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SEED LIPIDS FROM *Pulmonaria obscura*

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The compositions of lipids, fatty acids (FAs), and triacylglycerides (TAG) from seeds of Pulmonaria obscura *growing in the Republic of Bashkortostan were studied. The contents of neutral (NL) and polar lipids and the composition of FAs of all acyl-containing NL classes were identified and determined. The largest amounts of polyunsaturated FAs were concentrated in total NL (88.9%, including γ-18:3, 24.3; α-18:3, 10.4; and 18:4, 3.5%) and TAG (85.3%, including γ-18:3, 21.4; α-18:3, 10.7; and 18:4, 3.5%). The speciation (39 species > 0.5%) and type (three types) of TAG were established using pancreatic hydrolysis.*

Keywords: *Pulmonaria obscura* Dumort., Boraginaceae Juss., lipids, fatty acids, triacylglycerides.

Lipids from seeds of *Pulmonaria obscura* Dumort. that were collected in a sparse conifer–broadleaf forest in the vicinity of Zuyakovo, Beloretsk District, Republic of Bashkortostan (RB), were studied in continuation of a search in the RB for promising plant sources of lipids with ω-3, ω-6, and polyunsaturated fatty acids (FAs). Three species of the genus *Pulmonaria* (Boraginaceae Juss.) grow in Russia. Unspotted lungwort (*P. obscura*) occurs in central and mainly eastern Europe. The eastern distribution of *P. obscura* stretches to the Urals. The plant was declared endangered at certain British sites of northern Europe [1, 2]. The plant is widely used in folk medicine; possesses coating, anti-inflammatory, diuretic, expectorant, hemostatic, and analgesic activity; regulates endocrine glands; normalizes metabolism; and is used to treat lung diseases [3]. The chemical composition of the plant is insufficiently studied. Pyrrolizidine alkaloids were observed in the roots (0.026 mg/g of dry mass) and rhizomes (0.158). GC-MS identified in them lipoximine, intermedine, lycopsamine and their O^7-derivatives characteristic of *Borago* and *P. officinalis* (Boraginaceae) [4, 5].

Lipids from *P. obscura* seeds have not been reported except for the composition of FAs from seeds of plants grown in the botanical garden of the University of Goettingen in Germany [6]. Neutral lipids (NL) were extracted from *P. obscura* seeds by hexane; polar lipids, by $CHCl_3$–MeOH. Table 1 lists the lipid contents in the starting raw material.

Table 1 presents the FA compositions of NL and polar glyco- (GL) and phospholipids (PL) from *P. obscura* seeds. The set of FA components of the PL was more varied and numbered 17. NL had the greatest total amount (88.9%) of unsaturated FAs, including polyunsaturated FAs (PUFAs). The major saturated FA was 16:0, the contents of which varied from 7.6% in NL to 25.8% in PL. Acids α-18:2, γ-18:3, α-18:3, and 18:4 were identified in the PUFAs. The α-18:2 content was comparable in all three lipid groups (from 33.6% in NL to 36.6% in PL). NL had the greatest amounts of γ-18:3, α-18:3, and 18:4 acids.

The FA composition of seed oil from *P. obscura* growing in the botanical garden of the University of Goettingen was reported [6]. A comparison of our data (Table 1) and those in the literature showed that the oil content of seeds from plants growing in the RB was 1.4 times greater (35.0 vs. 24.3%). The oil contained 1.3 times more total unsaturated FAs (88.9 vs. 67.9%), including 1.4 times more PUFAs (71.8 vs. 51.0%).

Column chromatography, PTLC, specific reactions, and physicochemical and chemical methods isolated and identified from NL six lipid classes [7] with TAG having the greatest content. The FA composition was determined for all acyl-containing lipid classes (Table 2). A total of 18 acids were identified in free FAs (FFAs), diacylglycerides (DAG), and monoacylglycerides (MAG). Sterol esters (SEs) (47.6%) and FFAs (54.0%) contained the most saturated FAs with respect to total content; TAG (85.3%) and MAG (72.9%), unsaturated FAs. The main part of PUFAs occurred in TAG (α-18:2, 33.2; γ-18:3, 21.4; α-18:3, 10.7; and 18:4, 3.2%) (Table 2) and corresponded to their contents in the NL FAs (Table 1) in which the TAG fraction was 94.6% (Table 2).

