LITERATURE CITED

1. V. M. Dembitskii, G. V. Eplanova, N. I. Zharikova, and V. V. Zharikov, *Khim. Prir. Soedin.*, No. 2, 281 (1989).
2. E. S. Kaneshiro, K. B. Meyer, and D. E. Rhoads, *J. Protozool.* **34**, 357 (1987).
3. D. E. Rhoads and E. S. Kaneshiro, *J. Protozool.* **26**, 329 (1979).
4. G. A. Thompson, Jr., *Biochim. Biophys. Acta*, **6**, 2015 (1969).
5. E. S. Kaneshiro, *J. Lipid Res.* **28**, 1241 (1987).
6. J. D. Smith, in: *Role of Phosphonates in Living Systems*, R. L. Hildebrand (ed.), CRC, Boca Raton (1983), p. 31.
7. V. M. Dembitsky, *J. Chromatogr.*, **436**, 467 (1988).
8. M. C. Moschidis, *J. Chromatogr.*, **298**, 366 (1984).
9. M. C. Moschidis, *Z. Naturforsch.* **41C**, 369 (1986).
10. V. M. Dembitskii and N. I. Zharikova, *Khim. Prir. Soedin.*, No. 2, 294 (1988).
11. V. M. Dembitskii, *Bioorg. Khim.* **13**, 409 (1987).

LIPIDS OF ELAEAGNUS FRUIT

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The composition of the classes of lipids of the fruit of three morphological forms of the elaeagnus Elaeagnus angustifolia *L. have been studied. Their compositions were identical. The amounts of the main lipid classes of extracts of the seeds and pericarps, the fatty acid compositions of the acyl-containing classes of lipids, and the compositions of the carbohydrates and sterols have been determined. The fatty oil of the seeds contained linoleic acid, while the main fatty acids of the pericarp extracts were the 16:0, 18:1, and 18:2 acids. The 16:1 acid that is characteristic for sea buckthorn oil was detected in the elaeagnus fruit in insignificant amounts. The class of sterols, both in the free and in the esterified states, was represented by β-sitosterol. The main hydrocarbon of the pericarps and seeds was nonacosane.*

The family Elaeagnaceae includes three species of plants: *Hippophäe* L., *Elaeagnus* L., and *Sheferdia* L. Widespread on the territory of the Soviet Union are sea buckthorn (*Hippophäe rhamnoides* L.) and two species of elaeagnus — Russian olive (*Elaeagnus angustifolia* L.) and eastern elaeagnus (*Elaeagnus orientalis* L.) [1-3].

In folk medicine, plants of this family have long been known as medicinal. Elaeagnus fruit is used as an astringent, a tincture of the ripe fruit is used in homeopathy, and the leaves (in the form of a lotion) for rheumatism, while the essential oil stimulates the action of the heart [2]. The fatty oil of the sea buckthorn, which is a unique natural concentrate of vitamins and other biologically active compounds, is widely used for various types of tissue disorders. Industry is incapable of satisfying the ever-increasing demands for medicine in the form of sea buckthorn oil and, therefore, the problem of the search for a natural or synthetic analog of it is an urgent one.

It is known that the closer to one another individual plants are in the system, the larger are the amounts of similar chemical ingredients in their compositions [4]. Many studies have been devoted to the composition of the lipids of the sea buckthorn [5-8], but elaeagnus lipids have not previously been studied. The absence of information on the lipid composition and also the fact that elaeagnus belongs to the same family as the sea buckthorn has served as a reason for the study of this plant. Furthermore, this investigation is of interest also from the point of view of elucidating chemotaxonomic characteristics within the family.

