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Phytochemistry 65 (2004) 117–126
PHYTOCHEMISTRY
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Long-chain alkenones and related compounds in the benthic haptophyte *Chrysotila lamellosa* Anand HAP 17

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Received 6 June 2003; received in revised form 12 September 2003; accepted 23 September 2003

Abstract

The neutral lipid compositions of the coastal haptophyte *Chrysotila lamellosa* HAP 17 grown in batch culture at 10 and 20 °C have been determined. A comparison was also made between the lipid compositions of cells harvested in early and late stationary phase. This species contains a suite of very long-chain C_{37}–C_{40} alkenones and alkenoates as found in a few microalgae from the Haptophyta. The distributions of these compounds show some differences to earlier reports of different strains of this alga, which are only in part attributable to culture conditions. A suite of long-chain alkenols, the reduced form of the alkenones, was characterized for the first time. The abundance of these compounds was only 1.5% of that of the corresponding alkenones, and the relative proportion of C_{37}–C_{38} constituents depended on growth temperature. These data show that haptophyte algae are a possible source of the alkenols found in some marine sediments, but the small amounts found suggest that other sources such as bacterial reduction of alkenones are more likely in highly reducing sediments. A mixture of C_{29}–C_{33} *n*-alkenes, dominated by the $C_{31:1}$ monoene, was found in marked contrast to previous analyses of other strains which reported only the presence of a $C_{31:2}$ diene. The sterol distribution included the common haptophyte sterol 24α-methylcholesta-5,22*E*-dien-3β-ol (*epi*-brassicasterol) as well as significant amounts of Δ^5- and $\Delta^{5,22}$-C_{29} sterols.

Keywords: *Chrysotila lamellosa*; Haptophyte; Microalga; Lipids; Alkenones; Alkenols; Sterols; Long-chain

1. Introduction

Alkenones are typically amongst the most abundant extractable lipids in Quaternary marine sediments (Brassell, 1993), and the relative abundances of the C_{37} components with different degrees of unsaturation (U^{k}_{37}, $U^{k'}_{37}$) can be used as a proxy for paleo-sea surface temperature (Brassell et al., 1986; Prahl and Wakeham, 1987). Alkenones comprise an unusual class of long-chain unsaturated methyl and ethyl ketones that are synthesized by a limited number of microalgae from the Haptophyta (Volkman et al., 1980a; 1995; Marlowe et al., 1984a; Conte et al., 1994; 1995). Until now they were reported exclusively from the oceanic haptophytes *Emiliania huxleyi* and *Gephyrocapsa oceanica* (family Noelaerhabdaceae) and coastal species of *Chrysotila* and *Isochrysis* (family Isochrysidaceae) (Marlowe et al., 1984a, 1990; Volkman, 2000).

Alkenones possess several unusual characteristics for biolipids, including their very long chain-length (i.e. C_{35}–C_{40}) and the spacing (C_7) and configuration (*E*) of their positions of unsaturation (Marlowe et al., 1990). Haptophytes also contain significant abundances of methyl and ethyl alkenoates and alkenes exhibiting similar chemical features (de Leeuw et al., 1980; Volkman et al., 1980b; Marlowe et al., 1984a), although the alkenes also contain C_{31}–C_{33} components having double bonds with *Z* geometry (Rieley et al., 1998). Recently, trace amounts of alkenols (the reduced forms of alkenones) were also detected in *E. huxleyi*, *G. oceanica* and *Isochrysis galbana* (Rontani et al., 2001). During the latter study, relatively high amounts of these long-chain

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doi:10.1016/j.phytochem.2003.09.021

unsaturated alcohols (above 20% of the abundance of the corresponding alkenones) were also detected in some recent marine sediments from the Camargue (France) (Rontani et al., 2001). Since these alkenols might have been derived from benthic haptophytes present in the algal mats we undertook a study of the lipids of the widely distributed species *Chrysotila lamellosa* Anand.

Chrysotila lamellosa was first described from the chalk cliffs on the Kent coast (Anand, 1937), but since then it has been described from a variety of terrestrial and coastal environments (Marlowe et al., 1990). It is thought to be synonymous with *Ruttnera spectabilis* Geitler (Green and Parke, 1974) which was isolated from rocks and paths in a Vienna park. *Isochrysis maritima* from the chalk cliffs of the north coast of France (Billard and Gayral, 1972) is also believed to be a synonym of *C. lamellosa* (Green and Parke, 1975). The occurrence of alkenones, alkenoates and other lipids in two strains of *C. lamellosa* from the Plymouth Culture Collection was reported by Marlowe (1984) and Marlowe et al. (1984a,b). Strain 353 was isolated from rock scrapings from the estuary of the River Avon in south Devon, England (Green and Parke, 1975), while strain 528 was collected from the Neusiedlersee near Vienna (Austria) (Green and Course, 1983).

In the present study we examined the alkenones and derivatives of the marine benthic haptophyte *Chrysotila lamellosa* HAP 17. The aims of this work were to:

- determine whether benthic haptophytes might be potential sources of alkenols in marine sediments,
- compare the composition of a marine strain of *C. lamellosa* with those of two previously described strains (Marlowe et al., 1984a),
- examine if there is a relationship between temperature and alkenone distribution of *C. lamellosa*, in order to compare with that of *E. huxleyi*. Indeed, the degree of uncertainty associated with the use of an *Emiliania*-based calibration in environments where other species may be an important source of alkenones cannot be determined until additional species and strains have been examined systematically (Volkman, 2000).

