Chloroplast Fatty Acid Composition in Mediterranean Populations of the Marine Chlorophyte, *Anadyomene stellata*

Debra L. Bemis[a,*,‡], Vassilios Roussis[b], Constantinios Vagias[b], and Robert S. Jacobs[a]

[a] Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106

[b] Department of Pharmacy, Division of Pharmacognosy, University of Athens, Panepistimiopolis, Athens, 157 71, Greece

[‡] Present address: Department of Pharmacology, E-405, Weill Medical College of Cornell University, 1300 York Ave, New York, NY 10021. Fax: (212) 746–8835. E-mail: dlb2004@med.cornell.edu

* Author for correspondence and reprint requests

Z. Naturforsch. **55 c,** 569–575 (2000); received December 16, 1999/March 29, 2000

Anadyomene stellata, Chloroplast, Fatty Acids

Chloroplasts isolated from three populations of the tropical marine Chlorophyte *Anadyomene stellata* collected off the coast of Greece were analyzed for their fatty acid composition. Following the preparation of fatty acid methyl esters, GC-MS (EI) was utilized to identify the fatty acids present in each population. Including isomers, the fatty acid profile of all three algal populations was comprised of 19 fatty acids (4 saturated, 6 monounsaturated, 9 polyunsaturated) with palmitic acid present in the highest amounts (25–27% of total fatty acids). Analysis of variance revealed significant differences amongst the three populations in the percent of total fatty acids for twelve of the fatty acids. High levels of C_{20} PUFAs, an atypical observation in Chlorophytes, were observed in all three populations comprising approximately 17% of total fatty acids. Furthermore a 14:2 PUFA, apparently rare in marine macrophytic Chlorophytes, was identified in significant quantities. Surprisingly, we did not find any of the conjugated tetraene containing fatty acids that we previously identified in the *A. stellata* populations studied from the Florida Keys.

Introduction

The vast appearance of oxylipins, oxidative metabolites of arachidonic acid and its homologues, throughout the four kingdoms Protista, Eubacteria, Plantae, and Animalia suggests that these compounds have important physiological roles. This assumption has been illustrated quite extensively in mammals with the study of eicosanoids and their essential activity as signaling molecules in various physiological pathways. Less progress has been made in the clarification of biosynthetic pathways involved and physiological roles of oxylipins in other organisms. The ability to synthesize these compounds with various chain lengths has been observed throughout the major multicellular algal classes (Rhodophyceae, Phaeophyceae, and Chlorophyceae), in addition to the Cyanobacteria (Gerwick, 1994; Gerwick and Bernart, 1993; Moore, 1981). Biochemical pathways involved in the production of these compounds have been studied to some extent in Rhodophytes and Phaeophytes, however, less work has been done in Chlorophytes (Gerwick, 1994; Hamberg, 1992; Burgess *et al.*, 1991).

Previously, we reported the enzymatic production of a series of five unique conjugated tetraene containing polyunsaturated fatty acids (PUFAs) with chain lengths varying from 16–22 carbons occurring within the chloroplasts of the macrophytic Chlorophyte, *Anadyomene stellata* (Wulfen) C. Agardh (Mikhailova *et al.*, 1995). We reported these compounds to be 16:5Δ4,7,9,11,13; 18:4Δ6,8,10,12; 20:5Δ5,8,10,12,14 (bosseopentaenoic acid); 20:6Δ5,8,10,12,14,17; and 22:7Δ4,7,9,11,13,16,19 (stellaheptaenoic acid). Interestingly, formation of PUFAs with the conjugated tetraene moiety has only been observed in a few instances in nature, including α-parinaric acid found in certain seed oils (Noda *et al.*, 1980) and bosseopentaenoic acid which was first identified in the red algae *Bossiella orbigni-*

0939–5075/2000/0700–0569 $ 06.00

ana (Hamberg, 1995; Burgess *et al.*, 1991). The formation of the conjugated tetraene containing PUFAs in *A. stellata* appears to be moderated by a unique enzyme system independent of the lipid linked fatty acid desaturases present in both the chloroplast and the endoplasmic reticulum (Mikhailova *et al.*, 1995).

