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LIPIDS OF MICROALGAE. III. LIPIDS OF THE BIOMASS OF *Monocrysis lutheri* GROWN UNDER THE CONDITONS OF AN ACCUMULATION CULTURE

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The class composition of the lipids of the biomass of the microalga Monochrysis lutheri *grown under the accumulation regime of cultivation has been investigated for the first time. A predominance of triacylglycerols in the neutral lipids, of phosphatidylcholine in the phospholipids, and of digalactosyldiacylglycerols in the glycolipids has been established. New natural alkan-8-ones of the $C_{36}-C_{50}$ series have been detected.*

Continuing a study of the lipids of microalgae [1-4], we have investigated the lipids of the biomass of the microalga *Monochrysis lutheri* (crysophyta) grown under the accumulation regime of cultivation. The lipids were extracted from a paste of microalgae with a moisture content of 73.6% by means of a mixture of chloroform and methanol. The yield of lipids was 53.2% on the absolutely dry mass (a.d.m.). The lipid extract consisted of a resinous dark green mass with a specific odor. The lipids of *M. lutheri* were separated into classes by the CC method followed by TLC in systems 1-8. The quantitative evaluation of the classes of phospholipids (PLs) after their isolation by CC was made by a colorimetric method (Table 1).

More than half the total lipids of *M. lutheri* consisted of polar lipids, in which glycolipids (GLs) predominated, the ratio of these three groups of lipids – neutral (NLs), GLs, and PLs (without taking pigments into account) – being 1.7:1.5:1. The bulk of the NLs consisted of triacylglycerols (TAGs), a GL – digalactosyldiacylglycerol, and a PL – phosphatidylcholine.

In the IR spectrum of the hydrocarbons, in addition to the absorption bands of aliphatic chains (2980, 2920, 1465, 1375, and 720 cm^{-1}) there were characteristic absorption bands of double bonds in the *cis*- and *trans*-configurations (730 and 970 cm^{-1}).

On TLC in system 1, the hydrocarbons were revealed with I_2 vapor in the form of two spots corresponding to alkenes (R_f 0.90) and terpenoids (R_f 0.6). On treatment with H_2SO_4 followed by heating, the spot with R_f 0.66 assumed a pink-violet coloration. The assignment of this spot to the terpenoids was confirmed by the chromatography of the hydrocarbons on Ag^+-TLC in systems 1 and 2 [5]. Preparative TLC, using systems 1 and 2 successively, separated the hydrocarbons into two fractions – olefins and terpenoids. The compositions of these fractions were established from their mass spectra (Table 2). According to the results of analysis, among the hydrocarbons of *M. lutheri* there were no paraffins, a multicomponent fraction of olefins being observed.

The mass spectrum of the terpenoid fraction contained the M^+ peaks of only three hydrocarbon homologues (Table 2). They were represented by peaks of ions with *m/z* 423, 409, 395 $[M - 15]^+$, 367, 355, 341 $[M - 69]^+$, 273, 259, 165, and 151, showing that these compounds belonged to the acyclic triterpenes isolated previously from the alga *Botryococcus braunii* (Chlorophyta) [6]. The detection of terpene hydrocarbons in the lipids of *M. lutheri* has been reported previously [7], but their identification was not carried out.

It is known that in algae the hydrocarbon content may fluctuate within wide limits – from 0.1 to 36%, and their composition is more diverse than in higher plants [8]. On comparing literature facts with the results that we have obtained it is possible to conclude that the composition of the hydrocarbons of the biomass of *M. lutheri* is a specific one, since no

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TABLE 1. Composition of the Lipids of *Monochrysis lutheri*