2. Results and discussion

2.1. Hydrocarbons of C. lamellosa HAP 17

Alkenes are significant components (2.2% relative to alkenones for early stationary cells; Table 1) of the lipids in *C. lamellosa* HAP 17. The hydrocarbon fraction of this strain is mainly composed of straight-chain C_{31} and C_{33} alkenes with one, two or three double bonds (Table 2). One C_{29} diene, accounting for less than 0.5% of the total hydrocarbons (Table 2), is also present together with trace amounts of *n*-henicosapentaene. C_{31} and C_{33} alkenes have been detected previously in *E. huxleyi* (Grossi et al., 2000), *Isochrysis galbana* (Rieley et al., 1998), *G. oceanica* (Conte et al., 1995) and in the chlorophyte *Botryococcus braunii* (Gelpi et al., 1968). A previous study of the hydrocarbons of two strains of *C. lamellosa* (*C. lamellosa* 353 and *C. lamellosa* 528) (Marlowe et al., 1984a) reported the presence of only one C_{31} diene (Table 2). Large variations in the alkene composition of strains of *E. huxleyi* have been observed previously (Volkman et al., 1980b, Marlowe et al., 1984a; Rieley et al., 1998; Grossi et al., 2000), and our results indicate that significant variation also exists for *C. lamellosa* strains. These differences pose questions about the role and the biosynthesis of these compounds in microalgal cells. 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.

Table 1
Concentrations ($\mu g.mg^{-1}$ dry weight) of alkenones and derivatives detected in *Chrysotila lamellosa* HAP 17 grown at 20 °C

Compounds	Concentration[a]	
	Early stationary phase (15 days)	Late stationary phase (60 days)
Alkenones	9.10	9.21
Alkenoates	0.25	nd[b]
Alkenes	0.20	0.12
Alkenols	0.14	0.09

[a] Accuracy estimated to be ± 0.02 μg mg^{-1} dry weight.
[b] Not determined.

Table 2
Alkene composition of *Chrysotila lamellosa* HAP 17 and other, previously described, strains

Alkene	Relative percentage			
	C. lamellosa HAP 17 grown at 20 °C[a]	*C. lamellosa* HAP 17 grown at 10 °C[a]	*C. lamellosa* 353[b] grown at 15 °C[c]	*C. lamellosa* 528[b] grown at 15 °C[c]
$C_{29:2}$	0.5	tr[d]	–	–
$C_{31:1}$	68.6	54.6	–	–
$C_{31:2}$	18.3	33.3	100	100
$C_{31:3}$	4.1	10.3	–	–
$C_{33:2}$	7.5	1.8	–	–
$C_{33:3}$[e]	1.0	tr	–	–

[a] Early stationary phase.
[b] Marlowe et al. (1984a).
[c] Exponential growth period (15 days).
[d] Trace amounts.
[e] Several isomers.

Analysis of the hydrocarbon fraction of *C. lamellosa* HAP 17 shows a general trend to higher proportions of the more unsaturated C_{31} alkenes with decreasing temperature (Table 2). These changes certainly reflect a physiological adaptation of the alga to varying growth temperatures. The possibility that the alkenes in other haptophytes might respond to changes in temperature in a similar way as the alkenones was previously investigated by Sikes et al. (1997) and Grossi et al. (2000). Unfortunately, the non-linearity of the relationships obtained and the very low amounts of alkenes present in most sediments suggested that these compounds are of limited use as paleotemperature indicators in the marine environment.

2.2. Alkyl alkenoates of C. lamellosa HAP 17

In order to avoid coelutions with alkenones or alkenols, alkyl alkenoates were quantified in non-silylated $NaBH_4$-reduced fractions F_2 since the esters remained unaffected by these reaction conditions. The distributions of the methyl and ethyl esters of the polyunsaturated C_{36} acids are given in Table 3. Straight chain $C_{36:2}$, $C_{36:3}$ and $C_{36:4}$ ethyl esters dominate the ester fraction of *C. lamellosa* HAP 17, while the corresponding methyl esters are present in smaller amounts. Of the compounds thus recognized, only the ethyl esters have been previously reported in the case of *C. lamellosa* strains 353 and 528 (Marlowe et al., 1984a).

The proportion of more highly unsaturated ethyl and methyl alkenoates strongly increases with decreasing temperature (Table 3). A similar dependence of alkenoate unsaturation on growth temperature was previously noted by Volkman et al. (1995) in the case of *G. oceanica*. However, this result was not emphasized nor followed up in later studies, presumably because of the low abundance of alkenoates in most of the samples analysed.

Table 3
Alkenoate composition of *Chrysotila lamellosa* HAP 17 and other, previously described, strains

Alkenoate	Relative percentage			
	C. lamellosa HAP 17 grown at 20 °C[a]	*C. lamellosa* HAP 17 grown at 10 °C[a]	*C. lamellosa* 353[b] grown at 15 °C[c]	*C. lamellosa* 528[b] grown at 15 °C[c]
$C_{36:4}$ FAME[d]	2.8	7.9	–	–
$C_{36:3}$ FAME	8.1	3.4	–	–
$C_{36:2}$ FAME	2.9	nd[e]	–	–
$C_{36:4}$ FAEE[f]	4.8	50.3	36.0	41.5
$C_{36:3}$ FAEE	29.5	29.9	36.0	43.4
$C_{36:2}$ FAEE	51.9	8.5	28.0	15.1

[a] Early stationary phase.
[b] Marlowe et al. (1984a).
[c] Exponential growth period (15 days).
[d] Fatty acid methyl ester.
[e] Not detected.
[f] Fatty acid ethyl ester.

