Two Types of Volatile Polyenes in the Brown Alga *Sargassum thunbergii*

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Abstract: An essential oil from the brown alga *Sargassum thunbergii*, prepared by a simultaneous distillation extraction method, contained in two types of volatile polyenes with a terminal double bond such as (6Z,9Z,12Z,15Z,18Z)-1,6,9,12,15,18-henicosahexaene and (6Z,9Z,12Z,15Z)-1,6,9,12,15,18-henicosapentaene and with their saturated terminal structures such as (3Z,6Z,9Z,12Z,15Z,18Z)-3,6,9,12,15,18-henicosahexaene and (3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15,18-henicosapentaene. These volatile polyenes were identified by comparison with the GC-MS and NMR spectra of synthetics. The polyenes with the saturated terminal structures were found in the brown algae for the first time.

Key words: marine alga, essential oil, volatile, polyene, marine aroma

1 Introduction

Volatile components in seaweeds are released into the seawater. The volatiles play important roles as chemical communications in marine ecosystems. The compounds act as pheromones¹, chemical defenses such as deterrence against herbivores²–⁷, inhibition of bacterial and fungal fouling⁸–⁹, and suppression of competing neighbors². We investigated the pheromone-mediated mating process in brown algae *Dictyopteris* spp during sexual reproduction and elucidated the mechanistic pathway for the biosynthesis of characteristic volatiles¹⁰,¹¹. In the green alga *Bryopsis maxima*, one major volatile component was released by being a 8-heptadecene¹². It was suggested that the hydrocarbon act as an allelochemical. We demonstrated that long-chain aldehydes (C15, C17) are formed from fatty acids through the formation of the corresponding 2-hydroperoxy acids in the green alga *Ulva pertusa*, and that these hydroperoxides exist in a variety of marine algae¹³–¹⁵. We also found that short-chain aldehydes (C6, C9) and middle-chain aldehydes (C10) are formed from fatty acids (C20) in marine algae, whereas they are formed from C18 in higher plants¹⁶–¹⁸.

On the other hand, the marine aroma components in essential oils (EOs) from seaweeds are useful for flavors in foods and fragrances in cosmetics. The hydrocarbons and their oxygenated compounds in brown algae *Dictyopteris* spp, which act as pheromones, possess a “ocean” like aroma. We evaluated the long-chain aldehydes in the green alga *Ulva pertusa* as a “Ulva” or “boiled sea shells” like aroma¹⁹. We also evaluated debromolaurinterol as a “seashore” like aroma in the red alga *Laurencia okamurai*. Recently, we have analyzed the EOs from dried “wakame” *Undaria pinnatifida*, prepared by a simultaneous distillation extraction (SDE) method, indicating one major component of volatiles by GC-MS. After purification of the EOs, the volatile component was identified as (6Z,9Z,12Z,15Z,18Z)-1,6,9,12,15,18-henicosahexaene by comparison with the GC-MS and NMR spectra of synthetic. (6Z,9Z,12Z,15Z)-1,6,9,12,15-Henicosapentaene was also detected as a minor polyene in the EOs. The volatile polyenes showed a subtly “dried wakame” like aroma. It was suggested that these polyenes contribute to the characteristic aroma of the dried wakame. The henicosahexaene was first isolated from brown alga *Fucus vesiculosus* in 1971²⁰, then the polyenes were reported for the volatile components in brown and red algae and diatoms²²–²⁷. However, the exact composition and their roles of volatile polyenes in marine algae have remained unknown, because the polyenes were little interested in the EOs from marine algae. Furthermore, attempts to identify the position and geometry of the double bonds in the polyenes by using MS spectra and the NIST library have not been successful.

