5. M. Kates, Techniques of Lipidology, Elsevier North-Holland, Amsterdam (1962).
6. S. D. Gusakova, I. I. Vinokurov, and A. U. Umarov, Khim. Prir. Soedin., 288 (1981).
7. T. V. Khomova, D. T. Asilbekova, S. D. Gusakova, and A. U. Umarov, Khim. Prir. Soedin., 279 (1983).
8. T. K. Miwa, J. Am. Oil Chem. Soc., 40, 309 (1963).
9. R. G. Binder, T. H. Applewhite, M. I. Diamond, and L. A. Goldblatt, J. Am. Oil Chem. Soc., 41, 108 (1964).
10. E. N. Frankel, W. E. Neff, W. K. Rohwedder, B. P. S. Khrambay, R. F. Garwood, and B. C. L. Weedon, Lipids, 12, 901 (1977).
11. A. K. Sengupta, Chem. Ind. (London), 257 (1972).
12. L. J. Morris, and D. M. Wharry, J. Chromatogr., 20, 27 (1965).
13. F. D. Gunstone and H. R. Schuler, Chem. Phys. Lipids, 15, 174 (1975).

FATTY ACID COMPOSITION OF THE LIPIDS OF THE SEEDS OF *Helleborus abchasicus*

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The main class — the free fatty acids — of the lipids of the seeds of the second vegetation phase of *Helleborus abchasicus* A. Br. has been studied. The analysis of the composition and structure of the fatty acids in the lipids of the seeds showed the presence of a set of known but rarely encountered acids: 20:0; Δ^{11}-20:1; $\Delta^{11,14}$-20:2; and $\Delta^{11,14,17}$-20:3.

As we have reported, the main class of neutral lipids of the seeds of *Helleborus abchasicus* A. Br. (Abkhazian hellebore) of the second vegetation phase are the free fatty acids (FFAs) [1]. In the present investigation we have analyzed the composition and structure of these acids.

The IR spectra of the total lipids of the seeds and of the methyl esters (MEs) of the fatty acids isolated from them lacked the bands of trans double bonds and of any functional groups unusual for fatty acids. The same samples of lipids were transparent in the UV region.

The free acids were isolated from the total lipids by column chromatography and were converted into their MEs with diazomethane.

The absence of trans double bonds and of unusual functional groups in the acids was confirmed by PMR spectroscopy, where the nature of the signals of the olefinic protons at τ = 4.7 ppm and of the diallyl protons at 7.3 ppm also showed the absence of conjugation and the remoteness of double bonds from CH_3 and $COOCH_3$ groups.

According to GLC, the sum of the MEs consisted of the following acids (%): 14:0 (traces); 16:0 (9.3); 16:1 (0.1); 18:0 (2.6); 18:1 (5.6); 18:2 (21.5); 18:3 (52.2); 20:0 (traces); 20:1 (6.9); 20:2 (1.8); and 20:3 (traces).

On the analysis of the MEs by TLC on silica gel impregnated with $AgNO_3$ in system 1, spots were obtained which corresponded in their mobility to the esters of saturated (R_f 0.82), eicosenoic (R_f 0.73), oleic (R_f 0.70), and dienoic (R_f 0.58) and trienoic (R_f 0.41) acids.

To obtain the individual acids, the combined MEs were separated by preparative Ag^+-TLC in system 1.

Under these conditions each of the fractions of MEs of the monoenoic, dienoic, and trienoic acids appeared in the form of two zones, the top zone being colored more intensively.

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The fraction of the MEs of the monoenoic acids was separated into more distinct zones. The width of the intensively colored zones of the MEs of the dienoic and trienoic acids was small, and it was therefore impossible to separate the first zone of the trienoic compound from the main part in the pure state, and the two zones of the dienoic esters were removed from the plate together.

The quantitative proportions of the fractions isolated were as follows:

Fraction of MEs	Weight of the fraction, g	% of the weight of the total MEs
Saturated	0,133	14,8
Monoenes		
Zone 1	0,059	6,7
Zone 2	0,062	7,1
Dienes	0,176	20,4
Trienes		
Zone 1	0,102	11,6
Zone 2	0,346	39,4

An investigation of the fractions isolated by analytical Ag^+-TLC in system 1 showed that the MEs of the saturated acids contained no unsaturated components; the MEs of the monoenoic acids of zone 1 consisted of the component with R_f 0.8 and a component with R_f 0.73 corresponding to an 18:1 ME. A clearer separation of the 20:1 and 18:1 MEs was achieved by chromatographing the combined material on the same sorbent in system 2. In zone 2 of the monoenoic MEs the main compound was the 18:1 ME and an accompanying compound, a substance with R_f 0.8.

