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LIPIDS OF FOUR SPECIES OF FRESHWATER CHRYSOPHYTES

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Key Word Index—Chrysophyta, *Dinobryon divergenes*, *Mallomonas caudata*, *Syncrypta globosa*, *Synura uvella*, hydrocarbons, sterols, steryl esters, alkyl esters, triacylglycerols, fatty acids.

Abstract—Hydrocarbons, alkyl and steryl esters, free sterols, triacylglycerols and free fatty acids were extracted from four species of cultured freshwater chrysophytes. 21 6 and 21.5 alkenes were identified in two species which also contained the presumed C_{22} polyenoic fatty acid precursors Even carbon number (C_{28}–C_{38}) saturated and unsaturated alkyl esters occurred, together with esters of phytol, in all species The dominant free sterol was either 24-ethylcholesta-5,22*E*-dien-3β-ol or 24-ethylcholest-5-en-3β-ol, steryl esters showed a predominance of the same steryl moiety, identified by GC-MS analysis, as that dominant in free sterols from the same algal species The presence of unsaturated acyl groups linked to steryl esters from *Synura uvella* was demonstrated by trans-esterification. The acyl composition of triacylglycerols (TG), determined after transesterification, showed the absence of unsaturation in TG from *Mallomonas caudata* but *ca* 50% of unsaturated acyl groups in two species Determination of the molecular composition of intact TG by GC-MS revealed the presence of lower homologues containing a C_4 acyl group in *Dinobryon divergens* and *M caudata* Free fatty acids showed high relative abundances of 14·0 and polyenoic acids.

INTRODUCTION

The taxonomy of the phylum Chrysophyta has been clarified by the transfer of several genera, mainly marine organisms, into the Prymnesiophyta [1], some species of which have been the subject of recent chemotaxonomic studies [2]. Organisms remaining in the Chrysophyta are mainly freshwater genera that are important components of the nannoplankton in colder oligotrophic water bodies and are very sensitive to changes in the aquatic environment A characteristic feature of the phylum is the formation of a cyst or statospore; some families possess silicified scales. These microfossils may be preserved in sediments and thus provide evidence of changes in acidification [3] or trophic status [4] of lakes, based on the ecology of the source organism Volatile degradation products from chrysophyte blooms also cause odour problems in potable waters [5], but the lipid biochemistry of freshwater chrysophytes has been little studied apart from the total fatty acids [6] and sterols [7] recovered after saponification. Species belonging to the order Ochromonadales [8] (Chrysomonadales [9]), the largest order of the Chrysophyta, possess two flagella of unequal length The lipids of four species (Table 1) distributed between three families within this order have been analysed to obtain more information about freshwater chrysophytes and to facilitate recognition of sedimentary marker molecules typical of this phylum

RESULTS AND DISCUSSION

The lipid concentration data for the specific compound classes determined by GC-MS analysis are given in Table 2

Hydrocarbons

Hydrocarbons occurred in low abundance in all species except for *Synura* The latter contained two C_{21} polyenes, showing RI values [10] of 2033 and 2037 on an apolar 50 m GC column. Analysis on a polar phase (Supelcowax 10) showed RI values of 2290 and 2350, respectively, consistent with 21·5 and 21.6 alkenes [11, 12] but not allowing a distinction between isomeric structures, in the absence of authentic compounds.

Analogous studies on the hydrocarbons of *Syncrypta* showed the same two compounds as minor products in addition to the *n*-C_{17} alkane and a 17 1 alkene as major constituents The site of unsaturation in the latter may be *cis*-Δ^7 from comparison of RI values (1670 on DB-1, 1722 on Supelcowax 10) with published data [12]. Hydrocarbons of the remaining species showed, using a short GC column, an unresolved peak corresponding to the C_{21} polyenes but they were not studied in detail

Three marine species, now classified with the Prymnesiophyta [1], but previously regarded as chrysophytes, were reported to contain $C_{21\,6}$ as the dominant hydrocarbon [13]; its formation was attributed to the activity of a specific 22:6 fatty acid decarboxylase

