LIPIDS OF THE OILS OF THE FRUIT AND LEAVES OF *Hoppophaë rhamnoides*

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The analysis of some lipids of four forms of the common sea buckthorn has shown that they all differ with respect to the composition of the fatty acids of the triacylglycerols of the leaves. One of the forms is characterized by an infringement of the specificity of the composition of the fatty acids in positions 2 of the triacylglycerols of the oils of the seeds and the leaves.

Various forms of *Hippophaë rhamnoides* L. (common sea buckthorn) differ in the amounts and compositions of the carotenoids and the fatty acids of the oil of the juice (flesh) and seeds [1, 2]. The structure of the triacylglycerols of the sea buckthorn has not been studied although they are the main components of the oils of the fruit.

In studying various forms of sea buckthorn with the aim of elucidating possible differences in the chemical composition of the lipids, we have made an analysis of the total oil of the fruit (seeds and juice) and of the triacylglycerols of a petroleum ether extract of the leaves.

Sea buckthorn from the territory of the Lesser Caucasus (Kel'bzdzhar region) was studied. Fruit and leaves of these forms of sea buckthorn were collected at the end of September-beginning of October, 1980: form 1 in the environs of the village of Abdullaushagi, and forms 2, 3, and 4 in the flood plains of the R. Tutgun. An investigation of the sea buckthorn thickets revealed that these forms differ in the size, weight, color, and shape of the fruit, the size and shape of the seeds, and the yields of the petroleum extracts from the fruit (1 — 17.0; 2 — 24.5; 3 — 27.3; 4 — 21.8% on the air-dry weight) and of the leaves (5.9, 8.0, 10.1, and 12.2%, respectively).

The total material from each of the petroleum extracts was separated by column chromatography in solvent systems 1-7. Fractions of substances containing complex mixtures of components were subjected to rechromatography in a thin layer of silica gel in order to isolate chromatographically individual components. As a result, for each of the fruit oils of the four forms of sea buckthorn ten zones were detected with R_f 0.96, 0.90, 0.65, 0.43, 0.40, 0.29, 0.25, 0.16, and 0.05-0.00 in solvent system 3. Of these, hydrocarbons (R_f 0.96), triacylglycerols (R_f 0.65), free fatty acids (R_f 0.29), sterols (R_f 0.16), and chlorophyll *a* were identified. The lipids belonging to the first three classes of organic compounds were isolated and investigated in more detail.

The hydrocarbons as a class of compounds were identified by their R_f values in system 3 in comparison with authentic samples of hydrocarbons and from their IR and PMR spectra.

Assignment of the triacylglycerols to esters of glycerol and fatty acids was made from the results of TLC, IR and PMR spectroscopy, and alkaline and enzymatic hydrolysis. The composition of the fatty acids, total, and those esterifying positions 2, are given in Table 1.

The free fatty acids detected on a thin layer chromatogram by a comparison of their R_f values with those of authentic samples of fatty acids (16:0, 18:1, and 18:2) were methylated with diazomethane. Fatty acid methyl esters with R_f 0.72, corresponding to the rate of migration of the methyl ester of the fatty acids mentioned, were obtained. The composition of the free fatty acids found from a gas–liquid chromatogram of the corresponding methyl esters differed from that of the total fatty acids of the triacylglycerols by the presence of acids with 22-25 carbon atoms.

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TABLE 1. Compositions of the Fatty Acids, Free (FFAs) and Bound in the Triacylglycerols (total and in position 2), % of the Total

