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DISTRIBUTION OF 21:6 HYDROCARBON AND ITS RELATIONSHIP TO 22:6 FATTY ACID IN ALGAE

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Abstract—We report the distribution and quantitation of 21:6 hydrocarbon and 22:6 fatty acid within the major groups of algae in both marine and freshwater environments. The absence of 21:6 hydrocarbon in nonphotosynthetic dinoflagellates and diatoms suggested its localization within the chloroplasts. The hydrocarbon accounted for 2–15 per cent of the total lipid of diatoms, which have low (< 1 per cent of total fatty acids) amounts of the 22:6 fatty acid. In comparison the dinoflagellates have major amounts of the 22:6 fatty acid (20–30 per cent) and small quantities of 21:6 hydrocarbon (less than 2 per cent of the lipid). The synthesis of 21:6 hydrocarbon by a specific 22:6 fatty acid decarboxylase appeared probable, and the activity of this enzyme appeared to vary in different groups of algae.

INTRODUCTION

WE RECENTLY reported on the occurrence of heneicosahexaene (a straight chain hydrocarbon with 21 carbons and 6 double bonds) in diatoms.[1] The present study is concerned with the distribution and quantitation of this unique hydrocarbon within the major groups of algae in both marine and freshwater environments. Most previous work has been on the hydrocarbons of blue-green algae, which contain short chain saturated or monounsaturated hydrocarbons.[2–5] Other algae contain a series of saturated and monounsaturated hydrocarbons with the number of carbons ranging from 15 to 36.[6,7] Since most of the previous hydrocarbon work had been done on algae unrelated to diatoms, we felt a hydrocarbon survey of algae related to diatoms would be of value. Quantitation of the 22:6 fatty acid was carried out because structural similarity of 21:6 hydrocarbon to the 22:6 fatty acid suggested hydrocarbon synthesis by fatty acid decarboxylation.

RESULTS

The 21:6 hydrocarbon can be seen to have a widespread occurrence in many photosynthetic algae from both marine and freshwater environments, and occurs in field collections as seen by its presence in red tide (*Gonyaulax*) and *Codium* (Table 1). It is the major hydrocarbon (80–90 per cent) in photosynthetic diatoms, dinoflagellates, cryptomonads, phaeophytes, chrysophytes, and in the chlorophyte *Platymonas*, while it occurs in lesser amounts in the euglenids and the chlorophyte *Codium fragile*. The hydrocarbon was absent in cyanophytes, rhodophytes, xanthophytes, and most chlorophytes. Blumer and Mullin have also

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carried out a study of the distribution of 21:6 hydrocarbon in algae.[8] Their results also verify its presence in diatoms, dinoflagellates, chrysophytes, as well as its absence in cyanophytes, chlorophytes and rhodophytes.

The hydrocarbons 21:5 and 21:4 also appear to be important hydrocarbons in dinoflagellates and euglenids. The identification of 21:4 and 21:5 hydrocarbon is tentative, since it was based only on the relative retention time on the DEGS column, without the necessary polyunsaturated standards. Except for *Isochrysis*, none of the algae contained 22.4 or 22:5 fatty acid (Table 2).

In all algae we noted low amounts of a series of straight chain saturated and monounsaturated hydrocarbons ranging in carbon length from 15 to 36, and work by others also indicates the presence of this series in algae,[6,7] except for the blue-green algae in which the major hydrocarbons are 15:0, 16:0, 17:0, and 19:1.[2–5] The major hydrocarbons of the red algae examined (*Porphyridium cruentum, Gigartina* sp., and *Plocamium* sp.) were the saturated straight-chain hydrocarbons 17:0 and 15·0 as was previously noted by Clark and Blumer.[6] The freshwater xanthophyte, *Tribonema aequale*, and the marine xanthophyte, *Vaucheria* cf. *longicaulis*, contained no 21:6 hydrocarbon, but had large amounts of hydrocarbon (approximately 10% of the lipid—Table 1). The major hydrocarbon of *Tribonema* was 22:1, which cannot be correlated with the presence of a corresponding fatty acid (Table 2). The nonphotosynthetic dinoflagellates *Noctiluca scintillans* and *Oxyrrhis marina* were both high in hydrocarbon content, 21 and 7% of the lipids respectively (Table 1) while the green alga, *Dunaliella tertiolecta*, closely related to the alga they fed on (*D. viridis*), had a low hydrocarbon content (1% of the lipid). Thus these dinoflagellates may be either accumulating or synthesizing hydrocarbons from their dietary lipids. Since the major hydrocarbon of *Noctiluca* and *Oxyrrhis* was heptadecane, which is a minor hydrocarbon in *Dunaliella*, we suggest hydrocarbon synthesis is taking place, possibly from stearic acid, as shown previously in the blue-green alga, *Nostoc muscorum*.[9]