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TABLE 1. Content and Composition of Fatty Acids of Neutral and Polar Lipids from *P. obscura* Seeds, % of Total FAs

Acid	NL (35.0)*	GL (0.4)	PL (0.2)	Acid	NL (35.0)*	GL (0.4)	PL (0.2)
12:0	0.1	0.6	0.9	**α-18:3**	**10.4**	**7.4**	**6.1**
14:0	0.1	1.1	0.4	**18:4**	**3.5**	**2.2**	**1.3**
15:0	–	0.7	0.7	20:0	0.3	1.9	1.1
16:0	**7.6**	**14.9**	**25.8**	20:1	2.2	0.3	0.1
16:1	0.1	0.8	0.7	22:0	0.7	1.2	0.3
16:2	–	2.3	0.1	22:1	–	–	0.2
17:0	–	1.7	0.3	24:0	0.5	1.5	1.1
18:0	1.8	3.5	4.5	$\Sigma_{sat.}$	11.1	27.1	35.1
18:1	14.8	13.8	6.5	Σ_{unsat}	**88.9**	**72.9**	**64.9**
α-18:2	**33.6**	**30.1**	**36.6**	including			
γ-18:3	**24.3**	**16.0**	**13.3**	PUFAs	**71.8**	**55.7**	**57.3**

*Amounts of lipids calculated for air-dried seed mass.

TABLE 2. Content and Composition of Fatty Acids of Acyl-containing Neutral Lipids from *P. obscura* Seeds, % of NL Mass

Acid	SEs (0.5)	TAG (94.6)	FFA (1.4)	DAG + st. (3.2)*	MAG (0.3)
	% of total FAs				
12:0	0.3	–	0.6	0.8	0.4
14:0	0.4	0.2	3.6	0.6	1.1
15:0	1.2	Tr.	0.7	1.7	1.8
16:0	**7.9**	**7.6**	**30.3**	**17.7**	**14.5**
16:1	1.1	0.1	1.2	0.8	0.6
17:0	0.3	–	1.2	0.5	1.1
18:0	2.3	1.9	7.5	4.7	4.0
18:1	**11.2**	**16.3**	**32.9**	**24.3**	**18.7**
α-18:2	**7.3**	**33.2**	**4.3**	**24.9**	**31.8**
γ-18:3	**17.8**	**21.4**	**0.3**	**7.4**	**12.7**
α-18:3	**3.3**	**10.7**	**0.5**	**3.9**	**6.3**
18:4	**9.8**	**3.2**	**0.8**	**0.9**	**1.6**
20:0	3.3	2.7	4.4	4.1	2.3
20:1	1.9	0.3	1.7	0.2	0.3
22:0	3.5	1.1	1.0	1.1	0.3
22:1	–	0.1	4.3	3.2	0.9
23:0	–	–	3.2	0.9	0.3
24:0	28.4	1.2	1.5	2.3	1.3
$\Sigma_{sat.}$	47.6	14.7	54.0	34.4	27.1
$\Sigma_{unsat.}$	**52.4**	**85.3**	**46.0**	**65.6**	**72.9**
including PUFAs	**38.2**	**68.5**	**5.9**	**37.1**	**52.4**

*Contained 1.2% sterols.

Hydrolysis by porcine pancreatic lipase, which affects specifically the 1- and 3-positions of TAG to form 1,2- and 2,3-DAG and MAG was used to establish the FA composition in the middle position of TAG (Table 3) [8, 9]. The selectivity factor (SF) (Table 3) and speciation and type of TAG composition (Table 4) were calculated based on the results for the FA compositions in TAG, MAG, and DAG. Thus, FAs esterified in the TAG 2-position formed in the following order: γ-18:3 > 18:4 > 18:2 = 18:1 > 16:0 + 18:0 > α-18:3.

A total of 39 different TAG (0.5%, without accounting for isomers) were found (Table 4). Three TAG type compositions were identified, the greatest of which were triply unsaturated UUU at 76.5%. The total amount of types with an unsaturated acid in the TAG 2-position was 91.3%; TAG species with PUFAs in the 2-position, 79.2%; i.e., greater than two thirds of the species (or 86.7% of 91.3%). Our results agreed with those obtained earlier [10] in that the middle position of the plant TAG was occupied primarily by C_{18} unsaturated FAs, C_{18} PUFAs in our instance.

