PERGAMON

Phytochemistry 54 (2000) 393–399

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

The effect of growth temperature on the long-chain alkenes composition in the marine coccolithophorid *Emiliania huxleyi*

Vincent Grossi[a,*], Danielle Raphel[a], Claude Aubert[b], Jean-François Rontani[a]

[a]*Laboratoire d'Océanographie et de Biogéochimie (UMR CNRS 6535), Centre d'Océanologie de Marseille (OSU), Campus de Luminy, case 901, 13288 Marseille, France*

[b]*Laboratoire de Toxicocinétique et Pharmacocinétique, Faculté de Pharmacie, 13385 Marseille, France*

Received 16 December 1999; received in revised form 13 March 2000

Abstract

The hydrocarbon fraction of a pure culture of *Emiliania huxleyi*, composed of a mixture of C_{31}, C_{33}, C_{37} and C_{38} polyunsaturated *n*-alkenes, appeared strongly dependent on the growth temperature of the alga between 8°C and 25°C. The total hydrocarbon content increased linearly with decreasing temperatures. C_{37} and C_{38} alkenes (which accounted for more than 90% of the total hydrocarbons) showed distinct changes in distribution compared to C_{31} and C_{33} alkenes, suggesting different biological syntheses and/or functions for these two groups of compounds. C_{37} and C_{38} alkenes and C_{37} methyl ketones (alkenones) all showed a trend to lower proportions of the two diunsaturated isomers and to higher proportions of the corresponding trienes with decreasing temperature. Unlike the alkenone unsaturation ratio ($U^{k'}_{37}$), ratios based on the C_{37} and C_{38} alkadi- and trienes could be linearly related to the growth temperature of *E. huxleyi* only between 15°C and 25°C. The modifications in the distribution of alkenes induced by varying temperature appeared, however, to be twice as fast as the modifications undergone by the alkenones. Although structurally and biochemically related, the distinct evolutions of alkenes and alkenones in response to changes in growth temperature might indicate that these two classes of compounds play two distinct physiological functions. The non-systematic linearity of relationships to temperature of parameters based on alkenes distribution suggested that these compounds are of limited use as paleotemperature indicator in the marine environment in contrast with the alkenones.

Keywords: *Emiliania huxleyi*; Haptophyceae; Coccolithophorid; Growth temperature; Long-chain alkenes composition; Alkenones

1. Introduction

The coccolithophorid *Emiliania huxleyi* (Lohm.) Hay et Mohler is ubiquitous in all the world oceans where it constitutes a high proportion of the marine biomass (Conte et al., 1998). Among the neutral lipids of this alga long-chain (C_{37}–C_{39}) alkenones and alkenoates have attracted considerable attention since they occur widely in marine and lacustrine sediments where they are used as biological markers for inputs of haptophyte algae (Brassell, 1993 and references therein). The relative proportions of di- and triunsaturated C_{37} alkenones have been shown to be empirically related to the growth temperature of the alga and consequently, the alkenone unsaturation ratio ($U^{k'}_{37}$ which is the ratio of ($C_{37:2}$ alkenone)/($C_{37:2}$ + $C_{37:3}$ alkenones)) have been widely used as a proxy of sea surface temperature (e.g. Prahl and Wakeham, 1987; Brassell, 1993; Sikes and Volkman, 1993). C_{31} alkadienes, C_{33} alkadi- tri- and tetraenes, and C_{37} and C_{38} alkatrienes have also been reported to be present in different strains of *E. huxleyi* although their distributions show significant differences between strains (Volkman et al., 1980; Marlowe et al., 1984; Conte et al., 1995). For instance, some strains contain a mixture

* Corresponding author. Tel.: +33-4-91-82-96-23; fax: +33-4-91-82-65-48.

E-mail address: grossi@com.univ-mrs.fr (V. Grossi).

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00120-5

of C_{31}, C_{33}, C_{37} and C_{38} alkenes while others lack the C_{37} and C_{38} hydrocarbons. Interestingly, the stereochemistry of the internal double bonds in C_{31} and C_{33} alkenes have been established as *cis* while C_{37} and C_{38} alkatrienes internal double bonds exhibit a *trans* geometry (Rieley et al., 1998). This suggests distinct biosynthetic pathways for these two groups of compounds (Rieley et al., 1998) and may explain their different potential of preservation during early diagenesis (Wakeham et al., 1991).

The differences observed in the alkenes distribution of *E. huxleyi* may primarily separate coastal from open ocean strains but may also occur, in part, as a response to environmental or physiological variables (Conte et al., 1995). Field studies suggested that changes in water temperatures may influence the degree of unsaturation and the abundance of the alkenes in *E. huxleyi*, although this appeared to be limited to temperatures below 6°C (Sikes and Volkman, 1993; Sikes et al., 1997). In the present study, we report that changes within a wider range of growth temperatures (8–25°C) can induce qualitative and quantitative changes in the long-chain alkenes composition of a pure culture of *E. huxleyi*.