In the intertidal zone of Japan, a variety of seaweeds, green, brown and red algae, are widely distributed. The brown alga *Sargassum thunbergii* is very popular in the
temperate coastal waters throughout Japan and grows on underwater rocks in winter to spring to form a seaweed forest. The brown alga *S. thunbergii* contains alginate, mannitol, polyphenols and other useful compounds. In order to explore for a novel marine aroma from seaweeds, we have analyzed the EOs from the brown alga by the SDE method, and detected \( \delta 6Z,9Z,12Z,15Z,18Z \)-1,6,9,12, 15,18-henicosapentaene and \( \delta 6Z,9Z,12Z,15Z \)-1,6,9, 12,15-henicosapentaene as minor aroma components. Interestingly, the related polyenes of henicosahexaene and henicosapentaene were also found as relatively major components in the EOs. Thus, the aim of this study was to isolate and determine the structures of the related polyenes and to identify the composition of polyenes in the EOs by synthetic standards.

### 2 Materials and methods

#### 2.1 General procedure

All reactions were monitored by TLC carried out on 0.25mm E. Merck silica gel plates (60 F <sub>254</sub>). IR spectra were measured with a Nicolet FT-IR spectrometer. NMR spectra were recorded on Bruker AVANCE 400 spectrometer at 400 MHz for \( ^1H \) and 100 MHz for \( ^13C \). Chemical shifts were determined by using TMS or the residual solvent peak in CDCl<sub>3</sub> for \( ^1H \) and the peak for CDCl<sub>3</sub> at \( \delta 7.26 \) for \( ^1H \) and the peak for CDCl<sub>3</sub> at \( \delta 77.0 \) ppm: \( \delta 7.26 \) for \( ^1H \) and the peak for CDCl<sub>3</sub> at \( \delta 7.26 \) for \( ^1H \) and the peak for CDCl<sub>3</sub> at \( \delta 77.0 \) ppm.

#### 2.2 Materials

The brown alga *Sargassum thunbergii* was collected in Aio bay in Yamaguchi prefecture, Japan in December, 2015. The alga samples were stored at \(-20^\circ\text{C}\) until use. 2-Propyn-1-ol and trimethylsilylacetylene were purchased from Kanto Chemical Co. (Tokyo, Japan). 1-Heptyne was purchased from Sigma-Aldrich Co. (Tokyo, Japan). All other chemicals and solvents were commercially of the highest grade.

#### 2.3 Preparation of essential oil from *Sargassum thunbergii*

An essential oil of *S. thunbergii* was prepared by a simultaneous distillation extraction (SDE) method. The frozen sample (200 g) was homogenized with distilled water (200 mL) and then immediately subjected to the SDE apparatus for 2 hours with pentane-dichloromethane (2:1 v/v, 150 mL) as an extraction solvent. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* (700-293 mmHg) at 30°C. The total amount of essential oil (five times) was 25.7 mg in 2.6 \( \times 10^{-3} \) mL per 1 g of the sample. The essential oil was kept under N<sub>2</sub> at \(-25^\circ\text{C}\) until use.

#### 2.4 GC-MS analysis of essential oil from *Sargassum thunbergii*

The volatile components of the essential oil from *S. thunbergii* were analyzed by GC-MS with a DB-WAX column (0.25 mm i.d. \times 60 m). The oven temperature was programmed from 40°C (5 min) to 230°C (50 min) at a rate of 2°C/min. The injection port and ionizing source were kept at 240°C and 230°C respectively. Helium was the carrier gas at the flow rate of 1.5 mL/min. The sample \(1 \mu\text{L}\) was injected into the GC-MS, with the split ratio of the injector at 1:10. Ionization voltage was 70 eV.

