3. M. Kh. Malikova, D. A. Rakhimov, and Z. F. Ismailov, Khim. Prir. Soedin., 417 (1975).
4. M. N. Zaprometov, Biochemical Methods of Plant Analysis [in Russian], Moscow (1970), p. 296.
5. Yu. S. Ovodov and G. F. Solov'eva, Khim. Prir. Soedin., 299 (1966).
6. G. V. Buzina, O. F. Ivanova, and L. B. Sosnovskii, Khlebopek Konditersk. Promst., No. 4, 15 (1965).
7. M. P. Filippov, The Infrared Spectra of Pectin Substances [in Russian], Kishenev (1975), p. 32.
8. S. G. Kovalenko and O. D. Kurilenko, Ukr. Khim. Zh., No. 31, 175 (1965).
9. F. Smith and R. Montgomery, The Chemistry of Plant Gums and Mucilages and Some Related Polysaccharides, Reinhold, New York (1959).
10. A. G. Gorin, Khim. Prir. Soedin., 80 (1967).
11. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
12. D. McGarvie and H. Parolis, Carbohydr. Res., 88, 305 (1981).
13. Yu. S. Ovodov, Gas—Liquid Chromatography of Carbohydrates [in Russian], Vladivostok (1970).
14. H. Bouveng, H. Kiessling, B. Lindberg, and J. E. McKay, Acta Chem. Scand., 16, 615 (1962).

NEUTRAL LIPIDS OF THE SEEDS OF *Helleborus abchasicus*

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The neutral lipids of seeds of *Helleborus abchasicus* of the second vegetation phase have been studied. Fatty acid methyl esters have been found in the seed oil of *Helleborus abchasicus*. In a study of the triacylglycerols it has been established that the predominating acid in the acylglycerols is linolenic, and the main species of triacylglycerols are two-acid species with oleic and linolenic acids in position 2.

We have previously studied the chemical composition of the lipids of the roots of rhizomes of *Helleborus abchasicua* A Br. (Abkhazian hellebore; family Ranunculaceae), which is a plant endemic for the Georgian SSR and which in experiments on animals exhibits antitumoral activity [1, 2]. This plant vegetates twice a year. It has been shown that the compositions of the lipids of the epigeal organs of the two phases of vegetation are different [3].

In the present paper we give the results of a study of the chemical composition of the seeds of *H. abchasicus* from the second vegetation phase.

The seeds of the *H. abchasicus* contained 30% of lipids which consisted of an oily yellow liquid with a characteristic unpleasant odor. Some physicochemical indices of the lipids were determined: d_{20}^{20} 0.9019; acid No. 149 mg KOH; n_D^{20} 1.4729; iodine No. 152.0%.

To isolated the total fatty acids, the lipids were hydrolyzed, and the acids were converted into their methyl esters, which were analyzed by GLC on polar and medium-polar phases (Table 1).

It can be seen from Table 1 that the lipids of the *H. abchasicus* seeds have a fairly complicated fatty-acid composition. The polar phase gives good separation of the 18 : 2 and 18 : 3 components. On the moderately polar phase OV-101, the 18 : 2 and 18 : 3 components are superposed on one another but the 8 : 0, 9 : 0, 10 : 0, 11:0, 12 : 0, 13 : 0, 14 : 0, and 15 : 0 acids appear, in addition, and the total unsaturated acids decrease. We cannot explain the differences in the amounts of the 20 : 1 acid obtained with Reoplex and ethylene succinate nor the differences in the amounts of the 15 : 0, 18 : 1, and 18 : 2 acids obtained on the OV-101, as compared with the other stationary phases.

