COMPOSITIONS OF THE LIPIDS OF THE WHITEFISH *Coregonus peled* FROM DIFFERENT PARTS OF THE BODY

N. M. Storozhok and S. A. Storozhok

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The composition of the total, the neutral, and the polar lipids of the whitefish *Coregonus peled* (Gmelin) from different parts of the body — the white muscles of the back and abdomen, the red muscles, the brain, the internal organs, and the fish as a whole — have been studied. The amounts of lipids in these sections were 6.6, 16.1, 20.1, 13.8, 40.0, and 8.4%, respectively. It has been shown that the total and neutral lipids are similar with respect to the main groups of lipids, the bulk of them being represented by triacylglycerols (76.0-82.6%) and phospholipids (2.8-5.8%). The composition of the phospholipids depended substantially on their localization. In the lipids of the white muscles of the back, with a low level of choline-containing lipids, phosphatidylethanolamine and cardiolipin predominated. The lipids of the viscera were distinguished by a high content of sphingomyelin. A high level of choline-containing lipids and cerebrosides was found in the tissues of the brain.

Interest in the study of the composition of whitefish lipids is due to their inclusion in the diet of the bulk of the population of the Tumen' region, but information on their composition is sparse and contradictory [1]. The composition of the fatty acids of the total and neutral lipids from different parts of the whitefish has been studied previously [2, 3]. A high degree of unsaturation and a unique nature of the fatty acid composition of the lipids was reported.

The present paper gives the results of a study of the composition of the total, the neutral, and the polar lipids of the whitefish. For a preliminary analysis of the lipid composition, extracts of the total and neutral lipids were chromatographed on silica gel in system 1. The following classes of substances were detected qualitatively in the extracts: carbohydrates (R_f 0.98), sterol esters (R_f 0.75), fatty acid esters (R_f 0.54), normal triacylglycerols (R_f 0.32), free fatty acids (R_f 0.16), cholesterol (R_f 0.11), monoacylglycerols (R_f 0.08), and phospholipids (at the start). Identification was carried out with the aid of individual substances (markers) and from the R_f values corresponding to the migration paths of the individual fractions. The polar lipids were separated by chromatography in system 2 and also by two-dimensional chromatography in systems 3 and 4. The following pattern of distribution (system 2) was characteristic for the phospholipids: cardiolipins (R_f 0.92), cerebrosides (R_f 0.78), phosphatidylethanolamine (R_f 0.57), phosphatidylcholine (R_f 0.39), sphingomyelin (R_f 0.29), and lysophosphatidylcholine (R_f 0.21).

In the fish studied, depending on the localization, the amount of lipids ranged from 6.6 to 40% (Table 1). The bulk of the total lipids was represented by triacylglycerols, the amount of which was higher in the white muscle tissue. In the lipids of the brain, the viscera, and the red muscles, the amount of triacylglycerols was lower, but this was not accompanied by a rise in the level of phospholipids. The phospholipids were distributed fairly uniformly in the lipids from different positions, and a lower level was found only for the lipids of the viscera. The small amount of phospholipids calculated on the weight of the tissue of 0.5-1.1%, depending on the localization, was nevertheless sufficient, since it is known that this level permits the normal functioning of the organism [4, 5]. A number of authors have found higher levels of phospholipids (30-50%) in emaciated whitefish [1, 6-8].

The lipids of the internal organs and muscular tissue were the richest in cholesterol. The level of free fatty acids in the lipids of the viscera was more than three times higher than in the other tissues, in which their concentration did not appreciably differ. Sterol

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TABLE 1. Composition of the Lipids from Different Parts of the Whitefish (%), ±3%

Lipids	Abdomen as a whole	Red muscles	White muscles of the back	White muscles of the abdomen	Internal organs	Brain
1. Hydrocarbons	1.4	3.8	2.2	1.0	1.0	0.6
2. Sterol esters	2.3	1.4	1.3	1.5	2.0	5.3
3. Fatty acid methyl esters	5.2	4.8	2.0	Tr.	1.8	4.5
4. Triacylglycerols	78.5	77.6	81.0	82.7	77.6	76.4
5. Free fatty acids	1.4	1.4	1.8	1.7	6.6	2.0
6. Cholesterol	3.9	4.2	2.9	5.1	5.0	4.8
7. Monoacylglycerols	2.4	2.1	8.0	3.0	3.2	2.1
8. Phospholipids	4.8	4.7	5.8	5.0	2.8	4.3
Amount of lipids, % on the weight of the crude tissue	8.4	20.1	6.6	16.1	41.0	13.8

