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EPOXY AND HYDROXY ACIDS OF THE SEED OIL OF *Galeopsis bifida*

S. D. Gusakova, I. I. Vinokurov, and A. U. Umarov

UDC 547.915:665.33

The lipids of the seeds of the hemp nettle, which is toxic for ruminants have been studied. The composition of the fatty acids of five classes of lipids and the position-species composition of the triacylglycerols have been determined. The presence of epoxyacyl- and hydroxyacylglycerols in the oil and the structures of the acids of these lipids have been established. Squalene has been isolated from the oil.

Galeopsis L. (hemp nettle) is a widespread annual weed plant of the family Labiatae. Five species of hemp nettle are found on the territory of the USSR.

The amount of oil in the seed of this plant, which belongs to the semidrying group [1], is fairly high (42-46%). The high toxicity of hemp nettle oil is well known [2, 3].

It is not clear precisely what substances are responsible for the toxicity of the hemp nettle. A hypothesis exists according to which they are alkaloids, but the results of the analysis of several samples of seeds for the presence of alkaloids are contradictory [1]. However, it has been established that the poisonous components of the hemp nettle are stable on heating and accumulate in the fatty tissue [2].

The chemical composition of the fatty oil of the seeds of *Galeopsis* species has not been studied. Only some constants for hemp nettle oil are given in the literature [1, 4].

We have investigated the composition of the seed oil of *G. bifida* Boenn. The oil was extracted from the comminuted seeds with petroleum ether. The yield of extract was 42% on the weight of the seeds. A number of indices of the oil were determined by standard methods, and the total acids were isolated from it:

The qualitative reaction of the hemp nettle oil with tungstosilicic acid for the presence of alkaloids was negative [5]. From the defatted meal a base was isolated which was identi-

	Oil	Acids
Density, d_4^{20}, g/cm^3	0.9228	0.9003
Refractive index, n_D^{20}	1.4784	1.4685
Iodine No., % I_2	157.94	164.92
Acid No., mg KOH/g	2.0	–
Saponification No., mg KOH/g	190.09	–
Neutralization No., mg KOH/g	–	198.38
Amount of unsaponifables, %	1.07	–

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 288-295, May-June, 1981. Original article submitted November 18, 1980.

0009-3130/81/1703-0217$07.50

fied by comparison with a model sample as choline.* Choline has also been detected in other species of the family Labiatae [6].

In the UV spectrum of the oil and the fatty acids, there was absorption in the 229-234 nm region corresponding to a conjugated dienic system, and weak absorption of a carbonyl group at 265-285 nm.

In the IR spectrum of the oil and of the methyl esters (MEs) of the acids isolated from it bands were observed of the vibrations of an epoxy group at 850, 875, and 920 cm^{-1}, an inflection at 1280 cm^{-1}, the broad band of an ester carbonyl with inflections at 1700, 1710, and 1715 cm^{-1}, weak allene absorption (1960 cm^{-1}) and two bands of OH groups at 3470 and 3560 cm^{-1}.

To investigate the class composition of the lipids, 43.5 g of the oil was separated by column chromatography, the fractions being eluted with petroleum ether (40-60°C) — diethyl ether, pure diethyl ether, chloroform, and methanol. Mixed fractions were rechromatographed by column chromatography and TLC, and the classes of the same type were then combined.

The lipids were assigned to the corresponding classes on the basis of their chromatographic mobility and their IR, IV, and PMR spectra. In view of the spectral characteristics of the oil, the lipids more polar than the usual triacylglycerols (n-TAGs) were subjected to qualitative reactions for ketone and epoxy groups. The lipid composition of the oil is given below:

