Chemistry of Natural Compounds, Vol. 48, No. 4, September, 2012 [Russian original No. 4, July–August, 2012]

LIPIDS FROM SEEDS AND LEAVES OF *Solenanthus circinnatus*

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UDC 547.915/665.3

Lipids from seeds with pericarp and leaves of Solenanthus circinnatus *Ledeb. (Boraginaceae) were studied. The contents and class and fatty-acid compositions of neutral lipids, glycolipids, and phospholipids were established. The composition of unsaponified substances of lipids from seeds and leaves was determined. It was found that an insignificant part of* S. circinnatus *alkaloids was extracted with the lipids.*

Keywords: *Solenanthus circinnatus*, Boraginaceae, neutral lipids, glycolipids, phospholipids, fatty acids, unsaponified substances, alkaloids.

Solenanthus circinnatus Ledeb. is a perennial alkaloid-bearing plant that is distributed in Central Asia and the Altai [1]. Six species of the genus *Solenanthus* are indigenous to Uzbekistan. Toxic pyrrolizidine alkaloids were observed in five of these. Alkaloids were found in all organs of *S. circinnatus* predominantly in the aerial part (up to 2% in leaves) and were dominated by the alkaloid echinatin [2, 3].

Fatty acids (FAs) from seeds of numerous representatives of the family Boraginaceae have been studied systematically for more than 40 years. The presence in them of physiologically active γ-linolenic (all-*cis*-6,9,12-18:3) and octadecatetraenoic (all-*cis*-6,9,12,15-18:4, stearidonic) acids was established [4].

The FA composition of neutral lipids (NL) from seeds of *S. circinnatus* growing in Tien Shan was studied earlier and included 1.3% γ-18:3 and 6.1% 18:4 [5]. However, NL, glyco- (GL), and phospholipids (PL) from seeds and their FA compositions were not characterized. Data on lipids and FAs from leaves have not been reported.

In continuation of research on lipids from plants of the family Boraginaceae [6], we studied lipids from seeds with pericarp and air-dried leaves of *S. circinnatus* collected in Tashkent Oblast, Uzbekistan. Total lipids (TL) were extracted from ground samples by $CHCl_3$:MeOH (2:1, v/v) and purified of non-lipid impurities [7]. The chlorophyll content in the TL was determined by spectrophotometry. Alkaline hydrolysis isolated from the TL unsaponified substances (US). The parameters of the TL from *S. circinnatus* seeds and leaves are given below:

Parameter	*Seeds*	*Leaves*
Moisture, %	10.8	11.3
Lipid content, % of abs. dry wt.	7.1	2.3
chlorophyll a, mg%	171.0	1548.0
chlorophyll b, mg%	152.2	1117.1
unsaponified substances (US), %	5.6	24.6
Content of carotinoids in US, mg%	194.2	643.8.

The TL (7%) in *S. circinnatus* seeds were much less (by three times and more) than in seeds of other plants of the subfamily Boraginoideae [4]. The fraction of US and chlorophyll pigments was rather high. This was possibly due to the presence of the fruit husk (pericarp), which was difficultly separated from the fine seeds. As expected, the TL of leaves contained a significant fraction of chlorophyll and carotinoids pigments. The US comprised almost a quarter of the TL mass.

The group composition of the TL was established by separating them into NL, GL, and PL by column chromatography over silica gel with elution successively by $CHCl_3$, Me_2CO, and MeOH. A part of the chlorophyll pigments eluted with the NL. However, the main part of them was concentrated in the GL fraction.

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 0009-3130/12/4804-0548

TABLE 1. Composition of Total Lipids from *Solenanthus circinnatus* Seeds and Leaves

Lipids	Seeds, %		Leaves, %	
	of TL mass	of seed mass	of TL mass	of leaf mass
Neutral lipids, chlorophylls	72.3	5.13	46.1	1.06
Glycolipids, chlorophylls	15.2	1.08	42.0	0.97
Phospholipids	12.5	0.89	11.9	0.27

TABLE 2. Composition of Fatty Acids from *Solenanthus circinnatus* Seeds and Leaves, %, GC

Fatty acid	Neutral lipids		Glycolipids		Phospholipids	
	seeds	leaves	seeds	leaves	seeds	leaves
10:0	Tr.	0.1	Tr.	0.2	Tr.	0.5
12:0	0.1	0.5	2.2	0.7	0.3	1.0
14:0	0.2	5.3	Tr.	4.3	0.4	2.3
15:0	0.6	0.3	Tr.	0.5	0.2	1.7
16:0	10.4	35.6	20.8	24.7	17.7	20.7
16:1	1.7	4.8	3.8	5.7	–	7.7
17:0	0.8	–	2.3	–	1.7	–
18:0	1.6	5.2	6.3	5.8	2.8	10.2
18:1	27.9	6.5	30.7	7.9	32.0	6.9
18:2	12.9	5.5	11.9	9.4	24.2	15.3
γ-18:3	1.9	1.0	2.4	1.9	2.6	2.4
α-18:3	13.5	24.5	7.2	26.8	8.8	15.1
20:1+ 18:4	14.6	3.6	5.2	6.7	2.3	5.8
22:0	–	3.4	Tr.	Tr.	–	4.7
22:1	13.8	3.7	7.2	5.4	2.6	5.7
$\Sigma_{sat.}$	13.7	50.4	31.6	36.2	25.8	41.1
$\Sigma_{unsat.}$	86.3	49.6	68.4	63.8	74.2	58.9

Tr.: traces.

The ratio of these lipid groups was determined gravimetrically after removing solvent from the eluted fractions in a rotary evaporator. According to the results (Table 1), NL dominated in TL of *S. circinnatus* seeds. This was characteristic of reserve lipids. The PL content (0.89%) in *S. circinnatus* seeds was in the high range found in seeds of other species of the family Boraginaceae (PL 0.1–1.2%) [8, 9]. The fraction of NL in the TL of leaves was much less than in seeds even taking into account accompanying chlorophylls. The GL content was >3 times greater than the PL content.