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TABLE 1. Total Fatty-Acid Composition of the Lipids of *H. abchasicus* Seeds Collected in the 1979-1980 Season (%, GLC)

Acids	Methyl esters of the total fatty acids			
	1979 raw material		1980 raw material	
	Reoplex 400	OV-101	ethylene succinate	OV-101
8:0	—	0,4	—	—
9:0	—	0,9	—	—
10:0	—	0,9	—	0 7
11:0	—	0,7	—	1,2
12:0	—	0,4	—	1,3
13:0	—	Tr.	—	Tr.
14:0	—	1,2	—	1,2
15:0	—	5,6	—	Tr.
16:0	8,3	7,9	10 4	11,3
16:1	—	Tr.	0 2	0,4
18:0	2,8	1,3	1,9	1,8
18:1	5,1	5,1	7,7	10,1
18:2	20,1	69,1	25,4	65,3
18:3	54,9	—	53,1	—
20:0	—	Tr.	—	Tr.
20:1	7 2	5,2	1,3	5,2
20:2	1,6	1,3	Tr.	1,2
20:3	Tr.	Tr.	Tr.	0,3
Σ_{sat}	11,1	19,3	12,3	17,5
$\Sigma_{unsat.}$	88,9	80,7	87,7	82,5

The total lipids were analyzed by thin-layer chromatography (TLC) in system 1, the classes of lipids being identified on the basis of literature information and comparison with model samples.

The lipids were found to contain hydrocarbons (HCs, R_f 0.98), steroid esters (SEs, R_f 0.91), fatty acid methyl esters (FAMEs, R_f 0.89), triacylglycerols (TAGs, R_f 0.81), free fatty acids (FFAs, R_f 0.46), diacylglycerols (DAGs, R_f 0.29), free sterols (FSs, R_f 0.17), and unidentified fractions with R_f 0.33, 0.23, 0.21, and 0.14.

To isolate the classes of lipids, the combined material was chromatographed on a column of silica gel.

To obtain sharper separation, the coarse fractions obtained were subjected to two-dimensional preparative TLC in systems 2 and 3. This gave the following classes (% on the weight of the total lipids): HCs, 8.0; SEs, 1.6; FAMEs, 1.5; TAGs, 9.0; FFAs, 55.2; DAGs 2.4; FSs 4.2; total of the unidentified classes, 11.2.

It must be mentioned that the quantitative ratios of the individual lipids of the *Helleborus abchasicus* seeds are fairly unusual for the seeds of higher plants. The total material is distinguished by a high content of the metabolically active class — the FFAs — and of lipophilic compounds (HCs, SEs), while the amount of the main class of neutral lipids of the seeds, the TAGs, only slightly exceeds the amount of HCs.

Of the classes of lipids mentioned, the esters have been analyzed in detail.

The fatty acids from the individual fractions of the esters were isolated by hydrolysis and, in the form of their methyl esters, were analyzed by GLC on a polar phase. The composition of the acids of the esters is given in Table 2. It can be seen from the Table that the TAGs have the most diverse compositions in terms of the acids that they contain.

The TAG fraction was studied by enzymatic hydrolysis. As a result, 2-monoacylglycerols (2-MAGs) were obtained the composition of the acids of which (see Table 2) was used to calculate the species composition of the TAGs. For some simplification of the calculations, the 12 : 0, 14 : 0, 16 : 0, and 18 : 0 acids were combined into the "saturated" (S) group and the 18 : 1 and 20 : 1 acids into the "oleic" (O) group. The position-species composition of the TAGs of the lipids of *Helleborus abchasicus* is given in Table 3. The TAG species present in an amount of less than 0.1% are not shown in the Table.

