NEUTRAL LIPIDS OF THE SEEDS OF *Phlomis oreophilla* AND *Ph. regelii*

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The lipid and fatty acid compositions of the seeds of *Phlomis oreophilla* and *Ph. regelii* (family *Labiatae*) have been studied. The lipids of *Ph. oreophilla* are enriched with linoleic acid and those of *Ph. regelii* with oleic acid. Laballenic acid is present in the total acids of the triacylglycerides and the free fatty acids of both species, being concentrated in the free acids. The epoxy acids of *Ph. oreophilla* are represented by 9,11-epoxystearic, vernolic, coronaric, and 15,16-epoxylinoleic acids.

In the course of a systematic investigation of the lipids of plants of the family *Labiatae*, we have studied the lipids of the seeds of *Phlomis oreophilla* Kar. et Kir and of *Ph. regelii* M. Pop.

There is information in the literature on the composition of fatty acids of the lipids of of the seeds of twelve species of this family [1, 2]. Of them, the neutral lipids of three species were enriched with the 18:2 acid and those of the other with 18:1 acid. Almost all the species studied contained in their fatty acids, together with the 18:2(9, 12) acid (oleic), the 18:2(5, 6) acid isomeric with it (laballenic) in amounts of from 6 to 20%. The total fatty acids of eight of the species contained up to 4% of unidentified compounds [1].

The lipids of the seeds of *Ph. oreophilla* and *Ph. regelii* have not been studied previously.

The yields of hexane extracts from the comminuted seeds amounted 17.6% for *Ph. oreophilla* and 12.0% for *Ph. regelii*.

The IR spectra of the total lipids of the two species and of the methyl esters (MEs) of the fatty acids isolated from them contained the absorption bands of allenic groups (1970 cm^{-1}) and of hydroxy groups (3480 cm^{-1}), and the same samples of the lipids of *Ph. oreophilla* also showed the absorption bands of an epoxide ring (853, 890, 920, 1285 cm^{-1}).

The total lipids and MEs of the acids of the two species had maxima in their UV spectra at λ_{max}^{hexane} 232 nm, which characterizes the presence of a conjugated dienic system in the acids.

For a preliminary analysis of the lipid compositions, extracts were subjected to TLC on Silufol in system 1.

The following were detected qualitatively in hexane extracts of the two species: hydrocarbons (HCs, R_f 0.98), carotenes (R_f 0.95), normal triacylglycerols (n-TAGs, R_f 0.80), free fatty acids (FFAs, R_f 0.40), triterpene compounds (R_f 0.35), monohydroxyacyldiacylglycerols (hydroxy-TAGs, R_f 0.32), sterols (R_f 0.29), and glycolipids (GLs, R_f 0.03). The extract from *Ph. oreophilla* contained, in addition to the classes mentioned, monoepoxyacyldiacylglycerols (ep-TAGs), and monooxoacyldiacylglycerols (oxo-TAGs) (a single spot with R_f 0.46).

The individual classes of lipids were isolated by chromatographing extracts of *Ph. oreophilla* and *Ph. regelii* on a column of silica gel. This gave for *Ph. oreophilla* and *Ph. regelii*, respectively (%): 1.1 and 0.3 HCs; 91.1 and 92.4 n-TAGs in combination with carotenes; 2.1 and 2.4 of FFAs; 4.7 and 4.8 of hydroxy-TAGs together with triterpene compounds and sterols; and 0.6 and 0.1 of GLs.

The sum of the ep-TAGs and oxo-TAGs was isolated from the *Ph. oreophilla* extract in an amount of 0.4%.

The pigments of the lipids of *Ph. oreophilla* corresponded with respect to their absorption in the UV spectrum to α-carotene (λ_{max}^{hexane} 420, 445, 473 nm) and in the case of *Ph. regelii* to β-carotene (λ_{max}^{hexane} 425, 451, 478 nm) [3].