Class of lipids	Content	
	% on the mass of the lipids	mg/g a.d.m.
I NEUTRAL LIPIDS:	38.1	202.7
1. Hydrocarbons:	5.9	31.2
a) olefins	3.7	19.6
b) terpenoids	0.5	2.6
c) carotenoids	1.7	9.0
2. Sterol and triterpenol esters	0.5	2.6
3. Wax esters	1.0	5.2
4. Fatty acid methyl esters	0.4	2.2
5. High-molecular-mass ketones	0.3	1.6
6. Triacylglycerols	18.4	97.8
7. Free fatty acids	3.9	20.7
8. High-molecular-mass alcohols	0.3	1.6
9. Sterols and triterpenols	1.2	6.3
10. Chlorophylls	6.3	33.5
Polar Lipids	61.9	329.3
II. GLYCOLIPIDS:	43.9	233.6
1. Brown pigments	15.8	84.1
2. Sterol glycoside esters	5.4	28.8
3. Monogalactosyldiacylglycerols	7.9	42.4
4. Sterol glycosides	1.6	8.2
5. Digalactosyldiacylglycerols	9.5	50.5
6. Sulfolipids	3.7	19.6
III. PHOSPHOLIPIDS	18.0	95.7
1. Phosphatidylethanolamine	Tr.	Tr.
2. Phosphatidylglycerol	1.7	8.9
3. Phosphatidylcholine	8.6	45.7
4. Phosphatidylinositol	3.2	17.0
5. Unidentified	1.0	5.7
6. Lyso-phosphatidylcholine	1.1	5.7
7. Phosphatidylserine	1.5	7.9
8. Phosphatidic acid.	0.9	4.8

n-alkanes, as were found in the algae Bacillariophyta, Phaeophyta, and Rhodophyta, were detectd among them, and nor were the branched alkanes that are predominant in Chlorococcales.

The sterol and triterpenol esters and the wax esters and methyl esters of fatty acids were identified by their chromatographic mobilities, capacity for being saponified with the formation of sterols, triterpenols, high-molecular-mass alcohols, and fatty acids, respectively, and from the behavior in GLC of the fatty acid methyl esters on a polar phase. We have previously detected these classes of lipids in comparable amounts in the lipids of the biomass of *Chlorella vulgaris* [1] grown under the conditions of an accumulation regime of cultivation.

The aliphatic ketones (R_f 0.66 in system 3) gave a positive reaction with 2,4-dinitrophenylhydrazine. Their IR spectrum contained absorption bands of the groups $-CH_3$ (2980, 2375 cm^{-1}), $-CH_2$ (2920, 1465, 720 cm^{-1}) and $>C=O$ (1740 cm^{-1}). These results and the absence of a band in the IR spectrum at 2720 cm^{-1}, which is characteristic for the stretching vibrations of the C−H bond of an aldehyde group, enabled the compounds isolated to be assigned to the aliphatic ketones. In the UV spectrum, absorption was observed at λ_{max}^{hexane} 270 nm.

The mass spectrum of the ketones had a series of homologous M^+ ions with *m/z* from 520 to 716 of the general formula $C_{36+x}H_{75+x}O$, with values of *x* from 0 to 14. The presence in the mass spectrum of a high-intensity peak with *m/z* 128 (43%, rearrangement ion) formed on the cleavage of an α-C=O bond [9] showed that the C=O group was present at C-8.

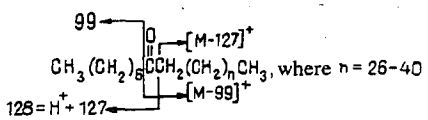

TABLE 2. Composition of the Hydrocarbons of *Monochrysis lutheri*

Hydrocarbon, general formula	Homologues	Molecular ions (M^+)
Fraction 1 (olefins):		
C*n*H2*n*	C22—C36	340—536
C*n*H2*n*—2	C20—C34	310—506
C*n*H2*n*—4	C16—C32	252—476
C*n*H2*n*—6	C16—C32	250—474
C*n*H2*n*—8	C20—C34	304—500
Fraction 2 (terpenoids);		
C*n*H2*n*—10	C30—C32	410, 424, 438

It is known that algae may be a source of carbonyl compounds. Thus, α-unsaturated aldehydes of the $C_{52}-C_{64}$ series have been detected in the alga *Botryococcus braunii* [10], and a series of *n*-$C_{37}-C_{39}$ alk-2- and -3-ones containing from 1 to 4 nonconjugated *cis*-double bonds in individual representatives of the family Prymneseophyceae [11]. Thus, the high-molecular-mass saturated ketones of the $C_{36}-C_{50}$ series that we have detected in the lipids of *M. lutheri* are new natural compounds.

