Methyl 3,6-dideoxy-β-D-ribo-hexopyranoside (III). Yield 0.09 g (24%), mp 50-51°C, $[\alpha]_D^{20}$ −58.8° (c 2.4; chloroform), R_f 0.22, R_T 1.08. According to the literature [9]: mp 51-53°C, $[\alpha]_D$ −64° (water). Methyl 2,4-di-O-acetyl-3,6-dideoxy-β-D-ribo-hexopyranoside (VII) had R_f 0.56, R_T 1.00 (6.9 min).

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HYDROXY ACIDS OF THE RESERVE LIPIDS OF Galeopsis bifida

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The structures of fatty monohydroxy acids of the lipids of the seeds of Galeopsis bifida Boenn. have been studied by the methods of chromatographic, spectral, and chemical analysis. (31) Acids with chain lengths of from 14 to 20 carbon atoms, with from 0 to 3 double bonds, and with the hydrolyls in positions characteristic for the products of the direct hydroxylation and the lipoxygenase and photosensitized oxidation of unsaturated fatty acids were detected. Of them, the 13-OH-9Z, 11E-17:2, the 15-OH-9, 12-18:2, the 16-OH-9, 12-18:2, and 17-OH-11, 14-20:2 acids were new, while this is the first time that the 15-OH-9Z, 12Z, 16E-18:3 acid has been described as a natural compound. The behavior of the TMS derivatives of the hydroxy acids on a polyester phase in GLC is discussed.

We have previously detected epoxy-, oxo-, and hydroxyacyldiacylglycerols with a complex set of oxygenated acyls in the lipids of the seeds of Galeopsis bifida Boenn. (family Lamiaceae) [1], and their detailed analysis has permitted the revelation of new fatty oxo acids [2] and epoxy acids [3]. Of the monohydroxy acids (HAs) of the hydroxyacyldiacylglycerols (H-TAGs) we succeeded in identifying two isomeric 12(9)-hydroxyoctadeca-9Z(12Z)-enoic and two isomeric 9(13)-hydroxyoctadeca-10E, 12Z(9Z-11E)-dienoic acids [1]. In the present paper we give the results of a further study of the composition and structure of the HAs of the reserve lipids of G. bifida and discuss possible routes of their biosynthesis.

The H-TAGs were isolated from the total lipids by a combination of CC and TLC, and their mild alkaline hydrolysis gave a mixture of unsubstituted and hydroxylated fatty acids. This mixture, in the form of methyl esters (MEs), was separated by preparative TLC into the MEs of unsubstituted acids and two fraction of HAMEs. The first fraction (1, R_f 0.56, 0.8% of the weight of the total lipids) consisted of isomeric saturated, monoenoic, and dienoic acids, and the second fraction (2, R_f 0.54, 1.5%) of isomeric dienoic and trienoic HAs. The ratio of the weights of the three fractions was 4.7:1.28:1.0 and corresponded to the structure of the initial H-TAGs with one residue of unoxidized and two residues of

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unsubstituted acyls. Because of their complex composition, it was impossible to separate the HAMES-1 and -2 into narrower fractions by using other solvent systems and by the addition of $AgNO_3$ to the sorbent.

The structures of the HAs were investigated by IR spectrometry, UV spectrophotometry, and the periodate-permanganate oxidation of their MEs. The hydroxy groups of the HAMES were protected by silylation with the formation of the trimethylsilyl derivatives (TMS-HAMEs), and their mass-spectrometric fragmentation and their GLC behavior on polar and nonpolar phases were analyzed. For a more accurate determination of the positions of the hydroxyls in the isomers, portions of the HAMEs-1 and -2 were hydrogenated, the completeness of hydrogenaltion being monitored by TLC with the revelation of the spots in I_2 vapor. The hydrogenation products were purified by preparative TLC using as a marker the ME of the 12-OH-9-18:1 acid (ricinoleic) hydrogenated under analogous conditions. Then the TMS derivatives of the esters of the hydrogenated HAMEs were obtained and these were analyzed in a similar way to the initial TMS-HAMEs.