The fatty acid composition of representatives from most groups of algae have been reported by a number of workers[10–16]. In Table 2 we report the fatty acid composition of some of the algal species which we analyzed, including several which have not previously been reported. Although a broad spectrum of fatty acids is seen among the organisms containing 21:6 hydrocarbon, it would appear that other fatty acids are not decarboxylated to any great extent. The 22:6 fatty acid was noted in the nonphotosynthetic dinoflagellate *Oxyrrhis marina*, which lacks the 21:6 hydrocarbon. These facts suggest a specific decarboxylase for the 22:6 fatty acid in photosynthetic species.

DISCUSSION

Function

Because of the absence of the 21:6 hydrocarbon in the nonphotosynthetic diatoms (*Nitzschia alba*) and dinoflagellates (*Oxyrrhis* and *Noctiluca*) we suggest that this hydrocarbon may have a function in the chloroplast membrane of certain groups of algae which

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TABLE 1. THE % LIPID, HYDROCARBON, CAROTENE, AND 22:6 FATTY ACID IN ALGAE AND PHOTOSYNTHETIC BACTERIA*

Organism and Strain	Lipid (% of dry wt.)	Hydrocarbon (% of lipid)	Carotene (% of total hydrocarbon)	21:6 Hydrocarbon (% of total hydrocarbon)	22:6 Fatty acid (% of total fatty acid)	Fresh water or marine	Culture media and purity
Photosynthetic bacteria—Pseudomonadales							
Chromatium sp. D	3·1	3·1	—	A	A	F	Ax Medium A[17]
Rhodopseudomonas spheroides 2.4.1	8·6	3·8	—	A	A	F	Ax[18]
Rhodosprillum rubrum S1	8·8	0·8	—	A	A	F	Ax[18]
Chlorobium thiosulfatophilum NCIB 8346	9·1	5·2	—	A	A	F	Ax[17]
Chloropseudomonas ethylicum 2K	11·7	2·2	—	A	A	F	Ax[17]
Photosynthetic bacteria—hyphomicrobiales							
Rhodomicrobium vannielii	10·6	2·1	—	A	A	F	Ax[18]
Blue-green algae—cyanophyta							
Anacystis nidulans IU 625	—	0·6	8	A	A	F	Ax[19]
Oscillatoria woronichinii Smayda, 1961	3·4	0·7	1·1	A	A	M	Ax[20]
Red algae—rhodophyta							
Gigartina sp.	—	0·2	—	A	A	M	—
Plocamium sp.	3·6	1·1	9	A	A	M	—
Porphyridium cruentum IU 161	1·7	0·2	39	A	A	M	Ax[20]

* —Not investigated, A absent, T trace, M marine, F freshwater, Ax axenic, Ag agnotobiotic, IU Indiana University Culture Collection, E in the experimental section.

