NEW EPOXY ACIDS OF *Galeopsis bifida*

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Twelve epoxy acids have been detected in the seed lipids of *Galeopsis bifida* Boenn. (family Lamiaceae). Their structures have been established by TLC, GLC, periodate-permanganate oxidation, hydrogenation, ORD,and UV, IR, and mass spectrometry. Three of them are known but this is the first time that they have been detected in this plant, and six of them are new. The absolute configurations of the two main epoxy acids have been determined.

Epoxyacyldiacylglycerols (ep-TAGs) have been detected previously in the seed lipids of *Galeopsis bifida* Boenn. (family Lamiaceae), which are toxic for warm-blooded animals, and as the main epoxyacyl components the following acids were identified: cis-12,13-epoxyoctadeca-cis-9-enoic (12,13-ep-9-18:1, V, Fig. 1), cis-9,10-epoxyoctadeca-cis-12-enoic (9, 10-ep-12-18:1, IV), and cis-15,16-epoxyoctadeca-cis-9,cis-12-dienoic (15,16-ep-9,12-18:2, VII) [1]. However, mass-spectrometric (MS) and gas-chromatographic (GLC) analyses of the mixtures of methyl esters of epoxy acids isolated from *G. bifida* showed the presence of a series of unidentified components, and its is the results of a study of these that are given in the present paper.

The ep-TAGs of the lipids of *G. bifida* were isolated in a manner similar to that described in [1] in an amount of 1.65% (on the weight of the lipids).

In the MS of these ep-TAGs, a series of M^+ peaks was observed, the most intense ions of which, with m/z 888-900, corresponded to triacylglycerols of the general formula $C_{57}H_{92-104}O_7$ with two residues of normal fatty acids (NFAs: 18:0, 18:1, 18:2, and 18:3) and a residue of one of the isologues of the C_{18} epoxy acids. Weak ions with m/z 866 and 864 were assigned to ep-TAGs with the same normal acyls and one C_{16} epoxyacyl, and ions with m/z 928, 926, and 924 to those with one C_{20} epoxyacyl. To each M^+ bundle corresponded $(M - 18)^+$ fragments.

The set of acyl residues in the ep-TAGs was also determined from the mass numbers of the $(R - CO)^+$ and $(R - CO - 1)^+$ fragments. Unoxidized acids were represented by the peaks of ions with m/z 239 and 238 (16:0); 267 and 266 (18:0); 265 and 264 (18:1); 263 and 262 (18:2); and 261 and 260 (18:3). To epoxyacyl residues were assigned the peaks of ions of appreciable intensity with m/z 279 and 278 (ep-18:1) and 277 and 276 (ep-18:2), and also weaker ones with m/z 281 and 280 (ep-18:0); 251 and 250 (ep-16:1); 249 and 248 (ep-16:2); 207 and 206 (ep-20:1); and 305 and 304 (ep-20:2). In MS, two series of homologous ions belonging to fragments of the $(M\text{-}RCOO)^+$ type were observed. Ions with m/z 593-587, 619-611, and 647-641 were formed as the result of the elimination of unsubstituted acyl radicals from the M^+ 866-874, 890-900, and 918-928 series. As a result of this splitting out of the epoxyacyl radicals, the peaks of ions with m/z 577-573 and 607-595 appeared. In the latter series, the high intensity of the peaks with m/z 597-605 showed that the epoxyacyl radicals were eliminated mainly from sn-1- and sn-3-positions of the ep-TAGs. At the same time, in MS there were the fragments $(RCO+74)^+$ and $(RCO+128)^+$ for the C_{18} epoxyacids (355, 353, 351, and 409, 407, 405) which could be formed if they were present in the sn-2-positons of the ep-TAGs. These results correlate with those of a stereospecific analysis of the ep-TAGs [2], and confirm the high specificity of the epoxyacyl radicals for the extreme sn-glycerol positions. A similar nature of the distribution of oxidized fatty acids has also been observed in the oxoacylglycerols of *G. bifida* [3].