TABLE 2. Fatty Acid Compositions of the Esters of the Seed Lipids of *Helleborus abchasicus* (in %, GLC)

Acids	SEs	FAMEs	TAGs	DAGs	2-MAGs
12:0	—	Tr.	Tr.	0,2	0,8
14:0	—	Tr.	Tr.	0,7	0,9
16:0	26,6	28,2	7,6	6,6	13,5
18:0	8,2	—	1 2	2,8	0,4
18:1	10,2	—	15,8	12,5	39,6
18:2	28,4	23,4	22,7	23 6	29,5
18:3	26,6	48,4	51,3	53,6	15 0
20:1	Tr.	Tr.	1,4	Tr.	0,3
20:2	—	—	Tr.	—	—
20:3	—	—	Tr.	—	—
20:4	—	—	Tr.	—	—
Σ_{sat}	34,8	28,2	8,8	10.3	15,6
$\Sigma_{unsat.}$	65,2	71,8	91,2	89,7	84,4

TABLE 3. Position-Species Composition of the Triacylglycerides (TAGs) of the Seed Lipids of *Helleborus abchasicus* (mole %)

TAGs with P in position 2	Amount	TAGs with O in position 2	Amount	TAGs with L in position 2	Amount	TAGs with Le in position 2	Amount
PPL	0,2	POP	0,1	PLP	0 1	PLeL	0,4
PPLe	1,2	POO	0 2	PLO	0,2	PLeLe	1,0
OPP	0,3	POL	0,8	PLL	0,6	OLeO	0,1
OPD	0,3	PULe	3,0	OLO	0,1	OLeL	0,4
OPL	0,2						
OPLe	1,2	OOO	0,1	OLL	0,6	OLeLe	3,3
LPL	0,6	OOL	1,0	OLLe	2,4	LLeL	0,6
LPLe	4,1	OOLe	3,2	LLL	1,1	LeLeL	2,0
LePLe	7 5	LOL	1,5	LLLe	8 0	LeLeLe	7,2
		LOLe	10,7	LeLP	2,2		
		LeOLe	19,3	LeLLe	14,2		

The results of the calculation show that the TAGs of this oil consist of 37 species among which the di-acid TAGs predominate: LeOLe, LeLLe, etc. With respect to type composition the TAGs are subdivided into SSUs (disaturated–monounsaturated), 1.7%; SUSs, 0.2% USUs, 13.9%, SUUs, 8.4%; and UUUs 75.8%. There were no acylglycerols of the SSS type. The predominating 18 : 3 acid was distributed in the TAGs mainly in positions 1 and 3, and the 18 : 1 and 16 : 0 acids mainly in position 2, while the 18 : 2 acid was present in all three positions. The amount of 18 : 2 acid in position 2 of the TAG molecules was 43.3% of its total amount.

According to TLC (system 1), the DAG fraction was the sum of the 1,3- and 1,2- (2,3-) isomers (R_f 0.28 and 0.29, respectively). According to the intensity of coloration of the spots, the 1,3-isomer predominated in the total DAGs. The assignment of a fraction to a class of DAGs was confirmed by IR and NMR spectral analyses [4]. In comparison with the TAGs, the acids of this class contained more saturated acids, including the 12 : 0 and 14 : 0 acids.

We have earlier detected the natural 16 : 0 and 18 : 2 methyl and ethyl esters in the lipids of the epigeal organs of *Helleborus abchasicus* [1]. The acid composition of the FAMEs of the seeds was characterized by six components and by a considerably higher amount of the 16 : 0 and 18 : 3 acids (Table 2). According to the literature, fatty acid methyl and ethyl esters have recently been detected in lipid extracts of the lower fungi [5], animal and human livers, maize pollen, insects [6], fresh-water algae [7], and bacteria [8]. They are present in rare cases in the seed lipids of higher plants.

Attention is attracted by the fact that in contrast to the roots and rhizomes, only FAMEs are synthesized in the seed lipids of *Helleborus abchasicus*.

The presence of this class was confirmed by NMR and also by IR spectral analysis where

the absence of a band of the vibrations of a trans double bond at 960 cm^{-1} showed the cis configuration of the olefinic groups.

After hydrolysis of the SE fraction and the separation of the fatty acids, the sterols were purified by crystallization. The mixture obtained was finally purified by preparative TLC on silica gel in system 4. Analysis of the sterols by GLC showed that they consisted of 72.4% of β-sitosterol and 27.6% of stigmasterol, while the lipids of the epigeal organs contained 82.1% of β-sitosterol and two unidentified compounds [1].

