The Very Long Chain Fatty Acids of the Green Alga, *Chlorella kessleri*

TOMÁŠ ŘEZANKA and **MILOSLAV PODOJIL***, *Institute of Microbiology, Czechoslovak Academy of Sciences, Prague 4, 142 20 Czechoslovakia*

ABSTRACT

Fatty acids of the fresh-water green alga, *Chlorella kessleri* (heterotrophically cultivated), were fractionated (as methyl esters) using high performance liquid chromatography (HPLC). A fraction of fatty acids with chain lengths longer than 28 carbon atoms was analyzed by gas chromatography-mass spectrometry (GC-MS). The very long chain fatty acids, ranging from 31 to 36 carbon atoms, were found for the first time in a green alga.
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INTRODUCTION

In our previous communication (1), we described the distribution of fatty acids of a collection of fresh-water green algae, e.g., fatty acids with up to C_{30} chain lengths in *Chlorella kessleri* cultivated heterotrophically. The detector response indicated the presence of higher homologues but they could not be precisely analyzed because of the low proportion.

This study was aimed at separating a fraction of very long chain fatty acids with more than 28 carbon atoms from the whole complex of fatty acids and determining the composition of fatty acids in this fraction.

EXPERIMENTAL METHODS

A sample of fatty acids (3,500 mg) from the fresh-water green alga, *C. kessleri*, was converted to methyl esters by boron trifluoride-methanol reagent (2).

High performance liquid chromatography (HPLC) of fatty acids was performed using a SP 8000 instrument (Spectra Physics, USA) with a Separon SI C1 column 50 cm × 6 mm i.d. (Laboratorní přístroje, ČSSR); mobile phase-linear gradient (2 ml/min) from the mixture methanol/water (80:20) into methanol (30 min), followed by methanol (60 min), then detection in a UV detector at 210 nm. The column efficiency was 8,700 theoretical plates, V_0 4 ml and t_R of the methyl ester of octacosanoic acid (28:0) was 27.13 min. In 7 runs (500 mg each), 3,500 mg of a mixture of very long chain fatty acids was chromatographed. The separation was achieved by collecting fractions with retention time of more than 27.13 min and pooling fractions from twice repeated HPLC. After evaporation of the mobile phase, the total yield of a mixture of fatty acids with the chain longer than 28 carbon atoms was 32.2 mg.

*To whom correspondence should be addressed.

These were separated and identified on a Hewlett-Packard 5992 B (USA) gas chromtograph-mass spectrometer (GC-MS) instrument with a SCOT glass capillary column (SGE, Australia) 77 m × 0.5 mm i.d., with SE-30 stationary phase. The operating conditions of the instrument were: ionization energy 70 eV, scan speed 690 amu/s, mass range 4-600 amu; data were processed with a HP 9825 A computer connected online with the GC-MS. For the separation of very long chain fatty acids, the capillary column oven temperature was programmed from 100-290 C (2 min isothermally, ballistically to 240 C, then at a rate of 2C/min to 290 C, and further isothermally 50 min). The flow of carrier gas (helium) was 1.5 ml/min. The t_R of methyl ester of octacosanoic acid was 9.15 min and that of the methyl ester of hexatriacontenoic acid (36:1) 57.21 min.

RESULTS AND DISCUSSION

The usual fatty acids of the fresh-water green algae (regardless to branching) range from C_{14} to C_{22} (3). Fatty acids with longer chains have been described in mycobacteria (up to 70 carbon atoms) (4); *Saccharomyces cerevisiae* (5,6); marine algae, *Botryococcus braunii* (7) and *Emiliana huxleyi*, (unsaturated fatty acids 36:2 and 36:3) (8); higher plants (fatty acid esters up to 60 carbon atoms) (9,10).

Table 1 shows the percentage representation of very long chain fatty acids in the whole complex of fatty acids of *C. kessleri*. The fatty acids found were saturated and monounsaturated from C_{29} up to C_{36}, odd and even numbered, most of them with a straight chain. Only one fatty acid (C_{29}) was branched. The ratio of saturated and unsaturated fatty acids changes with the number of carbons; in the C_{29} acids, the saturated fatty acids predominate; in the C_{30} acids, the unsaturated acid predominates and in the higher homologues, the saturated fatty acids are missing.

TABLE 1

Percentages of Very Long-chain Fatty Acids of the Alga, *C. kessleri* (Heterotrophically Cultivated), and MS Fragments Imp tant for Identification

Methyl ester	Percentage[a]	M	M-29	M-31	M-32	Other ions			Base peak
29:0 br_2	3.6	452(13)	423(1)	–	–	381(1)	325(1)	269(2)	88
29:1	6.3	450(1)	–	419(7)	418(18)	376(2)[b]	334(2)[c]	74(43)	55
29:0	9.9	452(17)	423(1)	421(1)	–	367(1)	311(1)	255(2)	74
30:1	60.0	464(1)	435(1)	433(5)	432(5)	390(1)[b]	348(1)[c]	311(1)	55
30:0	17.0	466(20)	437(1)	435(1)	–	423(4)[d]	367(2)	311(1)	74
31:1	3.9	–	–	–	446(13)	404(2)[b]	362(2)[c]	199(2)	55
32:1	14.6	–	–	461(5)	460(10)	418(1)[b]	376(1)[c]	199(2)	55
33:1	1.8	–	–	475(3)	474(5)	432(2)[b]	199(7)	143(10)	55
34:1	2.6	–	–	489(3)	488(6)	199(8)	143(12)	74(56)	55
35:1	2.0	–	–	503(2)	502(3)	199(7)	143(8)	74(32)	55
36:1	2.1	–	–	517(1)	516(3)	199(3)	143(12)	74(58)	55

[a]In the whole complex of fatty acids (10^{-2}).
[b]Ion M-74.
[c]Ion M-116.
[d]Ion M-43; br_2 –branching on carbon C_2; figures in parenthesis–percentage of intensity of the base peak.

The unsaturation of very long chain fatty acids might improve the transport across the membrane in Saccharomyces, as described by Welch and Burlingame (5), by bringing down the melting point; for example, the fatty acid with one double bond melts 22 C lower than the corresponding saturated acid. The difficulty in analyzing the very long chain fatty acids in the whole complex of fatty acids was eliminated by separating lower fatty acids (up to C_{28}) using the HPLC method. Even though the proportion of very long chain fatty acids (higher then C_{30}) drops dramatically, under the experimental conditions given, the C_{36} is also detectable although only 10^{-2}% of total fatty acids.

Table 1 also shows that in monounsaturated methyl esters of fatty acids above C_{31}, the molecular ions were not found. For their determination, the ions M-31 (M-MeO) and M-32 (M-MeOH) become important, as well as the ions M-74 and M-116. The intensity of the latter goes down with the increasing number of carbon atoms. The common peak, m/z 55 (C_4H_7) (11), was found in all monounsaturated methyl esters of fatty acids.

We preferred the identification of fatty acids as methyl esters rather than TMS derivatives for the following reason: at the operating column temperature (280 C), perceptible evaporation of stationary phase (methyl siloxane) already takes place, making the employment of the SIM method for ion 73 impossible (it originates by decomposition of stationary phase, SE-30). The use of SIM method for m/z 74 (MacLafferty rearrangement of methyl ester of fatty acid) enabled scanning the spectrum in the moment of maximum ion current.

The data summarized in Table 1, and by Řezanka et al. (1), indicate that the content of very long chain fatty acids was influenced by the mode of cultivation. In the autotrophically cultivated *C. kessleri*, only fatty acids up to C_{28} were found. Under these conditions the elongation of the carbon chain probably does not take place.

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