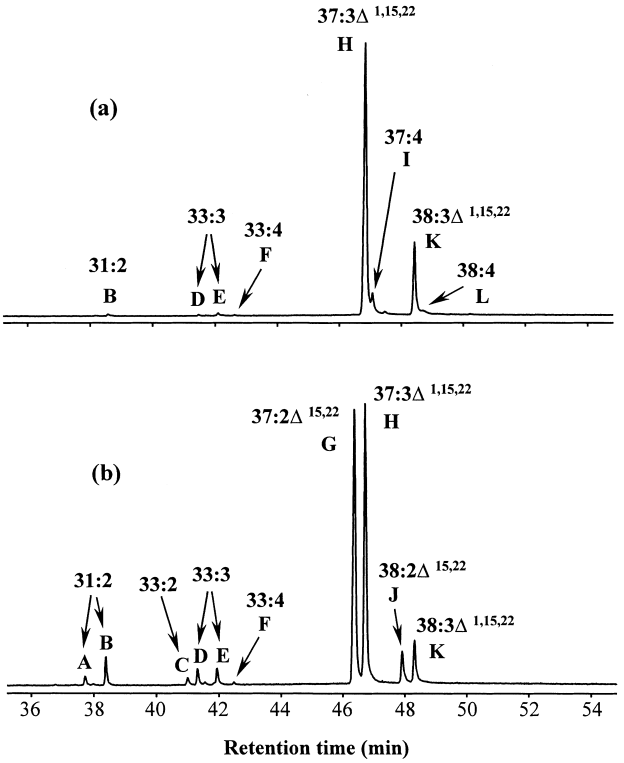

Fig. 1. Chromatograms (polar BPX-50 capillary column) of hydrocarbons from *Emiliania huxleyi* CS-57 grown at: (a) 8°C and (b) 20°C.

2. Results and discussion

2.1. Hydrocarbons of E. huxleyi CS-57

The hydrocarbon fraction of *E. huxleyi* CS-57 was mainly composed of straight chain C_{37} and C_{38} alkenes with two, three or four double bonds depending on growth temperature (peaks G–L, Fig. 1 and Table 1). Six minor components, two C_{31} dienes (peaks A and B, Fig. 1(b)), one C_{33} diene (peak C, Fig. 1(b)), two C_{33} trienes (peaks D and E, Fig. 1) and one C_{33} tetraene (peak F, Fig. 1), accounting in total for 2–10% of the total hydrocarbons (Table 1), were also present together with traces of *n*-heneicosahexaene (*n*-$C_{21:6}$) and squalene. Primary identification of the long-chain ($>C_{30}$) alkenes has relied upon interpretation of EI GC–MS traces obtained from a polar BPX-50 column. C_{37} and C_{38} dienes (peaks G and J, Fig. 1(b)) were not resolved from C_{37} and C_{38} trienes (peaks H and K, Fig. 1(b)) respectively on a non-polar HP-5MS column. The EI-mass spectra of the long-chain alkenes gave their M^+ ion and thus, their degrees of unsaturation. Their straight-chain nature was confirmed by catalytic hydrogenation of an aliquot of the hydrocarbon fraction and comparison of the products obtained with an *n*-alkane standard mixture. The identification of C_{37} and C_{38} alkadienes in *E. huxleyi* is apparently without precedent.

Table 1
Long-chain alkene distributions (relative % of total alkenes), total hydrocarbon contents and selected ratios comparing alkene and methyl ketone compositions at different growth temperatures of *E. huxleyi* CS-57 (average of triplicate cultures)

Alkenes	Peaks	Growth temperature (°C)			
		8	15	20	25
$C_{31:2}$	A–B	0.6	0.3	3.9	1.5
$C_{33:2}$	C	n.d.[a]	0.1	0.9	4.7
$C_{33:3}$	D–E	0.9	3.1	4	4
$C_{33:4}$	F	0.2	0.5	0.2	0.2
$C_{37:2}$	G	n.d.	0.2	41.7	53.5
$C_{37:3}$	H	78.4	79.9	38.2	21.4
$C_{37:4}$	I	1.2	0.4	n.d.	n.d.
$C_{38:2}$	J	n.d.	n.d.	5	10.1
$C_{38:3}$	K	17.3	15.2	6.1	4.6
$C_{38:4}$	L	1.4	0.3	n.d.	n.d.
ΣHC[b] (mg/g dry wt)		2.6	1.4	1.0	0.4
$\Sigma(C_{37} + C_{38})$Alk[c] /ΣHC		0.983	0.958	0.910	0.896
$\Sigma C_{37}/\Sigma(C_{37} + C_{38})$ Alk		0.810	0.839	0.878	0.836
$C_{37:3}/\Sigma C_{37}$ Alk		0.915	0.995	0.480	0.286
$C_{38:3}/\Sigma C_{38}$ Alk		0.916	0.978	0.560	0.315
$U^{k'}_{37} = C_{37:2}/(C_{37:2} + C_{37:3})$MK[d]		0.175	0.370	0.574	0.735
ΣC_{37} Alk/ΣC_{37} MK		0.780	0.808	0.448	0.230