TLC identified paraffinic, olefinic, and aromatic hydrocarbons; carotenes; xanthophylls; triacylglycerins; free FA (FFA); triterpenols; polyprenols; sterols; and chlorophylls in NL of seeds and leaves. Triterpene acids were also found in NL from leaves.

Analysis of GL from seeds and leaves by this same method detected sulfolipids, digalactosyldiacylglycerins, cerebrosides, sterylglycosides, and monogalactosyldiacylglycerins. Phosphatidylcholines, phosphatidylinositols, and phosphatidylethanolamines were found in PL from two samples.

Each lipid group was hydrolyzed by alcoholic base. The isolated FAs were purified (TLC) as methyl esters from impurities and analyzed by GC. Table 2 presents the analytical results.

The sets of FAs in all lipid groups from the two samples were almost identical with the exception of the absence of 17:0 acid in NL, GL, and PL of leaves and 22:0 acid in NL and PL of seeds. Palmitic acid (16:0) dominated in total FAs from NL and PL of leaves and was the principal saturated acid in all lipids from seeds and GL from leaves. All groups of seed lipids contained a significant fraction of 18:1 acid; lipids from leaves, α-18:3 acid. The essential FAs were enriched in PL from both seeds and leaves. Isomeric γ-18:3 acid was found in insignificant amounts (1.0–2.6%) in all lipid groups of the two samples. Stearidonic acid (18:4) under the analytical GC conditions used by us had the same retention time as eicosenoic (20:1) acid [10]. Therefore, its content was not established. However, it was shown earlier that 18:4 was present in NL from *S. circinnatus* [5]. The greatest total saturation of FAs was observed in NL from leaves; total unsaturation, in NL from seeds.

TABLE 3. Composition of Unsaponified Substances from *Solenanthus circinnatus* Seeds and Leaves

Class of lipophilic substances	Content, % of unsaponified substance mass	
	seeds	leaves
Paraffinic, olefinic hydrocarbons, carotenes	8.9	10.8
Aromatic hydrocarbons	2.5	4.3
Polyprenols, triterpenols	11.6	11.8
Triterpenols	–	30.8
Triterpenols, sterols	23.8	–
Sterols	36.7	10.8
Sterols, triterpene acids	–	11.2
Xanthophylls, unidentified classes	16.5	20.3

US isolated from TL of seeds and leaves were separated by preparative TLC over silica gel into crude fractions that were then rechromatographed. Table 3 presents the results for the class composition of lipophilic substances of the two samples.

The results showed an almost identical qualitative composition of US in *S. circinnatus* seeds and leaves with the exception of the presence of triterpene acids in US of leaves. A part of the US components from the two samples was not identified. The physiologically active classes polyprenols, triterpenols, and sterols made up almost 65% of US from leaves and 72% of US from seeds. Aromatic hydrocarbons, which were observed by us in *S. circinnatus*, are rarely encountered in plant lipids, including in representatives of the family Boraginaceae.

We showed earlier [6] that pyrrolizidine alkaloids, the main one of which was heliotrine, were co-extracted simultaneously with the lipids upon extraction of seeds of the alkaloid-bearing plant *Heliotropium lasiocarpum* (Boraginaceae) by $CHCl_3$:MeOH (2:1). Heliotrine had TLC R_f values that were close to those of digalactosyldiacylglycerins and phosphatidylcholines in solvent systems that separated GL and PL. We isolated trace quantities of alkaloids from *S. circinnatus* seeds and leaves by the reported method [6] and checked their TLC behavior. It was found that echinatin from *S. circinatus* had a mobility (R_f 0.76) in the system that separated PL that was comparable with that of *N*-acylphosphatidylethanolamines (R_f 0.80) [11], was detected by Dragendorff's solution, and did not give a qualitative reaction for PL with Vaskovsky's reagent [12]. Alkaloids in the extracts from seeds and leaves were not determined quantitatively.

EXPERIMENTAL

GC of FA methyl esters was performed on a Chrom-5 instrument with a flame-ionization detector using a column (2.5 m × 4 mm) packed with Chromaton N-AW with DEGS (15%) at column temperature 194°C and carrier-gas (N_2) flow rate 30 mL/min.

Quantitative analysis of chlorophyll and carotinoid pigments, column chromatography, analytical and preparative TLC of lipids and US, and isolation of lipids, FAs, US, and alkaloids were performed as before [6].

Aromatic Hydrocarbons. UV spectrum (hexane, λ_{max}, nm): 272, 225, 239, 245 (inflection); TLC over silica gel: R_f 0.95 [benzine (75–80°C):Et_2O, 98:1]; pink-lilac spot detected by H_2SO_4 (50%) at 110°C [13].

Triterpene acids on TLC using $CHCl_3$:MeOH (25:1) gave R_f 0.49 and appeared as a reddish spot after detection by H_2SO_4 (50%) and heating. This agreed with a standard of oleanolic acid.

The qualitative composition of chlorophyll and carotinoid pigments from leaves was determined by TLC over silica gel using hexane:Me_2CO:C_6H_6:*i*-PrOH (69.5:25:4:1.5). Spots of chlorophyll a (R_f 0.42), chlorophyll b (R_f 0.47), pheophytin a (R_f 0.52), pheophytin b (R_f 0.50), carotenes (R_f 0.69) and xanthophylls (R_f 0.38) were detected [14].

The chromatographic mobility of alkaloids was analyzed by TLC over silica gel using $CHCl_3$:MeOH:NH_4OH (28%) (65:35:5).

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