Class of lipids	R_f	Eluant	Amount g	Amount %
1. Hydrocarbons, squalene	front†	Petroleum ether	0.062	0.14
2. n-Triacylglycerols	0.78	Petroleum ether—diethyl ether (96:4)	39.2	90.1
3. Epoxyacylglycerols	0.46	Petroleum ether—diethyl ether (92:8)	0.709	1.63
4. Free fatty acids, Oxoacylglycerols	0.37	"	0.480	1.1
5. Hydroxyacyglycerols	0.33	"	0.922	2.12
6. Diacylglycerols, sterols	0.28	"	0.861	1.98
Polar acylglycerols:				
7. Fraction I	0.3	"	0.126	0.29
8. II	0.24	Petroleum ether—diethyl ether (80:20)	0.220	0.51
9. III	0.18	"	0.126	0.29
10. IV	0.12	Diethyl ether	0.217	0.50
11. V	0.08	Chloroform	0.048	0.11
12. VI	start	Methanol	0.522	1.2

As we can see, the hemp nettle oil has a complex composition, containing 14 classes of lipids. In the present paper we give the results of the analysis of five of them.

Squalene was freed from contamination with other hydrocarbons by TLC in system 3 and was identified spectrally. Its amount in the oil was 0.07%.

The acid composition of the acyl-contining lipids was studied after the isolation of the acids by mild alkaline hydrolysis and their conversion into the methyl esters (MEs). The fraction containing the free fatty acids (FFAs) was methylated, and the MEs of the free acids so formed were separated from the oxoacylglycerols by column chromatography, the MEs being eluted with system 4.

In addition to GLC analysis, the MEs of the acids, the n-TAGs, and the FFAs were separated on silica gel containing 20% of $AgNO_3$ (Ag^+-TLC) in system 7. It was found by this method that the total acids of the lipids of the two classes contained laballenic acid — the 18:2 (5, 6) acid — which is characteristic mainly for the linoleic-rich oils of the Labiatae seeds [7]. The amount of laballenic acid was calculated after the separation of the MEs of the acids of the lipids of the corresponding classes by preparative Ag^+-TLC in the same system and the gravimetric estimation of the isolated MEs of the saturated, allenic, monoenic, dienic, and trienic acids and subsequent GLC analysis. In narrow fractions of the MEs, on the basis of the GLC results we identified as minor components the 15:0, 17:0, and 20:1 acids. The amount of eicosenoic acid in the fractions of monoenic MEs from the n-TAGs was 1.6%, and the amount in the FFAs 1.4%.

*The choline was isolated and identified by S. Aripova.

†Chromatographic mobility 1-6 on Silufol in system 1; 7-12 in system 2.

The positions of the double bonds in the unsaturated MEs were established on the basis of the fragments from periodate—permanganate oxidation.

The compositions of the acids of the total lipid material and also of the individual classes were as follows (%, GLC):

Acid	Total lipids	n-TAG	FFAs	Epoxyacyl-glycerols	Hydroxy-acylglycerols	2-Monoacyl-glycerols
14:0	—	—	0,1	0,3	—	—
15:0	—	Tr.	Tr.	—	—	—
16:0	1,8	2,7	7,4	3,9	4,8	0,5
16:1 (9)	Tr.	0,1	0,3	0,3	Tr.	Tr.
17:0	Tr.	Tr.	Tr.	—	—	—
18:0	0,9	1,0	2,3	13,5	1,9	—
18:1 (9)	12,4	20,2	23,7	18,4	29,3	24,7
18:2 (9,12) }	50,6	49,1 }	45,6 }	44,3	50,1	55,6
18:2 (5,6) }		2,4 }	6,0 }			
18:3 (9, 12, 15)	33,4	24,5	14,6	19,3	13,9	19,2
20:1 (11)	Tr.	Tr.	Tr.	—	—	—
Unidentified	0,9	—	—	—	—	—

As can be seen from the figures given above, the total free fatty acids, more diverse in composition, includes larger amounts of the 16:0, 18:0, and 18:2(5, 6) acids than the n-TAGs.

The structure of the n-TAGs was investigated by enzymatic hydrolysis. Calculations showed that the set of n-TAGs included 51 species, with a predominance of LLLe (15%), LLL (13.6%), and OLL (9.6%).