EXPERIMENTAL

The seeds were collected in the period of the second ripening of seeds in the village of Tsebal'da in the Gul'ripshi area in 1979-1980.

The lipids were obtained from the air-dry freshly ground seeds by extraction with petroleum ether (40-70°C fraction) at room temperature by steeping for 6 h three times. The miscella obtained was filtered, and the solvent was distilled off in vacuum in a rotary evaporator.

The physicochemical indices were determined by standard methods [10].

For TLC we used the following systems: 1) heptane—methyl ethyl ketone—acetic acid (43 : 7 : 0.5); and petroleum ether—ethyl ether in ratios of (85 : 15) (2), (95 : 5) (3), and (60 : 40) (4).

For adsorption chromatography, 6 g of the total lipids was separated on a column (100 × 2 cm) containing 70 g of L 100/250 silica gel. Elution of the column successively with hexane and hexane—diethyl ether mixtures in ratios of 99 : 1, 97 : 3, 95 : 5, 90 : 10, 85 : 15, and 80 : 20 yielded 8.0% of HCs, 1.6% of SEs, 1.5% of FAMEs, 9.0% of TAGs, 55.2% of FFAs, 2.4% of TAGs, and 4.2% of FSs, and elution was carried out finally with diethyl ether. This gave compounds with R_f 0.33, 0.23, 0.21, and 0.14 in a total amount of 11.2%.

The fractions were purified by TLC on KSK silica gel (150-200 mesh) in systems 2 and 3.

Enzymatic hydrolysis of the TAG fraction was carried out as described by Markman et al. [11]. The position-species composition of the TAGs was calculated by a modification of Coleman's method [12], and the quantitative distribution of the FAs in the second positions of the TAGs was determined by means of formulas given by Panekina et al. [13].

The hydrolysis of the total lipids, FAMEs, TAGs, DAGs, and 2-MAGs was carried out with a 10% methanolic solution of KOH with vigorous shaking for 30 min. The methanol was distilled off in vacuum at 40°C on the water bath and the residue was diluted with water. The soap was decomposed under a layer of ether with 10% H_2SO_4. The combined ethereal extracts of the mixed fatty acids were washed and were dried with Na_2SO_4, and the solvent was driven off in a rotary evaporator. The fatty acids were converted into their methyl esters with diazomethane.

The fractions of fatty acids, SEs, FAMEs, TAGs, DAGs, and MAGs were analyzed on a Chrom-4 chromatograph with a flame-ionization detector in a column 2.5 m × 0.4 cm filled with Chromaton N-AW-DMES bearing 15% of Reoplex-400 (Chemapol) at a rate of flow of the carrier gas, He, of 62 ml/min, and of H_2 of 60 ml/min.

The total FAMEs were analyzed on the Chrom-4 chromatograph and on a Pye-Unicam series 105 chromatograph with a flame-ionization detector in an 8-m capillary column filled with 3% of OV-101 at 200°C with a rate of flow of the carrier gas, He, of 40 ml/min.

The acids were identified from C_{sp} relative to the 18 : 0 acid and from their equivalent chain lengths (ECLs) [14].

The alkaline hydrolysis of the SE fraction was performed with 25% methanolic sodium hydroxide solution at the boil with stirring under reflux for 3 h. The methanol was distilled off in vacuum, the residue was diluted with water, and the sterols were extracted three times with diethyl ether. The combined ethereal extracts were washed and were dried with Na_2SO_4, and the solvent was distilled off in a rotary evaporator. The residue was concentrated and was recrystallized three times from methanol. The sterols were analyzed on a Varian chromatograph with a flame-ionization detector using a 2.0 m × 0.4 cm column filled with 3% of SE-30 on Chromosorb W at 200°C with a rate of flow of the carrier gas, He, of 50 ml/min and of H_2 of 50 ml/min. IR spectra were taken on a UR-10 instrument using the substances in the form of films.