To progress towards elucidating the roles these PUFAs have within the whole alga and their subcellular mechanism(s) of formation, determination of the endogenous fatty acid substrates present in *A. stellata* chloroplasts is essential. Researchers have shown that fatty acid composition of lipids comprising biological membranes, such as the glycolipids and phospholipids of chloroplast thylakoids, can be effected by environmental conditions (Thompson, Jr., 1996; Harwood, 1996; Pohl and Zurheide, 1979). Decreased temperatures have long been demonstrated to result in increased levels of fatty acid unsaturation (reviewed in Harwood *et al.*, 1994; Marr, 1962). Growth limiting conditions such as decreased light intensity, increased salinity, increased cell concentration, suboptimal pH, and suboptimal temperature have also been indicated to alter algal fatty acid profiles (Cohen *et al.*, 1988). Preference towards PUFAs with fewer points of unsaturation has been observed under suboptimal growth conditions, as demonstrated in the red alga *Porphyridium cruentum* by a decrease in eicosapentaenoic acid and concurrent increase in arachidonate (Cohen *et al.*, 1988). Because environmental conditions can have such a significant impact on the fatty acid content of algal lipids, the contrarieties observed amongst fatty acid profiles obtained from species specific algal collections have often been attributed to these external factors, rather than the cause of genetic differences. For example, the controversial reports on the fatty acid content of the red algae *Gracilaria confervoides* are now thought to be resultant of differing ambient conditions (Cohen *et al.*, 1988; Araki *et al.*, 1986; Sato, 1975). Therefore, it is prudent to analyze the fatty acid composition of several populations of an algal species in varying environments prior to drawing firm biochemical, physiological, and taxonomic deductions.

The purpose of this study was two-fold: to determine the natural substrates present for the fatty acid oxidative enzyme system present, and to analyze chloroplast fatty acid composition from various populations of the algae during the same seasonal time frame. To obtain a statistically valid chloroplast fatty acid profile, we collected *A. stellata* from three geographically distinct populations off the coast of Greece and compared their chloroplast fatty acid compositions. The three sites included Daskalio on the southern coast, Ftelios located on the mid-eastern coast and Vagias Beach on Aegina Island, one of the Saronic Gulf Islands. The collection sites were chosen based on data reporting previous observations of this algae, and were quite different from each other in terms of wave energy, topography, and local environment (Diapoulis and Haritonidis, 1987). All collections were made within a six week period during the Fall.

Results and Discussion

A significant amount of data regarding either the fatty acid composition of whole cell or specific lipid preparations from a variety of marine macrophytic algae can be found in the literature (reviewed in Thompson, Jr., 1996; Pohl and Zurheide, 1979; Jameison and Ried, 1972; Vaskovsky *et al.*, 1996; Fleurence *et al.*, 1994; Khotimchenko, 1993; Dembitsky *et al.*, 1991). However, reports of isolated chloroplast fatty acid composition of these organisms are quite limited. The data presented in this study profiles and compares the chloroplast fatty acid composition of the *A. stellata* samples collected from the three locations off the coast of Greece. The majority of fatty acids identified in chloroplast preparations from all three *A. stellata* collections were found to be 16:0, 18:1 (n-9, n-7), 18:2, and 20:4 (n-6, n-3). In general, the data collected are similar to that reported for other whole cell Chlorophyte preparations with high amounts C_{16} and C_{18} fatty acids (Khotimchenko, 1993). The saturated fatty acid 16:0 is present in the highest amounts in all samples consisting of approximately 25 to 27% of total fatty acids. High levels of 16:0 and the 18:1 isomers are expected since these fatty acids are the endpoints of fatty acid synthesis and primary desaturation reactions in the chloroplasts (Stumpf *et al.*, 1982). The percent saturated fatty acid content was 33.7%, 33.7%, and 32.8% for the samples collected at Daskalio, Ftelios, and Vagias Beach, respectively. These values are quite comparable to data collected from a variety of Chlorophytes, Rhodophytes, and Phaeophytes which

yielded an average of 30% saturated fatty acids of total (Fleurence *et al.*, 1994).

