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LIPIDS OF MICROALGAE. IV. FATTY ACIDS OF THE BIOMASS OF *Monochrysis lutheri* GROWN UNDER THE CONDITIONS OF AN ACCUMULATION CULTURE

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In the lipids of the biomass of the microalga Monochrysis lutheri, 37 fatty acids have been detected; the main ones are the 16:0 and 16:1 species, the latter being represented by two isomers. The specific localizations of the 16:1, 20:4, and 20:5 acids in the triacylglycerols and the phospho- and glycolipids have been found.

The increasing demand of practical medicine for preparations of polyunsaturated fatty acids (PUFAs) makes it necessary to seek sources for their production. The use of the biomass of microalgae producing lipids with a high content of PUFAs, including eicosaenoic acids, which are biologically active substances [1, 2], is one of the current directions of modern biotechnology.

Continuing a study of the lipids of the microalga *Monochrysis lutheri* (Chrysophyta) growing under the conditions of an accumulation culture [3], we have investigated their fatty acid composition by the methods of UV, IR, and mass spectrometry, GLC, and Ag^+-TLC.

The UV spectrum of the FAs of the total lipids lacked the absorption of a conjugated system of dienic and trienic bonds, while the IR spectrum showed an absorption band at 980 cm^{-1} indicating the presence of isolated *trans*-double bonds in the FAs. A preliminary analysis of the GLC of the FA methyl esters (MEs) of the total lipids showed a high content of a 16:1 acid. To establish the complete composition and structure of the FAs of *M. lutheri*, their total MEs were separated according to degree of unsaturation with the aid of Ag^+-TLC in system 1. Four fractions were obtained and their compositions were determined by GLC (Table 1).

On analytical Ag^+-TLC in system 1, fraction 1 gave two spots: of saturated acids with R_f 0.86 and of monoenoic acids with R_f 0.83. Fraction 2 gave four spots with R_f 0.75, 0.73, 0.74, and 0.69, relating, respectively, to monoenoic and dienoic C_{16} and C_{18} acids; fraction 3 gave two spots of PUFAs with R_f 0.43 and 0.35, and fraction 4 one with R_f 0.35. The esters of fractions 3 and 4 corresponded in mobilities to model MEs of arachidonic (20:4) and eicosapentaenoic (20:5) acids.

GLC analysis showed that fraction 1 was rich in the 16:0 ester. In addition to the unsaturated FA esters shown in Table 1, it contained trace amounts of the 12:0. 15:0, 17:0, 20:0, 22:0, and 24:0 esters, and also of monoenoic 16:1 esters. In fraction 2, a 16:1 ester predominated, and it was accompanied by 16:2, 18:1, 18:2, and, in trace amounts, 14:1 esters. A 20:4 ester was concentrated in fraction 3 and a 20:5 ester in fraction 4. Fraction 3 also contained 14:3, 16:3, and 18:3 esters in trace amounts.

The 16:1 esters detected in fractions 1 and 2 differed not only by their R_f values in TLC but also by their retention times relative to the 16:0 ME (τ_{rel}) in GLC. Thus, the 16:1 ester with R_f 0.83 had τ_{rel} 1.18, while the 16:1 ester with R_f 0.75 had τ_{rel} 1.10 which permitted the first of them to be identified as the methyl ester of the 16:1(3,*trans*) acid and the second as a 16:1 isomer with a *cis*-olefinic bond remote from the carboxy end of the FA molecule – 16:1(9) or 16:1(7) [4]. These isomeric acids have been isolated previously from the lipids of the alga *Euglena gracilis* (Euglenophyta) [5].

The presence of two isomeric 16:1 esters was confirmed by the mass spectrometry of fractions 1 and 2. Appreciable differences were observed between the spectra of these fractions in the regions of fragments with high and medium mass numbers. In the spectrum of fraction 1 there were intense fragments with *m/z* 236 $[M - 32]^+$ and 194 $[M - 74]^+$, which is

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TABLE 1. Composition of Fractions of Fatty Acids, as Methyl Esters, of the Total Lipids of *Monochrysis lutheri* Obtained with the Aid of Ag^+-TLC

Fraction	Acid giving the methyl ester	Content, (%, GLC).
1	18:0	2.0
	16:0	69.3
	14:0	5.7
	16:1(3, trans)	23.0
2	16:1(cis)	65.3
	16:2	2.8
	18:1	3.6
	18:2	28.3
3	20:4	92.0
	20:5	9.0
4	20:5	100.0

characteristic for the 16:1(3,*trans*) ester [4], while in the spectrum of fraction 2 fragments with *m/z* 237 $[M - 31]^+$ and 195 $[M - 73]^+$, assigned to the breakdown under electron impact of a 16:1 ME with the double bond close to the middle of the chain, were more intense. The ratio of the intensities of the fragments differing by unity have been used previously as a characteristic index of the presence of isomers of hexadecenoic acids [4].