Esterified lipids

Alkyl esters, steryl esters and triacylglycerols were detected in each species by GC analysis of the lipid fraction showing appropriate TLC mobility. Variations in the relative abundance of total alkyl relative to total steryl esters, which co-elute during TLC separation, show that the organisms form two groups (Table 3). *Mallomonas* and *Synura*, belonging to the same family, Mallomonadaceae, contain a higher proportion of steryl esters than the other two species, but differ considerably in the

Table 1 Algae from the order Ochromonadales used in the study

Species	Family*	FBA No	CCap No†	Source‡	Features
1 *Dinobryon divergens* Imhof	Dinobryonaceae	L 143	917/1	Rydal Water	Colonial, loricate species
2 *Mallomonas caudata* Iwanoff	Mallomonadaceae	L 427	929/4	Esthwaite Water	Unicellular Siliceous scales on cell Long toothed spines radiate from body
3 *Synerypta globosa* (Schiller) Bourrelly	Ochromonadaceae	L 422	958/2	Elterwater	Colonial No scales Some mucilage present
4 *Synura uvella* Ehrenberg emend Korschikov	Mallomonadaceae	L 316	960/3	Priest Pot	Colonial Scales on cell and flagellar surface

*Using classification of Bourrelly [8]
†Culture Collection of Algae & Protozoa, Windermere Lab., The Ferry House, Ambleside
‡English Lake District

Table 2 Lipid composition of four freshwater chrysophytes

	Composition (mg/g dry alga)*			
	1	2	3	4
Solvent extractable lipids	148	135	120	129
Hydrocarbons (total)	0 6	1 7	1 1	10 6
Wax esters	14 3	3 2	4 1	4 8
Steryl esters	2 0	1 4	0 8	2 4
Triacylglycerols	6 5	11 2	4 5	4 1
Sterols	12 0	29 1	14 0	5 6
Alcohols	1 3	2 0	2 2	1 4
Monocarboxylic acids	16 7	14 6	19 9	6 5
Polar lipids†	77 5	48 5	57 0	88 0

*For species identification, see Table 1 Quantitation, based on weight of isolated lipid components, is the mean of two analyses
†For (4) includes Me_2CO and MeOH eluates from column chromatography, fucoxanthin present in the former, for other species lipids retained at origin during TLC

relative abundance of saturated alkyl esters (13 4 and 1 6%, respectively)

Alkyl esters

A series of even carbon number saturated and unsaturated n-C_{28}–C_{38} esters, together with phytyl esters eluting in the same GC retention range, were detected Unsaturated esters became more dominant and showed a greater degree of unsaturation with increasing M_r

GC-MS was used to confirm tentative identifications based on GC retention data and also to determine the molecular composition of co-eluting saturated esters (Table 4) from the relative intensities of the $[RCO_2H_2]^+$ ions in summation spectra obtained from each GC peak [14]. The data shows considerable similarity in molecular composition of corresponding homologues except for the C_{36} ester from *Dinobryon*

Monoenoic esters were identified from their $[M]^+$ and the presence of intense peaks corresponding with the $[RCO-1]^+$ and $[RCO]^+$ ions of unsaturated C_{16} and C_{18} acyl moieties Resolution within GC peaks identified as monoenoic esters suggested the presence of $\omega 7$ and $\omega 9$ unsaturation, previously confirmed in sedimentary esters by analysis of transesterified products [14], and similarly demonstrated in *Synura* (Table 5) The C_{36} and C_{38} dienoic esters occurring in *Mallomonas* and the C_{36} component from the remaining species showed alkyl and acyl fragment ions and also $[M]^+$ consistent with the major constituents being 20 1 16 1 and 22 1 16 1 for $C_{36\ 2}$ and $C_{38\ 2}$, respectively