An analysis of variance was employed to determine if differences between the populations exist for the contribution of each fatty acid (Table I). Including isomers, 19 fatty acids were identified in all samples: 4 saturated, 6 monounsaturated, and 9 polyunsaturated fatty acids. Twelve were found to have significant differences between either all locations or one other site. Although statistically significant ($P<0.05$), these differences were relatively minor, and were in the range of 2% or less with the exception of 20:4 (n-6) which was approximately 4% less at the Vagias Beach site than the other two sites.

Uncharacteristic of other Chlorophytes was the high levels of C_{20} PUFAs identified (approximately 17% of total fatty acids). Although many examples of C_{20} PUFAs are seen in Chlorophytes, the levels are generally below 10% of total fatty acids for whole cell preparations, and more commonly below 5% (Pohl and Zurheide, 1979; Dembitsky *et al.*, 1991). Such high levels of these PUFAs are considered to be more characteristic of Rhodophytes (Khotimchenko and Vaskovsky, 1990), and their presence, especially the essential fatty acid 20:4 (n-6), render *A. stellata* a unique model for future studies involving the use of macroalgae for nutritional and / or medicinal uses. The possibility that the chloroplasts of these organisms contain a higher percentage of C_{20} PUFAs than whole cell preparations must be taken into account and is a topic for further study.

The significant levels of 14:2 and 14:1 fatty acids found in the *A. stellata* chloroplast preparations were an unexpected observation in this study. The average levels of both of these fatty acids from data collected at all three sites was calculated to be 0.88% of total fatty acids for 14:2 and 0.25% for 14:1. Interestingly, no reports of the presence of 14:2 were found in the literature for a marine macrophytic Chlorophyte, and only a few reports of 14:1 were found (Jameison and Ried, 1972; Vaskovsky *et al.*, 1996; Dembitsky *et al.*, 1991; Khotimchenko, 1993; Pohl and Zurheide, 1979; Fleurence *et al.*, 1994). The application of density gradients during chloroplast isolation and concentration procedures made it highly

Table I. Chloroplast fatty acid composition from *Anadyomene stellata.*

Fatty acid	Average % of total fatty acids ± SEM		
	Daskalio	Ftelios	Vagias beach
14:2*	0.86 ± 0.05	1.00 ± 0.03†	0.76 ± 0.02
14:1 (n-5)	0.23 ± 0.02	0.27 ± 0.02	0.26 ± 0.01
14:0	5.05 ± 0.21†	4.75 ± 0.05†	3.98 ± 0.03
16:3 (n-3)	8.09 ± 0.31†	6.46 ± 0.11	7.58 ± 0.07†
16:3 (n-6)	0.91 ± 0.05‡	0.72 ± 0.006	0.83 ± 0.04
16:2 (n-6)	0.85 ± 0.06	1.04 ± 0.04	0.99 ± 0.02
16:1 (n-9)	1.39 ± 0.09	1.34 ± 0.09	1.60 ± 0.05
16:1 (n-7)	0.58 ± 0.04	0.58 ± 0.03	1.08 ± 0.02†
16:1 *trans* (n-13)	0.93 ± 0.06	0.95 ± 0.04	1.07 ± 0.03
16:0	26.04 ± 0.71	27:56 ± 0.16	26.97 ± 0.09
17:0	0.17 ± 0.04	0.13 ± 0.03	0.21 ± 0.01
18:3 (n-3)	7.62 ± 0.26‡ [0.69]§	6.40 ± 0.10 [0.55]§	6.31 ± 0.06 [0.48]§
18:2 (n-6)	11.90 ± 0.22†	13.15 ± 0.12†	14.13 ± 0.07†
18:1	3.55 ± 0.12	3.56 ± 0.06	5.25 ± 0.03†
18:1 (n-7)	11.04 ± 0.45	11.73 ± 0.11	13.03 ± 0.06†
18:0	2.78 ± 0.26†	1.63 ± 0.10	2.01 ± 0.06
20:4 (n-6)	14.82 ± 0.74†	14.10 ± 0.19†	10.80 ± 0.11
20:5 (n-3)	1.86 ± 0.23	3.45 ± 0.11†	1.66 ± 0.06
20:4 (n-3)	1.00 ± 0.08	0.88 ± 0.17	1.13 ± 0.10

n = 5 for Daskalio and Ftelios; n = 3 for Vagias Beach.
* Location of double bonds is unknown.
† Values significantly greater than values without † based on ANOVA analysis, $P<0.05$.
‡ Values that are significantly greater than lowest value of the group, but no significant difference from the mid-ranged value (ANOVA, $P<0.05$).
§ Ratio of % 18:3 to % 18:1.