Fatty acid	Fruit										Leaves						
	1			2			3			4	1	2	3			4	
	TAGs		FFAs	TAGs		FFAs	TAGs		FFAs	TAGs	TAGs	TAGs	TAGs			TAGs	
	total	position 2		total	position 2		total	position 2		total	position 2	total	total	position 2		total	position 2
$C_{8:0}$	—	—	—	0,5	—	—	0,6	—	—		—	—	—	—	—	—	—
$C_{10:0}$	—	—	—	—	—	—	—	—	—	—	0.6	0,4	—	—	—	—	—
$C_{12:0}$	0,2	4,3	—	0,8	—	0,3	0,4	—		0,4	0,3	0,7	3,8	—	Tr.	2,7	1.1	6,1
$C_{12:1}$	—	—	10,4	—	—	—	—	—	—	—	—	—	—	—	—	—	3,4	1,6
$C_{13:0}$	0.1	2.0	—	0,6	—	0,3	0.4	—	—	0,2	0,2	0.7	—	—			—	
$C_{13:1}$	—	—	—		—	—	—	—	—	—	—	—	—	—	—		Tr.	5.2
$C_{14:0}$	0,2	2,8	0,9	0.6	0,2	0,5	0,5	0,6	3,2	0,4	0,2	0,9	2,7	2,8	1,9	4,1	2,1	1,1
$C_{14:1}$	—	—	—	—	—	—	0,3	0.3	—	0,3	—	—	—	—	—	—	Tr.	7,1
$C_{15:0}$	Tr.	2,0	0,5	—	—	0,2	—	—	4,0	—	—	Tr.	2,8	2,5	1.2	2,2	0,7	—
$C_{16:0}$	33.2	34.4	23,5	32,2	1.7	30,8	28 7	5,0	10,4	36,2	2,5	14,7	27,2	11 8	21,1	3 .6	23,5	20,9
$C_{16:1}$	13.6	6,8	10,4	10,6	14,5	3,7	14,1	15,5	3,7	12,4	14,7	4 4	26 8	4,2	6,4	4,2	6,3	9,1
$C_{17:0}$	—	—	—	—	—	—	—	—	4,1	—	—	—	—	2,8	—			—
$C_{17:1}$	—	Tr.	—	—	—	2,4	—	—	—	—		0,7	—	—	—	—	—	—
$C_{18:0}$	1,5	9,4	1,4	1,7	0,5	—	1,1	1,9	5,3	1 1	0,5	2,3	4,4	3,4	3,1	22 2	4 1	9 0
$C_{18:1}$	29,8	23,1	22,1	40 0	66,3	2,0	30,1	49,2	9,4	38,2	66,7	17,1	9,8	8,7	28 0	6,8	20,1	16 9
$C_{18:2}$	12,9	11,4	10,6	8,4	13,6	8,9	12,8	17.2	6,1	7,1	10,7	25,0	6,0	7,5	4.2	4,3	7,1	10,1
$C_{18:3}$	3 7	3 3		4 6	3,9	—	10,6	10,3	—	3,4	4 9	32,9	5,5	—	—	—	—	—

TABLE 1 (continued)

Fatty acid	Fruit											Leaves						
	1			2			3			4		1		2	3		4	
	TAGs		FFAs	TAGs		FFAs	TAGs		FFAs	TAGs		TAGs		TAGs	TAGs		TAGs	
	total	position 2		total	position 2		total	position 2		total	position	total	position 2	total	total	position 2	total	position 2
$C_{19:0}$	—	—	—	—	—	—	—	—	Tr.	—	—	—	—	—	—	—	Tr.	3,6
$C_{20:0}$	—	—	—	—	—	—	0,4	—	—	—	—	—	—	9,8	4,8	—	—	—
$C_{20:1}$	2,8	Tr.	9,0	—	—	1,4	—	—	2,7	—	—	—	—	11,6	2,1	17,9	11,4	1,7
$C_{21:1}$	—	—	—	—	—	—	—	—	0,4	—	—	—	—	Tr.	—	—	2,9	—
$C_{22:0}$	—	—	3,2	—	—	—	—	—	0,8	—	—	Tr.	3,3	23,5	8,2	—	—	—
$C_{22:1}$	—	—	8,0	—	—	—	—	—	9,0	—	—	—	—	Tr.	—	—	14,3	—
$C_{23:1}$	—	—	—	—	—	—	—	—	1,9	—	—	—	—	—	—	—	—	—
$C_{24:0}$	—	—	—	—	—	9,5	—	—	—	—	—	Tr.	7,3	11,4	19,0	—	—	—
$C_{24:1}$	—	—	—	—	—	—	—	—	25,7	—	—	—	—	—	—	—	—	—
$C_{25:1}$	—	—	—	—	—	—	—	—	13,0	—	—	—	—	—	—	—	—	—
Unsaturated	64,8	45,1	70,5	63,6	97,6	58,4	67,9	92,5	71,9	61,7	96,3	80,1	49,1	32,0	40,7	33,2	65,5	59,9
Saturated	35,2	54,9	29,5	36,4	2,4	41,6	32,1	7,5	28,1	38,3	3,7	19,9	51,9	68,9	59,3	66,8	34,5	40,1

