n-Nonacosadienes from the marine haptophytes *Emiliana huxleyi* and
Gephyrocapsa oceanica

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ABSTRACT

The hydrocarbons in cultures of marine haptophytes *Emiliana huxleyi* NIES837 and *Gephyrocapsa oceanica* NIES1315 were analyzed, and nonacosadienes and hentriacontadienes were detected as the major compounds in both strains. We also detected C₂₉ and C₃₁ monoenes and di-, tri- and tetra-unsaturated C₃₃ alkenes as minor compounds but no C₃₇ and C₃₈ alkenes. The positions of the double bonds in the detected C₂₉ and C₃₁ alkenes were determined by mass spectrometry of their dimethyl disulfide (DMDS) adducts. Among the four C₂₉ alkenes identified, the most abundant isomer was determined to be 2,20-nonacosadiene, and the other three compounds were 1,20-nonacosadiene, 3,20-nonacosadiene and 9-nonacosene. This study provides the first identification of 2,20-nonacosadiene and 3,20-nonacosadiene as natural products. The double bond at the n-9 (ω9) position in these C₂₉ alkenes is hypothesized to be derived from precursors of unsaturated fatty acids possessing an n-9 double bond, such as (9Z)-9-octadecenoic acid. We propose the nonacosadienes have the potential for being used as distinct haptophyte biomarkers.

Keywords: Haptophyte, Nonacosadiene, *Emiliana huxleyi*, long chain alkene, biomarker

1. Introduction

Some microalgae are known to produce long-chain aliphatic hydrocarbons, and more recently, these hydrocarbons have been hypothesized to exhibit high potential for use in the biofuel industry (e.g., Chisti, 2007). Such microalgal hydrocarbons have been reported from green algae, such as *Botryococcus braunii* (e.g., Templier et al., 1991; Metzger, 1993), diatoms (e.g., Lee and Loeblich, 1971; Dunstan et al., 1994), haptophytes (e.g., Volkman et al., 1980, Marlow et al., 1984, Rieley et al., 1998), eustigmatophytes (Gelin et al., 1997; Mouzdahir et al., 2001), cyanobacteria (Shiea et al., 1991) and green non-sulfur bacteria (van der Meer et al., 1999). In particular, unique hydrocarbons, such as C₃₁, C₃₃, C₃₇, and C₃₈ alkenes, are known to be produced by cosmopolitan haptophyte algae belonging to the family Noëlaerhabdaceae, such as *Emiliana huxleyi* and *Gephyrocapsa oceanica*, and coastal haptophyte algae belonging to the family Isocrysidaceae, such as *Isochrysis galbana* and *Chrysotila lamellosa* (Volkman et al., 1980; Conte et al., 1995; Rieley et al., 1998; Grossi et al., 2000; Rontani et al., 2004; Sawada and Shiraiwa, 2004). The double-bond configuration of C₃₇ and C₃₈ alkenes is *trans* (Rechka and Maxwell, 1988), whereas that of fatty acid esters in

membrane-bound lipids, such as phospholipids, is generally *cis*. The double-bond configurations of C₃₇-C₃₉ unsaturated alkyl ketones (alkenones), which are well known as biomarkers for the reconstruction of past sea surface temperatures (Brassell et al., 1986; Prahl and Wakeham, 1987), are also *trans*, similarly to the C₃₇ and C₃₈ alkenes. Therefore, these C₃₇ and C₃₈ alkenes are considered to be closely related to long-chain alkenones. In contrast, the C₃₁ and C₃₃ alkenes produced by haptophytes contain double bonds with a *cis* configuration (Rechka and Maxwell, 1988). This finding suggests that the physiological and biochemical roles and metabolic pathway for the productions of C₃₁ and C₃₃ alkenes are different from those associated with the syntheses of C₃₇ and C₃₈ alkenes and C₃₇-C₃₉ alkenones. Through determination of the double-bond positions in the C₃₁ and C₃₃ alkenes, it has been found that most of these alkenes possess double bonds in the n-9 (ω9) position (Rieley et al., 1998). The n-9 (ω9) position and *cis* configuration of unsaturation indicate that the C₃₁ and C₃₃ alkenes are presumably derived from common unsaturated fatty acids, such as (9Z)-9-octadecenoic acid (Oleic acid), and are synthesized *via* chain elongation and decarboxylation (Rieley et al., 1998). However, it has also been noted that the usual mechanism for alkene biosynthesis via decarboxylation