2.3. Alkenones of C. lamellosa HAP 17

Alkenones were quantified in F_2 fractions after $NaBH_4$ reduction and subsequent silylation. This reduction-silylation technique (Rontani et al., 2001) affords a near-baseline resolution of the produced alkenols (Fig. 1). The silylated 2- and 3-alkenols display EI mass spectra exhibiting strong peaks at m/z 117 and 131, respectively, which are very useful to quantify alkenones in natural samples, particularly when the alkenones are present in only trace amounts or where coelution with alkenoates occurs (Fig. 1). Moreover, the $NaBH_4$ reduction step does not affect significantly the alkenone unsaturation ratio (Rontani et al., 2001). Examination of the F_2 fraction of *C. lamellosa* HAP 17 after this treatment led to the identification of $C_{37:1\text{-}4}$ and $C_{39:2\text{-}3}$ methyl alkenones and $C_{38:1\text{-}4}$, $C_{39:2\text{-}3}$ and $C_{40:2\text{-}3}$ ethyl alkenones (Fig. 1, Table 4). Of the compounds thus recognized, only $C_{37:2\text{-}4}$ methyl alkenones, $C_{39:3}$ ethyl alkenone and unresolved $C_{38:2\text{-}3}$ isomers have been previously reported in *C. lamellosa* strains 353 and 528 (Marlowe et al., 1984a) (Table 4).

Monounsaturated alkenones were recently detected and characterized in *E. huxleyi*, *G. oceanica* and *I. galbana* (Rontani et al., 2001). Surprisingly the double bond positions in these compounds were not in the same positions as those in their polyunsaturated analogues, implying that alkenone synthesis is more complicated than previously considered. Unfortunately, the amounts of $C_{37:1}$ methyl alkenone and $C_{38:1}$ ethyl alkenone present in *C. lamellosa* HAP 17 were not sufficient to allow the determination of the position of their double bond.

The C_{37} methyl alkenone unsaturation indices $U^{k'}_{37}$ and U^{k}_{37} defined as $U^{k'}_{37} = [C_{37:2}]/([C_{37:2}] + [C_{37:3}])$ and $U^{k}_{37} = ([C_{37:2}] - [C_{37:4}])/([C_{37:2}] + [C_{37:3}] + [C_{37:4}])$ decrease with decreasing growth temperature (Table 4). The range of $U^{k'}_{37}$ changes appears to be much more limited for *C. lamellosa* HAP 17 than in the case of *E. huxleyi* (Prahl et al., 1988) and *G. oceanica* (Volkman et al., 1995). Indeed, if we consider that $U^{k'}_{37}$ changes linearly with the growth temperature between 10 and 20 °C, we obtain a relationship of the form $U^{k'}_{37} = 0.01T + 0.0033$ for *C. lamellosa* HAP 17. This relationship is clearly not well constrained with only three data points, but it is clearly very different from those obtained for *E. huxleyi* ($U^{k'}_{37} = 0.033T + 0.043$) (Prahl and Wakeham, 1987) and *G. oceanica* ($U^{k'}_{37} = 0.049T - 0.520$) (Volkman et al., 1995). It is, in fact, quite similar to the relationship found for the coastal species *Isochrysis galbana* ($U^{k'}_{37} = 0.00932T - 0.0413$; Versteegh et al., 2001) and for lacustrine sediments from German lakes, the more brackish examples of which contain abundant *C. lamellosa* and other haptophyte algae (Zink et al., 2001). Sea