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TABLE 1. *cont.*

Organism and Strain	Lipid (% of dry wt.)	Hydrocarbon (% of lipid)	Carotene (% of total hydrocarbon)	21:6 Hydrocarbon (% of total hydrocarbon)	22:6 Fatty acid (% of total fatty acid)	Fresh water or marine	Culture media and purity
Yellow-green algae—xanthophyta							
Vaucheria cf. *longicaulis* West, No. 652	10·1	14·2	1	A	A	M	Ax^{20}
Tribonema aequale IU 50	17·9	12·2	4·5	A	A	F	Ax^{21}
Green algae—chlorophyta							
Acetabularia calyculus Baker, Puerto Peñasco	3·5	2·1	4·2	A	A	M	Ag sea water
Chlorella ellipsoidea IU 20	—	0·05	—	A	A	F	Ax^{22}
Codium fragile	2·2	3·6	60	20	—	M	—
Dunaliella tertiolecta Pintner	—	1	—	A	A	M	Ax^{23}
Dunaliella viridis Speer, D-24 1964	—	—	—	A	A	M	Ax^{20}
Platymonas sp. L. Loeblich, 1966	6·6	7·8	4	82	0·6	M	Ax^{20}
Euglenids—Euglenophyta							
Euglena gracilis IU 753	10·1	5·2	4	1	T	F	Ax^{24} autotrophic
Euglena gracilis IU 753	12·8	1·7	8	5	T	F	Ax^{24} heterotrophic
Eutreptia viridis Dodson, 1952	18·8	4·2	9	12	6·7	M	Ag^{20}
Dinoflagellates—pyrrhophyta							
Cachonina niei IU 1564	13·6	6·7	1	5	—	M	Ax^{20} no soil extract
Exuviaella cassubica Pintner, 1958	5·2	3·7	2	20	—	M	Ax^{20}
Gloeodinium montanum IU 1651	27·9	1·4	1	3	33·5	F	E,Ax

Gonyaulax polyedra-red tide	19·5	2·2	1·1	90	20·6	M	—
Gymnodinium splendens	16·2	0·4	1	80	26·7	M	Ag^{25}
Holmes, 1963							
Noctiluca scintillans	28	20·7	—	A	—	M	
Szladek, 1969							*D. viridis*^{20}
Oxyrrhis marina	17·3	7·1	—	A	15·2	M	
West							*D. viridis*^{20}
Peridinium sociale	16·2	0·9	1·8	80	23·5	M	Ax^{20}
Loeblich 133-4							
Cryptomonads—cryptophyta							
Cryptomonas ovata	18·3	12·3	2·2	20	1·4	F	Ax^{21}
var. *palustris*							
IU 358							
Rhodomonas lens	16·8	11·7	12	45	1	M	Ax^{20}
Lasker							
Diatoms—bacillariophyta							
Chaetoceros curvisetus	9·1	13·2	5	90	0·7	M	Ax^{25}
Jordan No. 15							
Cylindrotheca fusiformis	11·8	3·7	3	90	0·8	M	Ax^{26}
Watson, No. 13							
Ditylum brightwellii	—	2·7	9·1	90	0·8	M	Ax^{25}
Smayda							
Lauderia borealis	13·2	10·3	4	90	0·7	M	Ag^{25}
Jordan No. 14							
Navicula pelliculosa	8·2	1·0	—	80	—	F	E,Ax
IU 668							
Nitzschia alba	10 6	0·2	A	A	—	M	E,Ax
Tennant							
Skeletonema costatum							
Guillard							
3-day culture	6·2	4·3	—	91	2·5	M	Ax^{25}
8-day culture	8·6	15·7	4	91	0·9	M	Ax^{25}
8-day culture	9·2	15·0	—	90	0·6	M	Ax^{25}
8-day culture	8·1	12·2	—	90	0·9	M	Ax^{25}

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TABLE 1. *cont.*

Organism and Sytrain	Lipid (% of dry wt)	Hydrocarbon (% of lipid)	Carotene (% of total hydrocarbon)	21:6 Hydrocarbon (% of total hydrocarbon)	22:6 Fatty acid (% of total fatty acid)	Fresh water or marine	Culture media and purity
Golden algae—chrysophyta							
Cricosphaera carterae Parke 156	9·7	15·7	3	85	16·2	M	Ax^{20}
Isochrysis galbana IU 987	26·0	4·6	3·2	90	10·5	M	Ax^{20}
Prymnesium parvum Droop No. 65	4·2	7·6	2·2	80	1·4	M	Ax^{20}
Brown algae—phaeophyta							
Ectocarpus siliculosus Lewin, 1965	8·7	7·6	5	85	0·2	M	Ax^{20}
Laminaria sp.	—	5·8	6	30	0·9	M	—
Streblonema cf. *oligosporum* Lewin No. 115, 1965	5·6	8·7	4	85	0·2	M	Ax^{20}
Protozoan—ciliata							
Tetrahymena pyriformis 6L and HSM	—	1·6	A	A	A	F	Ax^{27}