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TABLE 1. Yields of Extracts of Elaeagnus Fruit

Form	Yield, % on the initial raw material	
	seeds (hexane)	pericarps (chlf–EtOH)
I	3,50	1,20
II	13,00	0,95
III	6,00	0,80

TABLE 2. Compositions of the Classes of Lipids of Elaeagnus Fruit

Class of lipids	Yield of lipids, % on the total					
	seeds			pericarps		
	I	II	III	I	II	III
Hydrocarbons	0,1	0,1	0,5	1,1	3,9	1,6
Esters	2,3	1,6	3,0	4,7	5,1	4,3
Triacylglycerols	90,3	93,7	86,2	18,2	12,7	15,3
Epoxyacylglycerols	1,9	Traces	0,9	—	—	—
Free fatty acids	1,2	0,1	1,4	2,5	7,0	2,9
Triterpenols	0,3	0,5	0,8	6,3	8,0	4,3
Sterols	1,9	2,9	6,2	14,9	19,5	15,0
Sterols + triterpene acids	—	—	—	11,2	11,1	17,0
Glycolipids + unident	2,0	1,1	1,0	41,1	32,7	39,6

We studied the lipids of three morphological forms of elaeagnus growing on the territory of Uzbekistan (fruit collected in September, 1986): I) small fruit with a brown-red color gathered in the environs of Keles (Tashkent province, Tashkent district); II) small fruit, silver-greenish, similar to the color of the leaves, gathered in the environs of Kapa-Sarai (Fergana province, Frunze district); and III) large brown fruit gathered in the mountain area of Chimgan (Tashkent province). The lipids were extracted separately from the seeds and pericarps. The highest yield of fatty oil from the seeds was given by plants growing in the Fergana Valley (Table 1). This is apparently due to differences in the climatic conditions.

The compositions of the classes of lipids were studied by using the methods of column and preparative chromatography (Table 2). Triacylglycerol predominated in the fatty oils of the seeds. In addition, in all forms there were hydrocarbons, esters, free fatty acids, triterpenols, sterols, and epoxyacylglycerols.

In the pericarps the percentage of triacylglycerols was low (from 18 to 30%, calculated on the neutral lipids). A considerable part of the pericarp extracts consisted of the classes of glycolipids, triterpenols, sterols, and triterpene acids.

The facts given show that these three morphological forms have no appreciable differences in the qualitative and quantitative compositions of the lipids.

The FA compositions of the acyl-containing classes are shown in Table 3. In the TAG fractions of the seed oils the main esterifying acid was linoleic while the FFAs had an increased amount of saturated acids (16:0 and 18:0) with the 18:2 acid still the main one. The same pattern (increase in saturation through the 16:0 and 18:0 acids, and also through short-chain, 8:0-15:0 acids) was also observed in the case of the esters.

In the TAGs of the pericarps the main acids were the 16:0, 18:1, and 18:2 species. The FA composition of the acyl-containing fractions of the pericarps differed from those of the seeds by the appearance of long-chain ($>C_{18}$) acids of which there was a particularly large amount in a plant of form II and the smallest amount in that of form III. The FAs were identified from a graph of the dependence of the logarithm of the retention time as a function of the number of carbon atoms, and also by a comparison of the relative retention times with respect to the 16:0 acid and the introduction of individual saturated FAs as internal standards.

On the whole, the FA compositions of the three forms of elaeagnus differed little from one another, and only for two specimens (the FFA fraction of the pericarps of III and the ester fraction of the seeds of II) were anomalously high amounts of lauric acid, 12:0, detected (49.8 and 22.6%, respectively).

In the hydrocarbon fractions of the fruits (Table 4), the main paraffin was nonacosane, $C_{29}H_{60}$, which is characteristic for the majority of plant fruits [9].