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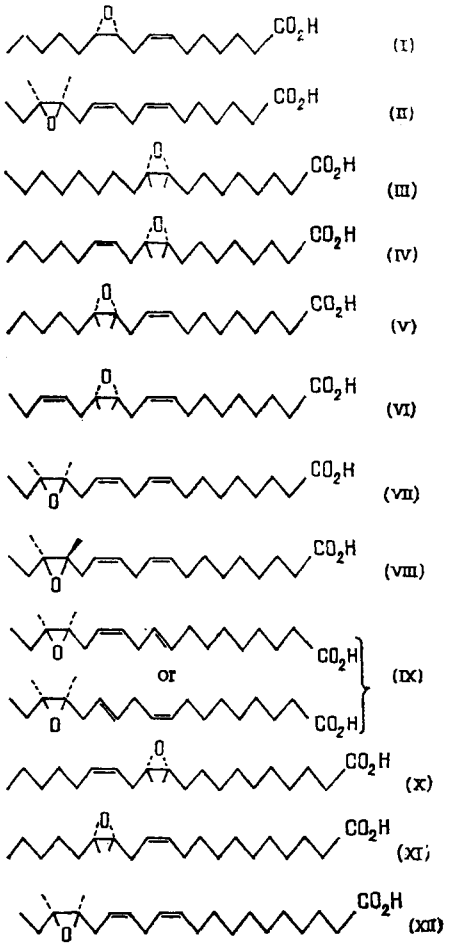


Fig. 1. Epoxy acids of *Galeopsis bifida*: 1) cis-10,11-ep-cis-7-16:1; II) cis-13,14-ep-cis,cis-7,10-16:2; III) cis-9,10-ep-18:0; IV) cis-9,10-ep-cis-12-18:1 (coronaric); V) cis-12,13-ep-cis-9-18:1 (vernolic); VI) cis-12,13-ep-cis-cis-9,15-18:2; VII) cis-15,16-ep-cis,cis-9,12-18:2; VIII) trans-15,16-ep-cis,cis-9,12-18:2; IX) cis-15,16-ep-cis,trans(trans, cis)-9,12-19:2; X) cis-11,12-ep-cis-14-20:1; XI) cis-14,15-ep-cis-11-20:1 (alchornoic); XII) cis-17,18-ep-cis,cis-11,14-20:2.

Thus, the mixture contained epoxyacids with 16 and 20 C atoms.

To determine their structures, the acids isolated from the ep-TAGs in the form of MEs were separated on a microcolumn of silica gel (CC). This gave the MEs of the unsubstituted acids and also of the epoxy acids enriched with monoenes and dienes. The retention of the epoxy group was checked on Silufol in system 1 by reaction with picric acid.

The composition of the MEs of the unsubstituted acids was determined by GLC (Reoplex 400), and the following results were obtained (%): 16:0-3.3; 18:0-1.7; 18:1-20.8; 18:2-57.6; 18:3-16.3

The methyl esters of the epoxy acid were rechromatographed on preparative Silufol plates in system 1, whereupon the epoxy acids enriched with monoenes were separated in to

fractions A-1 (R_f 0.43), and A-2 (R_f 0.41-0.34), and those enriched with dienes into fractions B-1 (R_f 0.41) and B-2 (R_f 0.34).

Some of the epoxy acid MEs of each fraction were converted into the MEs of the dihydroxy derivatives (DHDMEs). In their turn, part of the DHDMEs were hydrogenated after the trimethylsilyl (diTMS) derivatives of the initial and hydrogenated DHDMEs had been obtained, they were analyzed by MS and GLC on polar (Reoplex-400) and nonpolar (OV-1) phases. The peaks on the chromatograms were identified from the nature of the separation of the isomers, homologues, and isologues on the two phases. The quantitative ratios of the epoxy acids of *G. bifida* were determined by recalculating the results of the two chromatograms in the light of the weight ratios of the individual fractions.

In addition, the fractions of epoxy acid MEs were investigated by UVS, IRS, optical rotatory dispersion (ORD), and periodate-permanganate oxidation. The results of the analyses are given in Table 1.

All the epoxy acid ME fractions were transparent in the UVS. In the IRS, the A-1 fractions showed absorption bands only of a cis-oxirane ring (830, 850 cm^{-1}) and of isolated cis double bond (730 cm^{-1}) [1]. According to Ag^+-TLC in system 2, this fraction consisted of the ME of a C_{18} epoxymonoenoic acid (R_f 0.67) and a component with R_f 0.75. On GLC (OV-1), the diTMS ethers gave the peak of methyl vernolate (V) and the peak of compound (XI) having a chain two CH_2 groups longer than that of compound (V). In the MS of the diTMS derivatives, in addition to diagnostic fragments of the 12,13-diTMS-18:1 ME there were peaks (see Table 1) belonging to the ME of the 14,15-diTMS-20:1 acid. After the hydrogenation of fraction A-1, in the MS of the diTMS derivatives the peaks of the corresponding saturated analogues appeared.