The position-species compositions of the triacylglycerols of *G. bifida* were as follows (P — palmitic; S — stearic; O — oleic; L — linoleic; Le — linolenic acid; they are arranged according to the acid esterifying position 2 in the sequence P, O, L, and Le); the species amounting to 0.1% and more are shown:

TAG	Content, mole %	TAG	Content, mole %	TAG	Content, mole %
2-P		LOL	6,0	LLL	13,6
PPLe	0,1	LOLe	6,6	LLLe	15,0
LPL	0,1	LeOLe	1,8	LeLLe	4,1
LPLe	0,2	2-L		2-Le	
		PLP	0,1	PLeO	0,2
2-O		PLO	0,8	PLeL	0,8
POO	0,4	PLP	2,2	PLeLe	0,4
POL	1,0				
POLe	0,6	PLLe	1,2	SLeL	0,2
SOO	0,4	SLL	0,8	SLeLe	0,2
SOL	0,4	SLLe	0,4	OLeL	3,4
SOLe	0,2	OLS	0,2	OLeS	1,2
OOO	0,8	OLO	1,7	OLeLe	1,8
OOL	4,2	OLL	9,6	LLeLe	4,7
OOLe	2,4	OLLe	5,5	LeLeL	5,2
				LeLeLe	1,4

Of the main unsaturated acids the 18:1 acid esterified position 2 of the n-TAGs to the greatest extent [8].

Thus, in the FFAs and n-TAGs of *G. bifida*, amounting to 91% of the total lipids there are 20:1(11) and 18:2(5, 6) acids that are characteristic for some species of this family [7], and the distribution of the main unsaturated acids in the three positions of the n-TAGs, as we have found previously in a study of Central Asian species of Labiatae [8], differs from that most commonly found amoung the other plant families studied.

The epoxyacylglycerols (ep-TAGs) gave a positive test with picric acid for the epoxy group, and in the IR spectrum characteristic of the acylglycerols, there were bands of the vibrations of cis-epoxide group at 810, 825, 850, and 1280 cm^{-1}. The UV spectrum showed slight absorption at 234 nm. In addition to the usual signals of the protons of the hydrocarbon chain of the fatty acids and the glycerol residue, the PMR spectrum contained: a complex signal of the protons of methyl groups with τ 9.0-9.2 ppm showing a confusion of the signals of methyl groups of two or three types ($CH_3(CH_2)_n$-, $CH_3CH_2CH=$ and $CH_3CH_2CH—O$—), a multiplet of protons of diallyl methylene groups $=CHCH_2CH=$, and of an epoxide ring at 7.3 ppm, and a multiplet of olefinic protons at 4.3-4.9 ppm.

The acids were isolated from the ep-TAGs by mild alkaline hydrolysis at room temperature with preservation of the epoxy groups. After methylation, the MEs were separated by prepara-

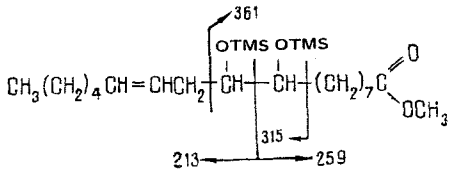
tive TLC in system 5 into the MEs of ordinary and epoxy acids, the ratio of the amounts of these classes being 2:1. The sum of the ordinary acids of the ep-TAGs, in contrast to those of the n-TAGs, contained about 18% of saturated acids, mainly the 18:0 acid.

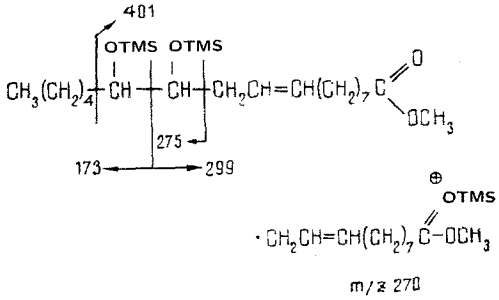
On chromatography on Silufol in system 5, the methyl esters appeared in the form of two spots with R_f 0.65 and 0.67.

To establish the structure of the epoxy acids we used the mass spectrometry of their derivatives. Acetylation of the total MEs of the epoxy acids gave acetoxy-hydroxy derivatives at the position of the epoxy ring, the acetoxy groups were eliminated by alkaline hydrolysis, and the dihydroxy acids so formed were converted into the trimethylsilyloxy derivatives (TMS derivatives), which were also analyzed by mass spectrometry.