The compositions of the free high-molecular-mass alcohols and those bound in wax esters were identical, according to their mass spectra. The presence of a series of peaks of homologous ions with *m/z* 326-606 $[M - 18]^+$ and their subsequent breakdown under electron impact in the mass spectrum that is characteristic of alcohols [9], together with their chromatographic mobilities and the nature of their interaction with I_2 vapor [2], permitted them to be assigned to pentaenols of the $C_{24}-C_{44}$ series.

According to their mass spectrum [12], the sterols were represented by both saturated and unsaturated components (Table 3). The set of free sterols was wider (8 components) than that of the bound sterols (5 components). The main sterol among the free species was cholesterol (see Table 3), which agrees with previous results [12]. In the mixture of sterols bound with fatty acids, campesterol predominated.

Information on the composition of the sterols of microalgae is fairly voluminous but the sterols of Chrysophyta have been studied inadequately. It has been reported that, depending on the conditions and phase of growth, 1.3-1.7% on the a.d.m. of free sterols containing from 5 to 16 components accumulate in the biomass of *M. lutheri* [12]. The authors concerned did not detect sterol esters in this algal species.

The pigments of *M. lutheri* were represented by carotenoids, chlorophylls, and brown compounds of unknown nature.

According to their IR spectrum, the carotenoids were a mixture of β-carotene and xanthophylls in which the β-carotene (λ_{max}^{hexane}, nm: 422, 445, 472) was predominant. Of native chlorophylls, four components, with R_f 0.43, 0.40, 0.32, and 0.28 (system 5), were detected in the sample under investigation. After their separation by preparative TLC in the same system, the compound with R_f 0.43 was assigned on the basis of its UV spectrum ($\lambda_{max}^{acetone}$, nm: 633) to chlorophyll *a* [13]. The UV spectra of the other green pigments had absorption maxima at 658, 656, and 653, respectively. The literature reports the presence in some species of Chrysophyta algae, together with chlorophyll *a*, of chlorophyll *c* [8]. However, the absorption maxima listed above are not characteristic for the latter and possibly relate to other forms of chlorophyll *a* [13].

A quantitative estimation of the pigments of *M. lutheri* by the spectrophotometric method [14] showed that 1 g of lipids of the sample of biomass contained 50.41 mg (26.82 mg/g a.d.m.) of chlorophyll *a* and 20.73 mg (11.02 mg/g a.d.m.) of β-carotene, which is comparable with the results of Table 1.

No quantitative evaluation of the individual classes of polar lipids of Chrysophyta algae has been made previously, while the GLs and PLs of Chlorophyta and Cyanophyta have been studied more deeply in connection with their determining role in the formation of the photosynthetic apparatus of photosynthesizing organisms [15]. We have established that almost one third of the mass of GLs consists of digalactosyldiacylglycerols, and approximately one half of the mass of the PLs is due to phosphatidylcholine (see Table 1).

On comparing the results given in the present paper and those obtained previously [2, 3], it is possible to conclude that the biomass of *M. lutheri* subjected to freeze-drying [3] contains a 1.3 times smaller amount of total lipids, mainly through decreased amounts of free fatty acids and their esters with sterols, triterpenols, and high-molecular-mass alcohols and of phosphatidylglycerols, and also a 1.8 times smaller amount of chlorophyll *a*.