Wax esters containing 20 1 and 22 1 alkyl groups occur in some species of marine zooplankton [15] In calanoid copepods these alkyl moieties were thought to originate from *de novo* biosynthesis of fatty acids from protein and carbohydrate dietary precursors The presence of these alkyl moieties in chrysophyte wax esters may serve as a dietary source for freshwater zooplankton, however, little is known about lipid constituents in the latter

Table 3. Percentage composition of wax/steryl esters in four freshwater chrysophytes

Constituent*	% Composition†			
	1	2	3	4
Alkyl esters				
*n*28:0	1 8	1.9	1 2	0 3
br/u 30	2.0	2 0	2 4	0 2
Phy-12 0	1 2	—	—	—
*n*30 0	3 7	3 6	3 1	0 3
br/u 32	3 7	4 9	6 1	0 9
Phy-14 0	25 8	3 7	3 9	4 8
*n*32 0	4.4	3 8	5 0	0 2
br/u 34	6 3	4 3	9 3	2.3
Phy-16:0	3 1	1 3	1 4	0 8
*n*34 0	6 0	2 8	5 8	0 5
*n*36 2	9 0	15.0	27 8	27 3
*n*36:1	6 8	14 5	5 4	23 4
Phy-18 0	1 9	1 8	1 9	—
*n*36 0	4 0	1 3	3 4	0 3
*n*38 2	5 0	2.5	—	1 5
*n*38 1	3.2	1 1	5 8	0 8
Unidentified ECL 38 85	—	5 2	—	1 6
Total %	87 9	69 7	82 5	65.2
Steryl esters				
$27\Delta^5$-14	1 0	1.3	1 2	tr
$29\Delta^{5,22}$-14	1 5	3 4	1 2	0 6
$27\Delta^5$-16/29 Δ^5-14	5 4	5 4	7 8	1 1
$29\Delta^{5,22}$-16	1 0	0 9	3 5	1.0
$29\Delta^5$-16/$27\Delta^5$-18	2 1	2 2	2.3	0.4
$28\Delta^5$-18	—	—	—	1 9
$29\Delta^{5,22}$-18	tr	10 2	tr	12 7
$29\Delta^5$-18	0 9	6 7	1 1	14.0
Total %	11 9	30 1	17 1	31 7

*Alkyl esters denoted by total number of carbon atoms double bonds or as alkyl-acyl chains in the case of phytyl esters *n* straight-chain; *br/u* iso-branched and *n*-monoenoic constituents unresolved ECL Equivalent chain length from GC elution time

Steryl esters denoted as steryl-acyl constituents, 28 and 29 steryl moieties possess 24-methyl and 24-ethyl substituents analogous to free sterols Acyl unsaturation not given as diagnostic ions not observed. tr Trace (0 5%)

†Species identification given in Table 1

Phytyl esters were recognised by mass fragmentography using the *m/z* 278 ion and confirmed by comparison of full mass spectra with those of authentic samples, as described previously [16]. The C_{14} phytyl ester was unusually dominant in *Dinobryon* (Table 3) and is the major phytyl ester in all species. Other algal sources of phytyl esters include dinoflagellates belonging to the family Peridiniaceae [17, 18] and also certain freshwater chlorophytes (Cranwell, P. A., unpublished results)

Steryl esters

The molecular composition of the steryl esters (Table 3) shows 24-ethylcholesterol and 24-ethylcholesta-5,22-dienol to be the major sterol moieties, based on mass spectral fragmentation Constituents having GC retention data indicative of C_{18} acylated sterols were dominant in *Mallomonas* and *Synura*. EI mass spectra of steryl esters show no diagnostic acyl ions [19], thus an alternative method of identifying acyl groups was necessary In the alkyl/steryl ester fraction isolated from *Synura* the five most abundant constituents totalled 82% of the fraction. Because of its relative simplicity, this mixture was subjected to base-catalysed transesterification using NaOMe Six constituents together represented 93% of the total fatty acid methyl esters (Table 5). Among the C_{18} acids, the predominance of mono- and dienoic constituents (50%) over 18·0 (2%) demonstrate that the major steryl esters in *Synura* were $29\Delta^{5,22}$–18·1/18:2 and $29\Delta^5$–18:1/18·2. The significant amount of 16.1 ester obtained is consistent with mass spectral evidence that the major 36·2 ester is 20·1–16:1.