TABLE 3. Selectivity Factor for Position 2 in Triacylglycerides from *P. obscura* Seeds

Sample	Calculated parameter	Acid, % of total FAs					
		P, 16:0 + 18:0	O, 18:1	L, 18:2	γ-Le (γ-18:3)	α-Le (α-18:3)	St., 18:4
TAG	a	7.7	15.3	34.1	26.6	12.0	4.3
MAG	b	4.3	13.8	32.3	39.1	5.0	5.5
1,3-Positions	3a–b/2	9.4	16.1	35.0	20.3	15.5	3.7
Selectivity factor (F), b/a		0.56	0.90	0.94	1.46	0.41	1.27

TABLE 4. Position-Speciation and Type Compositions of Triacylglycerides from *P. obscura* Seeds

TAG, species	Content, %	TAG, species	Content, %	TAG, species	Content, %
LeLe*L	11.4	LLP	2.0	PLSt	1.0
LLLe	8.2	LOL	1.7	POL	0.8
LeLeLe	5.7	LeOLe	1.8	LLSt	0.8
LLeL	5.4	LOO	1.6	LStL	0.7
LeLeO	5.0	LeOO	1.6	LeStLe	0.7
LLeO	5.0	LStLe	1.4	OLeSt	0.6
LLL	4.0	StLeL	1.2	OPLe	0.6
LeLLe	4.2	PLeO	1.2	LePLe	0.6
LeLO	3.8	StLeLe	1.2	OStL	0.6
OLL	3.6	OLeO	1.1	OStLe	0.6
LOLe	3.6	POLe	1.0	LPL	0.5
LLeP	2.8	PLO	1.0	OPL	0.5
PLeLe	2.8	StLLe	1.0	Total	93.5%
LeLP	2.2				

Speciation of TAG: UUU**, 76.5%; SUU + UUS, 14.8%; USU, 2.2%; SUS, USS + SSU, and SSS***, not detected. *Le = γ- + α-Le (Table 4); *U, unsaturated FA; **S, saturated FA.

Thus, the high oil content (up to 35%) and high PUFA content (up to 73%) with a ratio of ω-3 to ω-6 acids of 1:3.5 according to experimental results for the lipids of *P. obscura* seeds provide a basis to believe that the seed lipids are a rich source of PUFAs. The plant is promising for introduction as a renewable plant raw material, the processed products of which could be used in many areas.

EXPERIMENTAL

Equipment at the Khimiya CUC, UfIC, RAS, was used for the physicochemical studies, including GC analysis. GC analysis of FA methyl esters used a GC-2014 chromatograph (Shimadzu) with an Omegawax TM 250 capillary column (30.0 m × 0.25 mm, Supelco), poly(ethylene glycol) L stationary phase (0.25 μm), column temperature 205°C, vaporizer 250°C, detector 260°C, and He carrier gas at flow rate 30 mL/min. Seed lipids were extracted in a Soxhlet apparatus (NL, hexane; PL, $CHCl_3$–MeOH, 2:1). Non-lipid alcohol-soluble constituents (carbohydrates, amino acids, etc.) were removed by treating the condensed PL extract with NaCl solution (0.1%). GL and PL were separated by PTLC in Me_2CO dried over K_2CO_3. NL classes were separated, purified, and identified using hexane–Et_2O [9.5:0.5 (1), 9:1 (2), 8:2 (3), 7:3 (4)] on Silufol plates as reported before [11].

NL were separated into classes using CC over Silica gel L for chromatography (100–160 mesh, Lachema) with a 1:50 ratio (by mass) of extractant to adsorbent and a 1:20 ratio of column cross section to adsorbent layer height. The column was eluted by hexane–Et_2O (0–100%). Elution of NL classes was monitored by ATLC using solvent systems 1–4. Fractions were purified by preparative TLC on glass plates (20 × 20 cm) of MN-Kieselgel G silica gel (Macherey-Nagel GmbH & Co. KG) using solvent systems 1–4. The amount of each class was estimated gravimetrically.

Alkaline hydrolysis of acyl-containing lipid fractions used KOH (10%) in MeOH (1:10) at 60°C for 30 min; triterpene esters, KOH (20%) in MeOH (1:10) [12].

FA methyl esters were prepared via methylation by diazomethane [13].

Pancreatic hydrolysis of triacylglycerides was performed as before with lipase isolated from porcine pancreas (Sigma).

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