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TABLE 2. FATTY ACIDS OF SELECTED ALGAE

Fatty acid	*Tribonema aequale*	*Eutreptia viridis*	*Gloeodinium montanum*	*Gymnodinium splendens*	*Peridinium sociale*	*Cryptomonas ovata*	*Rhodomonas lens*	*Cylindrotheca fusiformis*	*Cricosphaera carterae*	*Isochrysis galbana*	*Prymnesium parvum*	*Ectocarpus siliculosus*	*Streblonema* cf. *oligosporum*
12:0	t*	1·2	t	t	t	0·2	0·7	0·5	t	t	t	0·6	t
12:1	t	t	t	t	t	t	0·1	0·4	t	t	t	t	t
13:0					t			0·4					
14:0	4·7	0·2	12·9	12·0	9·7	0·7	7·7	11·0	1·0	19·3	68·9	3·3	4·1
14:1	t	t		t	0·3	t	0·1	t	t			0·1	
15:0	0·5	t		t	0·6	t	0·2	0·5	t	0·2	t	t	1·0
15:1	t	t				t	0·3	2·6	t		t	t	
16:0	7·9	33·4	18·6	32·8	36·5	20·8	7·9	23·2	20·3	11·1	8·8	16·6	15·0
16:1	9·5	3·1	2·1	0·3	0·7	0·5	4·0	31·9	27·0	1·7	0·6	2·8	0·7
16:2	t	0·1		t		0·1		0·8		0·2	t		
16:3	0·2	0·4	0·6	2·0	3·5	7·1	0·7		1·3	0·4	0·2	0·5	0·6
17:0	t	t					0·3			t	t	t	t
18:0	0·4		t	t	t	t			t		t		
18:1	73·1	8·9	14·1	9·2	21·1	23·0	19·5	4·1	8·4	23·9	10·1	11·1	9·1
18:2	0·6	9·9	0·9	0·7	t	5·7	5·3	2·3	3·7	7·6	0·9	3·5	3·4
18:3	0·2	1·4	0·7	0·6	t	t	3·5	0·2	1·1	2·3		0·8	0·7
19:0							t						t
20:0									t			0·5	0·5
20:1	1·0	9·4		t		24·3	13·7	2·2	14·5	4·8	1·7	20·8	12·9
20:2		11·2	4·5	1·4		13·9	20·0	t	0·7	7·7	2·6	11·5	26·4
20:4	0·8	0·7		4·3			1·1	5·1		t	0·4	9·4	8·0
20:5	2·4	12·3	12·0	9·7	3·9	7·8	11·7	10·1	9·4	0·4	0·3	16·0	16·1
22:0									3·3			1·1	0·8
22:1								t	t				
22:2		1·2					1·0	0·3				0·9	0·1
22:3								1·8					
22:5										3·1			
22:6		6·7	33·5	26·7	23·5	1·4	1·0	0·8	16·2	10·5	1·4	0·2	0·2

*t, trace.

contain the hydrocarbon. Carotene although accounting for a small fraction of the total hydrocarbon (Table 1) apparently has an important function in the protection of algae from photooxidation.[28] Ji, Hess and Benson[29] have shown that a certain chloroplast protein binds carotene molecules, while Mohammadzadeh and co-workers[30] have shown that serum albumin can bind saturated straight chain hydrocarbons. In both cases the binding is probably due to the hydrocarbon entering the hydrophobic areas of the protein. Thus the 21:6 hydrocarbon could cause a change in the tertiary structure of chloroplast membrane protein.