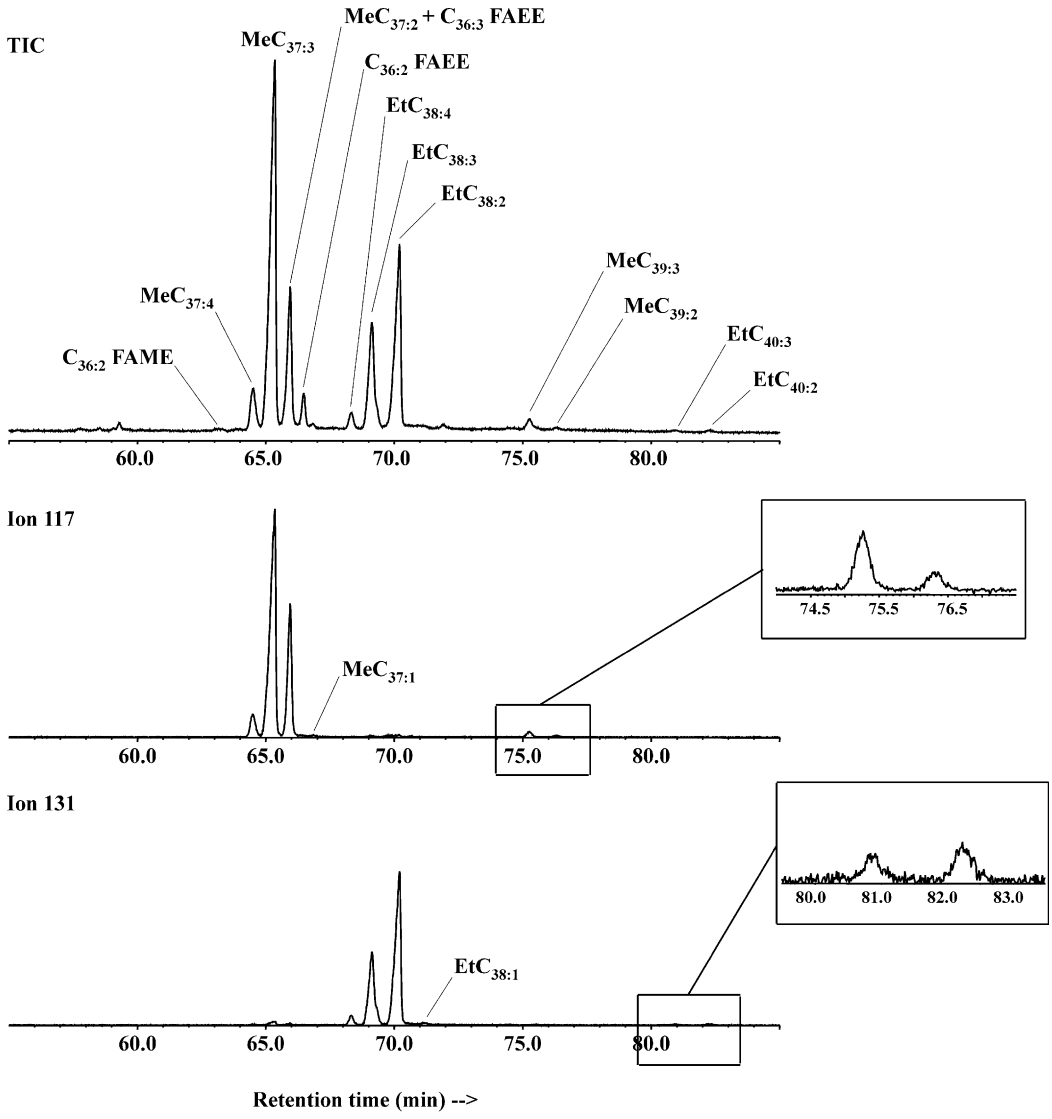

Fig. 1. Partial total ion chromatogram (TIC) and mass fragmentograms for m/z 117 and 131 of the reduced and silylated F_2 fraction of *C. lamellosa* HAP 17 grown at 20 °C (early stationary phase).

surface temperature reconstruction using an *Emiliania*-based calibration could thus be seriously biased in coastal or lacustrine sediments containing a high content of *Chrysotila* or *Isochrysis* cells (e.g. Versteegh et al., 2001). Volkman et al. (1995) suggested that it might be possible to use features of the distributions of alkenones and their derivatives to infer the likely source of alkenones in sediments. Though this idea has been contested by Conte et al. (1998) for distinguishing *Emiliania* and *Gephyrocapsa* contributions, the very low values of U^k_{37} observed in the case of the different strains of *C. lamellosa* (Table 4) (due to the production of a high proportion of the $C_{37:4}$ alkenone) could be of diagnostic value for detecting a contribution from such strains to coastal sediments.

Interestingly the alkenone content remains practically unchanged during aging of *C. lamellosa* HAP 17 (Table 1), while sterols are strongly degraded (see following section). These results provide support for the idea that alkenones are much more stable towards microbial (Teece et al., 1998) and photochemical (Rontani et al., 1997) degradation than other common phytoplankton lipids such as sterols and fatty acids.

2.4. Alkenols of *C. lamellosa* HAP 17

Examination of the F_3 fraction of *C. lamellosa* HAP 17 allowed the detection of significant amounts of natural alkenols (ca. 1.5% relative to the corresponding alkenones for early stationary cells) (Table 1, Fig. 2). Alkenols identified in this fraction were $C_{37:2\text{-}4}$ and $C_{39:2\text{-}3}$ methyl alkenols and $C_{38:2\text{-}4}$ ethyl alkenols (Table 5). As previously observed in the case of *E. huxleyi*, *G. oceanica* and *I. galbana* (Rontani et al., 2001), $U^{k'}_{37}$ values calculated from these natural alkenols (Table 5) are similar to those obtained from the corresponding alkenones

Table 4
Alkenone composition of *Chrysotila lamellosa* HAP 17 and other, previously described, strains

Alkenone	Relative percentage			
	C. lamellosa HAP 17 grown at 20 °C[a]	*C. lamellosa* HAP 17 grown at 10 °C[a]	*C. lamellosa* 528 grown at 15 °C[b,c]	*C. lamellosa* 353 grown at 15 °C[b,c]
$MeC_{37:4}$	5.0	37.6	36.5	29.9
$MeC_{37:3}$	44.8	29.7	49.4	54.1
$MeC_{37:2}$	11.3	3.3	1.9	3.9
$MeC_{37:1}$	tr[d]	–	–	–
$EtC_{38:4}$	1.6	10.9	–	–
$EtC_{38:3}$	13.1	9.7	8.4[e]	6.1[e]
$EtC_{38:2}$	21.7	5.7	1.9[e]	3.4[e]
$EtC_{38:1}$	tr	–	–	–
$MeC_{39:3}$	1.4	2.7	–	–
$MeC_{39:2}$	0.4	tr	–	–
$EtC_{39:3}$	tr	–	1.9	2.6
$EtC_{39:2}$	tr	–	–	–
$EtC_{40:3}$	0.3	0.4	–	–
$EtC_{40:2}$	0.4	tr	–	–
$U^{k'}_{37}$	0.20	0.10	0.04	0.07
U^{k}_{37}	0.10	−0.49	−0.39	−0.29
K_{37}/K_{38}[f]	1.4	3.3	8.5	9.2

[a] Early stationary phase.
[b] Exponential growth period (15 days).
[c] Marlowe et al. (1984a).
[d] Trace amounts (<0.2%).
[e] Unresolved C_{38} isomers.
[f] Relative abundance of total C_{37}–C_{38} alkenones (Prahl et al., 1988).