There are few previous reports of steryl esters in

Table 4 Molecular composition of saturated alkyl esters in four cultured freshwater chrysophytes

Carbon number	Alkyl–acyl	% Composition* 1	2	3	4
28	14–14	ND	50	80	79
	12–16		40	10	9
	16–12		10	10	12
30	16 14	46	58	65	57
	14 16	27	42	35	37
	18 12	27	-		
	12 18		-		2
32	16 16	65	ND	50	ND
	18 14	35		50	
34	18–16	79	72	81	ND
	20–14	11	20	16	
	16 18	10	8	3	
36	20 16	40	58	46	ND
	18 18	54	26	42	
	22 14	6	16	12	

*Organisms numbered as in Table 1 ND Molecular composition not determined
- Component absent

Table 5 Percentage composition of fatty acids in lipid components of four freshwater chrysophytes

Constituent*	% Composition‡ 1F	1TG	2F	2TG	3F	3TG	4F	4TG	4WE/SE
12 0	–	3 1	2 0	4 9		2 4	–		2 4
14 0	26 5	12 7	12 3	38 2	21 0	12 5	40 7	24 9	19 4
15 0	0 8	4 5	tr	5 9	0 6	3 5	0 5	1 5	
16 0	16 7	30 8	15 3	40 4	13 5	27 1	13 5	17 2	12 6
16 1ω9†	tr	9 1	tr			8 8	tr	3 0	11 3
16 1ω7	5 8	1 1	4 9	–	3 6	0 8	1 2	—	--
16 2(a)		8 6	-	—	—	14 0	2 4	0 8	
18 0	6 2	12 2	6 1	7 6	1 2	7 1	1 6	3 0	1 8
18 1ω9	2 0	14 9	6 0	—	2 1	19 3	5 9	16 4	10 4
18 1ω7	1 7	0 6	4 7	-	0 6	1 3	6 8	2 6	23 2
18 2ω6	4 7	1 7	4 9	–	22 6	2 6	12 4	17 6	16 6
18 3ω3	15 0	--	9 2	—	11 4	tr	2 4	4 6	2 1
18 4ω3†	12 8	—	14 1	-	4 2	-	0 6	2 4	-
20 0	—	0 5	tr	1 0	tr	0 5	–		--
20 4ω6	2 6		1 8	—	1 8		0 5	tr	
20 5ω3	2 3		4 3	--	1 2	—	tr	0 9	—
22 5ω3	2 3	---	7 3	- -	13 2	—	9 6	1 7	
22 6ω3	—	--	6 7	-	2 4	-	1 1	1 6	--
Total unsaturated	49 2	36 0	63 9	0 0	63 1	46 8	42 9	51 6	63 6
Total saturated	50 2	63 8	35 7	98 0	36 3	53 1	56 3	46 6	36 2

*Identification based on GC co-elution with authentic compounds, † tentative identification
‡Species identification given in Table 1 F Free fatty acids, TG fatty acids released from triacylglycerols, WE/SE fatty acids released from alkyl/steryl esters
tr trace (<0 5%), -- constituent absent

chrysophytes, however, an unidentified marine species contained 24E-*n*-propylidenecholesterol as the dominant esterified sterol [20]. Steryl esters containing unsaturated acyl groups were inferred by saponification of lipids from three members of the Xanthophyceae [21], previously considered as a class within the Chrysophyta, but now regarded as a class equal to the Chrysophyceae, within the Chromophyta [22]

Triacylglycerols

Constituents containing 32–52 acyl carbon atoms were detected by GC analysis but those below C_{42} were relatively abundant only in *Dinobryon* (Table 6). A non-polar phase capillary column gave some fine structure at each carbon number. This feature results either from isomers having saturated acyl groups varying widely in chain length or from differences in the number of unsaturated acyl groups [23, 24]. Two procedures were used to obtain further information, *viz* mass spectrometry and transesterification.

