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LIPIDS OF *Aconitum septentrionale* SEEDS

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Neutral, polar glyco-, and phospholipids of Aconitum septentrionale *K. (northern aconite) are studied. The fatty-acid composition of acyl-containing lipid classes is determined. The oil is demonstrated to possess biological activity. It stimulates reparative regeneration of skin and is more effective than dog-rose oil.*

Key words: *Aconitum septentrionale*, neutral lipids, glycolipids, phospholipids, biological activity.

The *Aconitum* genus includes 300 species that are widely distributed in the northern hemisphere. Extracts of the roots and aerial part of plants from this genus are used in folk medicine as a pain reliever and spasmolytic agent [1, 2]. Roots of *A. septentrionale* are a source of the diterpene alkaloid lappaconitine, which is used to prepare the antiarrhythmic preparation allapinine. The aerial part and seeds are processing wastes. We studied seed lipids of this plant in order to use the material fully. Seeds were collected after ripening in the vicinity of Ufa.

Neutral lipids (NL) were extracted from ground seeds by petroleum ether. The total NL content was 33.1% of their mass. Thus, the seeds have average oil content [3]. The individual components of NL were obtained by preparative TLC with subsequent rechromatography of the resulting fractions.

The pulp remaining after removal of NL was treated with $CHCl_3$—CH_3OH mixtures to isolate polar lipids (PoL), glyco- (GL), and phospholipids (PL). However, these solvent systems extracted from the seeds not only lipids but also alkaloids, which were separated from the PoL by extraction with dilute H_2SO_4. The PoL were purified from traces of NL by column chromatography (CC) over silica gel. The yield of PoL was 0.8% of the seed mass. GL were separated from PL by precipitation of PL from cold acetone. The GL were also divided into two groups by this operation: glycoglycerolipids remained in the acetone whereas sphingolipids precipitated with the PL. The sphingolipids were separated from PL using CC over silica gel. The lipids were further divided using preparative TLC. Each class was identified by IR spectroscopy, products of base and acid hydrolyses, and comparison of chromatographic mobility with that of model samples using specific developers.

The NL contain seven classes of compounds (Table 1). Like in seed lipids of other oil-bearing plants, the main component is TAG (89%). Chromatography-mass spectrometry identified sterols in the free-sterol (FS) fraction (Table 2) and sterols and triterpene alcohols in the triterpene-ester (TE) fraction after hydrolysis. The sterols in both fractions contain mostly β-sitosterol; the triterpene alcohols, α-amyrin. The 5α-stigmasta-3-one and 3-ketours-12-ene found in the TE may be formed during strong base hydrolysis or in the chromatograph source during GC-MS of the alcohols.

Table 1 shows that the GL consist of two groups of compounds: glycoglycerolipids (principal, 81.2%) and sphingolipids. The glycoglycerolipids contained Ac-MGDG and MGDG (significantly predominate). Cerebrosides (CS) and keramidoligohexosides (KOH) were present in equal amounts in the sphingolipids.

The PL contain seven components, mainly phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidylinosites (PI) (93% of PL mass). The PC contain half of the total PL mass. Furthermore, the PL contain four minor components, one of which (X_2) gave a positive qualitative test with ninhydrin and had a chromatographic mobility that corresponded with phosphatidylserine. The minor PL components could not be isolated and identified owing to their insignificant content.

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TABLE 1. NL and PoL in *A. septentrionale* Seeds

Lipids	Content, %	
	of lipid mass	of seed mass
I. Neutral lipids		33.12
Triterpene esters (TE)	1.1[a]	0.36
Fatty-acid methyl esters (FAME)	2.1[a]	0.70
Triacylglycerides (TAG)	88.9[a]	29.44
Free fatty acids (FFA)	3.2[a]	1.06
Diacylglycerides + free sterols (DAG+FS)	4.2[a]	1.39
Monoacylglycerides (MAG)	0.5[a]	0.17
II. Polar lipids		0.82
1. Glycolipids		0.11
Glycerolipids:		
Acylmonogalactosyldiacylglycerides (Ac-MGDG)	18.7[b]	0.02
Monogalactosyldiacylglycerides (MGDG)	62.5[b]	0.07
Sphingolipids:		
Cerebrosides (CS)	9.4[b]	0.01
Keramidoligohexosides (KOH)	9.4[b]	0.01
2. Phospholipids		0.71
X_4	1.0[c]	0.01
X_3	1.9[c]	0.01
Phosphatidylethanolamine (PE)	27.9[c]	0.20
Phosphatidylcholine (PC)	51.0[c]	0.36
Phosphatidylinosite (PI)	14.4[c]	0.10
X_1+X_2 (Phosphatidylserine)	3.8[c]	0.03

Of NL mass (a), of GL mass (b), of PL mass (c).