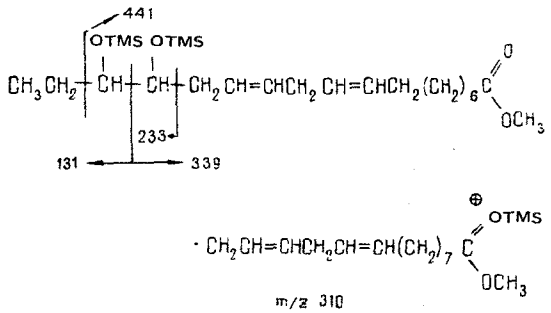
Before spectral analysis, the TMS derivatives were freed from accompanying impurities by preparative TLC in system 6. When they were chromatographed on Ag^+-TLC the derivatives freed from impurities were separated into two spots corresponding in mobility to monoenic (R_f 0.61) and dienic (0.43) esters. Just as in the case of the separation of the initial MEs of the epoxy acids, the more polar compound (dienic) predominated.

In the mass spectrum of the TMS derivatives of the monoenic dihydroxy acids there were the peaks of ions with m/z 472, which corresponds to the molecular weight of the MEs of octadecenoic acids with two CH—OTMS groups, m/z 457 $(M - 15)^+$ and 441 $(M - 31)^+$. The positions of the epoxide ring and of the double bond were established on the basis of fragments with m/z 231, 259, 315, and 361, and m/z 173, 270, 275, 299, and 401 [9]:





The mass spectrum of the derivatives of the dienic dihydroxy acids contains the peaks of the molecular ion with m/z 470 and ions with m/z 455 $(M - 15)^+$, 441 $(M - 29)^+$, 439 $(M - 31)^+$, 339, 310, 237, 233, and 131. Such fragmentation determines the structure of the epoxydienoate as the ME of 15,16-epoxyoxadeca-9,12-dienoic acid.



There were no fragments relating to any other isomers of the epoxydienoic acid in the mass spectrum. As stated above, in the IR spectrum of the ep-TAGs there were no bands of the vibrations of trans-epoxide groups and trans double bonds. Thus, hemp nettle oil contains cis-9,10-epoxyoctadec-12-enoic (coronaric), cis-12,13-epoxyoctadec-9-enoic (vernolic), and

cis-15,16-epoxyoctadeca-9,12-dienoic acids. We have reported the possible presence of epoxy acids in the seeds of Labiatae earlier [10]. *G. bifida* is the first plant of this family from the seed oils of which epoxy acids have been isolated. It must be mentioned that in addition to the epoxymonoenoic acids which are widespread in nature, the oil contains a rare epoxydienoic acid, which predominates quantitatively in the ep-TAGs. Coronaric and vernolic acids have been detected in the seed oils of a number of other plant families. Up to the present time, a 15,16-epoxydienoic acid had been found only in the seed oil of *Camelina sativa*, family Cruciferae [11].

The hydroxyacylglycerols gave a positive reaction with 2,4-dinitrophenylhydrazine and had absorption in the 225-235 and 260-290 nm regions in the UV spectrum. The IR spectrum of this fraction corresponded to the spectrum of ordinary TAGs, with exception of a complex band of C=O groups at 1600-1750 cm^{-1}. In view of the very small amount of the acid present in this fraction of the lipid, it was not studied in detail.

According to their mobility on TLC, the hydroxyacylglycerols (h-TAGs) corresponded to the hydroxyacyldiacylglycerols [12]. The IR spectrum showed bands of the vibrations of hydroxy groups at 3640-3200 cm^{-1}, and also a series of bands of low and medium intensity at 850, 870, 920, 955, and 990 cm^{-1}. The IR spectrum of this fraction had the absorption of a cis-trans-conjugated dienic chromophore at 233 nm.

Distinguishing features in the PMR spectrum of the h-TAGs were the signal of the protons of a CH—OH methine group at τ 5.83 ppm, coinciding with the multiplet of the protons of the glycerol residue (5.7-6.3 ppm), and a multiplet of the protons of the conjugated double bonds of a —CH=CH—CH=CH—C(OH)H— group in the 3.5-5.1 ppm region, corresponding in the nature of its splitting to that described in the literature [13].

