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LIPIDS AND ALKALOIDS FROM *Heliotropium lasiocarpum*

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Lipids of seeds with pericarp and an admixture of the air-dried aerial part (AP) of Heliotropium lasiocarpum *Fisch. et Mey. (Boraginaceae) were studied. The contents and component and fatty-acid compositions of neutral lipids, glycolipids, phospholipids, and strongly bound lipids were established. It was found that part of the alkaloids was extracted together with the lipids from these plant organs. The chromatographic mobility of the heliotrope alkaloids was determined under the lipid separation conditions.*

Keywords: *Heliotropium lasiocarpum*, total lipids, glycolipids, phospholipids, fatty acids, alkaloids.

Heliotropium lasiocarpum Fisch. et Mey. (Boraginaceae) is a perennial herbaceous alkaloid-bearing plant that is widely distributed in many regions of Uzbekistan [1, 2]. The aerial part of this heliotrope contains a mixture of the alkaloids heliotrine and lasiocarpine (0.4–0.5%) [3]; the seeds, a mixture of heliotrine, heliotrine *N*-oxide, and lasiocarpine *N*-oxide (~1%) [4].

The fatty-acid (FA) compositions of seeds with pericarp of the five species *H. amplexicaule*, *H. curassavicum*, *H. sirigosum*, *H. supinum* [5], and *H. europium* [6] are known. A characteristic signature of plants of the Boraginaceae family is the presence of reserve lipids in addition to the principal ordinary FAs 16:0, 18:1, and 18:2 and the rarely encountered γ-linolenic (all-*cis*-6,9,12-18:3) and octadecatetraenoic (all-*cis*-6,9,12,15-18:4) acids with high biological activity [5, 7]. Of the two biologically active FAs, only γ-linolenic acid was observed in trace quantities in seeds of *H. amplexicaule* whereas lipids of *H. europium* seeds contained octadecatetraenoic acid in insignificant (0.2%) quantities. It was reported that seeds of *H. lasiocarpum* contained up to 20% fatty oil [4], the composition of which was not studied.

We studied lipids of seeds with pericarp and an admixture of the aerial part of *H. lasiocarpum* collected in 2009 in Tashkent Oblast. Seeds of this heliotrope species are very small, 1.0 × 1.5 mm. They are difficult to separate from remains of the plant mass.

Total lipids (TL) were extracted from the ground sample by a $CHCl_3$:MeOH (2:1, v/v) mixture using the Folch method [8]. The yield of extract was 11.4% (per abs. dry weight). Strongly bound lipids (SBL) were isolated from the pulp remaining after extraction of TL using a $CHCl_3$:MeOH:HCl (conc.) (2:1:0.03, v/v) mixture [9]. The SBL cannot be isolated in the native state using only an organic extractant because they are covalently bound to protein in the seed kernel [10]. The HCl in MeOH hydrolyzes lipoproteins. The released lipids are readily extracted. The yield of SBL from heliotrope pulp was 0.92%.

The TL were green, indicative of the presence in them of chlorophyll. The content of chlorophyll "a" in the TL was determined by spectrophotometry [11] as 498 mg%; chlorophyll "b", 220 mg%; and carotinoids, 191.0 mg%.

Preparative TLC on silica gel using solvent system 1 separated the TL into neutral (NL) and polar (PoL) lipids. Then, the PoL were separated by this same method using solvent system 2 into glycolipids (GL) and phospholipids (PL), which remained at the chromatogram origin using this system. The amounts of NL, GL, and PL in the TL were 63.6, 22.7, and 13.7%, respectively.

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TABLE 1. Fatty-Acid Composition of Lipids from *Heliotropium lasiocarpum*, %, GC

Fatty acid	RT, min	NL	GL	PL	SBL
10:0	3.044	0.8	0.1	–	0.6
12:0	5.574	0.6	–	1.5	0.6
14:0	9.616	0.6	Tr.	1.5	0.9
16:1	13.716	0.4	0.3	–	–
16:0	14.210	11.3	7.1	36.4	16.3
X_1	15.047	2.0	0.3	4.9	2.0
18:2	18.011	51.0	62.5	16.5	46.4
18:1	18.136	24.1	22.1	29.2	24.6
X_2	18.247	4.8	4.0	1.9	4.0
18:0	18.698	3.1	2.8	5.4	3.8
20:1	22.351	0.8	0.3	1.3	–
20:0	22.895	0.5	0.5	1.4	0.8
Σsat.		23.7	14.8	53.0	29.0
Σunsat.		76.3	85.2	47.0	71.0

Tr.: traces.

TLC using system 1 detected in the NL paraffinic and olefinic hydrocarbons, carotinoids, triacylglycerides (TAG), tocopherols, free FAs (FFA), triterpenols, sterols, and chlorophyll-type pigments. The composition of the TL is given below:

Lipid group and class	*Content, % of mass*	*Lipid group and class*	*Content, % of mass*
Neutral lipids:		Free fatty acids	5.70
Paraffinic, olefinic hydrocarbons, carotinoids	2.60	Triterpenols, sterols, chlorophylls	8.50
Triacylglycerides	45.60	Glycolipids	22.70
Tocopherols	1.25	Phospholipids	13.60.

The main class of TL was TAG. NL of *H. lasiocarpum* seeds contained a significant amount of tocopherols. The GL/PL ratio was 1.6:1.