#### 2.5 Purification of polyenes in the essential oil from *Sargassum thunbergii*

The essential oil (25.7 mg) from *S. thunbergii* was purified by preparative TLC with pentane as an eluent solvent to afford colorless oils of polyene \( L \) (1.8 mg) and of polyene \( \Pi \) (0.9 mg), respectively. The isolated compounds were assigned by GC-MS and NMR spectra.

polyene \( L \): \( ^1\text{H-NMR} \) (400 MHz, CDCl<sub>3</sub>): \( \delta 5.41-5.33 \) (m, 12H), 2.85-2.80 (m, 10H), 2.12-2.01 (m, 4H), 0.98 (t, \( J = 7.6 \) Hz, 6H); \( ^13\text{C-NMR} \) (100 MHz, CDCl<sub>3</sub>): \( \delta 132.05 \) (2C), 128.57 (2C), 128.25 (2C), 128.13 (2C), 127.39 (2C), 127.02 (2C), 25.63 (3C), 25.54 (2C), 20.56 (2C), 14.27 (2C); GC-MS \( m/\text{z} \) 284 \( (M^+) \), 283, 259, 248, 247, 211, 198, 173, 159, 145, 133, 119, 103, 91, 79, 67, 55, 41, 39, 29.

polyene \( \Pi \): \( ^1\text{H-NMR} \) (400 MHz, CDCl<sub>3</sub>): \( \delta 5.39-5.30 \) (m, 10H), 2.87-2.80 (m, 8H), 2.10-2.01 (m, 4H), 1.35-1.20 (m, 6H), 0.98 (t, \( J = 7.5 \) Hz, 3H), 0.89 (t, \( J = 7.4 \) Hz, 3H); GC-MS \( m/\text{z} \) 286 \( (M^+) \), 271, 257, 243, 232, 215, 201, 187, 175, 159, 150, 133, 119, 105, 91, 79, 67, 55, 41, 39, 29.

#### 2.6 Syntheses of \((3Z,6Z,9Z,12Z,15Z,18Z)-3,6,9,12,15,18-henicosahexaene\) \((l)\)

2.6.1 2-propynyl-1-bromide \((4)\)

\[ \text{PBr}_3(62.7 \text{ g, 231 mmol)} \text{ was carefully added to 2-propyn-1-ol (1; 34.2 g, 610.9 mmol) containing a catalytic amount of pyridine at } -10^\circ\text{C}. \text{ After stirring for 2 hours at room temperature, the reaction mixture was distilled to give bromide } 4 \text{ (45.0 g, 61.0%)} \text{ as a colorless oil.} \]

\[ ^1\text{H-NMR} \text{ (400 MHz, CDCl}_3\text{): } \delta 3.88 \text{(d, } J = 2.6 \text{ Hz, 2H), 2.53 (t, } J = 2.6 \text{ Hz, 1H); } \]

\[ ^13\text{C-NMR} \text{ (100 MHz, CDCl}_3\text{): } \delta 78.72, 74.87, 13.39 \text{. IR (film) cm}^{-1}: \delta = 3300, 3009, 2980, 2933, 2877, 2122. \]

2.6.2 2-(2-propynloyloxy)tetrahydropryan \((5)\)

\[ \text{NaHSO}_4 \text{ solution (1.0 mL, 5.0 M) was added to the mixture of alcohol (1; 2.2 g, 39.3 mmol) and DHP (5.0 g, 59.0 mmol). After stirring for 24 hours at room temperature, the reaction mixture was quenched with NaHCO}_3 \text{ and diluted with ether. The mixture was washed with brine, then dried over Na}_2\text{SO}_4 \text{. The extract was concentrated } \text{in vacuo, and distilled to give TBP ether (4.4 g, 71.4% as a colorless oil.} \]

\[ ^1\text{H-NMR} \text{ (400 MHz, CDCl}_3\text{): } \delta 4.83 \text{(t, } J = 3.4 \text{ Hz, 1H), 4.26 (m, 2H), 3.89-3.81 (m, 1H), 3.57-3.50 (m, 1H), 2.42 (t, } \]

2.6.3 2-([2-propynloyl]methyloxy)tetrahydropryan \((6)\)
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\[ J = 2.4 \text{ Hz, } 1H \], 1.82-1.51 (m, 6H). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) ppm: \(\delta 96.83, 79.76, 73.98, 61.99, 53.99, 30.20, 25.32, 18.98\). IR (film) cm\(^{-1}\): \(\delta = 3315, 3292, 3259, 2956, 2873, 2846, 2248, 1733\).