In order to establish the positions of the double bonds, the MEs of the epoxy acids of fraction A-1 were oxidized with periodate-permanganate. The positive reaction of the oxidation products with picric acid showed that oxidative degradation had taken place at double bonds without affecting the epoxide ring. In the oxidation products the MEs of C_9- and C_{11}-dicarboxylic acids and a peak with C_{rel} = 0.777 (16:0), which was provisionally assigned to the ME of an epoxynonanoic acid, were detected (GLC).

For a proof of the structure of the epoxy-containing fragment, without isolation from the degradation products, it was converted by appropriate treatment first into the DHDME and then into the diTMS derivative.

The MS of the degradation product contained the peaks of the M^+, $(M - 31)^+$, and $(M - 73)^+$ ions for the C_9 (m/z 216, 185, 143) and C_{11} (244, 213, 171) dicarboxylic acids. In addition to these, 3,4-epoxynonanoic acid was identified from fragments with m/z 333 $(M - 15)^+$, 317 $(M - 31)^+$, 277, 275, 175, 173, and 74, this acid being formed in the oxidative degradation of (V) and (XI). The absence of other fragments in the MS and the formation of the C_{11} dicarboxylic acid on oxidation are proofs of the structure of (XI).

Thus, in the A-1 fraction, in addition to the epoxy acid (V) its homologue (XI) (alchornoic acid) was present. The same pair of epoxy acids has been detected in the seed lipids of *Alchornea cordifolia* (family Eurphorbiaceae) [4]. It was shown that the configuration of (+)-alchornoic aicd (14S, 15R) is identical with that of (+)-vernolic acid (12S, 13R).

The optical rotatory dispersion was measured for fraction A-1, containing 97.3% of (V) and 2.7% of (XI): $[\alpha]_D^{20}$ −2.7°, $[\alpha]_{560}$ −2.7°, $[\alpha]_{520}$ −2.7°, $[\alpha]_{480}$ −3.0°, $[\alpha]_{400}$ −3.3°, $[\alpha]_{360}$ −3.0°, $[\alpha]_{335}$ −1.8°, $[\alpha]_{310}$ 0°, $[\alpha]_{300}$ +0.9°, $[\alpha]_{280}$ +5.4°, $[\alpha]_{270}$ +6.7° (c 6.67 mg/ml; hexane).

A comparison of the ORD curve obtained with that of the methyl derivatives of (+)-(XI) and (+)-(V) [4] showed that they had an antipodal nature, on the basis of which may be assumed that fraction A-1 contained (−)-vernolic acid (12R, 12S).

In the IRS of fraction A-2, in addition to bands at 730 cm^{-1} (cis–CH=CH–) and 830 and 850 cm^{-1} (cis- $\underset{\diagdown\,O\,\diagup}{CH—CH}$—), there was the band of the vibrations of an isolated trans double bond at 960 cm^{-1}. On Silufol, this fraction gave two spots, the more polar component being the more brightly colored with picric acid.

TABLE 1. Results of Gas-Chromatographic Mass-Spectrometric Analyses of the Epoxy Acids of *Galeopsis bifida*

Acid*	$R_f \times 100$ for the epoxy acid MEs		GLC			Molecular ions and mass numbers of characteristic fragments of the diTMS derivatives of the DHDMEs, m/z						
			ECLs of the DiTMS derivatives		%	initial				hydrogenated		
	silufol	Ag^+-TLC	Reoplex 400	OV-1		M+	(M−15)+	(M−31)+	fragments	(M−15)+	(M−31)+	fragments
I	41	46	17,06	18,52	1,2	444	429	413	373, 275, 271, 173	431	415	375, 273, 275, 173
II	34	40	18,54	19,88	0,4	442	427	411	413, 311, 233, 131			417, 315, 233, 131
III	41	75	18,88	21,20	3,0	—	459	443	383, 315, 259, 215			391, 317, 233, 131
IV	39	67	19,19	20,82	2,0	472	457	441	391, 315, 259, 213			
V	43	67	19,19	21,00	29,3				401, 299, 275, 270, 173			
VI	34	42	20,50	21,00	7,2	470	455	439	401, 299, 273, 270, 171	459	443	403, 301, 275, 173
VII	34	42	20,50	21,93	52,8				441, 339, 310, 233, 131			445, 343, 233, 131
VIII	34	42	19,84	21,93	0,1							
IX	34	53	20,50	22,36	1,8							
X	34	75	21,37	22,71	0,5	500	485	469	389, 315, 287, 213	487	471	389, 317, 287, 215
XI	41	75	21,33	22,91	1,4				429, 327, 275, 173			431, 329, 275, 173
II	43	67	22,70	23,68	1,0	498	483	467	367, 333, 233, 131			371, 233, 131