(Table 4). This strongly suggests that the biosynthesis of the alkenols is closely related to that of the alkenones. However, it should be noted that the proportion of C_{38} homologues is significantly lower in the alkenols than in the alkenones (Tables 4 and 5).

The F_3 fraction (obtained after alkaline hydrolysis) also contained a variety of steroidal compounds (Fig. 3). Our data are compared with a previous analysis of this strain by Raederstorff and Rohmer (1984) and with analyses of two other strains by Marlowe (1984) (Table 6). Raederstorff and Rohmer (1984) reported 24α-methylcholesta-5,22*E*-dien-3β-ol (*epi*-brassicasterol; 49%), 24-methylcholesterol (campesterol; 6%) and 24-ethylcholesta-5,22*E*-dien-3β-ol (poriferasterol; 44%) in *C. lamellosa* grown at an unspecified temperature in enriched seawater. The same three sterols occur in similar proportions in the late stationary phase culture that we analysed although we also observed relatively small amounts of 24-ethylcholesterol, 24-methylenecholesterol and cholesterol (Fig. 3, Table 6). Interestingly, the proportion of *epi*-brassicasterol is much higher in the early stationary phase culture and 24-ethylcholesterol and 24-methylenecholesterol are only present in trace amounts. Changes in the proportions of sterols with culturing conditions are well documented although the responsible factors are still poorly elucidated (see Volkman, 1986 for a discussion). The sterol distribution reported by Marlowe et al. (1984a) for strains 353 and 528 of *C. lamellosa* from the Plymouth Culture Collection shows an even higher proportion of *epi*-brassicasterol (Table 6). This sterol is common in haptophyte microalgae and some diatoms (e.g. Volkman, 1986; Gladu et al. 1990; Conte et al., 1994).

Our GC–MS analysis technique does not separate C-24 sterol epimers, but previous work has shown that the C_{28} $\Delta^{5,22}$ sterol in haptophytes such as *Emiliania*, *Pleurochrysis*, *Dicrateria* and *Chrysotila* have the 24α stereochemistry (Maxwell et al., 1980; Raederstorff and Rohmer, 1984; Goad et al., 1983; Gladu et al., 1990). However, Raederstorff and Rohmer (1984) report that the C_{29} $\Delta^{5,22}$ sterol in *C. lamellosa* is poriferasterol (24β) rather than stigmasterol (24α), although this was based only on melting point differences. Poriferasterol also occurs in the haptophyte *Pavlova lutheri* (Véron et al., 1996). In contrast, Gladu et al. (1990) reported that *Pleurochrysis carterae* contains the 24α sterol epimer, stigmasterol, rather than poriferasterol. Although both C-24 stereochemistries are found in the sterols of diatoms and haptophytes, it is unusual to find one stereochemistry for the C_{28} sterols and the other stereochemistry for the C_{29} sterols in the same species (e.g. Gladu et al., 1990, 1991) as reported for *C. lamellosa*. Also, the occurrence of substantial amounts of C_{29} sterols in *Chrysotila* is not commonly observed in other haptophytes (Gladu et al., 1990).

Although the absolute amount of alkenols slightly decreases during aging of *C. lamellosa* HAP 17 (Table 1), their proportion relative to that of sterols in the F_3 fraction strongly increases (Fig. 3). This result shows that alkenols (and the corresponding alkenones) are much more stable towards degradation processes than other common phytoplanktonic lipids such as sterols. The presence of significant amounts of 24-methylcholesta-5,22*E*-dien-3β-ol-7-one and 24-ethylcholesta-5,22*E*-dien-3β-ol-7-one after aging (Fig. 3, Table 6) is indicative of intensive abiotic degradation. Indeed, it has been shown that such compounds result from the thermal degradation (in the GC injector or during alkaline hydrolysis) of photochemically-produced hydroperoxides (Rontani and Marchand, 2000). Analysis of the F_4 fraction of *C. lamellosa* HAP 17 (obtained after alkaline hydrolysis) provides additional evidence for hydroperoxide formation since we observed a large decrease in the proportion of the main polyunsaturated fatty acids ($C_{18:2}$, $C_{18:3}$ and $C_{18:4}$), which are the most sensitive to photochemical and autoxidative degradation (Frankel, 1998), during aging. In contrast, the very low amounts of *cis*-vaccenic and *iso*- and *anteiso*-C_{15} acids of bacterial origin (Volkman, 1986; Zegouagh et al., 2000) detected in this fraction indicate that bacterial processes are not important in the degradation observed.