Among the lipids of the fruit oil of form 4 and of the leaves of all four forms of sea buckthorn, there were only trace amounts of free fatty acids. Their low amount in the fruit of form 4 is a good advantage of this form above the other three.

The difference in the compositions of the fatty acids in position 2 of the triacylglycerols proved to be interesting. In form 1, in contrast to the other three, there was no specificity in the esterification of position 2 predominantly with unsaturated fatty acids [3], while in forms 2, 3, and 4 the total amount of unsaturated fatty acids in position 2 of the triacylglycerols 97.6, 92.5, and 96.3% respectively, for form 1 it was only 45.1%. The same feature is also characteristic for the structure of the triacylglycerols of the leaves of those forms of sea buckthorn in which the composition of the fatty acids in position 2 was determined (Table 1).

All the forms of sea buckthorn considered differed considerably in the composition of the fatty acids in the triacylglycerols of the leaves. In form 1, the main components of the fatty acids were linolenic acid (18:3) (~33% of the total)), which was not present in the fatty acids of the triacylglycerols of the leaves of the other forms, and linoleic acid (18:2) (~25%), which was found in considerably smaller amounts in form 4.

In addition, the linoleic acid behaved anomalously, there being less of this acid in positions 2 of the triacylclycerols (6%) than of palmitic, (16:0) (~27%) and palmitoleic (16:1) (~27%). Linoleic acid usually predominates over the other acids in the esterification of this position [3]. The main components of the fatty acids of the triacylglycerols of the leaves in form 2 were behenic (22:0) (~23%), in form 3 oleic (18:1) (~28%), and in form 4 palmitic (~23%) and oleic (~20%) acids.

EXPERIMENTAL

IR spectra were recorded on a UR-10 instrument with the samples in the form of films, and PMR spectra on a JNM-4H-100/60 MHz instrument using 10-14% solutions in carbon tetrachloride with hexamethyldisiloxane as internal standard.

Preparation of the Petroleum Ether Extracts. The comminuted fruit and leaves were subjected to 3-4 extractions with petroleum ether (40-60°C) by the method of steeping at room temperature.

The solvents were distilled off in a rotary evaporator at a water bath temperature of 40-50°C.

Silica gel L 100/160 μ was used for column chromatography. The size of the column of adsorbent was 2.5 × 60 cm. The sample:adsorbent ratio was 1:15. The volume of the eluates was 200-150 ml. The issuance of the fractions was monitored with the aid of thin-layer chromatography on glass plates and on Silufol in appropriate solvent systems.

Thin-layer chromatography was performed on silica gel LS 5/40 μ. The size of the plates for analytical purposes was 6 × 9 cm and for preparative purposes 18 × 24 cm.

Solvent systems: petroleum ether–diethyl ether: 1) 10:0; 2) 9:1; 3) 8:2; 4) 7:3; 5) 6:4; 6) 5:5; 7) 4:6; 8) 3:7.

The gas–liquid chromatography of mixtures of fatty acid methyl esters and the identification of individual components were carried out by a method described previously [4].

The fatty acids were methylated with diazomethane.

The alkaline and enzymatic hydrolyses of the triacylglycerols were carried out by known methods [4].