TABLE 2. Triterpene Content of FS and TE Fractions

Triterpene	Content, %
Free sterols:	
β-Sitosterol	51.6
Ergosta-7,22-dien-3β-ol	32.9
Stigmasterol	15.5
Triterpene esters	
1. Sterols:	
β-Sitosterol	54.6
Campasterol	18.0
5α-Stigmasta-3-one	27.4
2. Triterpene alcohols:	
α-Amyrin	69.8
β-Amyrin	21.8
3-Ketours-12-ene	8.4

The fatty-acid (FA) content of the separate NL classes (except TE), Ac-MGDG, and MGDG, and all PL classes was analyzed by mild base hydrolysis. TE were hydrolyzed under stronger conditions. CS and KOH were hydrolyzed by acid. The isolated FA were identified by GC as the methyl esters.

TABLE 3. Fatty-Acid Composition of Acyl-Containing Lipid Classes of *A. septentrionale* Seeds

Lipids	Acids												$\Sigma_{sat.}$	$\Sigma_{unsat.}$
	12:0	14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	21:0	22:0		
Neutral lipids:														
ΣNL	0.1	0.1	-	3.7	0.3	-	0.9	59.1	33.1	2.8	-	Tr.	4.7	95.3
FAME	0.1	0.2	-	5.9	2.1	-	1.4	73.6	16.7	-	-	-	7.6	92.4
TE	0.6	0.3	-	9.7	1.6	-	4.6	63.9	-	3.5	-	15.8	31.0	69.0
TAG	0.1	0.1	-	4.0	-	-	1.7	63.9	28.0	2.2	-	Tr.	5.9	94.1
FFA	0.3	0.7	-	10.0	0.7	-	2.3	68.1	16.7	1.2	-	-	13.3	86.7
DAG	-	3.7	-	20.3	1.5	-	1.0	60.5	11.9	0.3	-	0.8	25.8	74.2
MAG	1.1	1.1	-	7.6	-	-	2.7	53.7	29.0	4.8	-	-	12.5	87.5
Glycolipids:														
ΣGL	0.2	1.7	0.5	15.8	0.7	1.7	5.1	44.9	16.9	12.2	0.3	-	25.3	74.7
Ac-MGDG	Tr.	0.5	Tr.	5.3	Tr.	Tr.	1.7	62.4	26.1	4.0	-	-	7.5	92.5
MGDG	0.6	1.4	Tr.	13.1	-	2.7	5.0	41.8	17.2	18.2	-	-	22.8	77.2
CS	0.5	5.0	1.4	33.0	5.9	-	15.7	32.3	6.2	Tr.	-	-	55.6	44.4
KOH	Tr.	0.8	0.4	38.7	Tr.	-	3.2	47.8	3.3	2.5	3.3		46.4	53.6
Phospholipids:														
ΣPL	-	-	-	13.4	0.1	0.1	1.0	44.3	39.2	1.9	-	-	14.5	85.5
X_4	-	-	-	19.4	1.3	0.6	2.8	39.5	34.0	2.4	-	-	22.8	77.2
X_3	-	-	-	13.2	-	-	1.2	45.6	38.5	1.5	-	-	14.4	85.6
PE	-	-	-	13.0	-	-	0.6	34.3	51.0	1.1	-	-	13.6	86.4
PC	-	-	-	10.5	-	-	0.9	49.5	37.1	2.0	-	-	11.4	88.6
PI	-	-	-	22.2	-	-	1.1	36.6	38.7	1.4	-	-	23.3	76.7
X_1+X_2	-	-	-	19.8	1.9	1.2	2.9	60.1	12.2	1.9	-	-	23.9	76.1

Lipids of *A. septentrionale* contain 12 FA (Table 3). The qualitative set of these is typical of analogous lipids of other plants [3, 4]. The principal mass of the acids in the lipids consists of unsaturated FA (their content is maximal in NL, 95.3%), mainly oleic (18:1) and linoleic (18:2) acids. The exception is MGDG, which contain also linolenic (18:3) acid in an amount comparable with 18:1. Oleic acid dominates in all lipid classes except CS, where its content is comparable with that of palmitic (16:0), and PE, where the 18:2 acid predominates. The principal saturated acid in all lipid classes is palmitic; in TE, behenic (22:0). The high content of saturated FA in sphingolipids (CS and KOH) should be noted.