*I) cis-10,11-ep-cis-7-16:1; II) cis-13,14-ep-cis,cis-7,10-16:2; III) cis-9,10-ep-18:0; IV) cis-9,10-ep-cis-12-18:1; V) cis-12,13-ep-cis-9-18:1; VI) cis-12,13-ep-cis,cis-9,15-18:2; VII) cis-15,16-ep-cis,cis-9-12-18:2; VIII) trans-15,16-ep-cis,cis-9,12-18:2; IX) cis-15,16-ep-cis,trans-(trans, cis)-9,12-18:2; X) cis-11,12-ep-cis-14-20:1; XI) cis-14,15-ep-cis-11-20:1; XII) cis-17,18-ep-cis,cis-12,14-20:2.

Analytical Ag^+-TLC showed that fraction A-2 consisted of components with R_f 0.75, 0.67, and 0.53 corresponding to the MEs of epoxy saturated, epoxymonoenoic and epoxydienoic acids. The ME of the epoxydienoic acid (R_f 0.53) had a greater mobility than the ME of the (VII) detected previously (R_f 0.42) and was colored more intensely on the H_2SO_4/t treatment of the plates.

When the diTMS derivatives of the DHDMEs were chromatographed on a polar plate, peaks of saturated, monoenoic, and dienoic C_{18} epoxy acids were obtained. On a nonpolar phase, in addition to the peaks of compounds (III) and (IV) (see Table 1) there was a peak with a ECL of 22.36 which corresponded to the acid (IX) with cis,trans(trans,cis) π-bonds, while the peak of the cis,cis isomer (VII) had an ECL of 21.93.

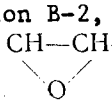
On the basis of certain characteristic fragments in the MS of the diTMS derivatives of fraction A-2, the first two peaks on the chromatogram were assigned to (III) and (IV) (see Table 1). In addition to these fragments, there were the peaks of ions characteristic only for the 15,16-ep-18:2 acid [1]. In the MS of the diTMS derivatives of the oxidation products, the peaks of the M^+, $(M-31)^+$, and $(M-73)^+$ ions were observed for the $C_{6:0}$ acid (with m/z 130, 99, 67) and the C_9 dicarboxylic acid, and also $(M-15)^+$ and $(M-31)^+$ and diagnostic fragments of the diTMS derivative of the DHDME of the undecomposed ME of (III). The presence of peaks of ions with m/z 175 and 277 indicated the formation of 9,10-epoxydodecanoic acid on the oxidative degradation of the ME of (IV). We were unable to detect 3,4-epoxyhexanoic acid, which is a fragment from the degradation of the ME of (VII) apparently because of the high losses of 3,4-dihydroxyhexanoic acid on its extraction from an aqueous solution of the products of the de-acetylation reaction. From the totality of the results, the compound of peak (IX) was identified as a cis-15,16-ep-cis,trans(trans,cis)-9,12-18:2 acid with the trans-π-bond either at C_9 or C_{12}.

In the IRS, fraction B-1 had the bands of a cis epoxide ring and of a cis double bond, and on Ag^+-TLC it was separated into three spots with R_f 0.75, 0.65, and 0.42. The fraction was distinguished by a complex composition, as shown on its analysis by GLC.

On a polar phase (Reoplex 400) the ep-18:0, ep-18:1, ep-20:1, and ep-20:2 isologues and homologues of the C_{12}-C_{20} epoxides were separated. Compounds (IV) and (V), isomeric with respect to the position of the epoxide group, were not separated under these conditions. On a nonpolar phase (OV-1), homologues and isomers with respect to the position of the epoxide group were separated, but the latter isomer issued together with (III).

In the MS of the diTMS derivatives of the DHDMEs the peaks of the M^+, $(M-15)^+$, and $(M-31)^+$ ions for the esters of the dihydroxy derivatives of hexadecenoic, octadecenoic, eicosenoic, and eicosadienoic acids were detected. The hydrogenation product lacked the peaks of the M^+ ions but had the peaks of the $(M-15)^+$ and $(M-31)^+$ ions of the corresponding saturated analogues of the acids. According to the MS results (see Table 1), the component with an ECL of 18.52 (OV-1) corresponded to structure (I). The presence of the C_7 dicarboxylic acid (m/z 188, 157, 115) in the oxidation products showed the C-7 position of the double bond in (I).