The molecular composition of co-eluting triacylglycerols was qualitatively determined by GC-MS operating in the EI mode (Table 6). The technique enables the molecular species of triacylglycerols containing saturated or monoenoic acyl groups to be determined, but is not applicable to triacylglycerols containing polyenoic acyl groups [25] The triacylglycerols of lower M_r obtained from *Dinobryon* showed the presence of a C_4 acyl group (Table 6), as recently reported for a dinoflagellate, *Woloszynskia coronata* [17]. In triacylglycerols above C_{44}, $[RCO]^+$ and $[M-RCO_2]^+$ ions having m/z values indicative of unsaturation were noted except for *Mallomonas*, consistent with the absence of fine structure in the GC trace of the higher triacylglycerols from this source. The molecular species resembled those previously reported in dinoflagellates [17].

As GC elution data suggested a dominance of unsaturated triacylglycerols in *Synura*, the acyl composition of methyl esters formed by transesterification was elucidated for all species by GC analysis using a polar liquid phase. The results (Table 5) confirm the presence of saturated acyl groups alone in *Mallomonas*, whereas, in the other three algal species, unsaturated moieties constitute 36–52% of esterified fatty acids. Only triacylglycerols isolated from *Synura* contained significant amounts of the polyenoic acids occurring in a free state in all these algal species

There are few previous studies of the molecular composition of intact triacylglycerols of algal origin, however, data for three freshwater dinoflagellates has been reported [17]. Alkenoic and alkanoic acids released from algal triacylglycerols by saponification have been reported for diatoms [26, 27], marine dinoflagellates [28, 29], and green algae [30]. Polyenoic acids were major constituents in diatoms and also in green algae only during exponential growth.

Variation of triacylglycerol content and composition with stage of growth, reported for green algae [30], was not studied; a similar content (4 1–6 5 mg/g) was obtained from three of the species analysed.

Free monocarboxylic acids

Among saturated fatty acids 14:0 was dominant in all chrysophyte species except for *Mallomonas*, in which 16.0 was only slightly more abundant (Table 5) Unsaturated acids consisted mainly of 18 2ω6 together with polyenoic acids belonging to the ω3 series, the latter being relatively more abundant than in the esterified lipids. Alkenoic acids belonging to the ω3 series have been previously noted in marine and freshwater chrysophytes [6, 31]. The presence of 22:6ω3 in low abundance is consistent with its postulated role as a precursor of the

Table 6 Chain length distribution and molecular species of triacylglycerols in four freshwater chrysophytes

	% Composition†				Acyl composition†‡		
CN*	1	2	3	4	1	2	4
32	3	3	2		8/12/12		
34	5	3	2		4/14/16		
36	10	7	6		4/16/16	4/16/16	
					12/12/12		
38	10	5	6		12/12/14		
					4/16/18		
40	6	4	4				
42	6	19	4	3	12/12/18	14/14/14	14/14/14
					12/14/16	12/14/16	
44	8	31	7	3	14/14/16	14/14/16	14/14/16
46u	5		6	24	14/16/16·1		14/14/18 1
							14/14/18 2
46	11	17	10	3	14/16/16		14/16/16
48u	11		15	32			
48	10	6	9	2	16/16/16		
					14/16/18		
50u	5		12	19	16/16/18 1		
50	4	3	3	1			
52	5		10	8			
54	1		3	4			

*Carbon number (excluding glycerol skeleton); u = unsaturated

†Species identification, see Table 1

‡Order of acyl groups does not reflect their position on the glycerol chain No data for components above C_{50} due to temperature limitations of GC-MS interface, or to presence of polyenoic acyl groups [25]