[a] n.d. = not detected.
[b] HC = hydrocarbons.
[c] Alk = alkenes.
[d] MK = methyl ketones.

The position and the stereochemistry of the double bonds in the long-chain hydrocarbons of *E. huxleyi* have recently been established by Rieley et al. (1998). However, the absence of C_{37} and C_{38} dienes in the two strains analysed by these authors led us to isolate (TLC) and identify precisely the major alkenes of *E. huxleyi* CS-57. ^{1}H and ^{13}C NMR analysis of a mixture of the C_{37} and C_{38} trienes (peaks H and K) fitted exactly with the NMR data reported by Rieley et al. (1998) for a C_{37} alkatriene possessing one terminal and two mid-chain double bonds with a *trans* configuration. The *trans* nature of the internal double bonds in our C_{37} and C_{38} trienes was further confirmed by analyzing the chemical shifts of the allylic carbons after selective irradiation of the allylic hydrogens (δ 1.95 ppm). Separate singlets were observed for the carbons allylic to the terminal double bond (δ 33.9 ppm), as observed in 1-heptadecene, and for those allylic to the internal double bonds (δ 32.6 ppm), as observed in (*E*)-7-tetradecene. The ^{1}H and ^{13}C NMR spectra of a mixture of the C_{37} and C_{38} dienes (peaks G and J) showed similar chemical shifts and splitting patterns than the NMR spectra of the corresponding trienes although no signal from a terminal double bond could be observed. Therefore, the C_{37} and C_{38} dienes have internal double bonds with a *trans* configuration.

The positions of the double bonds in the C_{37} and C_{38} alkadi- and trienes (peaks G, H, J and K, Fig. 1(b)) were determined by EIMS (direct insertion probe) of the dimethyl disulfide (DMDS) adducts (Vincenti et al., 1987). The mass spectra of the DMDS derivatives of the dienes matched exactly with the ones obtained by Rieley et al. (1998) after the selective reduction of the terminal double bonds of 1,15,22-hepta- and octatriacontatrienes and subsequent DMDS treatment. Peaks G and J were thus identified as (15*E*-,22*E*)-15,22-heptatriacontadiene and (15*E*-,22*E*)-15,22-octatriacontadiene, respectively. The mass spectrum of the adducted C_{37} alkatriene showed a low intensity M^{+} ion at *m*/*z* 796 and characteristic ions at *m*/*z* 61, 257, 301, 351 and 399 indicating the presence of double bonds at C-1, C-15 and C-22 positions (Fig. 2). Peak H (Fig. 1) was consequently identified as (15*E*-,22*E*)-1,15,22-heptatriacontatriene. The mass spectrum of the DMDS adduct of the C_{38} triene could not be recorded satisfactorily by EIMS; however, by analogy with the C_{37} and C_{38} dienes, it is likely that the $C_{37:3}$ and $C_{38:3}$ alkenes exhibit the same unsaturation patterns. Consequently, peak K was tentatively identified as (15*E*-,22*E*)-1,15,22-octatriacontatriene. Due to the low amounts of C_{31} and C_{33} alkenes and of C_{37} and C_{38} alkatetraenes present in *E. huxleyi* CS-57 (Table 1), the stereochemistry and the position of the double bonds in these compounds were not determined.