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TABLE 1. Fatty Acid Compositions of the Acyl-Containing Lipids of the Seeds of *Phlomis oreophilla* and *Ph. regelii* (%, GLC)

Sample	14:0	16:0	16:1	18:0	18:1	18:2 (9,12)	18:2 (5,6)	18:3
Phlomis oreophilla								
Sum of the lipids	Tr.	3,4	0,6	1,4	35.1	56,5		3,0
n-TAGs	Tr.	2,5	0,3	0,6	36.4	50,5	7,1	2,6
ep-TAGs + oxo-TAGs	Tr.	5,3	Tr.	1,2	27,9	60,0	—	5,6
FFAs	Tr.	4,8	Tr.	1,2	30,8	41,4	15,2	3,6
hydroxy-TAGs	Tr.	4,4	0,3	1,3	33,0	57,0	—	4,0
Phlomis regelii								
Sum of the lipids	Tr.	4,0	0.4	0,6	69,2	25,8		Tr.
n-TAGs	Tr.	4,6	Tr.	0,6	70,5	9,8	13,9	0,6
FFAs	Tr.	5,8	0,6	2,0	43,7	21,9	22,3	3,7
hydroxy-TAGs	0,5	5,7	0,9	1,5	50,8	39,5	—	1,1

The sterols were separated from the hydroxy-TAGs by precipitation from methanol [4] and were not studied further.

The qualitative reaction of the most polar fraction of the two extracts with the Vaskovsky reagent for phospholipids was negative, and that with α-naphthol for sugars was positive [5, p. 158]. In TLC on silica gel in system 2, the fraction had one spot with R_f 0.72 which appeared, after spraying with 50% H_2SO_4 followed by heating, in the form of a red spot which, in combination with the positive qualitative reaction permitted us to assign to it the glycosylsterols [5, p. 268].

From the main acyl-containing lipids, alkaline hydrolysis liberated fatty acids, which, in the form of their MEs were analyzed by chromatographic and spectra methods.

The amount of allenic acid was determined after the separation of the MEs of the fatty acids composing the TAGs and FFAs by preparative Ag^+-TLC on the basis of the weight ratio of the individual fractions and their subsequent GLC analysis [6].

The acids of the epoxy- and hydroxy-TAGs in the form of their MEs were separated by preparative TLC in system 3 into the MEs of the unsubstituted acids (R_f 0.90) and the sum of the MEs of the fatty epoxy and oxo acids (R_f 0.50). The compositions of the unsubstituted fatty acids of the main lipid classes are given in Table 1.

It can be seen from Table 1 that the lipids of the two species differed appreciably with respect to their contents of the 18:1 and 18:2 acids.

The lipids of *Ph. oreophilla* were enriched with the 18:2 acid, and those of *Ph. regelii* with the 18:1 acid. In the n-TAGs of *Ph. oreophilla*, of the two isomeric 18:2 acids, the 18:2(9, 12) isomer predominated, while in the analogous class of *Ph. regelii* it was the 18:2(5, 6) acid. It must be mentioned that the amount of the allenic acid in the FFAs of the two species is almost twice as great as in the n-TAGs..

The 18:2(5, 6) acid was not detected among the acids of the other lipid classes.

The composition and structures of the n-TAGs of the two species of plants were determined from the results of stereospecific analysis [7].

We were unable to separate the oxo acids from the epoxy acids because of their very small amount. The sum of the MEs of the epoxy and oxo acids was analyzed by GLC on a polar phase. Four peaks were detected on the chromatogram with the following characteristics:

MEs of the acids	RRT (to 16:0)	ECL	%
Epoxysterate (ep- 18:0)	7,36	23,57	27.1
Vernolate + coronarate (ep- 18:1)	8,54	24,14	53,3
Epoxylinoleate (ep- 18:2)	11,28	25,19	4.0
Oxooleate (oxo 18:1)	14,61	26,17	15,6

The two peaks with relative retention times (RRTs) of 8.54 and 11.28 were identified as the MEs of the ep-18:1 and ep-18:2 acids by comparison with the RRTs of the MEs of epoxy acids of known structure [6]. To identify the other peaks we calculated their equivalent chain lengths (ECLs) [8], and the ECLs were compared with literature figures. The peak with an ECL of 23.57 corresponds to epoxysterate [9]. The compound with an RRT of 14.61 was assigned provisionally to the ME of an oxo-18:1 acid, since its ECL of 26.17 is intermediate between the ECLs of the ME of the oxo-18:0 acid (24.50) and the oxo-18:2 acid (29.00) [9].