of an unsaturated fatty acid requires the biosynthesis of alkenes possessing a terminal double bond and the presence of an activating group, such as a β - γ double bond or a hydroxyl group in the α position in the fatty acid precursor, but these precursor compounds have not been found in haptophytes (Grossi et al., 2000; Rontani et al., 2006). Thus, the biosynthetic pathway of such long-chain alkenes in haptophytes remains unclear. Furthermore, shorter-chain alkenes, such as C₂₉ alkenes, have been found in *E. huxleyi* (Marlowe et al., 1984; Sawada and Shiraiwa, 2004) and *C. lamellosa* (Rontani et al., 2004; Nakamura et al., 2014), although these are found in markedly less quantities compared with the other alkenes.

In this manuscript, we report the mass spectral characteristics and contents of C₂₉ alkenes as one of the major alkenes in the marine haptophytes *Emiliania huxleyi* and *Gephyrocapsa oceanica* and discuss their significance as biomarkers for environmental science and organic geochemistry. This study provides the first determination of haptophyte-derived C₂₉ alkenes, which were identified by the positions of their double bonds, and the first identification of 2,20-nonacosadiene (**3**) and 3,20-nonacosadiene (**4**) as natural products.

2. Results and discussion

2.1 Mass spectral characteristics of DMDS derivatives of C_{29} and C_{31} alkenes

The C_{29} and C_{31} di-unsaturated alkenes (**2–4** and **6–8**) were detected as major compounds in apolar fractions of *E. huxleyi* NIES837 and *G. oceanica* NIES1315 (Fig. 1). Additionally, we detected a C_{31} mono-unsaturated alkene (**5**) and C_{33} di-, tri- and tetra-unsaturated alkenes (**9–14**) as minor compounds in these strains. However, C_{37} and C_{38} alkenes, which have been hypothesized to share a biosynthetic pathway with C_{37} and C_{38} alkenones, were not detected. The positions of the double bonds in these C_{29} and C_{31} alkenes (**1–8**) were determined by electron impact mass spectrometry (EIMS) of their dimethyl disulfide (DMDS) adducts (Carlson et al., 1989; Rieley et al., 1998). The mass spectra of the DMDS adducts indicate that the most abundant C_{29} alkadiene has two double bonds at C-20 (ions at m/z 173 (C^+) and 371 [$(AB - 48)^+$]) and C-2 (ions at m/z 75 (A^+), 469 [$(BC - 48)^+$] and 423 [$(BC - 94)^+$]) (Table 1, Figs. 2 and 3). The geometry of the double bonds in the alkadiene is presumed to be in the *cis* configuration, similarly to that of the C_{31} alkenes investigated in previous studies (Rieley et al., 1998; Grossi et al., 2000). Therefore, the major C_{29} alkadiene was identified as a (2Z,20Z)-2,20-nonacosadiene (**3**;

Fig. 4). The other two isomers of C₂₉ alkadiene possess the same double bonds at C-20 (ions at m/z 173 (C⁺) and 371 [(AB - 48)⁺]), but their second double bonds are located at different positions: C-1 (m/z 61 (A⁺), 483 [(BC - 48)⁺] and 437 [(BC - 94)⁺]) and C-3 (m/z 89 (A⁺), 455 [(BC - 48)⁺] and 409 [(BC - 94)⁺]) (Table 1, Figs. 3). Thus, these isomers were identified as (20Z)-1,20-nonacosadiene (**2**) and (3Z,20Z)-3,20-nonacosadiene (**4**) (Fig. 4). A C₂₉ monoene could be detected in the product of the DMDS adducts, although no peak of the compound was detected in the chromatograms of the apolar fraction before the DMDS adducts, as shown in Fig. 1, because of a trace amount of and coelution with **2**. The C₂₉ monoene possesses a double bond at C-9 (ions at m/z 173 (A⁺) and 327 [C⁺]) (Table 1, Figs. 2 and 3) and was thus identified as (9Z)-9-nonacosene (**1**; Fig. 4). According to a similar interpretation of the mass spectra of the DMDS adducts, the positions of the double bonds in the C₃₁ mono- and di-unsaturated alkenes were determined to be C-9 and C1, C2 or C3 and C-22, respectively. Thus, these alkenes were identified as 9-hentriacontene (**5**), 1,22-hentriacontadiene (**6**), 2,22-hentriacontadiene (**7**) and 3,22-hentriacontadiene (**8**). These three isomers of C₃₁ alkadienes (**6–8**) are identical to those found in *E. huxleyi* CCAP920/2, although the 2,24-hentriacontadiene found in *E.*

huxleyi CCAP920/2 (Rieley et al., 1998) was not detected in this study.