unlikely that these unique PUFAs were the result of contamination occurring in all three distinct habitats and in all samples at levels of approximately 4–5%.

The apparent lack of 16:4 is particularly important due to its recently proposed taxonomic significance for macrophytic green algae (Khotimchenko, 1993; Fleurence *et al.*, 1994). This PUFA has typically been considered to be a common chloroplast glycolipid constituent in a variety of macrophytic green algae (Johns *et al.*, 1979). However, observations made by Khotimchenko (1993) between algae of the two classes Chlorophyceae and Siphonophyceae, demonstrate that some of these macrophytes lack this fatty acid, and they suggest that specific differences involving the16:4 content can be used group algae into the appropriate classes. They reported that the algae in the class Chlorophyceae were able to synthesize 16:4 (n-3) and 16:3 (n-3), while algae in the class Siphonophyceae were found to either synthesize 16:3 (n-3) or 16:4 (n-3), but not both. Our data is in accordance with these findings since we identified only the presence of 16:3 in *A. stellata* and this algae is grouped in the class Siphonophyceae.

Data from a variety of plants and algae, including soybean, *ricinus communis* cell cultures and the red alga *Porphyridium cruentum*, suggest that increased light levels, decreased water temperatures, and overall optimal growth conditions result in higher levels of PUFA unsaturation and faster growth rates (Cohen *et al.*, 1988; Gemmrich, 1982; Pohl and Zurheide, 1979; Wilson *et al.*, 1978). Since the formation of 18:3 in plants and many algae is known to occur via a sequential desaturation of 18:1 by specific fatty acid desaturases (Harwood, 1996), the ratio of % 18:3/% 18:1 can yield useful information regarding the effects of environmental conditions on the alga's ability to produce the polyunsaturate. The ratios of % 18:3 to % 18:1, as bracketed in Table I, decrease from 0.69 at Daskalio to 0.48 at Vagias Beach. In addition, Vagias Beach was found to have a higher water temperature and greater salinity than the other two sites, as described in the Experimental section. The *A. stellata* population at Vagias Beach was also observed to be scarcest and in the worst condition in comparison to Daskalio and Ftelios. A consideration of the %18:3/%18:1, along with the quantitative and qualitative observations made the collection sites, suggests that Vagias Beach had the least optimal growth conditions.

In contrast to our previous studies of *A. stellata* (Mikhailova *et al.*, 1995), apparently no conjugated tetraene containing fatty acids were present in these chloroplast preparations. This data may offer important clues regarding the physiological roles of these unique PUFAs in *A. stellata*. If such compounds serve the purpose of signaling molecules, it is possible that the stimulus leading to their production was environmental and present at a much lesser extent or not at all in comparison to numerous previous studies conducted in the Florida Keys. Additionally, the concentration of conjugated tetraenes within the chloroplasts may have been quite small during this season and the half-life relatively short rendering them undetectable by the methods employed in this study. Although the samples were handled and processed with the same techniques, the algae analyzed from the Florida Keys were collected during the spring, while this current study was conducted during the fall.