Hydroxy Acids of Fraction I (R_f 0.56). In the UV spectra of the HAMEs-1 an absorption band was observed at λ_{max}^{hexane} 233 nm, which is characteristic for cis, trans-conjugated dienes [4, p. 435], and this was confirmed by the presence in the IR spectrum of strong bands of vibrations at 958, 994 cm^{-1} (s, cis-trans $(CH{=}CH)_2$), and 1668 cm^{-1} (w, trans $-CH{=}CH-$); the spectrum also contained bands at 1090 cm^{-1} (m, secondary OH groups) and 3470 cm^{-1} (s, associated OH groups) [4, p. 438].

The mass spectrum of the initial TMS-HAMEs-1 contained peaks of the M^+, $[M - 15]^+$, $[M - 31]^+$, and $[M - 47]^+$ ions with mass numbers corresponding to C_{15}–C_{18} monoenes (M^+ 342-384) and C_{16}–C_{18} dienes (M^+ 354-382), and the peaks of the same types of ions, except for the M^+ ions, for saturated C_{14}–C_{18} compounds.

The main fragments in the spectrum showed the presence of the 12-OH-9-18:1 (ricinoleic), 9-OH-12-18:1 (isoricinoleic), and 13-OH-9, 11-18:2 acids that had been established previously. The higher intensity of the peak of an ion with m/z 225 as compared with that of an ion with m/z 311 (Table 1) indicated the presence in the fraction of another OH-18:2 isomer – 9-OH-10, 12-18:2 [5].

TABLE 1. Compositions of the TMS Derivatives of the Methyl Esters of the Hydroxy Acids of Fraction 1

Structure of the acid	GLC						Mass spectrum	
	Reoplex 400			OV-1			characteristic fragments, m/z, %**	
	peak	RRT*	%	peak	RRT*	%	A***	B***
9-OH-14:0	1	0,45	0,5	1	0,51	0,3	173****	259
9-OH-15:0	2	0,54	0,6	2	0,69	1,1	187	•
9-OH-?-15:1	3	0,58	0,4				185	•
9-OH-16:0	4	0,74	0,2	3	0,93	0,9	201 (2)	•
9-OH-?-16:1	5	0,86	0,1				199	-
13-OH-9Z,11E-16:2	6	0,98	0,9				197 (4)	311
9-OH-12Z-17:1	7	1,10	0,5	4	1,25	1,2	213 (2)	259
12-OH-9Z-17:1							173 (8)	299
13-OH-9Z,11E-17:2							211 (3)	311
9-OH-18:0	8	1,31	5,0	5	1,68	96,5	229	259
10-OH-18:0							215 (4)	273 (2)
12-OH-18:0							187	301 (1)
9-OH-12Z-18:1	9	1,48	33,7				227 (13)	259 (6)
12-OH-9Z-18:1							187 (58)	299 (3)
9-OH-10E,12Z-18:2	10	1,84	58,1				225 (37)	311 (33)
13-OH-9Z,11E-18:2								
10-OH-8E,12Z-18:2							239 (6)	271 (3)
12-OH-9Z,13E-18:2							185 (12)	325 (0,4)

*Relative to the 18:0 ME.

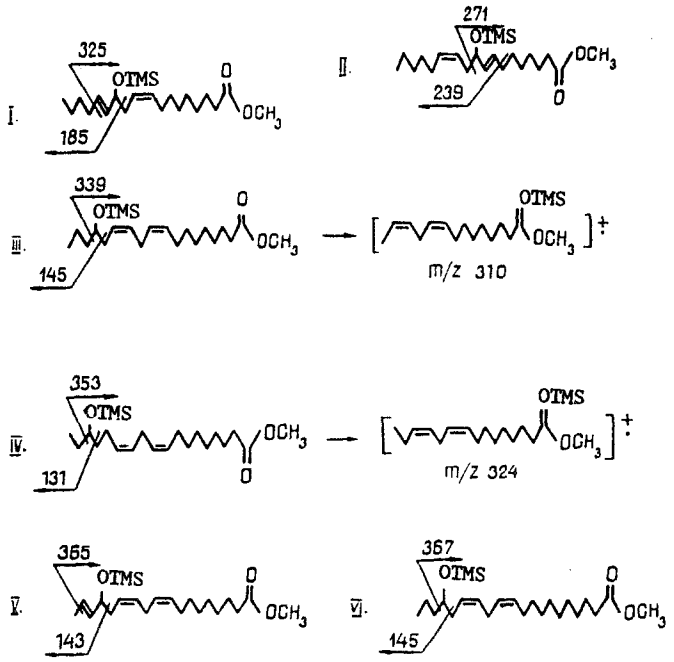
**Relative to m/z 73 (100%).