The peak with ECL 22.91 corresponded in the GLC behavior of its diTMS derivatives on a nonpolar phase to the C_{20} homologue of (IV). On the basis of the molecular ions and characteristic fragments in the MS of the diTMS derivatives, this peak was assigned to the derivative of the ME of (X). The results of MS and GLC in combination with the fact that the C_{11}-dicarboxylic acid was detected in the products of the oxidation of fraction B-1 gave grounds for determining the structure of the compound with ECL 23-68 (OV-1) as the 17,18-ep-11,14-dienoic acid (XII).

In the IRS of fraction B-2, bands were detected in the region of 730 cm^{-1} (cis–CH=CH–) and 830, 850 cm^{-1} (cis- ) together with a weak band at 890 cm^{-1}, and the presence of an isomer with a trans-oxirane ring was assumed.

The fraction was homogeneous on Ag^+-TLC (R_f 0.42). On GLC with a nonpolar phase, a peak corresponding to the diTMS derivative of the DHDME of the 15,16-ep-9,12-18:2 acid (ECL 21.93) together with the additional peaks of (II) (ECL 19.88), (VI) (ECL 21.00), and (IX) (ECL 22.36) were obtained. Analysis by GLC on a polar phase showed, in addition to the peaks mentioned above, an additional peak with an ECL of 19.84, which corresponded to the derivative of the trans-epoxy acid (VIII).

The MS of the diTMS derivatives contained the peaks of ions with characteristic fragments for (II), (VI), and (VII) (see Table 1). A comparison of the MSs of the initial hydrogenated samples permitted the peak with ECL 21.00 to be assigned to the diTMS derivative of the DHDME derivative of (VI).

The peak with ECL 19.88 corresponded to a derivative with a chain two CH_2 groups shorter than the di-TMS derivative of the DHDME derivative of (VII). In the MS of the silyl derivatives, this was represented by the peaks of ions (see Table 1) belonging to the structure of the ME of a 13,14-epoxyhexadecadienoic acid. In the products of the oxidation of this fraction, in addition to the main C_9 dicarboxylic acid the C_7-dicarboxylic acid was detected (GLC, MS), which permitted the peak to be assigned to the derivative of the ME of (II).

The ORD was measured for fraction B-2, consisting of 0.7% of (II) 11.9% of (VI), 87.2% of (VII), and 0.2% of (VIII) (c 5.83 mg/ml; hexane), with the following result: $[\alpha]_D^{20}$ +6.9°, $[\alpha]_{560}$ +6.9°, $[\alpha]_{480}$ +8.9°, $[\alpha]_{400}$ +15.5°, $[\alpha]_{360}$ +25.7°, $[\alpha]_{335}$ +35.4°, $[\alpha]_{300}$ +48.6°, $[\alpha]_{280}$ +79.5°.

The ORD curve had the same sign as the ORD curve of the MEs of the known acids (+)-vernolic (12S,13R) and (+)-alchornoic (14S, 15R) [4]. The asymmetric environment of the chiral centers in the epoxyoctadecadienoic acid under investigation was similar to that in vernolic and alchornoic acids. On this basis, it was concluded that the absolute configuration of the asymmetric centers in the (+)-(VII) acid was 15S, 16R.

The optically active (+)-cis-15,16-ep-cis,cis-18:2 acid has been detected previously in the lipids of *Camelina sativa*, and for the product of its hydrogenation, the 15,16-ep-18:0 acid, a value of $[\alpha]_D$ +3.3° (4%, C_2H_5OH) was given [5].

The acids (IV), (V), (VII) and a number of other hydroxy derivatives of unsaturated fatty acids have been detected in a variety of rice resistant to the fungal pathogen *Piricularia infestans* [6].

Thus, an analysis of the epoxy acids of *G. bifida* has shown the presence of 12 compounds, of which (I) and (XI) are homologues of the main monoenic epoxy acid — (12R, 13S) (V) — while (X) is a homologue of its position isomer (IV). (I) and (XII) are homologues of the epoxydienoic acid (VII). The position isomer (VII) and two geometric isomers (VIII) and (IX) of the dienoic acid (VII) were present in the mixture. Of them, the 9,10-ep-18:0, the 12,13-ep-9,15-18:2 and the 14,15-ep-11-20:1 acids were identified as known acids, while the cis-10,11-ep-cis-7-16:1, cis-13,14-ep-cis,cis-7,10-16:2, trans-15,16-ep-cis,cis-9,12-18:2, cis-15,16-ep-cis,trans(trans,cis)-9,12-18:2, cis-11,12-ep-cis-14-20:1, and cis-17,18-ep-cis,cis-12,14-20:2 acids are known.

