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OXYGENATED ACIDS OF THE LIPIDS OF *Onopordum acanthium* SEEDS

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The structures of the oxygenated fatty acids of the lipids of Onopordum acanthium *L. seeds have been studied by the methods of chemical, chromatographic, and spectral analyses. Triepoxy acids of the C_{18} series and 24 monohydroxy acids with chain lengths of $C_{14}-C_{18}$ have been identified, of which the 8-OH-9Z,12Z-18:2 and the 14-OH-9Z,12Z-18:2 acids have been isolated as natural compounds for the first time.*

We have previously established the composition of the the neutral lipids of the seeds of *Onopordum acanthium* L. (Scotch cotton thistle), family Asteraceae [1, 2]. Continuing a study of the lipids of this plant, we have investigated the composition and structure of the epoxy and hydroxy acids present in the lipids in the form of monoepoxy- and monohydroxyacylglycerols.

The epoxy and hydroxy acids were isolated in the form of methyl esters (MEs) from the products of the hydrolysis of the neutral lipids by CC on silica gel. The epoxy acid MEs (EAMEs) were analyzed by TLC on polar and nonpolar phases and, in the form of MEs of ditrimethylsilyl (diTMS) derivatives after the opening of the epoxy ring, by mass spectrometry.

According to the result of GLC analysis on a polar phase, among the EAMEs two peaks were detected with RRTs of 4.0 and 4.54 (relative to the 18:0 ME), and on a nonpolar phase one peak with a RRT of 2.4 also relative to the 18:0 ME), i.e., all the components of the total material had the same chain length but differed by the number of double bonds.

The mass spectrum of the MEs of the diTMS derivatives contained the peaks of ions with m/z 472 (M^+), 475, 459 $[M - 15]^+$, 441, and 443 $[M - 31]^+$, corresponding to the 18:1 and 18:0 ME derivatives. Ions with mz 173, 270, 275, 299, and 401 and with m/z 213, 259, 315, and 361 were assigned to known fragments of the diTMS ethers of the 12,13-epoxy-18:1(9) and 9,10-epoxy-18:1(12) acids, respectively, and ions with m/z 215, 259, 315, and 361 to those of the 9,10-epoxy-18:0 acid [3]. Consequently, according to the TLC results the peak on the chromatogram with a RRT of 4.0 corresponded to the 9,10-epoxy-18:0 acid in an amount of 20.9%, and the peak with a RRT of 4.54 to two isomers of the epoxy-18:1 acid in a total amount of 79.1% of the weight of the acids. The epoxy acids identified are extremely characteristic for plants of the Asteraceae family [4].

Analysis of the total MEs of the hydroxy acids (HAMEs) in thin layers of argentized and ordinary silica gel gave no clear separation of the substances. As models for this we used the MEs of ricinoleic acid from castor oil and the MEs of conjugated hydroxydienoic acids isolated from the seeds of *Artemesia absinthium* [5].

Characteristic for the IR spectrum of the total HAMEs were bands of the vibrations of associated OH groups at 3200-3260 and of conjugated cis,trans ethylene bonds at 955, 990 cm^{-1}. The intensity of the band at 990 cm^{-1} considerably exceeded that observed only for cis-trans conjugation, which showed the presence in the mixture of HAMEs of acids with trans-trans-conjugated ethylenic bonds [6], as well. According to the UV spectrum (λ_{max}^{hexane} 233), the amount of conjugated α-hydroxydienoic acids in the HAME mixture was ~76% [7].

The total HAMEs were separated by CC according to the polarities of the components shown in [8] into three fractions (I-III) making up 13.0, 77.7, and 9.3%, respectively, of the weight of the acids. Part of each fraction was hydrogenated and the HAMEs of fractions I and III and of the products of their hydrogenation were converted into TMS ethers, which were analyzed by GLC on polar and nonpolar phases and by mass spectrometry.