Synthesis

Kolattukudy feels that hydrocarbon synthesis in higher green plants occurs by elongation of a fatty acid and later decarboxylation of the longer chain.[31] Albro and Dittmer favor a condensation of two fatty acids followed by reduction to a hydrocarbon.[32,33] Blumer and associates have suggested that the isoprenoid hydrocarbons found in zooplankton and fish are synthesized by decarboxylation of the polyunsaturated isoprenoid acid,[34] while Han and co-workers showed that ^{14}C-stearic acid was decarboxylated to heptadecane in the blue-green alga, *Nostoc muscorum.*[9]

The all cis configuration and the position of the double bonds has been shown to be the same in the 21:6 hydrocarbon and the 22:6 fatty acid.[1,13] Thus we hypothesize the synthesis of 21:6 hydrocarbon by decarboxylation of the 22·6 fatty acid. The presence of 22·6 fatty acid was noted in all algae containing the 21:6 hydrocarbon (Table 1). The xanthophytes, rhodophytes, cyanophytes, most chlorophytes, and photosynthetic bacteria lacked both the 22:6 fatty acid and the 21:6 hydrocarbon. The 22:6 fatty acid is an important fatty acid of dinoflagellates (20–40 per cent) as shown by Patton,[35] Ackman,[12] and us, whereas in diatoms only a small percentage (less than 1%) is 22:6 fatty acid (Table 1). The 21:6 hydrocarbon accounts for less than 1% of the lipid of dinoflagellates, whereas this hydrocarbon in diatoms accounts for 1–15% of the lipid. Our conclusion is that dinoflagellates have a much less active 22:6 fatty acid decarboxylase than diatoms. Ackman has reported that the level of 22:6 fatty acid in the diatom, *Skeletonema costatum*, decreases with the age of cultures.[36] Since we have noted that the 21:6 hydrocarbon makes up less of the lipid in younger cultures of *Skeletonema costatum* (Table 1), the decarboxylase is either not active in young cultures, or is synthesized during the later part of the exponential phase of growth.

Distribution

The absence of 21·6 hydrocarbon in the prokaryotic blue-green algae and photosynthetic bacteria suggests that this hydrocarbon evolved in the eukaryotic algae. There is good correlation between the occurrence of 21.6 hydrocarbon and the photosynthetic pigments fucoxanthin and chlorophyll c. The dinoflagellates, the majority of which have peri-

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dinin[37] in place of fucoxanthin, are more distantly related morphologically and cytologically from those algal groups that contain fucoxanthin as the major accessory carotenoid. In this respect it would be interesting to examine the hydrocarbon content of the dinoflagellates containing fucoxanthin.[38] The cryptomonads, a group that has been related to the dinoflagellates, more closely resembles the diatoms, haptophytes, and brown algae with respect to the quantity of 21:6 hydrocarbon and 22:6 fatty acid present. The presence of 21:6 hydrocarbon and 22:6 fatty acid in euglenids would imply a closer relationship to the brown stock of algae. However, the presence of 21:6 hydrocarbon in the prasinophyte *Platymonas* and in *Codium* may indicate that the ability of an organism to produce this molecule may have arisen more than once. Absence of 21:6 hydrocarbon and 22:6 fatty acid in the xanthophytes implies a more distant relationship to the Chrysophyta than has previously been suggested.[39]

The absence of 21:6 hydrocarbon in copepods, crab larvae, sardines, and intertidal invertebrates examined by us indicates that the hydrocarbon does not pass up the food chain unchanged as in the case with pristane.[6,40] However, Blumer and Mullin recently found the 21:6 hydrocarbon in the copepod, *Rhincalanus*, indicating that this animal may be storing algal hydrocarbons derived from its diet.[8]

Some of the algae containing the 21:6 hydrocarbon, form dense blooms in various parts of the world's oceans, so that the release of large amounts of this hydrocarbon may be occurring when phytoplankton growth exceeds zooplankton grazing rates, as occurs in the Japan Sea.[41]

EXPERIMENTAL

Organisms and Culture Condition

Codium, *Plocamium*, *Laminaria*, and *Gigartina* were collected from the littoral zone at La Jolla, California. Red tide, which was 99% *Gonyaulax polyedra*, was collected off the pier at Scripps Institution of Oceanography. All other organisms were obtained from culture collections at Scripps Institution of Oceanography. All organisms except the following four were cultured in previously published media (see Table 1). The centric diatoms and *Gymnodinium splendens* were raised at 16° in 40 lx of fluorescent light; when not specified all other algae were grown at 20° at 80 lx.