This study represents a comparison of the fatty acid composition of chloroplasts isolated from a single species of algae, *A. stellata*, collected at three distinct sites of the coast of Greece. Although general trends remained the same between the three populations, minor differences in the percent of total fatty acids were observed. These differences are potentially resultant of the variations in growth conditions observed at the three sites, an example being variations in the levels of the 18:3 PUFA. It is possible, but less likely, that the differing fatty acid profiles are due to the presence of genetically distinct clones. However, the genetic data necessary to confirm this was not collected for this study. Interestingly, an apparently rare PUFA, 14:2, was found in samples collected from all three sites. Based on published reports dealing with a variety whole cell algal preparations, this PUFA has not been identified in other Chlorophytes. With the methods employed in this study, we did not detect any conjugated tetraene containing PUFAs in these Mediterranean samples. Whether the oxidative pathway involved in the formation of these compounds was not expressed in the Mediterranean samples during the time frame of this study or the conjugated tetraenes were not present in detectable levels can be the focus of future studies.

Experimental

Collection sites and materials

Daskalio can be described as a small bay with a rocky shoreline. This was the highest energy site of all three in terms of surge and wave action. *A. stellata* was found growing on rock substrate in 0.5 to 2 m of water. The average thallus size was approximately 1.25 cm in diameter. The abundance and physical condition of the algae collected at this location were superior to the other two collection sights. Algae were collected in October of 1997 with a water temperature of 17° and a salinity of 36.34 g/l.

Ftelios, a protected cove, was the lowest energy site regarding wave action and surge. *A. stellata* was found growing on an unidentified rod-like sponge, the brown algae *Cystoseira*, and rock substrate in 0.5 m of water. The alga was much less abundant at this site than Daskalio, but the average thallus diameter was 2.0 cm. A collection at this site was made during end of October, 1997 with a water temperature of 16.5° and a salinity of 36.07 g/l.

The last of the three sites, Vagias Beach on Aegina Island, is similarly characterized by large rock slabs entering the water. The distribution and scarcity of *A. stellata* at this site was quite unique. It was found growing only on one small rock located about 7 m off shore. The algae were covered in a thin layer of sediment. No sightings of *A. stellata* were made on the rock substrate lining the shore. The quantity present was by far the least of all three sites and the physical condition of the algae was poorest. Average thallus size was 1 – 1.5 cm in diameter. Algae were collected in November, 1997 with a water temperature 19° and salinity at 37.64 g/l.

Algae handling

Upon collection, algae was transported in local sea water, immediately cleaned of debris and other organisms including epiphytes, and the species were identified as described by Littler and Littler (1991). The samples were flash frozen in liquid nitrogen and stored at −70 °C until used.

Chloroplast isolation

Intact and viable chloroplasts were obtained as previously described (Mikhailova *et al.*, 1995; Gegenheimer, 1990; Bemis, 1998). Briefly, whole algae was ground using a mortar and pestle in an osmotically balanced HEPEs buffer (330 mM sorbitol, 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride, pH 7.8) filtered, and then subjected to several centrifugation steps. The resultant pellet was resuspended and applied to a continuous Percoll (Sigma) density gradient. The chloroplast fraction, sedimenting at a density of 1.054 g/ml, was recovered from the gradient, washed of excess percoll, and resuspended in the HEPES buffer described.

Fatty acid analysis

PUFAs in photosynthetic eukaryotic chloroplasts are mainly found acylated to the lipids comprising the chloroplast envelope and thylakoid membranes (Wood, 1974). Chlorophytes are reported to have roughly the same classes and proportions of galactolipids, phospholipids and sulpholipids as higher plants comprising these membranes, with the galactolipids accounting for the majority of the thylakoid lipids (Mazliak, 1977). Five independent chloroplast preparations from each site were analyzed for fatty acid content, except from Vagias Beach due to the limited amount of algae collected. In this case, only three samples were prepared. The fatty acid content of *A. stellata* chloroplasts was analyzed as follows. The isolated chloroplast preparations were first saponified, and then fatty acid methyl esters (FAMEs) were prepared by boiling the isolated chloroplast preparation in acidic methanol for 10 min at 100° (Sasser, 1997). The FAMEs were extracted in hexane: CH_2Cl_2 (1:1 v/v), and then washed in an aqueous solution of 0.3 M NaOH. Mass spectra were obtained from a Hewlett Packard 5973–6890 GC-MS system operating on EI mode (equipped with a HP 5MS capillary column, 30mx0.25m film thickness). The initial temperature of the column was 60° and then was heated to 280 °C with a 3°/min rate. All FAMEs were off the column within 45 min. The identification of the chemical constituents was based on comparison of the RRt values and mass spectra with those obtained from authentic samples and/or the NIST/NBS library spectra. Isomers were