The FA composition of the PL differs from that of the NL and GL both in the set of components and their content. It includes only seven components. This is much less than the NL and GL content owing to the absence of several saturated acids. The increased content of 18:2 acid in all PL classes compared with GL and NL is also interesting.

The sphingosine bases in solvent system 5 gave one spot. Their composition could not be found because of the insignificant content. The glyceroglycosides remaining from mild base hydrolysis of the Ac-MGDG and MGDG fractions were hydrolyzed by acid. The sugars were isolated. Sugars isolated from the glyceroglycosides and sphingolipids were silylated and identified by GC. The trimethylsilyl derivative of galactose was observed in all fractions.

The alcohol components from the principal PL classes were obtained via acid hydrolysis. The hydrolysis products contained choline, ethanolamine, and inositol according to qualitative reactions and chromatographic mobilities.

The biological activity of the oil (NL) from *A. septentrionale* seeds was checked in the "New Medicinal Substances" laboratory of the IOC, USC, RAS. The effect of this oil on reparative regeneration of skin in rats was investigated. Oil from *Rosa cinnamomea* (cinnamon rose) was used as a reference. The experiments were carried out on three model wounds: thermal and chemical burns and pannicular wounds [5]. The effectiveness of the preparations was judged from the decrease in area of the wound 10 and 15 days after treatment and the duration of the burn healing (Table 4). The significance of the results was estimated using the Student criterion for $P < 0.05$ [6].

TABLE 4. Wound-Area Decrease in Rats after Treatment with *A. septentrionale* Seed Oil

Oil	Wound-area decrease, %					
	Thermal burn		Chemical burn		Pannicular wound	
	10 days	15 days	10 days	15 days	10 days	15 days
Aconitum septentrionale	48	90	46	86	44	84
Rosa cinnamomea	30	76	35	70	30	69
Control	26	68	39	67	20	58

The experimental results show that oil of *A. septentrionale* seeds stimulates reparative regeneration of skin in rats that exceeds the effectiveness of rose oil.

EXPERIMENTAL

IR spectra were recorded on a Specord M80 spectrometer. Analytical TLC was performed on Silufol plates and glass plates with an applied layer of LSL 5/40 μm silica gel with 13% gypsum (Chemapol, Czech Rep.); preparative TLC, on glass plates with the same grade of silica gel. Plates were activated by heating in a drying oven for 60 min at 100°C before chromatography. The oil content of seeds was determined by the standard method [7]. GC analysis of methyl esters of FA was carried out in a Chrom-5/DIP chromatograph using a column (1.2 m × 3 mm) with PDEGS (5%) on Chromaton N-AW-DMCS at 170°C with He carrier gas (40 mL/min). GC analysis of silyl esters of sugars was carried out in a Chrom-5 chromatograph using a column (1.2 m × 3 mm) with SE-30 (5%) on Chromaton N-AW-DMCS, temperature programming (5-300°C), and He carrier gas (35 mL/min). GC-MS was performed in an HP 5890 chromatograph with an HP 5972A mass-selective detector using an HP-5 column (0.25 mm × 30 m), initial temperature 100°C, 20°C/min temperature increase, final temperature 270°C, and HP MS ChemStation data processing; MX-1320, ionizing potential 70 and 12 eV and ionization-chamber temperature 70-100°C.

The following solvent systems were used to separate, purify, and identify lipids: petroleum ether—diethylether (9:1, 1; 7:3, 2), $CHCl_3$—CH_3OH—NH_4OH (25%) (65:25:4, 3), $CHCl_3$—$(CH_3)_2CO$—CH_3CO_2H—CH_3OH—H_2O (65:20:10:10:3, 4), $CHCl_3$—CH_3OH—NH_4OH (25%) (40:10:1, 5; 65:35:5, 6), $CHCl_3$—CH_3OH—CH_3CO_2H—H_2O (14:5:1:1, 7), glacial CH_3CO_2H—*n*-propanol—H_2O (1:2:1, 8), butanol—glacial CH_3CO_2H—H_2O (5:4:1, 9).

Developers were iodine vapor (NL), H_2SO_4 (50%, triterpenes), α-naphthol [4] and diphenylamine [8] (GL), Ditmer—Lester—Vas′kovskii reagent, Dragendorff's solution [4], ninhydrin solution [8], and $KMnO_4$—CH_3CO_2H [9] (PL and hydrolysis products).

NL were extracted five times from air-dried ground seeds by petroleum ether (40-60°C) upon standing at room temperature.