The triacylglycerols of all eight extracts had qualitatively similar IR and PMR spectra, and the substances were transparent in the near ultraviolet.

IR spectrum ν_{max}^{film}, cm^{-1}: 3010 w, —CH=CH—; 2975 s, 2885 s, 1380 m, $-CH_3$; 2945 s, 2865 s, 1460 m, 730 m, $-CH_2-$; 1740 s, 1420 m, 1245 m, 1175 s, —OCOR.

PMR spectrum δ, ppm: t 0.86 (3 $-CH_3$); m 1.23, $-(CH_2)_n-$; m 1.55 ($-CH_2CH_2CH=$); m 2.02 ($-CH_2CH=$ and $-CH_2CH_2COO-$); t 2.23 ($-CH_2COO-$); m 2.68 ($=CHCH_2CH=$); m 4.10 ($-CH_2OCOR$, 4H); m 5.10 (>CHOCOR); m 5.22 (—CH=CH—).

Steroids. R_f 0.40, 0.16, 0.05. They gave a characteristic coloration on treatment with sulfuric acid. The component with R_f 0.16 migrated together with β-sitosterol (solvent system 8).

Chlorophyll *a*. UV spectrum, $\lambda_{max}^{acetate}$, nm: 410 max, 422 w, 504 w, 535 w, 570 w, 610 m, 656-668 [5].

SUMMARY

A comparative study of some lipids of four forms of common sea buckthorn has shown that:

1. All four forms differ sharply in the composition of the fatty acids of the triacyl glycerols from the leaves.

2. The sea buckthorn of form 1 differs from the other three by the fact that in the triacylglycerides of the oil of its fruit and leaves the law of the esterification of the positions 2 by unsaturated fatty acids is infringed.

3. The composition of the free fatty acids of the oil of the fruit differs quantitatively from that of the fatty acids of the triacylglycerols of the oils of the fruit and is close to the composition of the fatty acids of the triacylglycerols of the leaves through the presence of fatty acids with 22-25 carbon atoms.

LITERATURE CITED

1. A. D. Bukshtynov, T. T. Trofimov, and B. S. Ermakov, et al., Lesnaya Prom-st', 129 (1978); Kh. Yu. Yusufbekov, E. M. Glazunova, Yu. S. Korzinnikov, N. D. Gachechilidze, and N. B. Krumskaya, Rast. Res., 16, No. 1, 95 (1980).
2. T. G. Zhmyrko, É. I. Gigienova, and A. U. Umarov, Khim. Prirodn. Soedin., 313 (1978); E. E. Shishkina, Lesn. Promst., 173 (1978).
3. F. D. Gunstone, R. J. Hamilton, F. B. Padley, and M. J. Qureshi, J. Am. Oil Chem. Soc., 42, 965 (1965).
4. É. I. Gigienova, A. Akramova, and A. U. Umarov, Khim. Prir. Soedin., 26 (1976); N. T. Ul'chenko, É. I. Gigienova, K. L. Seitanidi, and A. U. Umarov, Khim. Prir. Soedin., 699 (1978).
5. R. F. Baufield, J. D. Barret, and R. H. Falk, J. Chromatogr., 28, 363 (1967).

UBIQUINONES OF MARINE INVERTEBRATES

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The quantitative and qualitative compositions of the ubiquinones of 28 species of marine invertebrates representing five main types have been studied. The amount of ubiquinones did not exceed 5 μg per 1 g dry weight. For all species the main component is Q_{10}, except for ascidians, which produce only Q_9.

The distribution of ubiquinones (Q_n) in living organisms is generally correlated with the aerobic metabolism of their tissues. In higher animals and plants Q_9 and, mainly, Q_{10} are found. Microorganisms are capable of synthesizing all natural ubiquinone homologs but they contain mainly Q_6-Q_9 [1].

Possessing a very broad spectrum of therapeutic action, ubiquinones are finding ever increasing use in medical practice. In view of this, intensive searches are being carried out for natural sources and synthetic routes for their production [2].

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