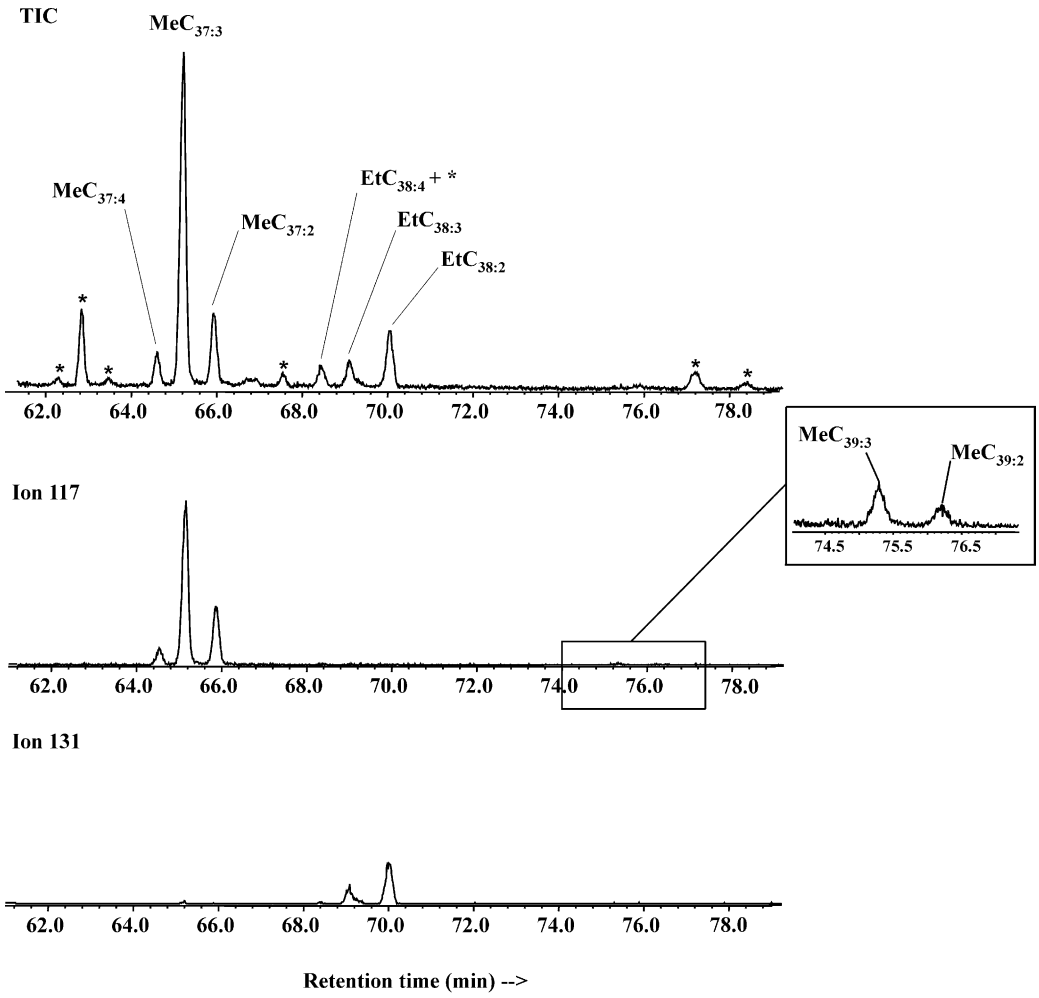

Fig. 2. Partial total ion chromatogram (TIC) and mass fragmentograms for m/z 117 and 131 of the silylated F_3 fraction of *C. lamellosa* HAP 17 grown at 20 °C (early stationary phase). (*alkenones not entirely eluted in the F_2 fraction).

Table 5
Alkenol composition of *Chrysotila lamellosa* HAP 17

Alkenol	Relative percentage	
	Growth at 20 °C[a]	Growth at 10 °C[a]
$MeC_{37:4}$	7.9	52.0
$MeC_{37:3}$	51.1	31.3
$MeC_{37:2}$	12.8	4.3
$EtC_{38:4}$	1.5	6.4
$EtC_{38:3}$	9.3	3.0
$EtC_{38:2}$	14.0	1.3
$MeC_{39:3}$	2.5	1.7
$MeC_{39:2}$	0.9	nd[b]
$U^{k'}_{37}$	0.20	0.12
U^{k}_{37}	0.07	−0.54
K_{37}/K_{38}[c]	2.9	8.2

[a] Early stationary phase.
[b] Not detected.
[c] Relative abundance of total C_{37} to C_{38} alkenols (Prahl et al., 1988).

3. Conclusions

Marlowe (1984) previously analysed the content of long-chain unsaturated lipids of two strains of *C. lamellosa* (strains 353 and 528). On the basis of the strong correlation observed between the lipid compositions of these two strains, this author concluded that *C. lamellosa* presents a greater intraspecific uniformity of biochemistry than is exhibited by *E. huxleyi*. The results obtained in the present work with the strain *C. lamellosa* HAP 17 do not support this conclusion, since this strain exhibits distinct alkene, alkenoate and alkenone distributions that differ from those of strains 353 and 528.