PoL were isolated from pulp remaining after removal of NL by $CHCl_3$—CH_3OH (2:1). The extraction was exhaustive. The extracts were combined. The solvents were evaporated. The solid was dissolved in $CHCl_3$. Alkaloids were separated by treating the $CHCl_3$ solution (1.5 L) three times with H_2SO_4 solution (200 mL, 0.5%), washing with water until the washings were neutral, and drying over Na_2SO_4.

The total NL were separated by preparative TLC on glass plates (20×20 cm) with an applied layer of silica gel using solvent systems 1 and 2.

The Pol and NL were purified by column chromatography (CC) over silica gel (1:10). The eluents were $CHCl_3$ (NL) and $CHCl_3$—CH_3OH (1-100%, Pol). TLC using solvent systems 1 and 6 was used for monitoring.

GL and PL were separated by placing an aliquot of the PoL solution (100 mg in 0.2-0.5 mL $CHCl_3$) in a centrifuge tube and adding acetone (5 mL). The mixture was stirred and cooled on an ice bath for 1 h. The resulting suspension was centrifuged for 3-5 min at 2500 rpm. The supernatant was removed with a Pasteur pipette. The solid was washed twice, suspended in cold acetone (1 mL), cooled on a ice bath for 1 h, and centrifuged. The acetone solutions were combined and evaporated.

The total PL and sphingolipids were separated using CC (lipid:silica gel ratio 1:15) with elution by $CHCl_3$—CH_3OH

mixtures (1-100%) and TLC monitoring using solvent systems 3 and 4. The isolated PL fractions were rechromatographed by preparative TLC using solvent systems 6 and 7 on glass plates (20×20 cm) with an applied layer of silica gel. Preparative TLC of individual GL fractions was performed on glass plates (10×20 cm) with an applied layer of silica gel using solvent system 4.

Base hydrolysis of TE and subsequent treatment followed the literature method [10]. Base hydrolysis of the acyl-containing lipid classes and subsequent treatment were carried out as before [11].

Acid hydrolysis of PL was performed by a previous method [9]. The products of PL hydrolysis were analyzed by TLC on plates with an applied layer of silica gel in solvent system 8. Paper chromatography was performed on Whatman No. 1 paper using solvent system 9 [9].

Acid hydrolysis of glyceroglycosides, CS, and KOH and subsequent treatment were carried out as before [4]. Solutions were filtered after acid hydrolysis of GL and separation of fatty acids, neutralized with Na_2CO_3, and evaporated. The resulting dry solid was treated by the literature method [12] to obtain silyl esters of sugars.

Ac-MGDG: R_f = 0.85. IR spectrum (ν, cm^{-1}): 3200-3600 (OH), 2940-2870, 1470, 1390 (–CH, $–CH_2$, $–CH_3$), 1740 (RCOOR), 1080 (C–O–C).

MGDG: R_f = 0.78. IR spectrum (ν, cm^{-1}): 3200-3600 (OH), 2940-2860, 1385 (–CH, $–CH_2$, $–CH_3$), 1740 (RCOOR), 1080 (C–O–C).

CS: R_f = 0.45. IR spectrum (ν, cm^{-1}): 3200-3600 (OH), 2940-2870, 1248 (–CH, $–CH_2$, $–CH_3$), 1650 (RCONHR), 1080 (C–O–C).

KOH: R_f = 0.39. IR spectrum (ν, cm^{-1}): 3200-3600 (OH), 2940, 2870, 1248 (–CH, $–CH_2$, $–CH_3$), 1650 (RCONHR), 1080 (C–O–C).

PI: R_f = 0.2 in solvent system 6. IR spectrum (ν, cm^{-1}): 3600-3336 (OH), 2928-2888, 1460 (–CH, $–CH_2$, $–CH_3$), 1740 (RCOOR), 1250 (P=O), 1200, 1112, 1040 (P–O–C–), 1080 (C–O–C).

PE: R_f = 0.58 in solvent system 6. IR spectrum (ν, cm^{-1}): 2992, 2928, 2880, 2856, 1460, 1376 (–CH, $–CH_2$, $–CH_3$), 1740 (RCOOR), 1250 (P=O), 1200, 1112, 1090, 1040 (P–O–C–), 1076 (C–O–C).

PC: R_f = 0.47 in solvent system 6. IR spectrum (ν, cm^{-1}): 3008 (OH), 2992, 2928, 2880, 1460, 1376 (–CH, $–CH_2$, $–CH_3$), 1736 (RCOOR), 1252 (P=O), 1200, 1112, 1080 (P–O–C–), 1096 (C–O–C).

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