In the present study, we confirmed that all isomers of the C₂₉ alkenes (**1–4**) possess double bonds at the n-9 (ω 9) position, similarly to the C₃₁ alkenes investigated by Rieley et al. (1998). This result clearly indicates that the C₂₉ alkenes (**1–4**) are closely related to the C₃₁ alkenes (**5–8**) and are also compounds within the same series in the biosynthetic pathway. The C₂₉ alkenes (**1–4**) are possibly derived from common unsaturated fatty acids possessing an n-9 double bond, such as 9-octadecenoic acid (Oleic acid), and are synthesized *via* chain elongation and decarboxylation (Rieley et al., 1998; Grossi et al., 2000).

2.2 Contents of long-chain alkenes

Table 2 shows the compositions and concentrations of C₂₉, C₃₁ and C₃₃ alkenes in *E. huxleyi* NIES837 and *G. oceanica* NIES1315. The relative abundances of C₂₉ alkenes (**1–4**) were 40.1 - 45.4% in *E. huxleyi* NIES837 and 30.8 - 40.9% in *G. oceanica* NIES1315, which are almost similar to those of C₃₁ alkenes (**5–8**) in these species. The most abundant (2Z,20Z)-2,20-nonacosadiene (**3**) accounts for 22 - 34.5% of the total

content in these strains. In contrast, C₂₉ alkenes have normally not been detected in cultures of *E. huxleyi* and *G. oceanica* (Conte et al., 1995; Marlowe et al., 1984) and were only found in minor amounts if present (Sawada and Shiraiwa, 2004). Similarly, C₂₉ alkenes are trace compounds if present in *I. galbana* and *C. lamellosa*, whereas C₃₁ alkenes were overwhelmingly abundant (>90%), as reported previously (Marlowe et al., 1984; Rontani et al., 2004; Nakamura et al., 2014). Based on these findings, the strains investigated in this study are characterized by high abundances of C₂₉ alkenes (**1-4**). Such variability in the alkene compositions may reflect intraspecific genetic differences, as described in *E. huxleyi* and *G. oceanica* (Marlowe et al., 1984; Conte et al., 1995; Rieley et al., 1998; Grossi et al., 2000) as well as *C. lamellosa* (Rontani et al., 2004). No systematic differentiations in the compositions of C₂₉ and C₃₁ alkene isomers were found between the strains grown at 15°C, 17°C and 20°C. However, we observed marked differences in the double-bond isomer compositions (e.g., significantly lower abundance of a (20Z)-1,20-nonacosadiene (**2**) and higher abundance of both (3Z,22Z)-3,22-hentriacontadiene (**8**) and (9Z)-9-hentriacontene (**5**) in a 100-L mass culture sample) between physiologically active growing cells harvested at the early

stationary growth phase and less active cells maintained in non-bubbling conditions for three days before harvesting in mass culture, even when *E. huxleyi* NIES837 cells were grown at the same temperature (20°C) (see first and second lines in Table 2). Thus, these results imply that the composition of double-bond positional isomers is closely associated with the physiological status of cells, such as growth phases and/or nutritional conditions, rather than the temperature.

The total content of C₂₉, C₃₁ and C₃₃ alkenes (**1–14**) in the mass culture sample of *E. huxleyi* NIES837 was 9.86 mg/g dry alga, whereas the total lipid content was 272.5 mg/g due to the rich production of long-chain alkenones (Table 2). The total alkene concentrations in batch cultures of *E. huxleyi* NIES837 and *G. oceanica* NIES1315 were 34.3 - 50.2 and 20.7 - 32.1 fg/cell, respectively (Table 2). Despite the distinct predominance of C₂₉ alkenes, the total alkene contents did not deviate from those found in other strains of *E. huxleyi* and *G. oceanica* (Conte et al., 1995), suggesting that the C₂₉ alkenes (**1-4**) may share same biosynthetic pathway with and are likely involved in similar physiological functions as C₃₁ and C₃₃ alkenes (**5–14**).

