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OXYGENATED FATTY ACIDS FROM NEUTRAL LIPIDS OF *Artemisia leucodes* SEEDS

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Three epoxy-, fifteen monohydroxy-, and three dihydroxyacids from lipids of Artemisia leucodes *Sch. seeds were identified using chemical, chromatographic, and spectral analyses.*

Key words: *Artemisia leucodes*, neutral lipids, oxygenated fatty acids, methyl esters, epoxyacids, hydroxyacids.

We found epoxy- and hydroxyacyldiacylglycerines in neutral lipids (NL) of white wormwood (*Artemisia leucodes* Sch., Asteraceae) seeds [1]. The composition and structures of the oxygenated fatty acids are given in the present article.

Hydrolysis of NL afforded the total unsubstituted fatty acids (FA) and three types of oxygenated acids. The latter were methylated and separated into pure fractions by column chromatography over silica gel using solvent systems 1-4. The FA methyl-ester (ME) fraction had R_f 0.75 on TLC; epoxyacid ME, 0.63; monohydroxyacid ME, 0.51; dihydroxyacid ME, 0.16 in solvent system 3. The yield of pure ME fractions was (mass %): FA ME, 97.1; epoxyacid ME, 1.0; monohydroxyacid ME, 1.2; dihydroxyacid ME, 0.7.

The composition of unsubstituted FA (mass %) as determined by GLC was as follows: 14:0, 0.3; 16:0, 7.8; 16:1, 0.4; 18:0, 1.7; 18:1, 12.4; 18:2, 77.4.

The epoxyacid ME contain according to GLC (mass %): epoxy-18:0, 11.3; epoxy-18:1, 88.7. The structures of epoxyacid ME were established by mass spectrometry of the trimethylsilyl (TMS) esters of their dihydroxy-derivatives. The mass spectra were interpreted using literature data [2, 3] and results obtained by us for epoxyacids of other specimens [4]. Thus, we identified 9,10-epoxy-18:0, 9,10-epoxy-18:1(12), and 12,13-epoxy-18:1(9) acids in the lipids of *A. leucodes* seeds. A strong peak with *m*/*z* 271 in the mass spectrum of the epoxyacid derivatives could not be assigned to a specific structure.

The structures of the monohydroxyacids were established by combined GLC and mass spectrometry (GLC-MS) of the TMS derivatives. We used TMS derivatives of ME of monohydroxyacids from seeds of *Galeopsis bifida* [5] and *Onopordum acanthium* [4] as models for the chromatography. The GLC analysis showed (Table 1) that the main components (almost 97%) are C_{18} acids, of which isomeric 18:2 isomers dominate, with conjugated ethylene bonds of *cis*- and *trans*-configuration (960, 995 cm^{-1}, IR spectroscopy).

Mass spectra of TMS derivatives of monohydroxyacid ME exhibit a strong peak for the molecular ion with *m*/*z* 382 (41.0%) and a weaker peak with *m*/*z* 384 (3.7%) that belong to hydroxyacids 18:2 and 18:1. Furthermore, additional peaks for fragments with *m*/*z* 343, 341, 371, 369, 367 $[M - 15]^+$, 327, 325, 355, 353, 351 $[M - 31]^+$, 311, 309, 339, 337, 335 $[M - 47]^+$ from monohydroxyacids 16:0, 16:1, 18:0, 18:1, and 18:2 were observed. The mass numbers and strengths of the peaks for the principal fragments corresponded to structures of known acids (Table 1). The strong peak with *m*/*z* 225 (100%) indicates that two isomeric acids, 9-OH-18:2(10,12) and 13-OH-18:2(9,11) are present [2].

The mass spectra also contain peaks with *m*/*z* 185 and 325 that may be formed by decomposition of two allyl isomers of hydroxylinoleic acids 12-OH-18:2(9,13) and 14-OH-18:2(9,12) [6, 7]. The presence in the chromatogram of a peak with relative retention time 2.12, like in the model TMS-derivatives of ME of hydroxyacids from *O. acanthium* and *G. bifida*, argues in favour of the latter component [4, 5].

