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OXYGENATED TRIACYLGLYCEROLS OF THE LIPIDS OF *Onopordum acanthium* SEEDS

N. T. Ul'chenko, S. D. Gusakova, and A. I. Glishenkova

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The results on the structure and composition of the epoxyacyl-, hydroxyacyl-, and epoxyacylhydroxyacylacylglycerols of Onopordum acanthium *L. seeds obtained by enzymatic hydrolysis have been supplemented with the aid of mass spectrometry. The desirability of using a combination of the two methods for these purposes has been shown.*

In a study of the lipids of the seeds of *Onopordum acanthium* L. (Scotch cotton thistle), family Asteraceae, we detected monoepoxyacyldiacylglycerols (e-TAGs), monohydroxyacyldiacylglycerols (h-TAGs, and epoxyacylhydroxyacylacylglycerols (e,h-TAGs). We established the distribution of the ordinary and oxygenated fatty acid (FA) acyls between the sn-2 and sn-1(3) positions in the molecules of the e-TAGs by the method of enzymatic hydrolysis and calculated their position-species composition [1, 2]. It was found that the oxygenated FAs esterify mainly one of the primary hydroxyls of the glycerol moiety, but 37% of the weight of the molecular species of the e-TAGs and 12.5% of the h-TAGs contain epoxy- or hydroxyacyl residues in the sn-2 position. We have previously determined the qualitative and quantitative compositions of the oxygenated Fas [3], which, together with the results of enzymatic hydrolysis, has enabled us to add to the possible isomeric compositions of the molecular species of TAGs (Table 1). As can be seen, the number of possible isomeric species with the main nonoxygenated and oxygenated FAs for the e-TAGs amounted to 23, for the h-TAGs to 27, and for the e,h-TAGs to 28.

In order to refine the position-species compositions of the TAGs investigated and to compare them with the analogous results from pancreatic hydrolysis, we have performed a mass-spectrometric analysis of the initial e-TAGs and of the TMS derivatives of the h-TAGs and e,h-TAGs.

In the mass spectra of the e-TAGs five peaks of M^+ ions corresponding to 15 main molecular species were observed (Table 2). For each species we detected breakdown fragments common for TAGs with unoxygenated FAs [4, 5]. Species with epoxyacyl residues in the sn-2 position were revealed by the fragments $(R_2CO + 74)^+$ and $(R_2CO + 128)^+$, and the peaks of the $(M - R_{1,3}COO)^+$ and $(M - R_{1,3}CO)^+$ were also used for diagnosis.

The choice of the most probable of the possible isomeric species was based on the quantitative ratio of the ordinary FAs [1] and e-FAs [3]. The latter consisted mainly of the e-18:1 with only ~20% of the e-18:0 species.

According to the results of the MS analysis, among the e-TAGs there were no species with the 16:0 acid in the sn-2 position and the amount of the unsaturated species 16:0–e-18:1–16:0 was obtained by calculation (Table 1).

Of the breakdown fragments of the epoxyacyls, the $[CH_3(CH_2)_4CHOCH]^+$ ion with m/z 113 formed on α-cleavage in the epoxy group of the 12,13-epoxy-18:1(9) residue had an appreciable intensity (10%). A correspondence was observed between the amount of the individual molecular species of e-TAGs (Table 1) and the relative intensities of the peaks of their molecular and high-mass ions (Table 2).

Neither pancreatic hydrolysis nor mass spectrometry has enabled us to establish the set of acyl residues in positions 1 and 3 of the molecule, but characteristic features of the structure of some unoxidized TAGs and some known hydroxy-TAGs of higher plants [4] give grounds for assuming that the 16:0 acids in the e-TAGs of *Onopordum acanthium* are concentrated in the sn-1 position, while the epoxyacids are concentrated in the sn-3 position. Before mass spectrometry, the hydroxy-

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent.
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TABLE 1. Possible Molecular Species of Oxygenated Triacylglycerols