It is known that the 16:1(3,*trans*) acid is an essential component of the photosynthetic apparatus of the majority of algae and higher plants and is specifically localized in the phospholipids (PLs), while some algae are incapable of synthesizing this acid [6].

In the mass-spectrometric analysis of the FAMEs of individual acyl-containing classes of *M. lutheri* [3] a high intensity of the peaks of fragments with *m/z* 236 and 194 was observed in the spectra of the triacylglycerols (TAGs), sterol and triterpenol esters (SEs + TEs), glycolipids (GLs) and PLs. The IR spectra of the the FAMEs of these lipids each had an absorption band at 980 cm^{-1} confirming the presence of *trans*-double bonds in them. In the IR spectra of the other classes (free FAs (FFAs), wax esters (WEs), and natural FAMEs) the band at 980 cm^{-1} was absent and in their mass spectra the fragments relating to the ME of a 16:1(*cis*) acid was stronger.

Thus, the hexadecenoic acid isomers of *M. lutheri* are specifically localized in definite lipid classes: 16:1(3,*trans*) – in the TAGs, SEs + TEs, GLs, and PLs, and 16:1(*cis*) in the FFAs, WEs and FAMEs. In addition to this, in contrast to other plant materials, in *M. lutheri*, the 16:1(3,*trans*) acid is a structural element not only of the PLs but also of other clases of lipids.

In the mass spectra of fraction 2, in addition to the fragments characteristic for the MEs of the acids listed in Table 1, we observed the M^+ peaks and also peaks of the characteristic fragments $[M - 31]^+$ and $[M - 74]^+$ with the appropriate mass numbers relating to 14:1 (M^+ 240, *m/z* 209, 166), 14:2 (M^+ 238, *m/z* 207, 164), 17:2 (M^+ 280, *m/z* 249, 206), 19:2 (M^+ 308, *m/z* 247, 234), and 20:2 (M^+ 322, *m/z* 291, 248) MEs.

The mass spectra of fractions 3 and 4 confirmed the correctness of the identification of the MEs of the 20:4 (M^+ 318, *m/z* 287, 244) and 20:5 (M^+ 316, *m/z* 285, 242) acids. In addition to these acids, the spectrum of fraction 3 permitted the detection, as minor components, of 15:3 (M^+ 250, *m/z* 219, 176) and 17:3 (M^+ 278, *m/z* 247, 204) MEs, and the mass spectrum of fraction 4 the identification of another three PUFAs — 16:4 (M^+ 262, *m/z* 231, 188), 22:4 (M^+ 346, *m/z* 315, 270), and 22:6 (M^+ 242, *m/z* 311, 268).

The complete composition of the SAs of *M. lutheri*, both of the total lipids and of the individual lipid classes, is given in Table 2. The number of FAs is large, ranging from 25 (TAGs) to 34 (PLs and GLs), and they include both low- and high molecular-mass acids, saturated and with degrees of unsaturation of from 1 to 6. In all the classes the 16:0 and 16:1 acids predominate.

In addition to the specific localization of the 16:1 isomers, definite regularities were observed in the distribution of the other FAs in the *M. lutheri* lipids. Thus, the PUFAs were localized predominately in the polar lipids. Of them, the 20:4 acid and also the PUFAs of the C_{22} series were concentrated in the PLs and the 20:5 acid in the GLs. Attention is attracted by the higher degree of unsaturation of the FFAs in comparison with that of the TAGs. *M. lutheri* is the second example of lower plants that we have studied, after *Chorella vulgaris* [7], in which we have not observed the enrichment of the FFAs with saturated acids revealed previously [8] in higher plants.

TABLE 2. Fatty Acid Composition of the Lipids of *Monochrysis lutheri* (wt. %)