Navicula pelliculosa was raised in freshwater medium[42] with 1 ml/l. of the following trace element solution: H_3BO_3—568 mg, $ZnCl_2$—624 mg, $CuCl_2 \cdot 2H_2O$—268 mg, $Na_2MoO_4 \cdot 2H_2O$—252 mg, $MnCl_2 \cdot 4H_2O$—360 mg, $CoCl_2 \cdot 6H_2O$—420 mg, $FeSO_4 \cdot 7H_2O$—2500 mg, Na_2tartrate$\cdot 2H_2O$—1760 mg/l.

Nitzschia alba was raised at 30° in the following medium (B. Hemmingsen, personal communication): Rila Marine Mix 40 g/l, $NaNO_3$—7·53 mM, K_2HPO_4—0·155 mM, Na_2HPO_4—4·2 mM, Na_3citrate—0·78 mM, Na_2SiO_3—0·713 mM, glucose—11·1 mM, EDTA—32·2 μM, H_3BO_3—6·47 μM, vitamin B_{12}—1 μg/l. vitamin B_1—0·5 mg/l. and 1 ml/l. of the trace element solution of *Navicula pelliculosa*. *Gloeodinium montanum* was cultured in natural light at 24° on the following medium: KNO_3—2 mM, K_2HPO_4—0·1 mM, $MgSO_4$—0·08 mM, soil extract (soil–water, 1:1 by weight)—50 ml/l. vitamin B_{12}—1 μg/l. vitamin B_1—2 mg/l. biotin—2 μg/l.,agar—14 g/l. H_3BO_3—46·1 μM, $CoCl_2$—0·17 μM, $FeSO_4$—9·0 μM, $MnCl_2$—9·1 μM, Na_2MoO_4—0·10 μM, $ZnCl_2$—0·15 μM, and Na_2tartrate$\cdot 2H_2O$—7·7 μM.

Extraction and Chromatography

Cells were collected by centrifugation at 2000 *g* for 10 min. (Sorvall Model RC-2 Centrifuge). The cells were then extracted with 200 ml of $CHCl_3$–MeOH–H_2O (1:2:1, by vol.). After lipid extraction a two phase separation was effected by adding 100 ml of $CHCl_3$–H_2O (1:1, v/v). The lipid phase was collected and the

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aqueous phase washed with $CHCl_3$ until the chlorophyll was completely extracted. The lipid phase was then concentrated to dryness (N_2) and weighed. The cell debris which was precipitated by the organic solvent was also collected, washed, and weighed for the dry weight calculation. The lipid was taken up in hexane and applied to an activated SiO_2 column.[43] The hydrocarbon fraction was eluted with 5 column vol. hexane, while the remaining lipid was eluted with 6 column vol. MeOH. The weight of the lipid fraction was determined on a Mettler Type S6 Analytical Balance. The carotene was determined spectrophotometrically in hexane at 451 nm (β-carotene), except 445 nm (α-carotene) was used for cryptomonads and *Codium*. For hydrocarbon determination we used a Loenco Model 70 Hi-Flex Apparatus fitted with a flame ionization detector operated isothermally and at 2·1 kg/cm^2 nitrogen carrier pressure. The two columns used were a 2·5m × 3mm (o.d.) column of 10% diethylene glycol succinate polyester (DEGS) and a 1·8m × 3·2mm (outside diameter) column of 3% OV-1 on 60–80 mesh Gas Chrom P (Applied Sciences, Inc). The 21:6 hydrocarbon isolated from the diatom, *Skeletonema costatum*, was used as a standard.[1] Lipid was transmethylated in a small vial with teflon liner by adding one ml of 5% H_2SO_4 and leaving at 60° for 2 hr, followed by extraction of the methyl esters with hexane.[44] The fatty acid methyl esters were analyzed by the OV-1 and DEGS columns used for the hydrocarbon work. The areas of the peaks were calculated by triangulation. A mixture of known saturated and unsaturated fatty acids were used as standards.

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