e-TAG species sn-1, sn-2, sn-3	Amount, % by weight*	h-TAGs species sn-1, sn-2, sn-3	Amount, % by weight*	e,h-TAG species sn-1, sn-2, sn-3	Amount, % by weight*
18:2—18:2—e18:1** 18:2—18:2—e18:0**	33,1	18:2—18:2—h18:2** 18:2—18:2—h18:1** 18:2—18:2—h18:0**	42,5	h18:2—18:1—e18:1 h18:1—18:1—e18:1 h18:0—18:1—e18:1 h18:2—18:1—e18:0 h18:1—18:1—e18:0 h18:0—18:1—e18:0	23,9
18:1—18:2—e18:1 18:1—18:2—e18:0	12,6	18:2—18:1—h18:2 18:2—18:1—h18:1 18:2—18:1—h18:0	12,2	h18:2—18:2—e18:1 h18:1—18:2—e18:1 h18:0—18:2—e18:1 h18:2—18:2—e18:0 h18:1—18:2—e18:0 h18:0—18:2—e18:0	56,1
18:2—18:1—e18:1 18:2—18:1—e18:0	8,2	18:1—18:2—h18:2 18:1—18:2—h18:1 18:1—18:2—h18:0	15,8	16:0—h18:2—e18:1	1,1
16:0—18:2—e18:1 16:0—18:2—e18:0	4,7	18:1—18:1—h18:2 18:1—18:1—h18:1	4,5	18:1—h18:2—e18:1 18:1—h18:1—e18:1 18:1—h18:2—e18:0 18:1—h18:1—e18:0	2,7
16:0—18:1—e18:1 16:0—18:1—e18:0	1,2	16:0—18:2—h18:2 16:0—18:2—h18:1	9,7	18:2—h18:2—e18:1 18:2—h18:1—e18:0 18:2—h18:2—e18:1 18:2—h18:1—e18:0	6,2
18:1—18:1—e18:1 18:1—18:1—e18:0	3,1	16:0—18:1—h18:2 16:0—18:1—h18:1	2,8	16:0—e18:1—h18:2	1,1
18:2—e18:0—e18:2 18:2—e18:1—e18:2	16,0	18:2—h18:2—18:2 18:2—h18:1—18:2	4,9	18:1—e18:1—h18:2 18:1—e18:1—h18:1 18:1—e18:1—h18:0	2,7
18:2—e18:1—e18:1	12,1	18:1—h18:2—18:2 18:1—h18:1—18:2	3,6	18:2—e18:1—h18:2 18:2—e18:1—h18:1 18:2—e18:1—h18:0	6,2
16:0—e18:1—18:2	4,5	16:0—h18:2—18:2 16:0—h18:1—18:2	2,3		
18:1—e18:1—e18:1	2,3	16:0—h18:2—16:0 16:0—h18:1—16:0	0,3		
16:0—e18:1—18:1	1,7	18:1—h18:2—18:1 18:1—h18:1—18:1	0,6		
16:0—e18:1—16:0	0,3	16:0—h18:2—18:1 16:0—h18:1—18:1	0,8		
18:1—16:0—e18:1 18:1—16:0—e18:0	0,1				
18:2—16:0—e18:1 18:2—16:0—e18:0	0,1				

*Results of enzymatic hydrolysis.

**The composition of the oxygenated fatty acids has been given in [3].

TABLE 2. Main Molecular Species of the e-TAGs of *Onopordum acanthium* According to Their Mass Spectrum

e-TAG species sn-1, sn-2, sn-3	Mass numbers of the characteristic fragments, m/z (rel. intensity, %)					
	M^+	$(M-18)^+$	$(M-R_1COO)^+$	$(M-R_3COO)^+$	$(R_2CO+74)^+$	$(R_2CO+128)^+$
16:0—18:2—e18:1	870(1)	852(2)	615(37)	575(10)	337(29)	391(4)
16:0—e18:1—18:2				591(11)	353(13)	407(1)
16:0—18:1—e18:1	872(1)	854(1)	617(29)	577(6)	339(27)	393(5)
16:0—e18:1—18:1			"	591	353	407
18:2—18:2—e18:1	894(4)	876(4)	615	599(16)	337	391
18:2—e18:1—18:2			615	615	353	407
18:1—18:2—e18:1			615	601(17)	337	391
18:2—18:1—e18:1			617	"	339	393
18:2—18:2—e18:0	896(3)	878(14)	"	599	337	391
18:1—e18:1—18:2			615	617	353	407
18:2—e18:0—18:2			617	617	355(5)	409(2)
18:1—18:1—e18:1			"	603(10)	339	393
18:1—18:2—e18:0	898(2)	880(3)	"	601	337	391
18:2—18:1—e18:0			619(1)	"	339	393
18:1—e18:1—18:1			617	617	353	407