The fraction of dienoic MEs contained, in addition to the main spot, with R_f 0.58 (18:2) a spot with R_f 0.7.

Zone 1 of the trienoic MEs also appeared in the form of two spots — a main compound with R_f 0.31 and a substance giving a spot of smaller area with R_f 0.52. The second zone of the trienoic compounds appeared as a single spot with R_f 0.31 (18:3).

The separated MEs were then analyzed by GLC and the following composition of the fractions was obtained (%, GLC):

Acid	Saturated MEs	Monoenic MEs		Dienic MEs	Trienic MEs	
		Zone *1*	Zone *2*		Zone *1*	Zone *2*
14:0	0,3	—	—	—	—	—
15:0	Tr.	—	—	—	—	—
16:0	83,1	1,5	—	—	—	—
16:1	—	—	1,2	—	—	—
17:0	Tr.	—	—	—	—	—
18:0	16,6	—	—	—	—	—
18:1	—	8,2	83,4	3,6	—	—
18:2	—	—	—	90,0	25,9	—
18:3	—	—	—	—	72,5	100
20:0	Tr.	—	—	—	—	—
20:1	—	90,3	15,4	—	—	—
20:2	—	—	—	6,4	—	—
20:3	—	—	—	—	1,6	—

For an accurate determination of the positions of the double bonds the isolated MEs were oxidized with the periodate-permanganate reagent.

In the products of oxidative degradation of each fraction of the MEs by GLC and TLC in system 3 we detected the following mono- and dicarboxylic acids (%, GLC):

MEs of unsaturated acids	MEs of monocarboxylic acids	MEs of dicarboxylic acids	Structures of the acids
Monoenes			
Zone 1	9:0 (100%)	9:0 (4.6%)	Δ^9-18:1
		11:0 (95,4%)	Δ^{11}-20:1
Zone 2	7:0 (0,9%)	9:0 (74,3%)	Δ^9-16:1
	9:0 (99,1%)	11:0 (25,7%)	Δ^9-18:1
			Δ^{11}-20:1

Dienes	6:0 (91.4%)	9:0 (92.8%)	Δ^{9}-18:1
	9:0 (8.6%)	11:0 (7.2%)	$\Delta^{9,12}$-18:2
Trienes			$\Delta^{11,14}$-20:2
Zone 1	3:0 (TCX)	9:0 (96.2%)	$\Delta^{9,12}$-18:2
	6:0 (100%)	11:0 (3.8%)	$\Delta^{9,12,15}$-18:3
			$\Delta^{11,14,17}$-20:3
Zone 2	3:0 (TCX)	9:0 (100%)	$\Delta^{9,12,15}$-18:3

The fraction of the MEs of the saturated acids was oxidized in a similar manner. The retention on the gas-liquid chromatogram of all the peaks that were present for the total saturated acids before oxidation showed the absence of unsaturated compounds among them.

Thus, in the free fatty acids of *Helleborus abchasicus*, simultaneously with unsaturated acids of the Δ^{9} series with 18 carbon atoms there are acids with a first double bond at Δ^{11} and a chain length of 20 carbon atoms, which shows the presence in the seeds of either two different or one "improved" enzyme system for the biosynthesis of fatty acids.

A qualitatively similar set of acids is also characteristic for the triacylglycerides of the seeds of *Helleborus abchasicus* [1], but the amount of acids of the Δ^{11} series in the FFAs is higher.

According to the results of R. Kleiman et al. [2], the oil of the seeds of *Ephedra campylopoda* also contains the Δ^{11}-20:1, $\Delta^{11,14}$-20:2, and $\Delta^{11,14,17}$-20:3 acids, in amounts of 0.1, 1.4, and 2.2%, respectively.

A comparison of the lipid compositions of the roots [3] and seeds of *Helleborus abchasicus* shows that in the generative organ of this plant the main class of lipids is represented by the FFAs, which is possibly connected with its capacity for vegetating twice a year.

It must be mentioned that the fatty acid composition of the lipids of the roots of *Helleborus abchasicus* is simpler, with a predominance of the 18:2 acid, and the absence of acids with a chain length of 20 carbon atoms.

In contrast to the lipids of the seeds of some other species of *Ranunculaceae* [4], we did not detect in the seeds of *Helleborus abchasicus* any acids with Δ^{5} unsaturation, although a small amount of the $\Delta^{5,10}$-18:2 acid is present in the lipids of the rhizomes [3].

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrometer in hexane, IR spectra on a UR-10 instrument in a film, and PMR spectra on a Varian XL-100 instrument in 10% CCl_4 solution with TMS as internal standard.