21 6 hydrocarbon co-occurring in these species (see above) As the polar lipids of freshwater phytoplankton are characterized by a high polyenoic acid content [32], free polyenoic acids (Table 5) may have been released from polar lipids by endogenous lipases during extraction; such lipase activity may account for the high levels of free fatty acids (Table 2) and their compositional differences relative to triacylglycerol or ester-bound fatty acids from the corresponding source

Sterols

Free sterols were major lipid components of *Mallomonas* (>25 mg/g), rather less abundant in the remaining species (5–14 mg/g) Either 24-ethylcholesta-5,22*E*-dien-3β-ol or 24-ethylcholest-5-en-3β-ol was dominant in all species (Fig 1) with minor amounts of cholesterol and 24-methylcholest-5-en-3β-ol also present No assignment of C-24 configuration in the major C_{29} constituents could be made

Previous studies on four *Ochromonas* species showed a dominance of 24*R*-ethylcholesta-5,22*E*-dien-3β-ol [33, 34] *Synura petersenii* contained cholesterol and 24-ethylcholesterol [35]; the configuration of the latter was not reported. Two marine chrysophytes of the Sarcinochrysidales contained a more complex free sterol composition among which 24*E*-*n*-propylidenecholest-5-en-3β-ol was the major product and 24*S*-ethylcholesta-5,22*E*-dien-3β-ol also occurred [20, 36]. In contrast the major sterol of two members of the Prymnesiophyta, *Chrysotila lamellosa* [2, 36] and *Emiliania huxleyi* [2, 37] is 24*S*-methylcholesta-5,22*E*-dien-3β-ol, the opposite C-24 configuration to the freshwater chrysophytes

The dominant sterol moieties in the steryl esters (Table 3) were also $C_{29}\Delta^{5,\ 22}$ and $C_{29}\Delta^{5}$, the C_{18} ester derivatives being particularly abundant in *Synura* and *Mallomonas* and showing a relative abundance parallel to that of the free sterols in the respective organisms, thus demonstrating a common biosynthetic origin of the steroidal constituents

Long-chain alkenones

TLC separation of extractable lipids gave bands corresponding in mobility to long chain ethyl or methyl ketones, as described previously [38], the constituents were analysed by GC. None of these freshwater species contained the novel di-, tri- and tetraenoic methyl and ethyl ketones occurring in a restricted number of marine prymnesiophytes belonging to the order, Isochrysidales [2] and also found in marine sediments [39]. These ketones have, however, been found in some lacustrine sediments [38] and may thus constitute potential biological markers of a specific algal input, as in marine sediments

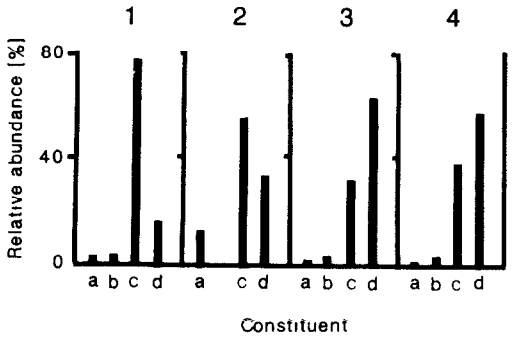

Fig 1 Percentage composition of free sterols in four chrysophyte species numbered as in Table 1 Key to constituents (a) cholest-5-en-3β-ol, (b) 24-methylcholest-5-en-3β-ol, (c) 24-ethylcholesta-5,22E-dien-3β-ol; (d) 24-ethylcholest-5-en-3β-ol

Chemotaxonomic aspects

Comparison between the data reported here and published data on algal lipid composition confirms a feature of the order Ochromonadales, namely sterol composition dominated by $29\Delta^{5\ 22}$ and $29\Delta^{5}$, that distinguishes these chrysophytes from the prymnesiophytes and xanthophytes formerly classified with them (see recent review of algal sterols [40])