***A – CH_3...CHOTMS fragments; B – CHOTMS...$COOCH_2$ fragment.

****Where a given ion belongs to more than one HA, its intensity is shown for the predominant HA.

The fragments with m/z 185 and 325 and m/z 239 and 271 observed in the spectrum (see Table 1) could be formed in the fragmentation of two allyl isomers of hydroxylinoleic acid, 12-OH-9, 13-18:2 (I) and 10-OH-8, 12-18:2 (II) [6, 7]. Additional information on the structures of the main and minor isomers and homologues was obtained from the mass spectrum of the hydrogenated TMS-HAMEs-1 which contained a series of three typical high-mass ions for OH-14:0–OH-18:0 esters and of fragments, combinations of which gave a set of isomers with the OH groups located between C-9 and C-13 (Table 2). Fragments corresponding to other positions of the hydroxyl in the molecules of the HAs-1 were not reliably detected.

When the initial and hydrogenated TMS-HAMEs-1 were subjected to GLC on a nonpolar phase, five peaks were obtained in each case. On the basis of the values of their relative retention times (RRTs), separation factors of homologous series [8], and information on the behavior of the TMS ethers of hydroxy acids under these conditions [9], peaks 1-5 were assigned to HAs with chain lengths of from 14 to 18 carbon atoms. It is known that, in packed columns, isologues (for example, 18:1 and 18:2) are not separated on polyester phases.



There is no information in the literature on the behavior of isomers and isologues of TMS_HAMEs on polyester phases; we analyzed these derivatives on a polar phase. As standards we used the TMS ethers of the 12-OH-9-18:1 ME and its hydrogenated product the 12-OH-18:0 ME. The initial TMS-HAMEs-1 were separated into ten, and the esters of the hydrogenated products into five, peaks. The assignment of the peaks to definite structures of the HAs was made by using separation factors for homologous unsubstituted acids for the given phase and the dependence of the order of emergence of the HAMEs and their acetoxy derivatives on the position of the OH group in relation to the methyl or carboxy end of the molecule [10] and on the geometry of the configuration of the ethylenic bond [11]. It was assumed that the conjugation of the double bonds in the dienes and the allyl position of the OH groups in the monoenic HAMEs decreases the retention time of the derivatives of these isomers in chromatography on a polar phase.

When the quantitative results of GLC and mass spectroscopy were compared, the relative intensities of the characteristic fragments were taken into account with a certain approximation, since the ions of methyl-containing fragment with lower molecular masses have higher intensities [12]. The results of the GLC analyses of the initial and hydrogenated TMS-HAMEs-1 on the polar phase are given in Tables 1 and 2.

As can be seen, under these conditions not only homologues but also isologues of the TMS-HAMEs were separated, except for isomers with respect to positions 9-13 of the hydroxyl. A correspondence of the quantitative amounts of the saturated homologues on the two phases was observed. Consequently, under the conditions of the separation of the TMS-HAMEs on a polyester phase no appreciable loss of them takes place.

TABLE 2. Compositions of the TMS Derivatives of the Hydrogenated Methyl Esters of the Hydroxy Acids of Fraction 1

Structure of the acid	GLC					
	Peoplex 400			OV-1		
	Peak	RRT*	%	Peak	RRT*	%
9-OH-14:0	1	0,45	0,5	1	0,51	0,4
9-OH-15:0	2	0,54	1,0	2	0,69	0,9
9-OH-16:0	3	0,75	1,0	3	0,95	1,3
13-OH-16:0						
9-OH-17:0	4	0,98	1,3	4	1,27	0,9
12-OH-17:0						
13-OH-17:0						
9-OH-18:0	5	1,40	96,2	5	1,76	96,5
10-OH-18:0						
12-OH-18:0						
13-OH-18:0						