2.2. Changes in long-chain alkene and alkenone compositions and biosynthetic relationships

The total hydrocarbons content of *E. huxleyi* CS-57 showed a linear increase with decreasing growth temperature (Table 1; $r^2 = 0.98$; $n = 12$). This trend has already been observed by Sikes and co-authors (Sikes and Volkman, 1993; Sikes et al., 1997) during field studies in polar regions, but the data showed strong scatter and seemed to be limited to temperatures less than 6°C. Our results clearly indicate that hydrocarbons tend to accumulate in the cells of *E. huxleyi* when the growth temperature decreases. We were unable to calculate the hydrocarbons content on a per cell basis since the formation of aggregates in some cultures

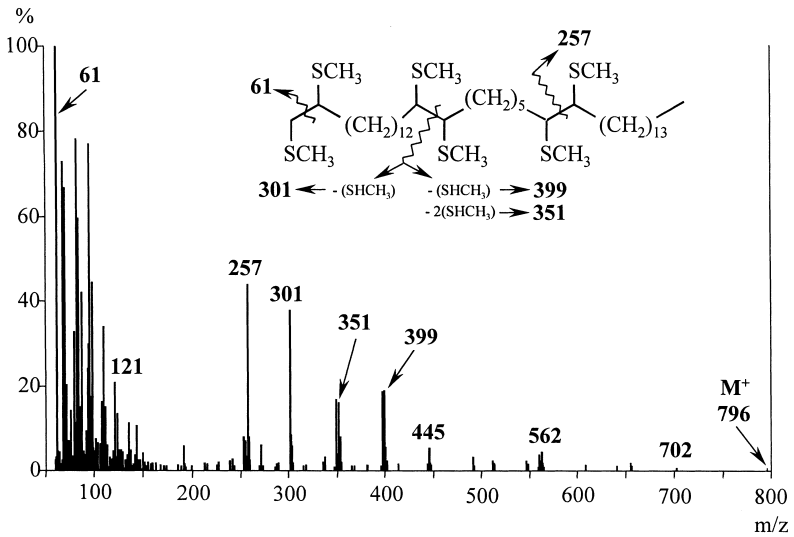

Fig. 2. EIMS (direct insertion probe) and fragmentation pattern of DMDS adduct of $C_{37:3}$ *n*-alkene from *Emiliania huxleyi* CS-57.

induced uncertainties in the quantitation. The proportion of C_{37} and C_{38} alkenes relative to the total hydrocarbons also showed a linear increase with decreasing temperature (Table 1; $r^2 = 0.95$; $n = 12$) which may indicate different strategies in alkenes biosynthesis depending on growth temperature. It is noteworthy that the proportion of C_{37} alkenes relative to the sum of C_{37} and C_{38} hydrocarbons did not change significantly with growth temperatures (Table 1)

The qualitative analysis of the hydrocarbon fraction of *E. huxleyi* CS-57 showed a general trend to higher degrees of unsaturation of C_{33}, C_{37} and C_{38} alkenes with decreasing temperature (Table 1). C_{31} alkadienes were present in varying amounts at all the temperatures but changes in growth temperature did not induce the appearance of any other C_{31} isomer. C_{33}, C_{37} and C_{38} alkadienes were absent at 8°C and became significant constituents of the cells at 20°C. The C_{37} diene showed a sharp increase from 15°C where it accounted for a minor proportion of the total hydrocarbons to 20°C where it constituted the major alkene of the alga (Table 1). This was accompanied by a twofold decrease in the concentration of the corresponding triene. To a lesser extent, the same trend to lower proportions of the diene and to higher proportions of the corresponding triene with decreasing temperature was observed for the C_{38} alkenes. Surprisingly, this was also true for the C_{33} alkadiene but not for the corresponding triene (Table 1). A different behaviour of the C_{33} hydrocarbons compared to the C_{37} and C_{38} alkenes was also observed for the tetraunsaturated isomers. While C_{37} and C_{38} alkatetraenes were detected only at 8°C and 15°C and increased with decreasing temperature, the C_{33} tetraene was observed at all the temperatures and its proportion showed little variation with changes in growth temperature. The distinct quantitative and qualitative evolutions with varying growth temperatures of C_{31} and C_{33} hydrocarbons on one hand, and of C_{37} and C_{38} alkenes on the other hand, implies distinct biological syntheses and/or functions for these two classes of hydrocarbons. This further supports the assumption made by Rieley et al. (1998) which was based on stereochemical data.