2.3 Significances of C₂₉ alkenes as haptophyte biomarkers

The predominant production of C₂₉ alkenes found in this study provides the first demonstration of these compounds in a haptophyte culture, even though trace amount of C₂₉ alkenes were previously observed in the cultures of *E. huxleyi* (Marlowe et al., 1984; Sawada and Shiraiwa, 2004) and *C. lamellosa* (Rontani et al., 2004; Nakamura et al., 2014). Abundant productions of C₂₉ alkenes have been reported only in the chlorophyte *Botryococcus braunii* (Gelpi et al., 1970) and the eustigmatophyte *Nannochloropsis salina* (Mouzdahir et al., 2001) and not in any other algae. In contrast, in natural environments, nonacosadienes were abundantly detected in sediments from Antarctica lakes, such as Lake Fryxell (Matsumoto et al., 1989; Jaraula et al., 2010), West Lake Bonney (Matsumoto et al., 1979) and Ace Lake (Volkman et al., 1986). Jaraula et al. (2010) found a high abundance of nonacosadienes in sediments of Lake Fryxell and therefore assumed that these alkenes may originate from specific haptophytes. Rare occurrences of C₂₉ alkenes and their frequent co-occurrence with long-chain alkenones in natural environments suggest that these alkenes are derived from distinct haptophytes reported in this study and/or haptophytes that grow under extreme environments. Hence,

we propose that C₂₉ alkenes, particularly nonacosadienes, have the potential for being used as distinct haptophyte biomarkers.

We note that a further survey of a wider collection of accessions for *E. huxleyi* and *G. oceanica* is necessary to elucidate the chemotaxonomic and physiological significance of the characteristics observed in the alkene compositions, including the predominance of C₂₉. However, even though different strains could possess different combinations of isomers (cf. Conte et al., 1995), most previous reports on the compositions of long-chain alkenes lack information on double-bond positions, inhibiting a proper comparison of the isomer compositions found in this study with previous references. Hence, we recommend that the method of DMDS adduct be run routinely for each haptophyte isolate or environmental sample in a future survey of long-chain alkenes.

3. Conclusions

In the marine haptophytes *E. huxleyi* NIES837 and *G. oceanica* NIES1315, nonacosadienes (**2–4**) and hentriacontadienes (**6–8**) were detected as major compounds, and C₂₉ and C₃₁ monoenes (**1** and **5**) and di-, tri- and tetra-unsaturated C₃₃ alkenes (**9–14**)

were found as minor compounds. According to the results obtained by the DMDS adduct method, the most abundant nonacosadiene was determined to be (2Z,20Z)-2,20-nonacosadiene (**3**), and in addition, two nonacosadiene isomers (**3** and **4**) were first identified in this study. The n-9 (ω 9) position of the double bonds in these C₂₉ alkenes is hypothesized to be derived from unsaturated fatty acids possessing an n-9 double bond, such as (9Z)-9-octadecenoic acid. Because nonacosadienes have been abundantly detected in sediments from several lakes in Antarctica, we propose that these compounds have the potential to be used as biomarkers of peculiar haptophytes.

4. Experimental

4.1. Algal cultures

The haptophytes *E. huxleyi* strain NIES837 and *G. oceanica* strain NIES1315 were obtained from the Microbial Culture Collection at the National Institute of Environmental Sciences (NIES Collection, <http://www.shigen.nig.ac.jp/algae/>), Japan. The *E. huxleyi* strain NIES837 was originally isolated from the Great Barrier Reef, Australia, during the November 1990 cruise of Sogen-maru of the Marine Biotechnology Institute, Japan (Sekino and Shiraiwa, 1994; Sawada et al., 1996), and the *G. oceanica* strain NIES1315

was isolated from Yaene Port of Hachijo-jima Island, Japan, in January of 2002. Both cells were grown in MA-ESM medium containing synthetic seawater, Marine Art SF (Osaka Yakken, Osaka, Japan) enriched with additional micronutrients and Erdschreiber's medium (ESM) containing 10 nM disodium selenite instead of soil extracts, according to the method described by Danbara and Shiraiwa (1999).

Two types of culture were conducted in this study. First, for batch cultures of *E. huxleyi* NIES837 and *G. oceanica* NIES1315, the algal cells were grown in a 70-mL flask under continuous illumination at $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ by fluorescent lamps. The temperature was maintained at 15°C, 17°C or 20°C in the respective experiments. The cells were harvested at the early stationary growth phase according to a growth curve constructed by monitoring the optical density at 750 nm (OD_{750}), with the exception of the sample of NIES1315 grown at 15°C (the exact growth phase at which it was harvested was not determined).