NMR spectra were taken on a Varian XL-100 spectrometer (CCl_4, internal standard TMS, τ scale).

SUMMARY

The neutral lipids of the second vegetation phase of *Helleborus abchasicus* are enriched with hydrocarbons and free fatty acids. Fatty acid methyl esters have been detected in the seed oil of this plant.

LITERATURE CITED

1. Ts. M. Dalakishvili and É. P. Kemertelidze, A Phytochemical Investigation of the Abkhazian Hellebore [in Russian], Tbilisi (1978).
2. N. G. Andguladze, M. D. Gedevanishvili, and I. S. Sikharlidze, Biologically Active Substances from the Georgian Flora [in Russian], Tbilisi (1976), pp. 14, 171.
3. Ts. M. Dalakishvili, Izv. Akad. Nauk GSSR, Ser. Biol., 3, 238 (1977).
4. T. V. Panekina, S. D. Gusakova, M. Ya. Tabak, and A. U. Umarov, Khim. Prir. Soedin., 44 (1978).
5. N. N. Stepanichenko, S. D. Gusakova, A. A. Tishchenko, S. Z. Mukhamedshanov, A. U. Umarov, and O. S. Otroshchenko, Khim. Prir. Soedin., 431 (1976).
6. J. M. Chu, M. A. Wheeler, and C. E. Holmlund, Biochem. Biophys. Acta, 18, 270 (1972).
7. V. M. Khasanova, S. D. Gusakova, T. T. Taubaev, Khim. Prir. Soedin., 49 (1978).
8. B. Maudinar and J. Villaureix, Phytochemistry, 16, 1299 (1977).
9. T. V. Panekina, S. D. Gusakova, and T. T. Taubaev, Khim. Prir. Soedin., 174 (1978).
10. Handbook on Methods of Investigation, Technical Control, and the Accounting of Production in the Oil and Fats Industry [in Russian], Leningrad, Vol. I, (1967).
11. A. L. Markman, T. V. Chernenko, and A. U. Umarov, Prikl. Biokhim. Mikrobiol., 5, 616 (1965).
12. M. N. Coleman, Biochim. Biophys. Acta, 67, 246 (1963).
13. T. V. Panekina, S. D. Gusakova, E. M. Zalevskaya, and A. U. Umarov, Khim. Prir. Soedin., 618 (1979).
14. R. V. Goloviya, V. P. Uralets, and T. E. Kuzmenko, Zh. Anal. Khim., 32, 340 (1977).

AROMATIC METABOLITES OF LICHENS OF THE FAMILY PARMELIACEAE.

I. DEPSIDONES

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(+)-Usnic acid, the depside atranorin, and the depsidones fumarprotocetraric, α-alectoronic, and α-collatolic acids, and also the phenoxyisocoumarin derivatives β-alectoronic and β-collatolic acids have been isolated by extraction with hexane and chloroform from lichens *Asahinea chrysantha, A. scholanderi,* and *Parmelia birulae*. This is the first time that β-alectoronic acid has been detected in lichens. The structures of the compounds have been established by spectral and chemical methods.

Continuing a chemical study of lichens of the family Parmeliaceae, from two of its representatives *Asahinea chrysantha* (Tuck.), W. Culb. et C. Culb. and *Parmelia birulae* Elenk., together with alectoronic acid (I) [1], we have isolated a previously undescribed compound isomeric with it. For this we propose the name β-alectoronic acid (III), to distinguish it from the acid known previously, which we propose to rename α-alectoronic acid.

We have also studied the lichen *Asahinea scolanderi* (Llano) W. Culb. et C. Culb, which, in addition to the α-collatolic acid (II) known for this species [2], also contained β-collatolic acid (VI). The latter was first obtained by Asahina by the alkaline hydrolysis of α-

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