

LIPIDS FROM *Atriplex dimorphostegia* LEAVES

D. T. Asilbekova,* F. M. Tursunkhodzhaeva,
and F. Yu. Gazizov

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The genus *Atriplex* belongs to the family Chenopodiaceae (Goosefoot) and includes 225 species [1]. Runners and young leaves of *Atriplex* species are valuable sources of vitamins and are used in spring (instead of spinach) as a green leafy vegetable. The leaves contain organic acids (*A. cana* L.), coumarins and flavonoids (*A. rosea* L.), alkaloids (*A. littoralis* L., *A. dimorphostegia* Kar. et Kir.) [2], and phosphatidylglycerol (*A. prostrata* Baucher ex DC.) [3]. Research on Chenopodiaceae has consisted only of isolated studies of lipophilic substances of *Halocnemum strobilaceum* (Pall.) Bieb. [3] and *Suaeda maritima* (L.) Dimort. [4]. Lipids of cultivated spinach *Spinacea olearacea* were investigated in one report [5].

We investigated lipids from fresh leaves of *A. dimorphostegia* Kar. et Kir., a salt-tolerant annual plant with pronounced xeromorphic structure. Leaves were collected during flowering in August, the driest time of year.

Enzymes of the leaves were inactivated before the lipids were extracted by immersing them for 1-2 min in boiling isopropanol. Tissues were ground in a mortar after the solvent was removed and extracted several times with CHCl₃:CH₃OH (2:1, v/v). The isopropanol and CHCl₃:CH₃OH extracts were combined and evaporated to dryness. The crude lipid extract was dissolved in CHCl₃ and purified of ballast compounds by multiple rinsings with CaCl₂ solution (0.04%).

The yield of lipids from fresh leaves of *A. dimorphostegia* was 2.8% of the dry mass. First total lipids were separated by column chromatography into separate groups of neutral (NL), glyco- (GL), and phospholipids (PL). Then, NL were rechromatographed over a microcolumn. Esters of alcohols, triterpenols, and sterols (ES) and monogalactosyldiacylglycerines (MGDG) were isolated using preparative TLC on silica gel with elution by hydrocarbons:Et₂O (9:1) and (CH₃)₂CO:C₆H₆:H₂O (91:30:8). Table 1 lists the results of the chromatographic separation.

NL, GL, and PL were identified; fatty acids were isolated and esterified; and GC of methyl esters was performed as before [6, 7]. Methyl esters of fatty acids obtained from homogeneous fractions of alcohol, triterpenol, and sterol esters in addition to acids isolated from MGDG were analyzed by GC/MS on an Agilent Technologies GC-MS/HP6890 chromatograph-mass spectrometer with an AT5973N mass-selective detector using a capillary column with phenylmethylsiloxane (5%) and programmed temperature from 150 to 280°C. Table 2 lists the composition of the fatty acids.

The usual spectrum of NL, GL, and PL components were identified in lipids from leaves of this species (Table 1). Esters of fatty alcohols, sterols, and triterpenols and free fatty acids dominated the NL whereas triacylglycerines were present in trace quantities. Polar lipids (GL and PL) were mainly galactolipids (monogalactosyldiacylglycerines and digalactosyldiacylglycerines), sulfolipids (sulfoquinovosyldiacylglycerines), glycerophospholipids (phosphatidylglycerines, phosphatidylethanolamines, phosphatidylcholines, phosphatidylinositols, and phosphatidic acids). Minor amounts of steryl glycosides, their fatty-acid esters, and two unidentified PL components were also found.

The fatty-acid composition of the NL, GL, and PL indicated that palmitic acid dominated the saturated acids of these groups (Table 2). The ES acids were high-molecular-weight even acids 20:0-30:0.

Unsaturated acids were mostly linolenic acid in GL and NL and oleic in PL. The highest content of linoleic acid was found in NL acids, its free form, and as esters of alcohols.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax: (99871) 120 64 75, e-mail: dasil@rambler.ru. Translated from *Khimiya Prirodnikh Soedinenii*, No. 6, pp. 618-619, November-December, 2008. Original article submitted July 7, 2008.

TABLE 1. Lipids from *Atriplex dimorphostegia* Leaves

Component	Content, % of total lipid mass
Neutral lipids, including:	50.4
Hydrocarbons and β -carotene	6.5
Fatty alcohol, sterol, and triterpenol esters in triacylglycerines	16.0
Free fatty acids	13.0
Fatty alcohols, sterols, and triterpenols	4.4
Sterols and triterpenols	10.5
Glycolipids, including:	26.4
Monogalactosyldiacylglycerines	12.3
Digalactosyldiacylglycerines, sulfoquinovosyldiacylglycerines, sterylglucosides and their esters	14.1
Phospholipids	16.3
Total chlorophyll pigments and unidentified substances	6.9

TABLE 2. Fatty-Acid Composition of Lipids from *Atriplex dimorphostegia* Leaves

Acid	NL	GL	PL	ES	FFA	MGDG
12:0	Tr.	Tr.	Tr.	Tr.	0.2	0.5
14:0	0.3	Tr.	2.0	Tr.	0.6	1.0
15:0	Tr.	-	-	Tr.	Tr.	0.5
16:0	29.5	31.4	38.3	20.5	35.7	29.3
16:1(9Z)	3.0	Tr.	5.6	5.0	Tr.	-
16:1(3E)	-	-	-	-	-	-
16:3(7Z,10Z,13Z)	-	1.5	-	-	-	2.5
18:0	4.8	2.6	5.1	4.4	4.9	3.4
18:1(9Z)	17.7	14.2	23.9	6.2	18.3	7.0
18:2(9Z,12Z)	16.7	9.5	12.9	10.8	14.1	5.6
18:3(9Z,12Z,15Z)	28.0	40.8	12.2	42.2	26.2	52.2
20:0	Tr.	-	-	Tr.	-	-
22:0	Tr.	-	-	Tr.	-	-
24:0	Tr.	-	-	1.7	-	-
26:0	Tr.	-	-	2.8	-	-
28:0	Tr.	-	-	2.1	-	-
30:0	Tr.	-	-	4.3	-	-
$\Sigma_{\text{Sat.}}$	51.9	34.0	45.4	35.8	58.6	32.7
$\Sigma_{\text{Unsat.}}$	47.4	66.0	54.6	64.2	41.4	67.3

The fatty acids of GL differed from the other lipid groups not only in the high content of linolenic acid ($[M]^+ 292$) but also the presence of hexadecatrienoic acid 16:3 (7Z,10Z,13Z) esterified in MGDG to 2.5% of the total acids. The 16:3 structure was established by GC/MS of the methyl esters of MGDG acids. The mass spectrum contained a peak for the molecular ion $[M]^+ 264$ and exhibited characteristic fragments with m/z 235, 233, 221, 209, and 195, which unambiguously confirmed the structure of the methyl ester of hexadecatrienoic acid. The results led to the conclusion that *A. dimorphostegia*, like species of *Nicotiana* [8], *Mandragora turcomanica* [9], and *S. olearacea* [5], belongs to a group of 16:3 plants. Apparently 16:3(7Z,10Z,13Z) acid and its homolog 18:3(9Z,12Z,15Z) play a determining role in photosynthetic processes occurring in chloroplasts of leaves of these plants.

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