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TABLE 1. Monohydroxyacids of Lipids from *Artemisia leucodes* Seeds

Acid	GLC, Reoplex-400			Mass spectrum of TMS-derivatives of monohydroxyacid ME, characteristic ions, *m/z* (I_{rel}, %)		
	Peak	Rel ret. time*	% of mass	A**	B***	Additional
9-OH-16:0	1	0.73	2.1	201(2.5)	259(14)	
9-OH-16:1(10)	2	0.85	0.7	199(4.5)	285(2.5)	
10-OH-18:0	3	1.30	1.2	215(6)	273(2)	169(6)
12-OH-18:0	3	1.30		187(4)	301(1)	
8-OH-18:1(9)	4	1.35	2.0	241(2.5)	-	
10-OH-18:1(8)	4	1.35		-	271(7.5)	
9-OH-18:1(10)	4	1.35		227(7)	-	294(2.5)
11-OH-18:1(9)	4	1.35		-	285	
9-OH-18:1(12)	5	1.50	1.8	227	259	230(1)
12-OH-18:1(9)	5	1.50		187	299(3.5)	270(1.5)
9-OH-18:2(10, 12)	6	1.85	77.7	225(100)	311(44.5)	292(20.5)
13-OH-18:2(9, 11)	6	1.85		225(100)	311(44.5)	292, 130(19)
10-OH-18:2(8, 12)				239(3.5)	271	
12-OH-18:2(9, 13)				185(4)	325(8.5)	
14-OH-18:2(9, 12)	7	2.12	14.5	185(4)	325(8.5)	

*Relative to 18:0 acid ME.

A = $CH_3\cdots$CHOTMS;*B = –CHOTMS$\cdots COOCH_3$.

TABLE 2. Dihydroxyacid Lipids from *Artemisia leucodes* Seeds

Acid	Mass spectrum of TMS-derivatives of dihydroxyacid ME, characteristic ions, *m/z* (I_{rel}, %)					
	M	$[M-15]^+$	$[M-31]^+$	Rearranged	A*	B**
9,10-di-OH-18:0	Отс.	459(0.3)	443(1)	332(7)	215(17), 317(1)	259(100), 361(28)
9,10-di-OH-18:1(12)	472(0.2)	457(2)	441(3.6)	332(7)	213(53), 315(1)	259(100), 361(28)
12,13-di-OH-18:1(9)	472(0.2)	457(2)	441(3.6)	270(2)	173(7), 275(7)	299(4), 401(0.2)

*Fragments: $CH_3\cdots$CHOTMS(1), $CH_3\cdots(CHOTMS)_2$ (2).

**Fragments: CHOTMS$\cdots COOCH_3$ (1), $(CHOTMS)_2\cdots COOCH_3$ (2).

Peaks for ions with *m/z* 147 and 146 were detected in mass spectra of TMS derivatives of ME of natural dihydroxyacids and are characteristic of the decomposition of these components [8]. Furthermore, peaks of the main and additional fragments occur in the spectrum (Table 2). These enable the three acids that were found previously in other samples to be positively identified [9, 10].

EXPERIMENTAL

IR spectra were recorded on a UR-10 instrument in films; mass spectra, in an MX-1310 instrument at ionizing-electron energy 40-50 eV and ionization-chamber temperature 180/100°C.

GLC of ME was carried out on a Chrom-4 instrument with a flame-ionization detector using a column (2000×4 mm) packed with Reoplex-400 (15%) on Chromaton-N-AW at 198°C with He carrier gas.

Neutral lipids were hydrolyzed as before [3]. Column chromatography of total ME of fatty acids was performed over silica gel L 100/250 μ; TLC, on silica gel L 5/40 μ with added gypsum (5-10%). The solvent systems were hexane—diethylether 9:1 (1), 8:2 (2), 1:1 (3), and 3:7 (4).

ME of epoxyacids were identified using ethanolic picric acid (0.05 M) [11]. ME of epoxyacids were converted to the dihydroxy derivatives by the literature method [12]. The TMS derivatives were prepared as before [13]. FA were methylated by diazomethane in diethylether. The diazomethane was prepared as before [14].

Mass spectra of TMS derivatives of ME of dihydroxyacids (obtained from epoxyacids), *m*/*z* (I_{rel}, %): 474 (1.5), 472 (1.5) $[M]^+$, 459 (0.3), 457 (1.5), 443 (1), 441 (2.6), 427 (1.3), 425 (1.5), 401 (1.0), 382 (4.1), 361 (20.5), 332 (5.8), 317 (1.2), 315 (2.9), 301 (1.7), 299 (6.5), 275 (6.2), 271 (64.7), 270 (2.5), 259 (100), 215 (17.6), 213 (58.8), 173 (20.5), 73 (65.0).

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