Unfortunately, although there have been several reports on the presence of long-chain alkenes in microalgae (reviewed by Volkman et al., 1998), the biosynthesis of these compounds has not been extensively studied (Templier et al., 1984) and their exact physiological role is still unknown. A usual mechanism for alkene biosynthesis is via decarboxylation of an unsaturated fatty acid containing one carbon atom more than the alkene (Harwood and Russell, 1984). This mechanism seems to be especially involved in the biosynthesis of alkenes possessing a terminal double bond, although this may require the presence of an activating group (e.g., a $\beta - \gamma$ double bond or a hydroxyl group in α position) in the fatty acid precursor (Templier et al., 1984). However, we could not detect any unsaturated long-chain fatty acid that could have served as direct precursors for the long-chain alkenes of *E. huxleyi* CS-57. This does not exclude fatty acid decarboxylation but it is possible that other mechanisms for alkene biosynthesis may be involved. The changes observed in the long-chain alkenes composition of *E. huxleyi* CS-57 certainly reflect a physiological adaptation of the alga to varying growth temperatures. It is well known that aquatic organisms are able to modify their lipid composition to match environmental conditions of stress (Harwood and Russell, 1984). Specifically, they maintain the fluidity of their membranes by changing the molecular composition of the lipid bilayer, either in chain length or in unsaturation. Such a role of regulator of membrane fluidity has already been proposed for the alkenones present in *E. huxleyi* and related species (Marlowe et al., 1984; Conte et al., 1995). Therefore, the response of *E. huxleyi* CS-57 to changes in growth temperature, in term of alkenones content, was determined by calculating the alkenone unsaturation ratio ($U^{k'}_{37}$; Prahl and Wakeham, 1987) (Table 1). This ratio appeared linearly related to the growth temperature of the alga in the entire range of temperatures studied (Fig. 3). The relationship can be written $U^{k'}_{37} = 0.033T–0.107$ ($r^2 = 0.97$; $n = 12$) which is in good agreement with previous calibrations obtained either with different strains of *E. huxleyi* or with field samples (reviewed by Brassell, 1993).

The possibility that the alkenes of *E. huxleyi* CS-57 might respond to changes in temperature in a similar way as the alkenones was investigated by calculating the same ratios as those studied by Sikes et al. (1997) for natural seawater samples. By plotting the abundance of the C_{37} or C_{38} alkatriene against the sum of the alkenes with the same chain length (e.g., $C_{37:3}$ / ΣC_{37}Alk), linear relationships to growth temperature could be obtained within the range 15–25°C ($r^2 = 0.94$ and 0.98 for C_{37} and C_{38} alkenes respectively; $n = 9$) but, unlike the alkenones, these relationships became non-linear between 8°C and 15°C (Fig. 3; Table 1). A comparison of the slopes of the relationships to temperature between 15°C and 25°C indicated that the rate of change of the alkenes was twice that of the alkenones ($k = 0.033$, 0.071 and 0.066 for C_{37} alkenones, C_{37} alkenes and C_{38} alkenes, respectively). Thus, although C_{37} and C_{38} alkenes and C_{37} alkenones showed a concomitant increase in their degrees of unsaturation with decreasing temperature, the responses of *E. huxleyi* CS-57 to variations in temperature, in term of alkenes and alkenones contents, differed significantly. The sum of the C_{37} alkene abundances was always lower than the sum of the C_{37} methyl ketones but their relative abundances (ΣC_{37}Alk

/ΣC_{37}MK) increased linearly with decreasing temperatures from 25°C to 15°C ($r^2 = 0.98$; $n = 9$) and seemed to stabilise between 15°C and 8°C (Table 1). Sikes et al. (1997) observed a similar increase in the abundance of alkenes relative to alkenones with decreasing temperature from 12°C to 0°C, but alkenes became minor constituents above 12°C while they were still significantly present in the culture grown at 25°C (Table 1). This variation in magnitude probably reflects differences in lipid composition and/or in modes of response to varying temperatures from one strain of *E. huxleyi* to another. On the other hand, Sikes et al. (1997) also observed a poor relationship of the alkenones to temperature below 6°C which led them to suggest that the C_{37} and C_{38} alkenes could fulfil the role of regulator instead of the ketones at low temperatures. Alternately, they suggest that the accumulation of alkenes in the cell may result from an incomplete response in alkenone synthesis from the organism to physiological stress caused by extreme growth temperatures. The results obtained with *E. huxleyi* CS-57 are somewhat in contrast with these statements. Indeed, although this strain was not grown at temperatures below 8°C, the concomitant variations of alkenes and alkenones compositions in response to changing temperatures rather suggested that these two classes of compounds play complementary physiological functions although differences between strains may again be responsible for these contrasting results. From our results, it seems also that changes in the abundance of a specific class of compounds constitute a distinct mode of response of the alga to varying growth temperature than changes in the degree of unsaturation or in the chain-length of these compounds.

Although it is well established that C_{37} and C_{38} alkenes and alkenones of *E. huxleyi* are structurally and biochemically related, the biological functions and the modes of synthesis of these compounds are still unknown and deserve further attention.

2.3. Usefulness of the long-chain alkenes of E. huxleyi as biomarkers

Long-chain alkenes have been detected in several microalgae (reviewed by Volkman et al., 1998); C_{37}–C_{39} polyunsaturated hydrocarbons seem to be, however, exclusively biosynthesised by oceanic strains of *E. huxleyi* (Conte et al., 1995). C_{31} and C_{33} alkenes are less specific of *E. huxleyi* since they also appear in the haptophytes *Isochrysis galbana* (Rieley et al., 1998) and *Gephyrocapsa oceanica* (Conte et al., 1995), and in the chlorophyte *Botryococcus braunii* (Gelpi et al., 1968).