The absence of long chain alkenones in the species analysed is consistent with a reported correlation with sterol composition in the marine Prymnesiophyta Only members of the order Isochrysidales that showed 24-methylcholesta-5,22*E*-dien-3β-ol as dominant sterol were found to contain long chain alkenones [2], these were absent in prymnesiophytes containing the sterols noted in these freshwater organisms A more extended survey of freshwater chrysophytes might reveal organisms having $28\Delta^{5\ 22}$ as dominant sterol and also containing long chain alkenones, thus constituting putative sources of the alkenones detected in lacustrine sediments

The presence of 21 6 alkene is consistent with a reported correlation in its co-occurrence with the pigments fucoxanthin and chlorophyll c that are characteristic of several algal divisions, including the Chrysophyta

CONCLUSION

The lipid composition of four species of freshwater chrysophytes showed the following features in common (i) Presence of 21 6 hydrocarbon (ii) Combined dominance of $C_{29}\Delta^{5\ 22}$ and $C_{29}\Delta^{5}$ sterols (iii) Steryl esters showing a distribution of sterol moieties parallel to that of the free sterols from the same organism (iv) Presence of ω3 polyenoic free fatty acids possibly derived from polar lipids by lipase activity during extraction (v) High relative abundance of 14.0 among free saturated acids (vi) Triacylglycerols showing a fatty acid composition different from that of the free fatty acids (vii) Absence of long chain alkenones

EXPERIMENTAL

Algal cultures were inoculated into medium, modified from Chu as described in ref [41], and incubated at 20 under continuous illumination from daylight fluorescent tubes giving 200 μE (400–700 nm)/m^2/s at the base of the flasks Growth was stimulated by briefly bubbling CO_2 through the solns at 3 day intervals to reduce pH to 6 5, cell densities were monitored daily Cells in late log-phase were harvested after 8–10 days using a continuous-flow centrifuge head at 12 000 rpm Typical cell densities when harvested were *ca* 18 10–13, 45–80 and 60–90 $\times 10^3$ cells/ml for species 1–4, respectively The wet alga was extracted with $CHCl_3$ MeOH followed by $CHCl_3$ (Soxhlet), as described previously [16] The wt of residual algal matter was determined

after drying at 90°. Lipids were sepd by prep. TLC on silica gel G (thickness 500 μm) pre-eluted with EtOAc. Development with hexane–Et_2O–HOAc (89:10:1) gave a series of bands corresponding in mobility to hydrocarbons, alkyl/steryl esters, alkan-2-ones, triacylglycerols/free fatty acids and alcohols/sterols, respectively, by comparison with authentic samples sepd on the same plate. Constituents were recovered by elution with Et_2O (bands 1–3) or EtOAc (bands 4, 5). The triacylglycerol/free fatty acid fraction was esterified with CH_2N_2 and the product re-sepd by TLC using the above solvent system to isolate fatty acid Me esters and triacylglycerols. Triacylglycerols (and the alkyl/steryl ester fraction from *Synura*) were transesterified by treatment with NaOMe–MeOH at 75° for 20 min and the fatty acid Me esters recovered by extraction with Et_2O. These lipid components were analysed by GC using open tubular fused silica columns (15 m × 0.3 mm or 50 m × 0.2 mm) coated with an apolar phase, DB-1 (15 m) or SE-30 (50 m) with H_2 as carrier. Unsaturated fatty acid Me esters and alkenes were identified from GC R_t data obtained on a column (30 m × 0.24 mm) coated with a polar phase, Supelcowax 10. Sterol fractions were treated with BSTFA to produce the corresponding TMSi ethers prior to GC analysis. For each lipid class, the percentage composition was determined by integration of GC peak areas, assuming equal FID response factors for all constituents.

Constituents other than alkenes and alkenoic acids were identified by GC/MS analysis using the shorter apolar column (above) mounted in a chromatograph fitted with an on-column injector and coupled to a quadrupole filter MS operating in the EI mode. The ion source was operated at 40 eV with an ionization current of 350 μA. MS data were acquired and edited using a data system.

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