TABLE 3. Fatty Acid Compositions of the Acyl-Containing Lipids of the Three Forms of Elaeagnus

Class of compounds	Form of the plant	Acid content, %, GLC							
		10:0	12:0	13:0	14:0	15:0	16:0	16:1	17:0
Pericarps									
TAGs	I	—	0,2	—	0,5	0,4	27,9	2,2	0,5
	II	3,6	0,4	—	0,7	0,4	23,7	10,1	—
	III	—	3,4	1,5	1,4		14,5	3,3	—
FFAs	I	—	0,3	—	1,5	0,9	48,2	3,4	1,8
	II	—	1,1	0,8	1,7	1,2	31,8	2,5	1,7
	III	—	4,8	7,0	8,3	—	8,0	7,0	—
Esters	I	—	1,9	—	2,8	1,9	41,2	3,5	2,5
	II	—	—	—	2,6	3,7	21,5	3,3	2,7
	III	—	1,3	0,6	3,7	1,4	32,9	4,4	—

Class of comp.	Form of the plant	Acid content, %, GLC							
		18:0	18:1	18:2	18:3	21:0	22:1	23:0	24:1
Pericarps									
TAGs	I	4,6	20,7	32,5	9,6	—	—	0,9	—
	II	3,6	23,5	19,8	4,7	—	4,5	—	5,0
	III	3,3	24,6	32,3	15,7	—	—	—	—
FFAs	I	9,3	11,5	8,9	9,2	—	5,0	—	—
	II	5,3	9,0	4,7	7,6	2,8	11,6	4,1	14,1
	III	8,0	6,6	5,3	—	—	—	—	—
Esters	I	7,0	7,9	6,2	14,5	—	10,6	—	—
	II	7,6	7,1	6,8	13,3	2,5	19,7	5,5	3,7
	III	9,8	11,4	16,2	10,9	3,0	4,4	—	—

Class of comp.	Form of plant	Acid content, %, GLC										
		8:0	10:0	12:0	14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3
Seeds													
TAGs	I	—	—	—	0,5	—	3,7	—	—	2,7	22,3	54,2	16,6
	II	0,7	0,3	—	0,3	0,5	1,8	—	0,5	1,3	23,1	59,1	12,4
	III	—	—	—	—	—	4,3	0,5	1,0	1,9	25,7	56,6	10,4
FFAs	I	—	—	2,2	1,7	—	20,7	3,2	—	7,7	25,9	32,6	6,0
	II	0,9	0,5	1,7	1,5	0,5	18,8	2,7	1,2	7,5	24,6	35,3	4,8
	III	—	0,2	0,1	0,7	—	21,3	5,6	0,3	6,9	26,9	34,1	3,9
Esters	I	—	1,1	3,4	3,0	0,9	27,3	3,1	3,2	6,9	24,2	13,6	13,3
	II	1,0	1,2	22,6	0,9	0,5	3,6	—	2,5	13,8	21,9	27,0	5,0
	III	—	0,9	3,3	1,5	—	29,2	1,8	1,2	3,8	27,2	20,7	10,4

TABLE 4. Composition of the Hydrocarbons of the Fruit of Three Forms of Elaeagnus

Plant form	Hydrocarbons, %, GLC													
	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}
Pericarps														
I	—	—	2.7	1,4	1,1	1,2	0,9	0,8	0.9	4.8	1,7	79.1	2,9	2 5
II	1,3	1,7	2.0	2 8	3,5	2,5	3,1	2.2	2,8	4.1	3,2	55,8	3.8	11.2
III	—	—	1,1	1,3	1,1	1,4	1,0	1,5	0,9	4,1	1,6	76.7	2,6	6,7
Seeds														
I	1,5	1,6	1,5	1,8	1,7	1,6	1,6	1,7	1,4	1,8	1,4	63,2	6.0	8,2
II	1,0	1,3	1,7	1,9	2,1	2,2	1,8	1,4	1.1	1.3	1.5	70.1	6.1	6,5
III	—	—	0,9	1,2	1,5	1,3	0,9	1.2	1.0	5.3	4,2	75,4	3,1	4,0

Among the free sterols and also the sterols obtained by the severe hydrolysis of their ester fractions, only β-sitosterol was detected by the methods of GLC and mass spectrometry (M^+ 414, one peak in GLC).