We have found no information on the mobilities of the MEs of oxooleic acids under GLC conditions in the literature.

The correctness of the identification of the acids was confirmed by the mass-spectrometric analysis of the MEs of the trimethylsilyloxy derivatives of the dihydroxy acids (di-TMS derivatives) that were obtained from the epoxy acids [6]. The MEs of the oxo acids were not separated from the di-TMS derivatives.

The mass spectrum of the di-TMS derivatives contained weak peaks of molecular ions with m/z 474, 472, and 470, which correspond to initial MEs of the ep-18:0, ep-18:1, and ep-18:2 acids.

The sum of the isomeric MEs of the 9,10-epoxyoctadec-12-enoic and 12,13-epoxyoctadec-9-enoic acids agrees with the mass numbers of the main fragments of the MEs of the ep-18:1 acid, and the ME of the 15,16-epoxyoctadeca-9,12-dienoic acid agrees with the mass numbers of the ME of the ep-18:2 acid [6].

Fragments with m/z 215 and 259 are characteristic for the ME derivative of epoxystearic acid in the chain of which the epoxy ring is located between carbon atoms 9 and 10 [10].

The presence in the spectrum of a strong peak of a rearrangement ion with m/z 310 formed in the decomposition of the di-TMS derivative of the ME of the dihydroxy-18:2 acid [6] prevented the detection of the molecular ion of the ME of the oxo-18:1 acid ($M^+ - 310$). However, the spectrum contained the peaks of ions with m/z 295 ($M^+ - 15$), 279 ($M^+ - 31$) 278 ($M^+ - 32$), and 250 ($M^+ - 60$) containing no Si atoms, which we assigned to the ME of an oxooctadecenoic acid.

Intensive fragments with m/z 293, 225, and 185, likewise not containing Si, give grounds for assuming that the oxo group and the double bond are located in the molecule of the ME of the oxo-18:1 acid between atoms 9 and 13 of the chain, counting from the carboxy group.

Since the IR spectrum of the sum of the lipids and of the MEs of the acids lacked the absorption bands of trans-olefinic bonds while the absorption bands of an oxirane ring were detected in the isolated ep-TAGs at 810, 825, and 850 cm^{-1}, we concluded that the double bonds and the epoxide rings in the acids have the cis configuration.

Thus, the lipids of the seeds of *Ph. oreophilla* contain cis-9,10-epoxyoctadecanoic, cis-9,10-epoxyoctadec-12-enoic (coronaric), cis-12,13-epoxyoctadec-9-enoic (vernolic), and 15,16-epoxyoctadeca-9,12-dienoic (epoxylinoleic) acids.

It must be mentioned that the weight percentages of epoxy acids given above do not reflect the true amounts of these compounds in the total material quite accurately, since it is known from literature sources that about 4% of epoxy acids undergoes change in the process of GLC analysis [11]. Nevertheless, these results permit us to compare not only the qualitative set but also the quantitative ratios of the individual epoxy acids of the lipids of *Ph. oreophilla* and of another plant from the same family – *Galeopsis bifida*.

In the lipids of the seeds of *G. bifida* we likewise detected oxo fatty acids and a qualitatively similar amount of unsaturated epoxy acids [6]. The amount of ep-18:2 in the total material was almost twice as great as that of the isomeric ep-18:1 acids. The saturated analog (ep-18:0) has not been reliably detected in the lipids of *G. bifida*.