TABLE 2. Composition of the Neutral Lipids from Different Parts of the Whitefish (%), ± 3%

Lipids	Abdomen as a whole	Red muscles	White muscles of the back	White muscles of the abdomen	Internal organs	Brain
1. Hydrocarbons	1.5	4.0	2.2	1.2	1.0	1.0
2. Sterol esters	1.8	3.0	1.0	1.2	Tr.	3.8
3. Fatty acid methyl esters	1.4	2.0	1.8	Tr.	1.7	1.8
4. Triacylglycerols	84.5	81.6	84.5	84.5	81.9	81.8
5. Free fatty acids	1.5	1.7	2.1	3.0	5.2	2.2
6. Cholesterol	4.6	4.6	3.1	5.0	4.1	5.0
7. Monacylglycerols	3.4	1.5	4.0	3.0	4.5	3.6
8. Phospholipids	1.3	1.6	1.3	2.1	1.6	0.8

TABLE 3. Composition of the Phospholipids from Different Parts of the Whitefish (%), ± 4%

Tissue or organ	Lysophosphatidylcholine	Sphingomyelin	Phosphatidylcholine	Phosphatidylethanolamine	Cerebrosides	Cardiolipin
Abdomen as a whole	27.5	16.0	13.5	16.0	13.5	13.5
Red muscles	24.0	15.5	10.0	16.0	26.0	8.5
White muscles of the back	6.0	8.5	12.5	23.0	21.5	18.5
White muscles of the abdomen	27.5	12.0	16.0	17.0	11.0	16.5
Internal organs	21.5	43.0	5.5	11.0	6.0	3.0
Brain	30.0	15.0	15.0	5.5	32.5	2.0

esters predominated in the brain lipids. The least polar fraction of hydrocarbons was present in large amount in the lipids of the muscular tissue and in smaller amount in the lipids of the internal organs and brain.

Fatty acid esters in an amount of 1.8-5.3% were detected in the majority of lipid extracts isolated by the binary solvent chloroform–methanol (1:2) using the Bligh–Dyer method [9]. In the lipids obtained by extraction with a mixture of chloroform and diethyl ether (1:1) [10] and in chloroform extracts [11] the methyl ester fraction was absent. In these extracts the amount of free fatty acids had risen. Thus, the methyl esters in whitefish lipids are not native and their formation is due to the esterification of the free fatty acids in the process of extracting the lipids with solvent mixtures containing methanol.

In comparison with the total lipids, the ratio between the main classes in the neutral lipids was different: The proportion of triacylglycerols had increased by 5-10% and the amount of phospholipids had decreased by a factor of 3 and more (Table 2). The concentration of monoacylglycerols and hydrocarbons had increased somewhat and the proportion of fatty acid esters and sterol esters had fallen. The concentration of free fatty acids and cholesterol changed slightly with no kind of regularity.

The polar fraction insoluble in acetone consisted to the extent of 28.5-45% of choline-containing lipids: phosphatidylcholine and lysolecithin (Table 3). At the same time, the lipids of the white and red muscles, and also the lipids of the internal organs, were characterized by a low level of them (28.5-37%), and the lipids of the whole carcass, the lipids of the white muscles of the abdomen, and the lipids of the brain by a higher level (41-45%). Phosphatidylethanolamine and cardiolipin predominated in the lipids of the white muscles of the back and abdomen and the smallest amounts of these lipids were found in the tissues of the internal organs and the brain.

The amount of cerebrosides changed substantially according to their localization, the maximum amount being found in the brain. Characteristic for the lipids of the internal organs was a high content of sphingomyelin (more than 40%), and for the lipids of the white muscles of the back a low amount of sphingomyelin.