Despite their occurrence in different strains of *E. huxleyi* (Volkman et al., 1980; Marlowe et al., 1984; Conte et al., 1995) C_{31} to C_{39} alkenes have not often been reported in marine particulate matter, especially where alkenones occur in high abundance (Volkman et al., 1998). These apparent absences are usually explained by a differential degradation between compound classes (Brassell, 1993). The relationship to temperature observed for the amount of hydrocarbons relative to alkenones in *E. huxleyi* CS-57 (Table 1) may also suggest that, at low latitudes, alkenes would be much less abundant than the other lipids, so that they could simply have been overlooked.

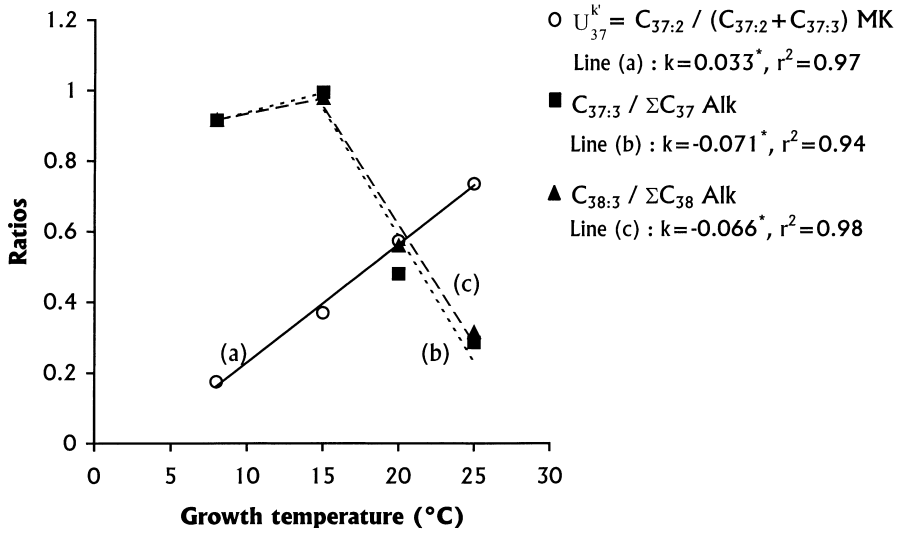

Fig. 3. Relationships of the unsaturation ratio of the C_{37} methyl ketones, C_{37} alkenes, and C_{38} alkenes to growth temperature in *Emiliania huxleyi* CS-57. Lines are approximations of the relationships to temperature. Symbols are average of triplicate analyses. * Slopes were calculated within the ranges 8–25°C and 15–25°C for methyl ketones (MK) and alkenes (Alk), respectively.

To date, the Southern Ocean (Sikes and Volkman, 1993; Sikes et al., 1997) and the Black Sea (de Leeuw et al., 1980; Wakeham et al., 1991; Eglinton et al., 1997) are the only places where long-chain alkenes derived from *E. huxleyi* have been observed as significant hydrocarbons of both the water column particulate matter and the underlying sediments. In contrast to the polar waters where C_{37} and C_{38} alkenes were the only long-chain alkenes detected (Sikes and Volkman, 1993; Sikes et al., 1997), C_{31} and C_{33} alkenes were dominant in the water column particles from the Black Sea where they co-curred with smaller amounts of C_{37}–C_{39} alkenes (Wakeham et al., 1991). Depth profiles in the water column and the sediments suggested, however, a higher potential of preservation and thus a better role of biomarker of *E. huxleyi* in the sedimentary record for C_{37}–C_{39} alkenes.

Previous attempts to use the long-chain alkenes of *E. huxleyi* as a proxy of paleotemperatures in a similar way as the alkenones have been rather unsuccessful (Sikes et al., 1997). There had been, however, no report from laboratory experiments on a possible relationship that would link C_{37} and C_{38} alkenes to the growth temperatures of their source organisms. The non-systematic linear relationships to growth temperature of *E. huxleyi* CS-57 of the relative amounts of $C_{37:3}$ and $C_{38:3}$ alkenes on one hand, and of the abundance of C_{37} hydrocarbons relative to C_{37} alkenones on the other hand, reinforced the idea that parameters based on long-chain alkenes are of limited use as paleotemperature proxies. Nevertheless, the linear relationships to temperature obtained for the total hydrocarbon content of *E. huxleyi* CS-57 and for the sum of C_{37} and C_{38} alkenes relative to the total hydrocarbons, suggested that such data may occasionally be useful in paleoclimate studies. This needs to be tested by further studies of sedimentary materials.