At the same time, of the three epoxy acids of *Ph. oreophilla* those present in largest amount are the isomeric ep-18:1 and ep-18:0 acids.

The hydroxyacylglycerols of the two species of plants were saponified by alkaline hydrolysis and the acids were isolated without preliminary separation of the triterpene compounds. According to TLC on Silufol in system 4, the hydrolysates of both samples contained triterpene compounds (R_f 0.80) and unhydroxylated (R_f 0.78) and hydroxylated (R_f 0.52) fatty acids. The triterpene compounds gave a specific coloration on treatment with 50% H_2SO_4 [11]. By prepara-

tive TLC on silica gel in the same system, two types of fatty acids were isolated which were then converted into their MEs. The compositions of the unhydroxylated acids of the hydroxy-TAGs are given in Table 1.

The methyl esters of the hydroxy acids of the two species of *Phlomis* were revealed on TLC in system 3 in the form of two spots corresponding to the MEs of hydroxymonoenoic acids (R_f 0.76) and hydroxyoctadecadienoic acids with conjugated systems of double bonds (R_f 0.73) [12]. The presence of a conjugated system of double bonds in the MEs was, as stated above, confirmed by UV spectroscopy. The structures of the hydroxy acids were not studied in detail.

Thus, the differences in the composition of the unsubstituted fatty acids of the lipids of the seeds of the two species of *Phlomis* are small. Common for them is the presence of laballenic acid. However, these species differ profoundly with respect to the quantitative ratio of the 18:1 and the isomeric 18:2 acids. The neutral lipids of both species contain hydroxy-TAGs. Epoxy- and oxo-TAGs are present only in the lipids of *Ph. oreophilla*.

EXPERIMENTAL

The seeds of *Ph. oreophilla* and of *Ph. regelii* were collected in the environs of Santash and of Darbaza (KazSSR) in 1980.

IR spectra were taken on a UR-10 instrument in a film, UV spectra on a Hitachi spectrophotometer in hexane, and mass spectra on a MKh-1310 instrument.

GLC analysis was performed on a Chrom 4 instrument with a flame-ionization detector, using a 2500 × 4 mm column filled with 17% of ethylene succinate on Chromaton N-AW-DMCS. The temperature of the column was 194°C for the MEs of the unsubstituted fatty acids and 215°C for those of the substituted fatty acids.

Column chromatography was performed on type L 100/160 silica gel as described previously [6].

For TLC we used type L 5/40 silica gel with the addition of 10% of $CaSO_4$ and the following solvent systems: 1) hexane–diethyl ether–CH_3COOH (70:30:1); 2) chloroform–methanol–H_2O 65:25:4); and hexane–diethyl ether; 3) (8:2); 4) (6.5:3.5).

The lipids were extracted and hydrolyzed by a known method [6].

The main lipid classes were identified by comparison with known samples from their chromatographic mobilities and spectral characteristics.

The qualitative reactions for epoxy groups with picric acid and for oxo groups with 2,4-dinitrophenylhydrazine were carried out as described previously [6], and for phospholipids and GLs as described by Kates [5].

The opening of the epoxide ring and the production of trimethylsilyloxy derivatives were performed as described by Gunstone and Shuler [13].

SUMMARY

1. The neutral lipids of the seeds of *Phlomis oreophilla* are similar with respect to the set of their main components and the presence of monohydroxyacyldiacylglycerols to the lipids of *Ph. regelii*, but unlike the latter include monoepoxyacyl- and monooxoacyldiacylglycerols.

2. The lipids of *Ph. oreophilla* are enriched with linoleic and those of *Ph. regelii* with oleic acid. Laballenic acid is present in the total acids of the triacylglycerols and in in the free fatty acids of both species, being concentrated in the free acids.

3. The epoxy acids of *Ph. oreophilla* are represented by 9,10-epoxystearic, vernolic, coronaric, and 15,16-epoxyoctadeca-9,12-dienoic acids.

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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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