Structure of the acid	Mass spectrum				
	main ions, m/z, %**				
	$[M-15]^+$	$[M-31]^+$	$[M-47]^+$	A***	B***
9-OH-14:0	315	299 (2)	283 (0,1)	173	259
9-OH-15:0	329 (1)	313 (3)	297 (0,3)	187	-
9-OH-16:0	343 (6)	327 (2)	311 (3)	201 (3)	-
13-OH-16:0				145 (3)	315
9-OH-17:0	357 (5)	341 (0,2)	325 (0,1)	215	259
12-OH-17:0				173	301
13-OH-17:0				159 (7)	315
9-OH-18:0	371 (7)	355 (6)	339 (11)	229 (17)	259 (11)
10-OH-18:0				215 (6)	273 (5)
12-OH-18:0				187 (39)	301 (30)
13-OH-18:0				173 (28)	315 (27)

*Relative to the 18:0 ME.

**Relative to m/z 73 (100%).

***A – CH_3...CHOTMS fragment; B – CHOTMS...$COOCH_3$ fragment.

In order to refine the localization of the olefinic bonds in the hydroxy groups, we carried out the oxidative degradation of the HAMEs-1 by periodate-permanganate. The following dicarboxylic fragments were obtained (%, GLC): hexanedioic acid, 3.4; heptanedioic, 94.5; octanedioic, 0.9; and dodecanedioic, 1.2. Hexanedioic acid is formed on decomposition of a compound with a C-8 OH group or olefinic bond but since the 8-OH-18:0 isomer was absent from the hydrogenated HAMEs-1 (see Table 2), this fragment was a product of the oxidative cleavage of the Δ^8 olefinic bond of the 10-OH-8, 12-18:2 acid. The octanedioic and dodecanedioic acids were products of the cleavage of 10-OH-18:0 and 12-OH-18:0 acids, respectively. The oxidation of the other HAMEs-1 (see Table 1) took place with the formation of heptanedioic acid.

Thus, more than half the HAMEs-1 consisted of dienic compounds with a predominance of the 13-OH isomers, one third of ricinoleic and isoricinoleic esters, and the other 8% of a mixture of other known compounds, except for the 13-OH-17:2 species.

The conjugated α-hydroxydienoic, 9-OH- and 13-OH-18:2, acids, together with ricinoleic, are most frequently found in plant tissues [13], while the homologous 13-OH-18:2 acid, 13-hydroxyheptadeca-9Z, 11E-dienic, has not previously been detected in plant lipids.

The two allyl isomers 10-OH-18:2 and 12-OH-18:2 were known as the products of the reduction of the corresponding hydroperoxides formed in the modeled photosensitized oxidation of the 18:2 acid [6, 7, 12, 14]. It has been proposed to use these isomers with specific structures as markers of this type of nonenzymatic oxidation in vitro [6].

The allyl OH-18:2 isomers, together with the 9-OH-10E-18:1 and 10-OH-8E-18:1 acids, have recently been detected in the leaves of a Phleum pratense plant subjected to infection stress [15].

In agreement with the mechanism of photosensitized oxidation, the allyl addition of O_2 with respect to a CH=CH bond does not change the configuration of the olefinic bond, while the addition of O_2 to one of the olefinic carbon atoms is accompanied by the appearance of a new transolefinic bond shifted in a terminal allyl hydroperoxide by one carbon atom

towards the CH_3 or the HOOC end of the molecule [6]. Since the mutual positions of the double bonds and the hydroxyl in the allyl OH-18:2 isomers of the HAs-1 of G. bifida do not differ from those in the 10-OOH- and 12-OOH-18:2 acids and the products of the reduction of the OH groups and the IR spectra do not exclude the presence of trans–CH=CH– bonds in the components of the fraction, it was concluded that the configurations of the corresponding double bonds in the molecules of these isomers were similar.

Because of the smaller amounts of the 9-OH-15:1 and 9-OH-16:1 species, the positions of the olefinic bonds in these HAs were not established accurately, but the most probable position is Δ12. The other HAs listed in Table 1 have been isolated previously from reserve [13, 16] or cuticular [17, p. 1] lipids of plants from several families.