Second, algal mass cultivation was performed to prepare large amounts of the samples for molecular structure analysis using a mass spectrometer. For this purpose, only *E. huxleyi* NIES837 was grown in a 100-L mass culture system at 20°C under continuous

illumination by fluorescent lamps and continuous bubbling of air at a rate of 100 mL min⁻¹. One day after air bubbling was stopped after five days of culture, the algal cells were harvested by sedimentation. Then, 10 L of medium with the condensed cells was harvested from the bottom of the culture tank (culture 1). The algal culture was then restarted after the addition of fresh ESM-enrichment medium. After 13 days, the cells were harvested by sedimentation for three days (culture 2). The harvested cells (culture 2) were lyophilized and then used for the analysis (sample shown in the first line in Table 2).

4.2. Lipid extraction and separation

The extraction and separation of lipids were performed according to Sawada et al. (1996) and Sawada and Shiraiwa (2004). The lipids were extracted from the cells with dichloromethane and methanol. Upon the addition of distilled water, the combined extracts were shaken and centrifuged to separate the lipid fraction. The resulting organic solvent layer was siphoned off and passed through an anhydrous Na₂SO₄ column. The extract was dried in a rotary evaporator and then re-dissolved in hexane. The lipid-containing hexane extracts were separated using a silica gel column into three

fractions with hexane, hexane/ethyl acetate (9/1, v/v) and ethyl acetate/methanol (1/1, v/v) to yield hydrocarbons, alkenones, and more polar lipids (e.g., sterols and fatty acids), respectively. These fractions were analyzed by GC and GC-MS. An internal standard (*n*-hexatriacontane) was added prior to analysis for the quantification of alkenes and the other compounds.

4.3. Formation of DMDS adducts

As described by Carlson et al. (1989) and Rieley et al. (1998), an aliquot of the hydrocarbon fraction was dissolved in hexane and allowed to react at 40°C (12 h) with DMDS and a solution of iodine in diethyl ether. The reaction was quenched with Na₂S₂O₃, and the adducts were extracted into hexane (x3).

4.4. GC and GC-MS analyses

The lipids were identified by gas chromatography-mass spectrometry (GC-MS) under the following GC-MS conditions: Agilent 6890N GC instrument; 30 m x 0.25 mm i.d. DB-5HT fused silica column (J&W scientific) directly coupled to an Agilent 5975 inert

XL MSD quadrupole mass spectrometer (electron ionization, 70 eV; emission current, 350 μ A; m/z 50-650 in 1.3 s). The GC-MS temperature program was the following: 50°C (maintained for 4 min) to 310°C at 4°C/min (maintained for 20 min). For the analysis of DMDS adducts, the temperature program was the following: 80°C (maintained for 3 min) to 250°C at 12°C/min and then to 350°C (maintained for 20 min) at 3°C/min. The separated fractions were quantified using a Shimadzu GC-2025 GC-FID equipped with 50-m $\times$ 0.32-mm i.d. CPSil5CB fused silica column (Chrompack) using following temperature program: 80°C (maintained for 3 min) to 180°C at 15°C/min and then to 310°C (maintained for 20 min) at 10°C/min.

Acknowledgments

The study was supported by a fund of CREST/JST, Japan. We thank Ms. C. Kanou of Hokkaido University for helping with the lipid extraction and separation. We are grateful to two anonymous reviewers for constructive comments.

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Captions

Table 1 Mass-to-charge ratio (% relative abundance) of key ions in the mass spectra of the DMDS derivatives of C₂₉ and C₃₁ alkene isomers from *E. huxleyi* NIES837.

Table 2 Carbon number distributions and concentrations of alkenes in *E. huxleyi* NIES837 and *G. oceanica* NIES1315.

Fig. 1 Total ion chromatograms showing the distribution of C₂₉–C₃₃ alkenes in the apolar fractions from *E. huxleyi* NIES837 (a) and *G. oceanica* NIES1315 (b) grown at 20°C before the formation of DMDS adducts. •: steroids, †: co-eluted with (9Z)-9-nonacosene (1).

Fig. 2 Fragmentation scheme for the formation of DMDS adducts of alkenes.

Fig. 3 Mass spectra of 9-nonacosene (1), 1,20-nonacosadiene (2), 2,20-nonacosadiene (3) 3,20-nonacosadiene (4) and from *E. huxleyi* NIES837 and of their DMDS adducts.

Fig. 4 Possible structures of C₂₉ and C₃₁ alkenes.