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrometer, ORD curves on a Jasco spectropolarimeter in hexane, IR spectra on a UR-10 instrument in a film, and mass spectra on MKh-1303 and MKh-1310 instruments (150°C, 50 eV, 0.5 mV).

Gas-liquid chromatography was performed on a Chrom-4 instrument with a flame-ionization detector, using a 4 × 3700 mm column filled with 15% of Reoplex 400 and a 4 × 2500 mm column filled with 3% of OV-1. In both cases the support for the stationary phase was Chromaton N-AW-DMCS, and the rate of flow of the carrier gas, He, was 0.7 kg/cm^2.* The composition of the NMEs and MEs of dicarboxylic acids in the oxidation products were determined on 15% of Reoplex 400 at temperatures of 196 and 180°C, respectively. The diTMS derivatives of the DHDMEs were chromatographed on 15% of Reoplex 400 at 198°C and on OV-1 at 200°C. The ELCs were calculated as described by Miwa [7].

Column chromatography of the MEs of the fatty acids from the ep-TAGs was performed on silica gel L 100/160 mesh; the NMEs were eluted from the column by the solvent system hexane—diethyl ether (96:4) and the MEs of the epoxy acids by a mixture of the same solvents in a ratio of 92:8. The ME fraction of the epoxy acids was rechromatographed on a preparative Silufol plate in system 1 [hexane—diethyl ether (4:1)] with a 20 cm rise of the solvent front.

*As in Russian original — Publisher.

Analytical Ag^+—TLC was performed on silica gel L 5/40 mesh with the addition of 6.5% of $CaSO_4$ and 30% of $AgNO_3$ in system 2 — benxene—chloroform—diethyl ether (50:50:15).

The isolation of the ep-TAGs, the preapration of the DHDMEs and of the diTMS derivatives, and the Rudlov oxidation of the MEs of the acid with periodate-permanganate were carried out as in [1], and the hydrogenation of the MEs of the epoxy acids as described in [8].

SUMMARY

The structures of twelve epoxy acids from the seed lipids of *Galeopsis bifida* Boenn. (family Lamiaceae) have been established. Three of them were acids of known structure — cis-9,10-ep-18:0, cis-12,13-ep-cis,cis-9,15-18:2, and cis-14,15-ep-cis-11-20:1, detected in this plant for the first time, while the six epoxy acids cis-10,11-ep-cis-7-16:1, cis-13,14-ep-cis,cis-7,10-16:2, trans-15,16-ep-cis,cis-9,12-18:2, cis-15,16-ep-cis,trans(trans,-cis)-9,12-18:2, cis-11,12-ep-cis-14-20:1, and cis-17,18-ep-cis-cis-11,14-20:2 were new. The configuration of the main components have been determined as (—)-12(R),13(S)-ep-cis-9-18:1 and (+)-15(S),16(R)-ep-cis,cis-9,12-18:2.

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NEUTRAL LIPIDS OF FREON EXTRACTS OF THE FRUIT OF *Hippophaë rhamnoides*

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The composition of Freon extracts of the fruit of two samples of sea buckthorn has been studied — from the Western Pamir and from Biisk. The presence of 16 classes of lipids has been shown. The distribution of the fatty acid residues between the 2- and 1,3-positions of the triacyl glycerols of these extracts have been studied by the method of lipase hydrolysis, and their position-species compositions have been established.

Sea buckthorn oil, obtained by extracting the comminuted pulp (a mixture of seeds and flesh), of the sea buckthorn with Freon-12 [1] possesses a high anti-ulcer activity [2]. For a more complete evaluation of this oil, we have studied the composition of the neutral lipids of Freon extracts of sea buckthorn from the Western Pamir and Biisk. The sea buckthorn oil was obtained from an experimental plant in the Lenin Tadzhik University.

The Freon extracts were separated by column chromatography (CC) into individual classes of lipids. Complex fractions were separated additionally by thin-layer chromatography (TLC). The lipids were identified by qualitative reactions, chromatographic mobilities, comparison with model samples, and IR, UV, PMR, and mass spectroscopy. The ratio between the classes of lipids was determined gravimetrically (Table 1).

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