Hydroxy Acids of Fraction 2 (R_f 0.54). The UV and IR spectra of this fraction differed little from those of the HAMEs-1. UV spectrum of the HAMEs-2: λ_{max}^{hexane} 233.5 nm. UV spectrum ν^{film}, cm^{-1}: 958 (m), 975 (inflection, isolated trans–CH=CH–); 995 (s), 1662 (w), 3010 (s), 1090 (m), 3470 (s).

The mass spectrum of the initial TMS HAMEs-2 contained a series of peaks M^+ ions and of typical high-molecular-mass fragments for the monoenoic C_{18} and C_{20} (M^+ 384 and 412), the dienoic C_{18} and C_{20} (M^+ 382, 410), and trienoic C_{18} (M^+ 380) acids, and also of ions with m/z 294, 292, and 290 $(M - 90)^+$. In the region of medium masses there were fragments from the breakdown of the esters of the two isomeric OH-18:1 species and of the four isomeric OH-18:2 species that had been detected in the spectrum of the HAs-1 (see Table 1), but with different values of the relative intensities of the peaks, namely: the peaks of the A ions of the 12-OH-18:1 and 10-OH-18:2 esters had lower intensities and the analogous A ions with m/z 227 (9-OH-18:1) and 225 (9-OH-18:2) substantially higher intensities.

On the basis of the known difference the ratios of the relative intensities of the peaks of the A, B, and [M –90[$^+$ fragments for the isomeric 9-OH-10-18:1 and 9-OH-12-18:1 acids [5] and the ratio of these intensities in the spectrum under discussion, it was concluded that the HAs-2 contained, in addition to the 9-OH-12-18:1 isomer the allyl 9-OH-10-18:1 isomer. Likewise, the increased intensity of the B ion with m/z 271 did not correlate with the fragmentation of the 10-OH-18:2 ester alone (see Tables 1 and 3) but gave grounds for assuming the presence of the allyl 10-OH-8-18:1 ester, while the homologous ion with m/z 285 possessing an appreciable intensity suggested the presence of the 11-OH-9-18:1 ester. It is appropriate to mention that a feature of the fragmentation of the allyl 18:1-TMS MEs is predominant α-cleavage with respect to the TMSOCH group [5, 18].

Other intense ions present in the TMS spectrum of the HAME-2 derivatives permitted the identification of the 15-OH-18:2 (III) and the 16-OH-18:3 HAs, while ions of lower intensity corresponded to the 16-OH-18:2 (IV), 15-OH-18:3 (V), 14-OH-18:2, and 9(13)-OH-18:3 HAs (Table 3).

The presence of an ion with m/z 145 excluded the C-13 position of the double bond in the 15-OH-18:2 and C-15 position in the minor 17-OH-20:2 species, while the absence of the fragment $[CH_3(CH_2)_2CHOTMC\ (CH{=}CH)_2]^+\cdot$ with m/z 197 excluded the conjugation of the double bonds in these dienes. Furthermore, fragmentation under the action of electron impact of the derivative of the 15-OH-9, 12-18:3 HA may be accompanied by the formation of a rearranged ion [5] with m/z 310 which was present in the spectrum of the TMS-HAMEs-2 with a relative intensity of 10%.

Evidence in favor of the analogous breakdown of the double bond in the 16-OH-18:2 compound was provided by fragments with m/z 353 $[M - 29]^+$ and 131 and of a rearranged ion with m/z 324 (2.0%).

The mass spectrum of the TMS ethers of the hydrogenated HAMEs-2 contained, in addition to the $[M - 15]^+$, $[M - 31]^+$, and $[M - 47]^+$ ions with mass numbers of the M^+ ions of 386 (the 18:0 TMS-HAME) and 414 (the 20:0 TMS-HAME), the ions of fragments of eight isomeric hydroxystearates and of two hydroxyarachidates (Table 4). In this spectrum, the ions of fragments A and B with m/z values 2, 4, and 6 mass units greater, respectively, than in the spectrum of the initial esters were observed (see Tables 3 and 4), which confirmed the different degrees of unsaturation of the fragments mentioned.