Table 1

Mass-to-charge ratio (% relative abundance) of key ions in the mass spectra of the DMDS derivatives of C₂₉ and C₃₁ alkene isomers from *E. huxleyi* NIES837.

Compound	x [#]	y [#]	z [#]	<i>m/z</i> (% abundance relative to base peak)									
				[A] ⁺	[C] ⁺	[BC - 48] ⁺	[BC - 94] ⁺	[AB - 48] ⁺	[AB - 94] ⁺	[M - 141] ⁺	[M - 94] ⁺	[M - 48] ⁺	[M] ⁺
<i>Alkadiene</i>													
(20Z)-1,20-nonacosadiene, 2	0	17	8	61 (20)	173 (51)	483 (1)	437 (10)	371 (100)	325 (10)	451 (14)	498 (3)	544 (1)	592 (4)
(2Z,20Z)-2,20-nonacosadiene, 3	1	16	8	75 (24)	173 (69)	469 (18)	423 (26)	371 (100)	325 (49)	451 (26)	498 (5)	544 (2)	592 (7)
(3Z,20Z)-3,20-nonacosadiene, 4	2	15	8	89 (50)	173 (100)	455 (47)	409 (56)	371 (99)	325 (74)	451 (39)	498 (8)	544 (1)	592 (12)
(22Z)-1,22-hentriacontadiene, 6	0	19	8	61 (24)	173 (57)	511 (1)	465 (12)	399 (100)	353 (12)	479 (15)	526 (4)	572 (1)	620 (3)
(2Z,22Z)-2,22-hentriacontadiene, 7	1	18	8	75 (28)	173 (81)	497 (18)	451 (30)	399 (100)	353 (57)	479 (26)	526 (7)	572 (1)	620 (5)
(3Z,22Z)-3,22-hentriacontadiene, 8	2	17	8	89 (47)	173 (100)	483 (26)	437 (59)	399 (63)	353 (63)	479 (23)	526 (14)	-	620 (3)
<i>Alkene</i>													
(9Z)-9-nonacosene, 1	9	18		173 (58)	327 (100)							453 (6)	500 (11)
(9Z)-9-hentriacontene, 5	9	20		173 (60)	355 (100)							481 (6)	528 (11)

#: x, y, z represent partial chain lengths shown in the chemical formula H(CH₂)_xCH=CH(CH₂)_yCH=CH(CH₂)_zH in alkadiene and H(CH₂)_xCH=CH(CH₂)_yH in alkene for interpretation (See Fig. 2).

Table 2Carbon number distributions and concentrations of alkenes in *E. huxleyi* NIES837 and *G. oceanica* NIES1315.

Species (Strain ID)	Temp. (°C)	Relative abundances (%)									Total alkene		
		C ₂₉				C ₃₁				C ₃₃	concentrations		
		Compound 2	4	3	subtotal	6	8+5	7	subtotal	subtotal	(µg/mL)	(fg/cell)	(mg/g)
<i>Emiliana huxleyi</i> (NIES837)	20*	15.9	5.8	23.8	45.4	9.7	23.7	16.6	50.0	4.6	-	-	9.86
	20	6.3	2.9	30.9	40.1	9.6	4.5	36.1	50.3	9.7	0.77	50.2	-
	17	5.6	3.6	34.5	43.7	7.0	3.4	36.5	46.8	9.5	0.40	34.3	-
<i>Gephyrocapsa oceanica</i> (NIES1315)	20	8.9	1.3	30.7	40.9	11.8	6.8	25.2	43.8	15.3	0.26	20.7	-
	15	7.0	1.7	22.0	30.8	12.2	3.2	33.8	49.3	20.0	0.41	32.1	-

*: Mass culture sample

Fig. 1

Fig. 2

(9Z)-nonacosene, 1

DMDS adduct of 1

(20Z)-1,20-nonacosadiene, 2

DMDS adduct of 2

(2Z,20Z)-2,20-nonacosadiene, 3

DMDS adduct of 3

(3Z,20Z)-2,20-nonacosadiene, 4

DMDS adduct of 4

Fig. 3

Fig. 4

n-Nonacosadienes from the marine haptophytes *Emiliana huxleyi* and *Gephyrocapsa oceanica*

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Cultured *Emiliana huxleyi* NIES837 and *Gephyrocapsa oceanica* NIES1315 abundantly produce C_{29:2} alkenes. The 2,20-nonacosadiene and 3,20-nonacosadiene were firstly identified.

Graphical Abstract