Occasional mismatches between alkenone-derived sea surface temperatures and those derived from other proxies such as oxygen isotopes, trace element ratios, or foraminiferal assemblages indicate that the $U^{k'}_{37}$ index,

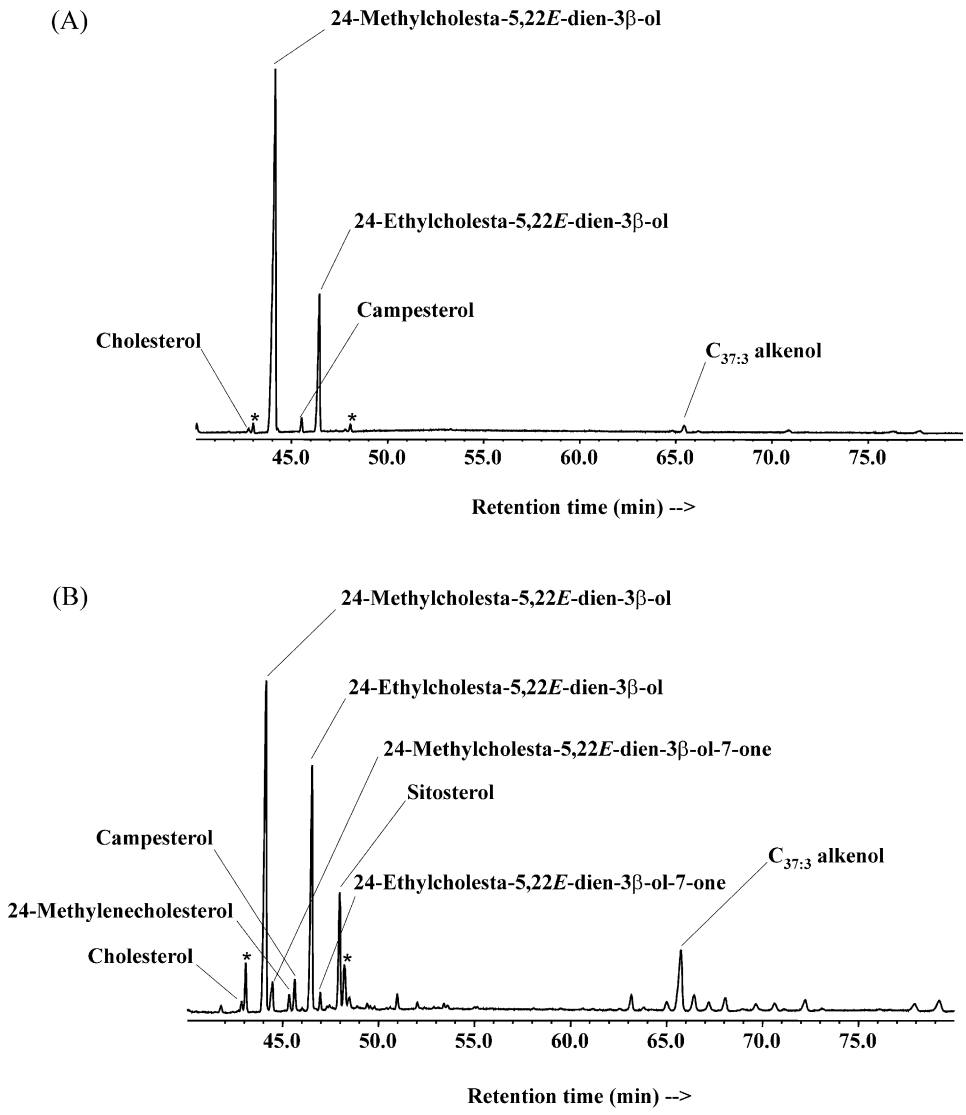

Fig. 3. Partial total ion chromatogram (TIC) of the silylated F_3 fraction (obtained after alkaline hydrolysis) of *C. lamellosa* HAP 17 grown at 20 °C (A) early stationary phase (15 days), and (B) late stationary phase (60 days). (*contaminants).

Table 6
Sterol composition[a] of *Chrysotila lamellosa* HAP 17 and other, previously described, strains[d]

Compound	*C. lamellosa* Caen HAP 17 (early stationary phase) 20 °C	*C. lamellosa* Caen HAP 17 (late stationary phase) 20 °C	*C. lamellosa* Caen HAP 17[b] enriched seawater	*C. lamellosa* Plymouth 528[c] (exponential growth period) 15 °C	*C. lamellosa* Plymouth 353[c] (exponential growth period) 15 °C
Cholest-5-en-3β-ol	0.5	1.2	–	12.6	4.0
24-Methylcholesta-5,22*E*-dien-3β-ol	76.4	45.1	49	78.2	86.1
24-Methylcholest-5-en-3β-ol	1.6	3.5	6	1.7	0.1
24-Methylcholesta-5,24(28)-dien-3β-ol	tr[c]	2.0	–	–	–
24- Ethylcholesta-5,22*E*-dien-3β-ol	21.5	30.3	44	6.25	9.6
24-Ethylcholest-5-en-3β-ol	tr	12.2	–	1.25	0.2
24-Methylcholesta-5,22*E*-dien-3β-ol-7-one	–	4.1	–	–	–
24-Ethylcholesta-5,22*E*-dien-3β-ol-7-one	–	1.6	–	–	–

[a] Relative percentage.
[b] Raederstorff and Rohmer (1984).
[c] Marlowe et al. (1984a).
[d] Trace amounts (<0.1%).

like all proxies, must be applied with due recognition of its limitations (Volkman, 2000). Questions have been raised about the appropriateness of *Emiliania*-based calibrations in some environments (particularly in lacustrine, near-shore or older (that predate the first appearance of *Emiliania* about 250 ky BP; Marlowe et al., 1990) marine sediments; Volkman, 2000). Sea surface temperature reconstruction using such calibrations could also be seriously biased in those coastal sediments that have a high content of *Chrysotila* cells since the relationship between temperature and $U^{k'}_{37}$ observed for *C. lamellosa* HAP 17 ($U^{k'}_{37} = 0.01T + 0.0033$), is significantly different from those obtained for *E. huxleyi* ($U^{k'}_{37} = 0.033T + 0.043$) (Prahl and Wakeham, 1987) and *G. oceanica* ($U^{k'}_{37} = 0.049T - 0.520$) (Volkman et al., 1995).