The dicarboxylic acids produced by the oxidative cleavage of the HAMEs-2 consisted of (%, GLC): propanedioic acid, 3.4; hexanedioic, 5.5; heptanedioic, 85.8, and nonanedioic, 5.3. Their quantitative ratio showed that in the C_{18} HAs of this fraction the functional groups began in the main at C-9, while in the minor, C_{20}, HAs then began at C-11.

TABLE 3. Compositions of the TMS Derivatives of the Methyl Esters of the Hydroxy Acids of Fraction II

Structure of the acid	GLC						Mass spectrum	
	Reoplex 400			OV-1			characteristic ions, m/z, %	
	peak	RRT*	%	peak	RRT	%	A*	B*
9-OH-10E-18:1	1	1,34	19,6	1	1,72	71,5	227 (41)	
10-OH-8E-18:1							—	271 (13)
9-OH-12Z-18:1	2	1,48	6,3				227	259 (11)
11-OH-9Z-18:1							—	285 (15)
12-OH-9Z-18:1							187 (17)	299 (3)
9-OH-10E,12Z-18:2	3	1,82	30,2				225 (50)	311 (28)
13-OH-9Z,11E-18:2								
10-OH-8E,12Z-18:2							239 (2)	271 (13)
12-OH-9Z,13E-18:2							185 (17)	325 (4)
9-OH-10E,12E-18:2	4	1,86**					225	311
14-OH-?,15-18:2							157 (13)	353
15-OH-9,12-18:2	5	2,13	19,3				145 (61)	339 (10)
16-OH-9,12-18:2	6	2,25**					131 (30)	353 (5)
9-OH-10E,12Z,15Z-18:3	7	2,68	1,8				223 (14)	311
13-OH-9Z,11E,15Z-18:3								
14-OH-11Z-20:1							187	327 (2)
15-OH-9Z,12Z,16E-18:3	8	3,03	5,5	2	2,26**		143 (23)	365 (0,2)
15-OH-9,12-18:2***								
16-OH-9Z,12Z,14E-18:3	9	3,44	17,3	3	2,33	26,0	183 (52)	351 (15)
16-OH-9,12-18:2***							—	—
17-OH-11,14-20:2				4	2,81	2,5	145	367
14-OH-11Z-20:1***							—	—

*See note to Table 1.

**Partially separated peaks.

***Order of emergence of the corresponding TMS-HAMEs on OV-1.

TABLE 4. Compositions of the TMS Derivatives of the Hydrogenated Methyl Esters of the Hydroxy Acids of Fraction 2

Structure of the acid	GLC						Mass spectrum	
	Reoplex 400			OV-1			Characteristic fragments, m/z, %	
	peak	RRT*	%	peak	RRT	%	A	B
9-OH-18:0	1	1,40	57,1	1	1,75	79,5	229 (64)	259 (77)
10-OH-18:0							215 (26)	273 (10)
11-OH-18:0							201 (1)	287 (4)
12-OH-18:0							187 (54)	301 (21)
13-OH-18:0							173 (36)	315 (18)
14-OH-18:0							159 (19)	329 (6)
15-OH-18:0	2	1,59	19,8	2	1,96**		145 (54)	343 (17)
16-OH-18:0	3	1,80	20,4	3	2,10	18,4	131 (82)	357 (23)
11-OH-20:0	4	2,15	1,9	4	2,81	2,3	187	329
17-OH-20:0	5	3,14	0,8	5	3,13	0,8	145	371 (4)

*See note to Table 1.

**Partially separated peak.

From a combination of the results, the C_{20} HAs had the structures of the 14-OH-11-20:1 acid (lesquerolic) and the 17-OH-11, 14-20:2 acid (VI).

When the TMS-HAMEs-2 were separated by the GLC method on a nonpolar phase, three completely and one partially separated peaks were obtained, while the chromatography of the hydrogenation products gave five peaks. On the polar phase the hydrogenated esters were clearly separated into five peaks, while the initial esters formed nine peaks of which two were separated only partially (see Tables 3 and 4).