The acids were isolated from the h-TAGs by alkaline hydrolysis and, in the form of the MEs, were separated by column chromatography, the MEs of the ordinary acids being eluted with system 4 and the MEs of the hydroxy acids with diethyl ether. The composition of the ordinary acids was similar to that of the set of the acids from the n-TAGs. Appreciable differences were observed only in the ratio of the amounts of 18:3 and 18:1 acids.

The methyl esters of the hydroxy acids were converted into the TMS derivatives and were purified by TLC in a similar manner to that described above. The results of the separation of the resulting TMS derivatives on Ag^+-TLC showed that the total hydroxy acids consisted of not less than 6 components with different degrees of unsaturation.

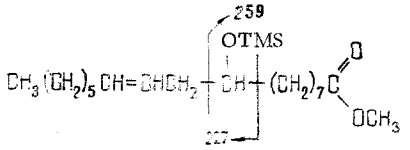
The mass spectrum of the silyl derivatives of the MEs of the hydroxy acids contained in the high-mass region fragments with m/z 371, 369, 367, 365 $(M-15)^+$, 355, 353, 351, and 349 $(M-31)^+$, which corresponds to monohydroxy acids of the C-18 series with degrees of unsaturation of from 0 to 3. The peaks of the molecular ions were detected only for the hydroxydienic (M^+ 382) and hydroxymonoenic (M^+ 384) derivatives.

The main components of the mixture were the MEs of ricinoleic (12-hydroxyoctadec-9-enoic) acid and of two isomeric 9(13)-hydroxyoctadeca-trans,cis-12-(cis-9,trans-11)-dienoic acids. The mass numbers and intensities of the main fragments in the spectrum corresponded to those described in the literature [14].

In addition to those mentioned, isoricinoleic acid was identified in the mixture from fragments with m/z 227 and 259.

Because of the very low concentration of hydroxy saturated and hydroxy trienic acids, the positions of the hydroxy groups in them were not determined.

With the exception of the isoricinoleic acid, the hydroxy acids isolated from the oil of *G. bifida* are specific components of the lipids of a number of plant families. This is the first time that they have been found in the oils of Labiatae seeds.



EXPERIMENTAL

IR spectra were recorded on a UR-10 instrument using films of the substances, UV spectra on a Hitachi spectrophotometer in hexane, PMR spectra on a Varian XL-100 instrument in CCl_4 with TMS as internal standard and mass spectra on a MKh-1310 instrument.

Gas-liquid chromatography was performed on a Khrom-4 instrument with a flame-ionization detector using a 2.5 m × 4 mm column filled with 17% of ethylene succinate on Chromaton N-AW-DMCS at 198°C for the high-molecular-weight and dicarboxylic acids and at 132°C for the low-molecular-weight acids.

Column chromatography was carried out on silica gel L100/160, and thin-layer chromatography on Silufol and silica gel L5/40 with the addition of 10% of $CaSO_4$ in the following solvent systems: hexane—diethyl ether—CH_3COOH (70:30:1) (1), and hexane—diethyl ether (8:7) (2), (99:1) (3), (96:4), (4), (7:3) (5), and (9:1) (6) on silica gel L5/40 with the addition of 10% of $CaSO_4$ and 20% of $AgNO_3$ in benzene (system 7).

The hemp nettle seeds were collected in the forest-steppe zone of the Bashkir Predural' on grey forest soils in August 1978 in the stage of ripeness.

The oil was extracted by five steepings with petroleum ether (40-60°C) of the comminuted seeds at room temperature.

The indices of the oil and of the combined acids were determined by standard methods [15]. The qualitative reactions with picric acid and with 2,4-dinitrophenylhydrazine were carried out as described in the literature ([16] and [17], respectively).

Mild alkaline hydrolysis was performed with a 10% aqueous methanolic solution of KOH (H_2O—MeOH, 1:9, v/v), and enzymatic hydrolysis and the calculation of the position-species composition of the n-TAGs as described previously [19]. The acids were methylated with diazomethane.