identified from one another by comparison to ECL data (Christie, 1988). Fatty acids are listed as percentage of total fatty acids based on TIC. To standardize the fatty acid percentages, known concentrations of a series of saturated and unsaturated fatty acids (Sigma Chemical) with the same chain lengths as those identified in our samples, were injected into a Hewlett Packard 6890 GC-MS using FID. The calculated percentages of the saturated fatty acids were found to be 1% higher than actual amounts, while that of the unsaturated fatty acids were found to be 1% lower than actual concentrations. Therefore, all values listed in Table 1 were multiplied by the appropriate factor to correct for this minor discrepancy.

Acknowledgements

This paper is funded by a grant from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U. S. Department of Commerce, under grant number NA66RG0477, project number R/MP-71 through the California Sea Grant College System, and in part by the California State Resources Agency. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. The U. S. Government is authorized to reproduce and distribute for governmental purposes.

Araki S., Sakurai T., Oohusha T., and Kayama M. (1986), Component fatty acids of lipid from *Gracilaria verrucosa*. Bull. Jap. Soc. Sci. Fish. **52**, 1871.

Bemis D. L. (1998), Fatty acid oxidation in isolated chloroplasts from the tropical marine Chlorophyte, *Anadyomene stellata*. Ph.D. Thesis, University of California, Santa Barbara.

Burgess J. R., de la Rosa R. I., Jacobs R. S., and Butler A. (1991), A new eicosapentaenoic acid formed from arachidonic acid in the coralline red algae *Bossiella orbigniana*. Lipids 26, **162–165.**

Christie, W. W. (1988), Equivalent chain-lengths of methyl ester derivatives of fatty acids on gas chromatography. J. Chromatogr. **447**, 305.

Cohen Z., Vonshak A., and Richmond A. (1988), Effect of environmental conditions of fatty acid composition of the red alga *Porphyridium cruentum*: correlation to growth rate. J. Phycol. **24**, 328–332.

Dembitsky V. M., Pechenkina-Shubina E. E., and Rozentsvet O.A (1991), Glycolipids and fatty acids of some seaweeds and marine grasses from the black sea. Phytochemistry, **30**, 2279–2283

Diapoulis A. and Haritonidis S. (1987), A qualitative and quantitative study of the marine algae in the Saronikos Gulf (Greece). Mar. Ecol. **8**, 175.

Fleurence J., Gutbier G., Mabeau S., and Leray C. (1994), Fatty acids from 11 marine macroalgae of the French Brittany coast. J. Appl. Phycol. **6**, 527–532.

Gegenheimer P. (1990), Preparation of extracts from plants. In: Methods in Enzymology, vol. 182 (M. P. Deutscher, ed.). Academic Press, New York, NY, pp. 174–193.

Gemmrich A. R. (1982), Effect of light on lipid metabolism of tissue cultures. In: Biochemistry and Metabolism of Plant Lipids. Elsevier Biomedical Press, New York, NY, pp. 213–216.

Gerwick W. H. (1994), Structure and biosynthesis of marine algal oxylipins. Biochim. Biophys. Acta. **1211**, 243.

Gerwick W. H. and Bernart M. W. (1993), Eicosanoids and related compounds from marine algae. In: Marine Biotechnology, Vo. 1: Pharmaceutical and Bioactive Natural Produces (D. H.Attaway and O. R. Zaborsky, eds.). Plenum Press, New York, NY, pp. 101–152.

Hamberg M. (1992), Metabolism of 6,9,12-octadecatrienoic acid in the red alga *Lithothamnion corallioides*: mechanism of formation of a conjugated tetraene fatty acid. Biochem. Biophys. Res. Commun. **188**, 1220–1227.

Hamberg M. (1995), Biosynthesis and degradation of alpha-paranaric acid and related conjugated tetraene fatty acids. Adv. Prostaglandin Thromboxane Leukot. Res. **23**, 193–8.