For the interpretation of the peaks of the hydrogenated esters of the HAs-2 we used Tulloch's result [10]: on analyzing the GLC behavior of acetoxy derivatives of hydroxy-

stearates he established that the isoemric 8-OH–14-OH-18:0 HAs were scarcely separated on any of the phases but, beginning with the ME of the 15-acetoxy-18:0 acid, separation of the following isomers took place thanks to the more pronounced polarity of the $CH_3(CH_2)_nCH(OCOCH_3)$ end of the carbon chain (where $n \leq 2$). According to this rule, peaks 2-5 of the TMS derivatives of the stearates of the fraction related to isomers with the OH group localized close to the CH_3 end of the HA.

It can be seen from Table 4 that the proportion of each of the isomeric 15-OH-18:0 and 16-OH-18:0 HAs was about 20%.

The peaks on the chromatograms of the initial TMS-HAMEs-2 were identified with the assistance of their mass spectra. The chromatography of these esters showed that on a polar phase the allyl and the ethylene-interposed isomers of the monoenoic C_{18} acids having the same position of the hydroxyl were separated (peaks 1 and 2, Table 3); the cis, trans- and trans, trans- isomers of the OH-18:2 acids (peaks 3 and 4) and of the 15-OH-18:2 and 16-OH-18:2 acids (peaks 5 and 6) were separated partially. Isologues of the last two isomers, namely the 15-OH-18:3 and 16-OH-18:3 acids, were separated more sharply (peaks 8 and 9).

According to the mass spectra of the initial and hydrogenated TMS-HAMEs-2, in the 14-OH-18:2 acid one double bond was located at C-15, in view of which this allyl isomer may have a RRT of 2.1-2.2. The position of the second, isolated, double bond, localized between the TMSOCH– and $-COOCH_3$ groups has not been accurately established.

As was to be expected, on the nonpolar phase no separation of the isologues of the TMS-HAMEs-2 with the same position of the hydroxyl took place (peak 3, Table 3) while the 15-OH and 16-OH isomers of the dienic and trienic esters were separated partially (peaks 2 and 3). The C_{20} TMS esters behaved similarly. From the results of GLC analysis on the two phases it was calculated that the amount of the 15-OH-18:2 derivative in the HAMEs-2 was 12-14%, and that of the 16-OH-18:2 derivative 5-7%.

The configurations of the olefinic bonds in the 15-OH-9, 12-18:2, 16-OH-9, 12-18:2, 14-OH-?, 15-18:2 and 17-OH-11, 14-20:2 acids were not established. It is most likely that they are the all-cis isomers, since in their 18:3 (9, 12, 15) and 20:2(11, 14) biogenetic precursors the corresponding bonds are in the cis form [19].

Thus, the HAs-2 consisted of a mixture of 19 components of which the 15-OH-9, 12-18:2, the 16-OH-9, 12-18:2, the 14-OH-?, 15-18:2, and 17OH-11,14-20:2 HAs were new. Of the four isomeric OH-18:3 acids the 9-OH, 13-OH, and 16-OH isomers were known as rice phytoalexins [20] and as metabolites of certain green microalgae [21]; this is the first time that the 15-OH-18:3 hydroperoxide is a known product of the photosensitized oxidation of the 18:3 acid in model systems [12, 14]. The 11-OH-9Z-18:1 hydroxymonoenoic acid is a component of the lipids of the leaves of a wilt-resistant variety of the cotton plant [22].

According to the results of the analyses, 31 HAs were esterified in the H-TAGs of G. bifida. In spite of the complexity of the set, these acids can be combined into three groups on the basis of similarities of their structure and the mechanism of their biosynthesis. One of them consists of hydroxymonoenoic and hydroxydienoic acids formed enzymatically from the 17:1–20:1 and 18:2 and 20:2 acids by hydroxylation in the β or γ position to a cis-olefinic bond (similarly to ricinoleic of isoricinoleic acid) [17, p. 72]). The specificity of the hydroxylation of the unsaturated FAs in the seeds of G. bifida is expressed in the predominant oxidation of the β-carbon atom between a double bond and the CH_3-end of the molecule (see Tables 1 and 3). The second group includes HAs having in their structure the $-CHOH(CH{=}CH)_2-$system, mainly in cis, trans conjugation, and synthesized enzymatically from the 16:2, 17:2, 18:2, and 18:3 acids by the lipoxygenase route [23]. The third combines the monoenoic, dienoic, and trienoic HAs the structures of which include the –CHOHCH=CH– fragment where the nonconjugated double bond has the trans configuration. The most probable route of the formation of these HAs is the direct oxidation of the corresponding saturated acids by singlet oxygen ($^1O_2$) without the participation of the lipoxygenase enzyme system.