3. Experimental

3.1. Culturing and harvesting

An axenic culture of *Emiliania huxleyi* strain CS-57 was obtained from the CSIRO Collection of Living Microalgae and 500 ml batch cultures were grown in 1 l glass flasks capped with sterile stoppers. Triplicates cultures were grown at 8°C, 15°C, 20°C and 25°C under 100 μEin $m^{-2}s^{-1}$ (PAR) of fluorescent light with a 12 h light/12 h dark regime in f/2 medium (Guillard and Ryther, 1962). The cultures were gently hand shaked regularly and the growth as well as the purity of the cultures were monitored by flow cytometry. The cultures were harvested at the beginning of the stationary phase (reached after 10 to 15 days) by filtration on precombusted and preweighted GF/F paper.

3.2. Lipid extraction and separation

The filtered cultures were dried at 40°C for 12 h, weighed and then directly saponified with 1 N KOH in $MeOH/H_2O$ (1:1). After cooling, the unsaponifiable lipids were extracted with *n*-hexane (×3), dried over Na_2SO_4, and concentrated by means of rotary evaporation. The organic residue obtained was chromatographed over a wet packed (*n*-hexane) column of silica gel (3% H_2O) and two fractions were eluted with *n*-hexane and chloroform yielding, respectively, hydrocarbons and more polar lipids (e.g., alkenones, sterols).

3.3. TLC isolation of hydrocarbons

Hydrocarbons were separated by TLC on silica gel (Kieselgel 60) as previously described by de Leeuw et al. (1980). Bands were visualised under 256 nm UV light after spraying with 2′,7′-dichlorofluorescein, and extracted into *n*-hexane (×3). Fraction A ($R_f = 0.79$) contained the C_{37} and C_{38} alkatrienes and fraction B ($R_f = 0.85$) the dienes equivalents.

3.4. Catalytic hydrogenation

An aliquot of the total hydrocarbon fraction was suspended in MeOH and stirred (12 h) under an atmosphere of hydrogen in the presence of $Pd/CaCO_3$ (5%).

3.5. Formation of DMDS adducts

Following the method of Vincenti et al. (1987), an aliquot of the hydrocarbon fraction was dissolved in *n*-hexane and allowed to react at 50°C (24 h) with DMDS and a solution of iodine in Et_2O. The reaction was quenched with $Na_2S_2O_3$ and the adducts were extracted into *n*-hexane (×3). Under these conditions, the reaction was quantitative; the use of higher temperatures is not recommended due to the possible formation of by-products of lower molecular weight (Francis and Veland, 1981).

3.6. Gas chromatography

Hydrocarbons were quantified by GC using a Girdel series 30 gas chromatograph equipped with a Ross injector, an FID and a BPX-50 bonded phase capillary column (30 m; 0.25 mm i.d.; 0.25 μm film thickness). Alkenones were analysed on a HP-5MS bonded phase capillary column (30 m; 0.25 mm i.d.; 0.25 μm film thickness). The oven temperature was programmed from 150°C to 300°C at 5°C min^{-1}; the injector and detector temperatures were 290°C and 280°C, respectively; N_2 was used as the carrier gas (1.0 bar). Quanti-

tative determinations were based on the response factor of a GC external standard (hexatriacontane).

3.7. Mass spectrometry

EI GC–MS was performed on a HP 5890 series II plus gas chromatograph coupled with a HP 5972 mass spectrometer operated at 70 eV with a mass range m/z 50–700. The gas chromatograph was equipped with an on-column injector and the BPX-50 bonded phase capillary column, and helium was used as the carrier gas (1.04 bar). Samples were injected at 60°C and the oven temperature was programmed to 130°C at 30°C min^{-1} and then at 3°C min^{-1} to 310°C at which it was held for 10 min.

EIMS analyses (direct insertion probe) were carried out on an HP 5987 mass spectrometer operated at 70 eV with a mass range m/z 58–850. The temperature was programmed from 50°C to 220°C at 30°C min^{-1}; the temperature of the source was 200°C.

3.8. Nuclear magnetic resonance spectroscopy

NMR spectra were recorded on a Brucker AM400-X spectrometer in $CDCl_3$. Chemical shifts were determined in δ units relative to TMS according to classical relationships. The frequency of the instrument was 400 and 100 MHz for ^1H and ^{13}C NMR, respectively.

Acknowledgements

We thank Dr. Gerald Gregori for flow cytometry analyses. NMR analyses were provided by Dr. Robert Faure. This work was supported by grants from the Centre National de la Recherche Scientifique (CNRS) and Elf Society (groupement de recherche Hycar n°1123).