EXPERIMENTAL

For the investigation we used freshly trapped yearling whitefish from the lakes in the south of the Tumen' province, winter catch. The procedure for preparing the tissues has been previously described [2]. Lipids were isolated by the method of Bligh and Dyer [9]. The total lipids were separated into neutral and polar functions on the basis of their solubility in cold anhydrous acetone [13]. The acetone was freed from impurities and water by the method of obtaining an unstable crystalline derivative with dry sodium iodide [14]. The chromatography of the lipids was performed on glass plates (128 × 235 mm) with a thin layer of Chemapol LS5/40 silica gel containing 10% of gypsum. The following solvent systems were used: for separating the total and neutral lipids (system 1), petroleum ether (bp 30-60°C)—diethyl ether—acetic acid (90:10:1 by volume) [15]; for separating the polar lipids (system 2), chloroform—methanol—water (65:30:5 by volume) [16]. The two-dimensional chromatography of the polar lipids was performed successfully, beginning in system 3 (chloroform—methanol—7 N ammonia solution (65:30:5 by volume)), and then in system 4 (chloroform—methanol—water (70:30:5 by volume)) [16]. Qualitative reactions were performed with ninhydrin and Dragendorff's solution [12].

The quantitative treatment of the chromatograms was carried out in a scanning densitometer using previously prepared calibration curves for the individual fractions.

The amounts of phospholipids in the total lipid extracts and in the individual fractions of the polar lipids were judged from the amount of phosphorus after mineralization. The chromatograms obtained by separating the substances in systems 3 and 4 were treated with 10% sulfuric acid and were heated to 180°C to reveal the spots of the fractions of the polar lipids. The sections of the thin layer of silica gel corresponding to them were transferred to test tubes and were digested with concentrated perchloric acid. The amount of phosphorus in each spot was determined spectrophotometrically from the absorption of the ascorbic-acid-reduced "molybdenum blue" complex at a wavelength of 815 nm [12, 15]. The results were expressed in percentages of the total amount of phosphorus in all the fractions of phospholipids of the given sample.

SUMMARY

1. The total and neutral lipids of whitefish tissues from different parts of the bodies are similar in relation to the set of the main groups of substances. The absolute amounts of the triacylglycerol and phospholipid fractions are higher in the lipids of the white muscles.

2. The composition of the phospholipids depends substantially on their localization. In the lipids of the white muscles of the back at a low level of choline-containing lipids, phosphatidylethanolamine and cardiolipin predominate. The lipids of the internal organs are distinguished by a high level of sphingomyelin, and the tissues of the brain have the highest level of choline-containing lipids and cerebrosides.

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COUMARIN GLYCOSIDES OF *Haplophyllum perforatum*.

STRUCTURES OF HAPLOPEROSIDES C, D, AND E

M. P. Yuldashev, É. Kh. Batirov,
A. D. Bdovin, V. M. Malikov, and M. R. Yagudaev

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New coumarin glycosides — haploperosides C, D, and E — have been isolated from the epigeal part of the *Haplophyllum perforatum* (MB) Kar et Kir. On the basis of chemical transformations and spectral characteristics, haploperoside D has the structure of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-2H-benzopyran-2-one, and haploperoside C that of 6-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 6)-(2-O-acetyl-β-D-glucopyranosyloxy)]-2H-benzopyran-2-one. The structure of haploperoside E has been established as 7-[O-α-L-rhamnopyranosyl-(1 → 2)-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]-2H-benzopyran-2-one. The structures of haploperosides A and B have been refined. An assignment has been made of the carbon signals in the ^{13}C NMR spectra of haploperosides A, D, C, and E.

We have previously reported the isolation of two coumarin glycosides from *Haplophyllum perforatum* [1-3]. Continuing this investigation we have isolated another three glycosides, which we have called haploperosides C, D, and E. In the present paper we give proofs of their structures.

The UV spectrum of haploperoside D (I) is characteristic for 6,7-di-O-substituted coumarins and is similar to the spectra of scopolin and of haploperoside A [1]. It was established by the GLC method that the molecule of (I) contained D-glucose and L-rhamnose residues in a ratio of 1:1. The acid hydrolysis of (I) gave, in addition to the monosaccharides mentioned above, an aglycone identified as scopoletin. The acetylation of haploperoside D led to a hexaacetate with the composition $C_{34}H_{40}O_{19}$, M^+ 752. Consequently, haploperoside D is a scopoletin bioside.

The mass spectrum of the acetate of (I) contained strong peaks of ions with m/z 273, 213, and 153, showing that in the haplopenoside D molecule the L-rhamnose is the terminal sugar residue [4]. This was confirmed by the production, on partial hydrolysis, of a monoglucoside which was identified as scopoletin 7-O-β-D-glucopyranoside (scopolin) [1]. To determine the structure of the carbohydrate chain we performed the Hakomori methylation of glycoside (I) [5]. In a hydrolysate of the methylation product we identified by GLC

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