The heliotrope GL consisted of sulfolipids, digalactosyldiacylglycerides, sterylglycosides, monogalactosyldiacylglycerides, and an unidentified compound according to analytical TLC using system 3.

Two-dimensional TLC on silica gel using systems 4 and 5 found in the PL phosphatidylcholines, phosphatidylinositols, phosphatidylethanolamines, and two unidentified components.

Analysis of the SBL by TLC using system 1 showed that they contained TAG, FFA, and sterols.

Unsaponified substances (US, 7.1%) were isolated from the TL. Hydrocarbons, carotinoids, tocopherols, triterpenols, sterols, and FA were detected in them (TLC on silica gel, system 1). The FAs were removed by neutralizing the alcohol solution of US with KOH solution (0.02N). Then, the true amount of US was calculated as 5.7% [12].

The FAs from the individual lipid groups were analyzed as methyl esters using GC. Table 1 presents the analytical results. The acids of heliotrope lipids consisted of 12 components, two of which were unidentified. The qualitative composition of the *H. lasiocarpum* FAs differed from those of the other described species [5, 6] by the presence of medium-weight acids 10:0–14:0 and eicosanoic acid 20:0 and the absence of acids α-18:3 (9,12,15) and γ-18:3 (6,9,12). The NL, GL, and SBL FAs were dominated by 18:2; the PL, by 18:1. The principal saturated acid of FAs from all lipid groups was palmitic 16:0, the content of which in the PL acids reached 36%. The highest content of total unsaturated FAs was found in GL; of total saturated acids, in PL.

Because seeds and the aerial part of heliotrope contain alkaloids, we hypothesized that the five unidentified compounds present in GL and PL and giving a positive reaction with Dragendorff's solution might be alkaloidal in nature. Therefore, experiments on the extraction of alkaloids from starting raw material and from TL by known methods were carried out.

During the isolation of alkaloids from the raw material and TL, the preliminary extraction of alkaloids by an organic extractant containing water and subsequent work up of the extract by solutions of bases and acids caused partial destruction of labile PoL, especially phospholipids [13]. Therefore, the TL and alkaloids were isolated in parallel in order to preserve the integrity of the PoL. Then, alkaloids were extracted from the resulting TL. As a result, alkaloids were isolated from the starting raw material (0.13%) and TL (0.037%).

The alkaloids were analyzed by TLC on silica gel using the solvent systems that were used to separate NL, GL, and PL. The chromatograms were visualized using Dragendorff's solution. The alkaloids remained at the origin using system 1. The principal alkaloid heliotrine, which represented about 70% of the total heliotrope alkaloids [3], had R_f 0.30 that was comparable with that of digalactosyldiacylglycerides (R_f 0.27) using system 3, which was used to separate the GL. Heliotrine had R_f 0.4 using system 4, which separated the PL. This was slightly greater than for phosphatidylcholine (R_f 0.33–0.35).

Thus, part of the alkaloids, depending on the structural features, can be extracted together with the lipids during extraction of TL from alkaloid-bearing plants. This must be considered when estimating the lipid content in raw material and analyzing separate lipid classes.

EXPERIMENTAL

GC analysis of FA methyl esters was performed on an Agilent Technologies 6890 N instrument with a flame-ionization detector using a capillary column (30 m) with HP-5 deposited nonpolar phase at 60–250°C. FAs were methylated by diazomethane for the GC analysis and additionally purified from impurities by PTLC using system 1. The contents of chlorophyll and carotinoid pigments in acetone were determined on an SF-46 instrument [11]. Preparative and analytical TLC was carried out on silica gel and Silufol UV-254 plates using the following solvent systems: Et_2O:benzine (bp 70–75°C) (2:3) (1); Me_2CO:MeC_6H_5:HOAc:H_2O (60:60:2:1) (2); $CHCl_3$:Me_2CO:MeOH:HOAc:H_2O (65:20:10:10:3) (3); $CHCl_3$:MeOH:NH_4OH (28%) (65:35:5) (4); $CHCl_3$:MeOH:HOAc:H_2O (14:5:1:1) (5).

Spots of compounds were detected in I_2 vapor and H_2SO_4 (50%) with subsequent heating. Known components of plant lipids and qualitative reactions with specific reagents were used to identify the compounds [14].

The TL were isolated from ground raw material using $CHCl_3$:MeOH (2:1, v/v) by extraction (4×) on standing for 6 h at room temperature. Nonlipid impurities were removed from the combined extracts by washing with aqueous $CaCl_2$ solution (0.04%). SBL were extracted (3×) from pulp on standing for 6 h in $CHCl_3$:MeOH:HCl (conc.) (2:1:0.03, v/v) also at room temperature [9]. US were isolated by the literature method [12].

Isolation of Alkaloids from Lipid Extract. TL (2 g) were dissolved in $CHCl_3$ (100 mL), treated with H_2SO_4 solution (5%, 30 mL, 4×). The acid extracts were checked by a qualitative reaction with silicotungstic acid for the presence of alkaloids. The combined acid extracts were made basic by NH_4OH solution (17%). Alkaloids were extracted as the bases by $CHCl_3$ (4×, 30 mL). The $CHCl_3$ extract was washed with H_2O and dried over anhydrous Na_2SO_4. The solvent was removed in a rotary evaporator. The solid was dried at 70°C.

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