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TABLE 1. Composition of the TMS Derivatives of Hydroxy Acid Methyl Esters

Acid	GLC (Reoplex 400), % by weight					Mass spectrum	
	Peak	RRT	Fraction			Characteristic ions, m/z (% rel.)	
			I	II	III	A*	B*
9-OH-14:0	1	0,42	3,5	—	—	173(5)	259
9-OH-15:0	2	0,55	5,6	—	—	187(12)	«
9-OH-16:0	3	0,73	3,8	—	—	201(2)	«
9-OH-17:0	4	0,98	3,5	—	—	215	«
9-OH-12Z-17:1	5	1,08	0,8	—	—	213(1)	«
12-OH-9Z-17:1						173(5)	299(1)
9-OH-10E, 12Z-17:2	6	1,18	0,5	—	—	211(2)	311
13-OH-9Z, 11E-17:2						«	«
10-OH-18:0	7	1,30	2,7	—	—	215(4)	273(2)
12-OH-18:0						187	301(1)
8-OH-9Z-18:1	8	1,33	—	6,3	7,3	241(5)	—
9-OH-10E-18:1			—			227(10)	—
10-OH-8E-18:1			—			—	271(3)
11-OH-9Z-18:1			—			—	285(6)
9-OH-12Z-18:1	9	1,44	4,1	—		227(8)	259(43)
12-OH-9Z-18:1	10	1,49	—	5,7	—	187(20)	299(3)
13-OH-9Z-18:1	11	1,51	—	—	2,3	173(6)	313(2)
9-OH-10E, 12Z-18:2	12	1,84	75,5	88,0	78,0	225(100)	311(50)
9-OH-10E, 12E-18:2						«	«
13-OH-9Z-11E-18:2						«	«
8-OH-9Z, 12Z-18:2						239(9)	—
10-OH-8E, 12Z-18:2						—	271(10)
12-OH-9Z, 13E-18:2						185(33)	—
14-OH-9Z, 12Z-18:2	13	2,05	—	—	12,4	—	325(5)

In the case of GLC on a nonpolar phase (OV-17), no clear separation of the OH-18:0, OH-18:1, and OH-18:2 acids took place. In view of the low informativeness of these chromatograms the results of GLC on a polar phase will be discussed in more detail. The assignment of the peaks of the individual components was made on the basis of a model mixture of the MEs of the TMS derivatives of the hydroxy acids of *Galeopsis bifida* and ricinoleic acid. GLC analysis showed (Table 1) that all three fractions were enriched with isomeric OH-18:2 acids having conjugated ethylenic bonds (~75–88%) and also included the OH-18:1 acid with a nonallyl position of the olefinic bond. However, saturated HAs with chain lengths of $C_{14}-C_{18}$ were concentrated in fraction I, and the allylic OH-18:1 and OH-18:2 acids in fractions II and III.

In the mass spectra of the initial HAs (I-III), the peaks of the M^+ ions and the main peaks, including characteristic fragments relating to the structures of known $C_{14}-C_{18}$ saturated acid and monoenic and dienic C_{17} and C_{18} acids [3, 4, 8, 9] were observed. A comparison of the results of GLC and the mass spectra enabled the distribution of the HA derivatives on chromatograms in accordance with their structural features to be shown. Thus, in the mass spectrum of fraction I the peaks of fragments of the 9-OH-18:1 (12) acid had a considerable intensity, in the mass spectrum of II the peaks of fragments of four allyl isomers of the OH-18:1 and 12-OH-18:1 (9) acids, and in (III) four allyl isomers of the OH-18:1, and four of the OH-18:2 and 13-OH-18:1 (9) acids. In all the fractions fragment from the breakdown of derivatives of the 9-OH-18:2 (10,12) and, to a smaller degree, of the 13-OH-18:2 (9,11) acids predominated.

