal tissues may bear definite biological information.

The present paper reports the presence of a whole series of low-molecular-weight triacylglycerols in the seed oil of *Artemisia absinthium* (family Asteraceae) of the 1979 harvest which differ from those mentioned above by the fact that they contain propanoyl, butanoyl, pentanoyl, hexanoyl, heptanoyl, octonoyl, and nonanoyl radicals.

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