Acid	Total lipids	SEs + TEs	WEs	Natural FAMEs	TAGs	FFAs	GLs	PLs
10:0	0.2	0.2	0.7	Tr.	0.2	0.3	0.4	0.4
11:0	-	0.3	Tr.	Tr.	0.1	0.1	Tr.	Tr.
12:0	0.2	0.4	0.6	0.6	0.4	0.8	0.4	Tr.
13:0	-	0.3	0.2	Tr.	Tr.	0.1	Tr.	0.4
14:0	3.9	2.8	7.1	5.5	5.9	9.7	4.5	2.5
14:1	0.2	Tr.	Tr.	Tr.	0.2	Tr.	Tr.	0.3
14:2	0.3	0.3	Tr.	0.4	0.4	0.5	Tr.	0.2
14:3	Tr.	0.5	Tr.	Tr.	Tr.	0.2	Tr.	Tr.
15:0	Tr.	Tr.	0.4	Tr.	Tr.	Tr.	Tr.	Tr.
15:3	Tr.	-	-	-	-	-	Tr.	Tr.
16:0	29.5	31.8	39.0	23.7	35.5	28.3	25.4	21.8
16:1(cis)	32.1	27.0	27.0	50.0	38.2	39.9	34.0	32.9
16:1(3, trans)								
16:2	0.9	2.1	0.3	1.2	2.2	0.1	0.7	Tr.
16:3	0.3	0.8	0.1	0.8	0.4	0.1	0.5	Tr.
17:0	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	-	-
17:2	Tr.	-	-	-	-	-	Tr.	Tr.
18:0	0.7	4.5	5.7	1.6	1.7	1.1	4.4	2.3
18:1(9)	2.8	11.6	7.3	5.9	2.8	3.9	5.0	6.2
18:2(9, 12)	3.6	8.3	4.8	2.6	1.4	1.6	3.4	10.8
18:3(9, 12, 15)	0.2	Tr.	0.1	Tr.	Tr.	Tr.	1.3	2.8
X(18:2(6, 9)?)	1.7	2.3	Tr.	Tr.	Tr.	Tr.	1.0	0.6
19:0	Tr.	1.5	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
19:2	Tr.	-	-	-	-	-	Tr.	Tr.
20:0	Tr.	0.9	1.8	0.9	Tr.	0.2	0.8	0.8
20:1	0.3	-	0.4	1.5	-	-	0.7	Tr.
20:2	Tr.	0.8	-	-	-	0.1	0.9	0.9
20:4(5, 8, 11, 14)	5.9	0.9	1.2	0.8	2.2	1.0	0.4	5.3
20:5(5, 8, 11, 14, 17)	17.2	2.4	1.3	1.8	3.4	1.0	15.0	10.8
21:0	-	Tr.	Tr.	Tr.	-	-	-	-
22:0	Tr.	-	1.0	1.0	Tr.	Tr.	Tr.	1.0
22:1	-	-	1.6	-	-	-	1.2	Tr.
22:2	-	Tr.	Tr.	-	-	-	-	-
22:3	-	-	-	-	-	-	Tr.	Tr.
22:4	Tr.	-	-	-	-	-	Tr.	Tr.
22:5	Tr.	-	-	-	-	-	Tr.	Tr.
22:6	Tr.	Tr.	-	-	5.0	11.0	Tr.	Tr.
24:0	Tr.	Tr.	0.9	Tr.	-	Tr.	Tr.	Tr.
Σ_{sat}	34.3	41.8	57.9	33.3	43.8	40.6	35.9	29.2
Σ_{unsat}	65.7	58.2	42.1	66.7	56.2	59.4	64.1	70.8

Taking into account the moisture content and the yield of lipids [3], 1 g of the lipids of the biomass of *M. lutheri* grown in the continuous regime of cultivation contained 64.8 mg of PUFAs (34.5 mg/g a.d.m.), including 16.6 (8.8) mg/g of a 20:4 acid and 48.2 (25.6) mg/g of a 20:5 acid, which are almost identical with the same indices of the freeze-dried biomass that we studied previously [9]. However, the 22:6 acid present in the moist paste in trace amounts was not detected in the freeze-dried biomass. The results obtained show the absence of significant autooxidative changes in the PUFAs during the freeze-drying of the biomass of *M. lutheri*.

In the biomas grown in a controlled regime of cultivation [10] there was only half as much PUFAs as in the sample used in this investigation. Thus the continuous regime of cultivation is more promising for growing a *M. lutheri* biomass with a high content of PUFAs.

EXPERIMENTAL

The conditions for recording the UV, IR, and mass spectra and for growing the algae and the method for extracting the lipids and isolating individual lipid classes have been described previously [3], and the conditions for GLC in [11].

The TLC of the FAMEs was conducted on silica gel L5/40 (Czechoslovakia) with the addition of 10% of $CaSO_4$ and 20% of $AgNO_3$ in system 1: chloroform–benzene–diethyl ether (50:50:25, v/v) using as models the FAMEs from cottonseed oil and 20:4 and 20:5 MEs obtained from the Pacific Ocean Institute of Bioorganic Chremistry, Far Eastern Division of the Russian Academy of Sciences, Vladivostok. The saponification of the lipids was carried out as in [11].

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