The presence of the 13-OH-18:1 (9) acid was confirmed on the chromatogram of fraction III by peak 11 with a RRT of 1.51, and in the mass spectrum by fragments with m/z 173 and 313. Ions with m/z 325 and 239 corresponded to the isomeric 14-OH-18:2 and 8-OH-18:2 acids. The configurations of the olefinic bonds in the last two isomers were shown (Table 1) in accordance with the mechanism of the formation of these acids in the reactions of the 18:2 acid with singlet oxygen [10]. It must be mentioned that the seeds of *Onopordum acanthium* L. form the first natural source of the dienic 8-OH-18:2 (9,12) and 14-OH-18:2 (9,12) acids, which have been described previously as products of the reduction of the corresponding hydroperoxides formed in the photosynthesized oxidation of the 18:2 acid in model systems [11].

On a chromatogram of the TMS ethers of the MEs of the hydrogenated HAs, five peaks were observed for the acids of fraction I and one each for fractions II and III (Table 2). The mass spectrum of the hydrogenated esters I—III contained fragments of isomeric HAs, confirming the positions of the OH groups in the acids shown in Table 1.

TABLE 2. Composition of the TMS Derivatives of Hydrogenated Hydroxy Acids

Acid	GLC (Reoplex 400), % by weight					Mass spectrum	
	Peak	RRT	Fraction			Characteristic ion, m/z	
			I	II	III	A*	B*
9-OH-14:0	1	0,42	2,8	—	—	173	259
9-OH-15:0	2	0,55	4,9	—	—	187	«
9-OH-16:0	3	0,73	4,5	—	—	201	«
9-OH-17:0	4	0,98	5,1	—	—	215	«
12-OH-17:0						173	301
13-OH-17:0						159	315
8-OH-18:0	5	1,35	82,7	100,0	100,0	243	245
9-OH-18:0						229	259
10-OH-18:0						215	273
11-OH-18:0						201	287
12-OH-18:0						187	301
13-OH-18:0						173	315
14-OH-18:0						159	329

*See Table 1.

Thus, the hydroxyacylglycerols of the reserve lipids of *Onopordum acanthium* include 24 monohydroxy acids with chain lengths of from C_{14} to C_{18} and 0-2 double bonds, of complex isomeric composition, in which seven isomers each of octadecaenoic and octadecadienoic acids have been identified. Only three acids, 9-OH-10E,12Z-18:2, 9-OH-10E,12E-18:2, and 13-OH-9Z,11E-18:2 have been detected previously in the seed lipids from the family Asteraceae. This is the first time that the other acids have been found in the reserve lipids of this family and it is the first time that two hydroxy acids, 8-OH-9Z,12Z-18:2 and 14-OH-9Z,12Z-18:2 have been found in plant material.

EXPERIMENTAL

The UV spectra of the HAMEs were taken on a Hitachi spectrophotometer in hexane, the IR spectra on a UR-10 instrument in a film, and the mass spectra on a MKh-1310 instrument at an ionization energy of the electrons of 40/50 eV with a temperature of the ionization chamber of 100/80°C.

GLC was conducted on a Chrom-4 instrument with a flame-ionization detector. A 4 × 2000 mm column filled with 15% of Reoplex-400 on Chromatom N-AW was used at 198°C, and a 4 × 1000 mm column filled with 2% of OV-17 on Chrom W at 200°C, the carrier gas being helium.

The neutral lipids were saponified with 10% KOH in methanol in a ratio of 100 ml of solution to 10 g of sample. The CC of the total MEs was conducted on silica gel L 100/250, the EAMEs being eluted with the solvent system hexane–ether (4:1) and the HAMEs with hexane–ether (1:1). The TLC of the total HAMEs was conducted on silica gel L 5/40 with the addition of 5% of gypsum and 20% of $AgNO_3$ in the solvent system benzene–chloroform–diethyl ether (50:50:15). The EAMEs were converted into dihydroxy derivatives as described in [12].

The TMS derivatives were obtained by the method of [13]. The HAMEs (25 mg) were hydrogenated in ethanol (2.5 ml) at 60°C using Pd (2.5 mg) on aluminium powder (5 mg) with the constant passage of H_2 for 4 h [14].

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