Oxidation by the von Rudloff periodate—permanganate reagent was carried out in a mixture of tert-butanol (20 ml) and water (30 ml) containing 25 mg of K_2CO_3 in the boiling water bath under reflux for an hour. The ratio of KIO_4 to $KMnO_4$ to MEs was 10.7:0.1:1. After cooling, the reaction mixture was acidified with H_2SO_4, the excess of oxidizing agent was destroyed with dry $Na_2S_2O_5$, the acid fragments were converted into soaps by the additon of KOH, the alcohol was distilled off, the soaps were decomposed with 15% HCl and the acids were isolated by extraction with diethyl ether after the saturation of the solution with NaCl.

After the hydrolysis of the ep-TAGs, the epoxy acids were isolated by decomposing the resulting soap with 15% HCl under titration conditions until the medium was weakly acidic. The opening of the epoxide rings and the formation of the silyl derivatives were performed as described by Gunstone and Shuler [18].

Squalene (R_f 0.87 in system 3, a transparent almost colorless oily liquid) does not absorb in UV light. In its IR spectrum there are strong bands at 840, 1675 (—CH=C—) and 1380 cm^{-1} ($(CH_3)_2C=$). The PMR spectrum was similar to that described in the literature [20]. Its mass spectrum contained the peaks of ions with m/z 410 (M^+), 395 ($M^+ - 15$), 367 ($M^+ - 43$), 341 ($M^+ - 69$), and 69 (100%).

SUMMARY

1. The acid composition of the main lipid classes and the composition of the triacylglycerols of the seed oil of *Galeopsis bifida* Boenn. have been established.

2. From the total lipids, epoxy- and hydroxyacylglycerols have been isolated and the structures of the acids present in them have been determined.

3. The terpenoid hydrocarbon squalene has been detected in the oil.

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ARYLNAPHTHALENE LIGNANS OF *Haplophyllum dauricum*. THE STRUCTURE OF DAURINOL

D. Batsuren, É. Kh. Batirov,
V. M. Malikov, V. N. Zemlyanskii,
and M. R. Yagudaev

UDC 547.99

The arylnaphthalene lignans justicidin B and daurinol I have been isolated from the epigeal part of *Haplophyllum dauricum* (L.) G. Don.

We have previously established the presence of arylnaphthalene lignans in plants of the genus *Haplophyllum* Juss. (family Rutaceae) [1]. In the present paper we report the results of a study of the lignans of *Haplophyllum dauricum* (L.) G. Don.

The plant was collected at the stage of incipient fruit bearing in the Uvurkhangaiskii aimak, Mongolian People's Republic. The roots and epigeal part were studied. From a chloroform fraction of the ethanolic extract of the epigeal part we isolated two individual compounds by adsorption chromatography on a column of silica gel. Both substances exhibited bright blue fluorescence in UV light, and with concentrated sulfuric acid they formed dark brown colorations. These properties are characteristic of arylnaphthalene lignans [2]. The lignan with the composition $C_{21}H_{16}O_6$ (I) mp 234-235°C (methanol) was, according to its IR and PMR spectra, identical with justicidin B [3, 4]. This was confirmed by a direct comparison of (I) with an authentic sample of justicidin B isolated from *Haplophyllum obtusifolium* [1].

Lignan (II) with the composition $C_{20}H_{14}O_6$, M^+ 350, $\lambda_{max}^{C_2H_5OH}$ 227, 262, 296, 324, 352 nm (log ε 4.55, 4.68, 4.07, 4.09, 3.41) proved to be new, and we have called it daurinol. The peak of the molecular ion (m/e 350) is the strongest in the mass spectrum of (II). Strong peaks of ions with m/e 321 (M − CHO), 305 (M − CO_2H), 292 (M − $C_2H_2O_2$), 291 (M − CH_3O − CO), 277, 263, and others were also observed.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 295-297, May-June, 1981. Original article submitted February 3, 1981.

0009-3130/81/1703-0223$07.50