TABLE 3. Composition of the Sterols of *Monochrysis lutheri* According to Their Mass Spectrum

Sterol	M+ (rel. %*, %**)	Characteristic fragments, *m/z*	Form in which present	
			free	bound
5,22-Cholesta-5,*trans*-22-dien-3β-ol (22-dehydrocholesterol)	384 (6.5*, 3.2**)	369, 301, 300	+	+
5α-Cholesten-3β-ol (cholesterol)	386 (100*, 6.2**)	371, 368, 353	+	+
5α-Cholestan-3β-ol	388 (5.9**)	373, 355, 248	-	+
24-Methylcholest-5-en-3β-ol (campesterol)	400 (4.3*)	385, 382, 367, 315	+	-
24-Methylcholestan-3β-ol (campestanol)	402 (40**)	400, 387, 217, 165	-	+
24-Ethylcholesta-5,22-dien-3β-ol (stigmasterol)	412 (6.5*)	413, 379, 351, 300	+	-
24-Ethylcholest-5-en-3β-ol (sitosterol)	414 (3.9*)	399, 396, 329, 303	+	-
4,4,14α-Trimethyl-9β,19-cyclo-5α-cholest-24-ene-3β-ol (cycloartenol	426 (4.3*)	411, 408, 393, 365	+	-
24-Ethyl-4α-methylcholest-8(14)-en-3β-ol	428 (4.3*)	413, 410, 395, 287	+	-
24-Ethyl-4α-methylcholestan-3β-ol	430 (13.5*, 2.6**)	397, 394, 247	+	+

*In relation to *m/z* 386 (100%) for the free sterols.

**To *m/z* 55 (100%) for the bound sterols.

In addition, the biomass of *M. lutheri* grown under a controlled regime of cultivation [2] contained 8.5 mass-% less total lipids, a 1.5 times lower amount of chlorophyll *a*, and 1.3 times less β-carotene than the sample under investigation.

A continuous regime of cultivation permits a *M. lutheri* biomass to be obtained with a high content of lipids and biologically active lipophilic compounds.

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrophotometer (in acetone and hexane), IR spectra on a UR-20 spectrophotometer (in a film), and mass spectra on a MKh-1310 spectrometer with a system for direct injection into the ion source, at an ionizing voltage of 70 V, a collector current of 80 μA, and a temperature of the ionizing chamber of 190°C for ketones and sterols and 150°C for the other classes of lipids.

The *M. lutheri* biomass was provided by G. S. Skotnikova of VNIIbiotekhnik [All-Union Scientific-Research Biotechnical Institute] (Moscow). The algae were grown on a nutrient medium containing all the biogenic elements, including 230-300 mg of N_2/liter, with day-long illumination by DNAT-400 lamps (illuminance at the surface of the radiation receiver 100 W/m^2 at a temperature of 26 ± 2°C, pH 7 ± 0.5, for 8 days [2].

The lipids were extracted from the moist paste by steeping with a mixture of chloroform and methanol (2:1, v/v) at a ratio of raw material and extractant of 1:4.

CC was carried out on a 30 × 70 cm column of silica gel L 100/160 (Czechoslovakia), the NLs being eluted as described in [5], the GLs with acetone, and the PLs with methanol. TLC was conducted on silica gel L 5/40 with the addition of 10% of $CaSO_4$ and 20% of $AgNO_3$ on Silufol (Czechoslovakia) in the systems: 1) hexane; 2) benzene; 3) hexane–diethyl ether–acetic acid (80:20:1); hexane–diethyl ether: 4) 9:1; 5) 7:3; 6) 7:8; 7) chloroform–methanol–25% ammonia (65:25:5); 8) direction 1: chloroform–methanol–25% ammonia (8:3:1); direction 2: chloroform–methanol–acetic acid–water (8:3:1:1); 9) chloroform–benzene–diethyl ether (50:50:25). The chromatograms were revealed as described in [5].

The composition of the PLs was determined as in [16].

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