GLC conditions: "Chrom 4" chromatograph with flame-ionization detector, 2.5 m × 3 mm stainless steel column filled with Chromaton N-AW-DMCS impregnated with 15% of Reoplex 400 (Chemapol) with a rate of flow of the carrier gas, helium, of 62 ml/min and of hydrogen of 60 ml/min, at a temperature of 196°C. The MEs of the monocarboxylic acids were analyzed at a temperature of 130°C.

For PTLC we used L 5/40 silica gel impregnated with 13% of $AgNO_3$, and for the TLC analysis of the low-molecular-weight monocarboxylic acids – cellulose.

Solvent systems: 1) hexane–diethyl ether (8:2); 2) benzene–petroleum ether (4:6); and 3) tert-butanol–NH_4OH–H_2O (20:1:4) [5].

To isolate the FFAs, the sum of the lipids was separated on a column containing silica gel. The fractions were eluted from the column successively with hexane and mixtures of hexane and diethyl ether in ratios of 99:1, 97:3, and 95:5, and, finally the FFA fraction was obtained with a 90:10 mixture.

As model samples for TLC and GLC we used the MEs of the acids of cottonseed and sunflowerseed oils, and methyl propionate. To reveal the spots, the analytical TLC plates were treated with iodine vapor and, in parallel, with 50% H_2SO_4 followed by heating, and the preparative plates with 50% H_2SO_4 and heating.

Oxidation with Von Rudloff's periodate-permanganate reagent was carried out as described previously [6].

For the degradation of the MEs we used 0.118 g of the saturated compounds; 0.04 g of zone 1 and 0.047 g of zone 2 of the monoenoic compounds; 0.132 g of the dienoic compounds;

and 0.045 g of zone 1 and 0.093 g of zone 2 of the trienoic compounds. Part of the fragments isolated was methylated with diazomethane and analyzed by GLC, and another part was converted by treatment with NH_4OH into the ammonium soaps and subjected to TLC on cellulose.

SUMMARY

A set of known but rarely encountered acids — the 20:0, Δ^{11}-20:1, $\Delta^{11,14}$-20:2, and $\Delta^{11,14,17}$-20:3 acids — has been found in the lipids of the seeds of *Helleborus abchasicus*, family *Ranunculaceae*.

LITERATURE CITED

1. Ts. M. Dalakishvili, Khim. Prir. Soedin., 9 (1983).
2. R. Kleiman, G. F. Spencer, F. R. Earle, and T. A. Wolff, Chem. Ind. (London), No. 31, 1326 (1967).
3. Ts. M. Dalakishvili, S. D. Gusakova, and É. P. Kemertelidze, Izv. Akad. Nauk GSSR, Ser. Khim., 1, No. 2, 146 (1975).
4. C. R. Smith, Prog. Chem. Fats Other Lipids, 16, Part 1, 139 (1970).
5. S. D. Gusakova, A. L. Markman, and A. U. Umarov, Maslo-Zhir. Promst., No. 4, 21 (1969).
6. V. M. Khasanova, S. D. Gusakova, and T. T. Taubaev, Khim. Prir. Soedin., 49 (1978).

CIRCULAR DICHROISM OF QUINOID PIGMENTS FROM FAR EASTERN REPRESENTATIVES OF THE FAMILY BORAGINACEAE

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The electronic absorption and circular dichroism (CD) spectra of ten natural and modified quinoid pigments are described. An assignment has been made of the dichroic absorption in the 250-600 nm region of the CD spectra of the pigments investigated. It has been shown that in the solution of stereochemical questions for such compounds the most informative region of the CD spectrum is the 250-350 nm interval.

It is known that the naphthoquinones found in plants of the family Boraginaceae possess a high physiological activity. From nine Far Eastern representatives of the family we have isolated and identified eleven quinoid pigments forming derivatives of shikonin [1]. In the present paper we describe the electronic absorption and circular dichroism (CD) spectra of ten natural and modified quinoid pigments.

The parameters of the electronic absorption spectra of the pigments investigated are given in Table 1. Assignment of the bands in the 250-600 nm range of the absorption spectra is known for compounds having a naphthazarin system in their structure [2]. Small bands due to electronic transitions of the quinoid and benzenoid chromophores appear in this region (Fig. 1a). An intense broad band in the visible region of the spectrum (400-600 nm), which is responsible for the color of the naphthoquinone pigments, is due to the excitation of the $p-\pi^*$ transitions of the unbound electrons of the 5,8-hydroxyls of the benzenoid chromophore. The position of the maximum of this band depends on the nature of the substituent of the side chain. Thus, the replacement of the hydrogen at C-11 by a hydroxy, a chlorine atom, or an acetoxy group shifts the maximum by 2, 6 and 9 nm, respectively, into the red region (Table 1, compounds I, II, VII, and III). The passage of the 5,8-hydroxyls into the anionic form

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