In a comparison of the fatty oils of elaeagnus and sea buckthorn seeds [6], their FA compositions were found to be identical and both were found to contain oxidized lipids. The composition of the elaeagnus pericarp lipids was not similar to that of the sea buckthorn lipids. The amount of the unique fatty oil of sea buckthorn pericarps amounted to 8-10% on the dry weight, and 90% of the oil was represented by TAGs; the flesh of the elaeagnus fruit was poor in lipids and, accordingly,

in the amount of the TAG class. The amount of palmitoleic, 16:1, acid that is characteristic for sea buckthorn pericarps is 40-50% on the mass of the acid, but in elaeagnus its amount was only 3-10%. Apparently the 16:1 acid cannot be considered as a chemotaxonomic indication of this family. The 22:1 and 24:1 acids have been found in the fruit of Central Asian forms of sea buckthorn [7].

EXPERIMENTAL

Extraction of the Lipids. The ripe fruit of *Elaeagnus angustifolia* L. was separated mechanically into pericarps and seeds. The seeds were ground in an electric mill after treatment with liquid nitrogen in view of their extreme hardness. The extraction of the seeds was carried out with hexane while, because of its poor wettability by hexane, the extraction of the dry ground pericarp was performed with a 1:1 mixture of chloroform and ethanol by repeated steeping at room temperature. The combined extracts of the pericarps after the solvents have been driven off were washed with water to eliminate sugars.

The separation of extracts of the neutral lipids of the seeds, the chromatographic isolation of the pure classes, the alkaline hydrolysis of the alkyl-containing fractions, the esterifications of the FAs, and the GLC of the FA methyl esters were carried out as described in [6].

The separation of extracts of the pericarp lipids was carried out by the methods of column chromatography on silica gel L 100/250 (Czechoslovakia). The total lipids were added to the column in hot hexane, washing with hot hexane was continued to elute the wax esters, and elution was then carried out at room temperature with the following solvents in succession: mixtures of hexane and diethyl ether of increasing polarity, diethyl ether, chloroform, and mixtures of chloroform and methanol with increasing concentrations of methanol. The other operations were analogous to those described for the seeds.

The GLC of the hydrocarbons and sterols was effected on a Chrom-41 chromatograph with a flame-ionization detector using a 1.2-m column containing 5% of SE-30 on Chromosorb W; t_{evap} – 280°, t_{therm} – 250°.

Mass Spectrometry. MKh 1310 mass spectrometer, direct introduction of the sample, temperature of the heater bulb and of the ionization chamber 150-200°C, collector current 40 μA, ionizing voltage 50 V.

LITERATURE CITED

1. *Atlas of Areas and Resources of Medicinal Plants of the USSR* [in Russian], Moscow (1980), p. 272.
2. *Flora of the USSR* [in Russian], Vol. 15, Moscow (1949), p. 515.
3. Kh. Kh. Khalmatov, *Wild-Growing Medicinal Plants of Uzbekistan* [in Russian], Meditsina, Tashkent (1964), p. 156.
4. N. I. Sharapov, *Plant Chemistry and the Climate* [in Russian], Izd. Akad. Nauk SSSR, Moscow–Leningrad (1954).
5. V. A. Mironov, G. S. Vasil'ev, V. S. Matrosov, T. M. Filippova, V. A. Zamurenko, V. V. Mishchenko, V. G. Maironovskii, I. I. Kas'yanenko, and L. M. Maksimova, *Khim.-farm. Zh.* **17**, 1242 (1983).
6. T. G. Khmyrko, N. P. Goncharova, É. I. Gigienova, and A. I. Glushenkova, *Khim. Prir. Soedin.*, 300 (1984).
7. S. Sh. Mamedov, É. I. Gigienova, A. U. Umarov, and S. M. Aslanov, *Khim. Prir. Soedin.*, 710 (1981).
8. O. V. Ozerinina, G. A. Berezhnaya, I. P. Eliseev, and A. G. Vereshchagin, *Prikl. Biokhim. Mikrobiol.*, **24**, 422 (1988).
9. P. Capella, E. Fedeli, M. Cirimele, and C. Jacini, *Riv. Ital. Sostanze Grasse*, **40**, 603 (1963).