TABLE 3. Main Molecular Species of the h-TAGs of *Onopordum acanthium* According to the Mass Spectrum of the TMS Derivatives

h-TAGs sn-1, sn-2, sn-3	Mass numbers of the characteristic fragments, m/z (relative intensity, %)				
	M^+	$(M-15)^+$	$(M-90)^+$,a	$(a-R_1CO+1)^+$	$(M-R_{3(f)}COO)^+$ $(a-R_3COO)^+$
16:0—18:2—h18:2	942(2)	927(0,5)	852(4)	614(100)	575(12)
16:0—h18:2—18:2				"	573(3)
16:0—18:1—h18:2	944(1)	929(0,2)	854(2)	616(40)	577(6)
16:0—18:2—h18:1				"	575
16:0—h18:2—18:1					573
18:2—18:2—h18:2	966(4)	951(2)	876(13)	614	599(52)
18:2—h18:2—18:2				"	597(10)
18:1—18:2—h18:2	968(4)	953(1)	878(11)	614	601(28)
18:2—18:1—h18:2				616	"
18:2—18:2—h18:1				"	599
18:1—h18:2—18:2				614	"
18:1—18:1—h18:2	970(3)	955(1)	880(5)	616	603(10)
18:1—18:2—h18:1				"	601
18:2—18:2—h18:0				618(9)	599
18:1—h18:2—18:1				616	"

containing TAGs were converted into TMS derivatives. The mass spectrum of the TMS-h-TAGs exhibited the ions characteristic for the breakdown of ordinary TAGs [5] and of TMS-h-TAGs [6]. The authors of the last paper referred to showed that the mass spectrum of the TMS derivatives of the h-TAGs do not contain fragments permitting the sn-2-hydroxyacyl species to be detected.

The molecular species with hydroxyacyl residues in the sn-2 position shown in Table 3 were revealed in a comparison of the results of mass spectrometry with those of enzymatic hydrolysis [2]. The other fragments in the spectrum mainly corresponded to those described in the literature [6], with the exception of a cluster of ions of appreciable intensity with m/z 577-573 and 547-541. As in the case of the e-TAGs, the position-species composition of the h-TAGs obtained as a result of the analysis of the mass spectrum differed somewhat both in the number and structure of the species from those indicated by pancreatic hydrolysis.

The mass spectrum of the h-TAG derivatives lacked the ions of the disaturated species 16:1−h-18:1(h-18:2)−16:0 while in the five sn-2-h-TAG species, in agreement with the quantitative composition of the hydroxy acids [3], the most probable acyl in this position was OH-18:2. The set of molecular species of the h-TAGs differed little from that of the e-TAGs, apart from the nature of the oxygenated acyl.

TABLE 4. Main Molecular Species of e,h-TAGs of *Onopordum acanthium* According to the Mass Spectrum of the TMS Derivatives

e,h-TAG species sn-1, sn-2, sn-3	Mass numbers of the characteristic fragments, m/z (relative intensity, %)					
	M^+	$(M-15)^+$	$(M-18)^+$	$(M-90)^+$	$(M-15-90)^+$	$(R_{2,2(3)}CO+74)^+$
16:0—e18:1—h18:2 16:0—h18:2—e18:1	958(2)	943(1)	940(0,5)	868(4)	853(1)	353(33)
h18:2—e18:1—18:2 18:2—h18:2—e18:1 h18:2—18:2—e18:1	982(9)	967(4)	964(2)	892(14)	877(6)	353 337(30)
18:2—e18:1—h18:1 18:1—e18:1—h18:2 h18:2—18:2—e18:0						353 353 337
18:2—h18:2—e18:0 18:1—h18:2—e18:1 18:2—h18:1—e18:1 h18:2—18:1—e18:1 h18:1—18:2—e18:1	984(7)	969(2)	966(2)	894(10)	879(3)	
18:1—e18:1—h18:1 18:1—h18:1—e18:1 h18:1—18:1—e18:1	986(3)	971(1)	968(2)	896(5)	881(2)	353 339

The mass spectrum of the TMS derivatives of the e,h-TAGs contained the peaks of the ions observed in the mass spectrum of the e-TAGs and the analogous h-TAG derivatives. For the purposes of the present investigation we used the ions listed in Table 4. A position-species composition of e,h-TEGs consisting of 16 species was obtained, among which there were no species with saturated oxygenated and unoxygenated FAs in the sn-2 position.

It follows from the results that with the aid of enzymatic hydrolysis it is possible by experiment to detect and to estimate quantitatively the species of oxygenated acyls in the sn-2 position, while mass-spectrometric analysis permits the set of molecular species to be refined and for some TAGs — as for example, the e-TAGs — it enables the species with oxygenated acyls in the sn-2 position to be found.

Thus, more complete information on the composition and structure of oxygenated TAGs can be obtained by using a combination of enzymatic hydrolysis and spectrometry.

EXPERIMENTAL

Mass spectra were taken on a Mkh 1310 instrument at a temperature of the ionization chamber of 170-150°C, an ionizing voltage of 50 V, and a collector current of 40 μA. Oxygenated TAGs were isolated as described in [2] and were silylated by the procedure of [7].

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