The detection of significant amounts of natural alkenols in the strain *C. lamellosa* HAP 17 demonstrates that benthic haptophytes may contribute to the presence of these compounds in marine sediments. However, the very high proportion of alkenols previously detected in some sediments from the Camargue (more than 20% of the abundance of the corresponding alkenones; Rontani et al., 2001), suggests an additional source of alkenols. Since there are no reports of haptophytes containing a high content of alkenols, it is likely that bacterial reduction of alkenones to the corresponding alcohols occurs in these sediments.

4. Experimental

4.1. Microalgae

Chrysotila lamellosa HAP-17 was isolated by C. Billard in 1968 from Octeville (France) and was obtained from the Caen Algobank (France). Batch cultures (500 ml) were grown at 10 and 20 °C under 200 μmol of photons m^{-2} s^{-1} (PAR) of cool-white fluorescent light with a 12 h light/12 h dark regime in f/2 medium (Guillard and Ryther, 1962). The cultures were gently hand shaken regularly and harvested at the beginning of the stationary phase (after 26 days at 10 °C and 15 days at 20 °C) and at late stationary phase (60 days at 20 °C) by centrifugation (in 250 ml bottles, ×5000 *g*) for 15 min.

4.2. Lipid extraction

The wet or freeze-dried pellets were extracted with chloroform–methanol–water (1:2:0.8, v/v/v) using ultrasonication for 15 min. Chloroform and purified water were added to the combined extracts to give a final chloroform–methanol–water ratio of 1:1:0.9 (v/v/v), to initiate phase separation. The upper aqueous phase containing salts and water-soluble material was discarded and total solvent-extractable lipids were recovered in the lower chloroform phase, after drying over anhydrous Na_2SO_4, filtration and solvent evaporation under vacuum.

4.3. Alkaline hydrolysis

The wet or freeze-dried pellets were directly saponified with 1 N KOH in MeOH/H_2O (1:1) (50 ml for 1.0 g of wet pellet) by refluxing for 2 h. After cooling and acidification with hydrochloric acid (pH = 1), the content of the flask was extracted three times with dichloromethane. The combined dichloromethane extracts were dried over anhydrous Na_2SO_4, filtered and concentrated as above to give total solvent-extractable lipid compounds.

4.4. Separation of lipid classes

The organic residues obtained after extraction or alkaline hydrolysis were chromatographed over a wet-packed (*n*-hexane) column of silica gel (Kieselgel 60 + 0.5% H_2O). Four fractions were eluted with *n*-hexane, toluene, dichloromethane and methanol yielding, respectively: F_1 (hydrocarbons), F_2 (alkenones and alkenoates), F_3 (sterols and alkenols) and F_4 (fatty acids). These fractions were concentrated by rotary evaporation. The F_2 fractions were analysed before and after $NaBH_4$ reduction.

4.5. Reduction of alkenones

F_2 fractions were reduced (20 min) in diethyl ether:-methanol (2:1, v/v) (5 ml) with excess $NaBH_4$ (10 mg/mg of extract). After reduction, 10 ml of a saturated solution of ammonium chloride were added cautiously; the mixture was shaken and extracted three times with dichloromethane. The combined dichloromethane extracts were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under nitrogen.

4.6. Derivatization

After evaporation of solvent, each residue to be derivatized was taken up in 400 μl of a mixture of pyridine and BSTFA (Supelco) (3:1, v/v) and silylated for 1 h at 50 °C. After evaporation to dryness under nitrogen, the residue was taken up in a mixture of ethyl acetate and BSTFA and analyzed by GC–EIMS.

4.7. Gas chromatography

Hydrocarbons were quantified by GC using a Girdel series 30 gas chromatograph equipped with a Ross injector, a FID detector and a BPX-50 bonded capillary column (SGE; 30 m×0.25 mm i.d.; 0.25 μm film thickness).

4.8. Mass spectrometry

GC–EIMS analyses were carried out with a HP 5890 series II plus gas chromatograph connected to a HP 5972 mass spectrometer. The following operating conditions were employed: 30 m×0.25 mm (i.d.) column coated with Solgel-1 (SGE; film thickness, 0.25 μm); oven temperature programmed from 60 to 130 °C at 30 °C min^{-1}, from 130 to 250 °C at 5 °C min^{-1} and then from 250 to 300 °C at 2 °C min^{-1}; carrier gas (He) maintained at 1.04 bar until the end of the temperature program and then programmed from 1.04 bar to 1.5 bar at 0.04 bar min^{-1}; injector (on-column with retention gap) temperature, 50 °C; electron energy, 70 eV; source temperature, 170 °C; cycle time, 1.5 s. Structural assignments were based on interpretation of mass spectrometric fragmentations and confirmed by comparison of retention times and mass spectra with those of authentic compounds, when these were available. Quantitative determinations in Total Ion Current (TIC) and Selective Ion Monitoring (SIM) modes were based on integrator data that had been calibrated using external standards.

Acknowledgements

This work was supported by grants from the Centre National de la Recherche Scientifique (CNRS).

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