It is considered that the main source of $^1O_2$ in plant tissues is chemical reactions in the chloroplasts taking place with the participation of photosensitizing substances (chlorophylls or their derivatives, some secondary metabolites, herbicides) that are capable of activating molecular oxygen directly or indirectly [24].

To the products of the photosensitized oxidation of the lipids of photosynthetic tissues may be assigned the 9-OH-10E-18:1, the 10-OH-8E-18:1, and the allyl OH-18:2 acids isolated from the leaves of a wilt-resistant variety of the cotton plant [22] and from the leaves of timothy plant infected by a fungal pathogen [15]. One of the toxins of this pathogen possesses photodynamic properties [24].

The generation of active forms of oxygen does not take place only in the chloroplasts. It has been reported that the superoxide oxygen radical $O_2^{\cdot}$ [25] is formed in the peroxysomes of the seeds of oil plants in the course of biochemical reactions, and this, as is well known, may the spontaneously dismutate to $^1O_2$ [26]. The formation of $^1O_2$ from 18:2 peroxide radicals is possible [27].

In some plant species allyl nonconjugated hydroxy unsaturated acids are synthesized in response to an infection stress, and in others (for example the fern Azolla fileculoides Lamark [28] and the seeds of Dyospiros montana [29]) they are present in healthy tissues and probably also fulfill the functions of substances involved in the self defense of the plant against pests. It must be mentioned that the weed plant G. bifida (particularly its seeds and influorescences) are toxic for warm-blooded animals [1].

Thus, according to the combined results of our investigations it has been established that the seeds of G. bifida contain oxidized lipids the structures of which include the residues of not less than 52 oxygenated fatty acids. Analysis of their structures gives grounds for concluding that in healthy tissues of one plant a complex association of enzymatic and nonenzymatic oxidation reactions of unsaturated fatty acids by molecular O_2 and $^1O_2$ can exist simultaneously. The products of these reactions are toxic for pests and parasites to different degrees, but the plants themselves that are capable of synthesizing such HAs in appreciable amounts apparently possess a flexible mechanism for the protection of the corresponding tissues form their harmful action.

EXPERIMENTAL

The UV spectra of the HAMEs were recorded on a Hitachi spectrophotometer in hexane, and their IR spectra on a UR-10 instrument using films. The mass spectra of the TMS-HAMEs and also of the products of their hydrogenation were taken on a MKh-1310 mass spectrometer under the following conditions: ionizing voltage 40-50 V, collector current 50 μA, temperature 90-130°C.

The GLC analysis of the samples was carried out on a Chrom-4 instrument (Czechoslovakia) with a flame-ionization detector in the isothermal regime using a stainless steel column (4 × 2500 mm) filled with Chromaton N-AW-DMCS (0.160-0.200 mm) with 15% of Reoplex 400 (at 198°C) and with 3% of OV-1 (at 200°C).

For TLC we used silica gel L 5/40 (Czechoslovakia) containing 10% of $CaSO_4$ with preliminary activation of the layer at 105°C for 1 h, the solvent system being hexane–diethyl ether (7:8).

The isolation of the H-TAGs from the lipids of the seeds of G. bifida, the preparation of the HAs, the HAMEs, and the TMS-HAMEs and the period-permanganate oxidation of the HAMEs by von Rudloff's method have been described in [1]. In the oxidation of the HAMEs the ratio of the reagents was taken in the light of the number of multiple bonds and the presence of the OH group. The HAMEs (20 mg) were hydrogenated in ethanol (2 ml) at 40-60°C with a palladium catalyst (2 mg) on aluminum dust (50 mg) obtained by Ginzberg's method [30], with the constant bubbling of H_2 for 3 h.

Ricinoleic acid was isolated from castor oil and was purified in a similar way to the HAMEs.

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