Harwood J. L. (1996), Recent advances in the biosynthesis of plant fatty acids. Biochim. Biophys. Acta **1301**, 7–56.

Harwood J. L., Jones A. L., Perry H. J., Rutter A. R., Smith K. L., and Willikams M. (1994), In: The Temperature Adaptation of Biological Membranes (A. J. Coussins, ed). Portland Press, London, pp. 107–118.

Jameison G. R. and Ried E. H. (1972), The component fatty acids of some marine algal lipids. Phytochemistry **11**, 1423–1432.

Johns R. B., Nichols P. D., and Perry G. J. (1979), Fatty acid composition of ten marine algae from Australian waters. Phytochemistry **18**, 799.

Khotimchenko S. V. (1993), Fatty acids of green macrophytic algae from the sea of Japan. Phytochemistry **32**, 1203–1207.

Khotimchenko S. V. and Vaskovsky V. E. (1990), Distribution of C_{20} polyenoic fatty acids in red macrophytic algae. Bot. Mar. **33**, 525–528.

Littler D. S. and Littler M. M. (1991), Systematics of *Anadyomene* species (Anadyomenaceae, Chlorophyta) in the tropical western Atlantic. J. Phycol. **27**, 101–118.

Marr A. G. (1962), Effect of temperature on the composition of fatty acids in *E. coli*. J. Bacteriol. **84**, 1260–1268.

Mazliak P. (1977), Glyco- and phospholipids of biomembranes in higher plants. In: Lipids and Lipid Polymers in Higher Plants (M. Tevini and H. K. Lichtenthaler,-eds). Springer, Berlin, pp. 48–74.

Mikhailova M. V., Bemis D. L., Wise M. L., Gerwick W. H., Norris J. N., and Jacobs R. S. (1995), Structure and biosynthesis of novel conjugated polyene fatty acids from the marine green algae *Anadyomene stellata*. Lipids **30**, 583–589.

Moore R. E. (1981), Constituents of blue-green algae. In: Marine Natural Products: Chemical and Biological Perspectives (P. J. Scheuer,ed). Academic Press, New York, pp. 1-52.

Noda M., Ohga K., Nakagawa Y., and Ichihara K. (1980), Biosynthesis of parinaric acid (9,11,13,15-octadecatetraenoic acid) from *Impatiens balsamina* seed oil. Dev. Plant Biol. **6**, 215–218.

Pohl P. and Zurheide F. (1970), Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Marine Algae in Pharmaceutical Science (H. A. Hoppe, T. Levring, and Y. Tanaka, eds.). Walter de Gruyter, Berlin, pp. 472–523.

Sasser M. (1997), Identification of bacteria by gas chromatography of cellular fatty acids. In: Midi Technical Note (Internal Technical Report), Midi, Newark, DE. **101**, 1–7.

Sato S. (1975), Effects of environmental factors on the lipid components of *Porphyra* species. Bull. Jap. Soc. Sci. Fish. **41**, 1173.

Stumpf P. K., Shimakata T., Eastwell K., Murphy D. J., Liedvogel B., Ohlrogge J. B., and Kuhn D. N. (1982), Biosynthesis of fatty acids in a leaf cell. In: Biochemistry and Metabolism of Plant Lipids (J. F. G.M. Wintermans and P. J. C. Kuiper, eds.). Elsevier Biomedical Press, New York, NY, pp. 3-12.

Thompson Jr. (1996), Lipids and membrane function in green algae. Biochim. Biophys. Acta **1302**, 17–45.

Vaskovsky V. E., Khotimchenko S. V., Xia B., and Hefang L. (1996), Polar lipids and fatty acids of some marine macrophytes from the yellow sea. Phytochemistry **42(5)**, 1347–1356.

Wilson A. C., Kates M., and de la Roche A. I. (1978), Incorporation of [1-^{14}C] acetate into lipids of soybean cell suspensions. Lipids **13**, 504.

Wood B. J. B. (1974), Fatty acids and saponifiable lipids. In: Algal Physiology and Biochemistry) (W. D. P. Stewart,ed). University of California Press, Berkely, pp. 236–265.