References

Brassell, S.C., 1993. Applications of biomarkers for delineating marine paleoclimatic fluctuations during the Pleistocene. In: Engel, M.H., Macko, S.A. (Eds.), Organic Geochemistry: principles and applications. Plenum Press, New York, pp. 699–738.

Conte, M.H., Thompson, A., Eglinton, G., 1995. Lipid biomarker diversity in the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae) and the related species *Gephyrocapsa oceanica*. Journal of Phycology 31, 272–282.

Conte, M.H., Thompson, A., Lesley, D., Harris, R.P., 1998. Genetic and physiological influences on the alkenone/alkenoate versus growth temperature relationship in *Emiliania huxleyi* and *Gephyrocapsa oceanica*. Geochimica et Cosmochimica Acta 62 (1), 51–68.

de Leeuw, J.W., van de Meer, F.W., Rijpstra, W.I.C., Schenck, P.A., 1980. On the occurrence and structural identification of long chain unsaturated ketones and hydrocarbons in sediments. In: Douglas, A.G., Maxwell, J.R. (Eds.), Advances in Organic Geochemistry 1979. Pergamon Press, Oxford, pp. 211–217.

Eglinton, T.I., Benitez-Nelson, B.C., Pearson, A., McNichol, A.P., Bauer, J.E., Druffel, E.R.M., 1997. Variability in radiocarbon ages of individual organic compounds from marine sediments. Science 277, 796–799.

Francis, G.W., Veland, K., 1981. Alkylthiolation for the determination of double-bond positions in linear alkenes. Journal of Chromatography 219, 379–384.

Gelpi, E., Oró, J., Schneider, H.J., Bennett, E.O., 1968. Olefins of high molecular weight in two microscopic algae. Science 161, 700–702.

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. Part I: *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Canadian Journal of Microbiology 8, 229–239.

Harwood, J.L., Russell, N.J., 1984. Lipids in Plants and Microbes. George Allen & Unwin, London.

Marlowe, I.T., Green, J.C., Neal, A.C., Brassell, S.C., Eglinton, G., Course, P.A., 1984. Long chain (n-C_{37}-C_{39}) alkenones in the Prymnesiophyceae. Distribution of alkenones and other lipids and their taxonomic significance. British Phycology Journal 19, 203–216.

Prahl, F.G., Wakeham, S.G., 1987. Calibration of unsaturation patterns in long-chain ketone compositions for palaeotemperature assessment. Nature 330, 367–369.

Rieley, G., Teece, M.A., Peakman, T.M., Raven, A.M., Greene, K.J., Clarke, T.P., Murray, M., Leftley, J.W., Campbell, C.N., Harris, R.P., Parkes, R.J., Maxwell, J.R., 1998. Long-chain alkenes of the Haptophytes *Isochrysis galbana* and *Emiliania huxleyi*. Lipids 33 (6), 617–625.

Sikes, E.L., Volkman, J.K., 1993. Calibration of alkenone unsaturation ratios ($U^{k'}_{37}$) for paleotemperature estimation in cold polar waters. Geochimica et Cosmochimica Acta 57, 1883–1889.

Sikes, E.L., Volkman, J.K., Robertson, L.G., Pichon, J.-J., 1997. Alkenones and alkenes in surface waters and sediments of the Southern Ocean: implications for paleotemperature estimation in polar regions. Geochimica et Cosmochimica Acta 61 (7), 1495–1505.

Templier, J., Largeau, C., Casadevall, E., 1984. Mechanism of non-isoprenoid hydrocarbon biosynthesis in *Botryococcus braunii*. Phytochemistry 23, 1017–1028.

Vincenti, M., Guglielmetti, G., Cassani, G., Tonini, C., 1987. Determination of double bond position in diunsaturated compounds by mass spectrometry of dimethyl disulphide derivatives. Analytical Chemistry 59, 694–699.

Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L., Gelin, F., 1998. Microalgal biomarkers: a review of recent research developments. Organic Geochemistry 29 (5–7), 1163–1179.

Volkman, J.K., Eglinton, G., Corner, E.D.S., Forsberg, T.E.V., 1980. Long-chain alkenes and alkenones in the marine coccolithophorid *Emiliania huxleyi*. Phytochemistry 19, 2619–2622.

Wakeham, S.G., Beier, J.A., Clifford, C.H., 1991. Organic matter sources in the Black Sea as inferred from hydrocarbon distributions. In: Izdar, E., Murray, J.W. (Eds.), Black Sea Oceanography. Kluwer Academic Publishers, The Netherlands, pp. 319–341.