2.6.3 2-pentyn-1-bromide (6)

PBBr\(_2\) (25.9 g, 93.8 mmol) was carefully added to 2-pentyn-1-ol (22.0 g, 256.0 mmol) containing a catalytic amount of pyridine at \(-10^\circ C\). After stirring for 3 hours at room temperature, the reaction mixture was poured into an ice-water. The mixture was extracted with ether, and washed with water, saturated NaHCO\(_3\) solution and brine. After drying over Na\(_2\)SO\(_4\), the extract was concentrated \textit{in vacuo}. The concentrate was distilled to give bromide 6 (26.0 g, 66.9%) as a colorless oil. \(^{1}\)H-NMR (400 MHz, CDCl\(_3\)) ppm: \(\delta 3.93, 2.3 \text{ Hz, } 2H\), 2.26 (qt, \(J = 7.5 \text{ Hz}, 2.3 \text{ Hz, } 2H\), 1.14 (t, \(J = 7.5 \text{ Hz, } 3H\)). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) ppm 89.44, 74.63, 15.72, 13.48, 12.66. IR (film) cm\(^{-1}\): \(\delta = 2967, 2937, 2911, 2233, 1444, 1209\).

PbBr\(_2\) solution (50.0 ml, 50.0 mmol) in THF at 10\(^\circ C\), and the mixture was stirred for 1 hour at 65\(^\circ C\). After adding C\(_6\)H\(_6\) (40.0 mg, 0.2 mmol) at room temperature, the mixture was stirred for 10 min, and then bromide 4 (6.4 g, 53.0 mmol) was added. After stirring for 5 hours at 65\(^\circ C\), the reaction mixture was carefully poured into a cooled saturated NH\(_4\)Cl solution, and the mixture was extracted with ether, and washed with saturated NaHCO\(_3\) solution and brine. After drying over Na\(_2\)SO\(_4\), the extract was concentrated \textit{in vacuo}. The concentrate was purified by column chromatography to give triynol \(\delta (4.0 \text{ g, } 64.7\%)\) as a yellow oil. \(^{1}\)H-NMR (400 MHz, CDCl\(_3\)) ppm: \(\delta 4.26, 2.1 \text{ Hz, } 2H\), 3.21 (tt, \(J = 2.2 \text{ Hz, } 2.3 \text{ Hz, } 2H\), 3.14 (tt, \(J = 2.3 \text{ Hz, } 2H\), 2.17 (m, 2H), 1.12 (t, \(J = 7.5 \text{ Hz, } 3H\)). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) ppm: 82.88, 80.09, 78.70, 75.52, 73.68, 72.92, 51.21, 13.81, 12.34, 9.88, 9.70. IR (film) cm\(^{-1}\): \(\delta = 3337, 2982, 2939, 2913, 2871, 2246, 2207\).

2.6.6 2,5,8-undecatriyn-1-bromide (9)

A solution of 1M PBBr\(_2\) (8 ml, 8.0 mmol) in ether was slowly dropped to alcohol (8.0 g, 22.0 mmol) in ether (20 ml) containing a catalytic amount of pyridine below \(-10^\circ C\). After stirring for 2 hours at 0\(^\circ C\), the reaction mixture was carefully poured into an ice-water. The mixture was extracted with ether, and washed with saturated NaHCO\(_3\) solution and brine. After drying over Na\(_2\)SO\(_4\), the extract was concentrated \textit{in vacuo}. The concentrate was purified by column chromatography to give bromide \(\delta (1.6 \text{ g, } 29.1\%)\) as a yellow oil. \(^{1}\)H-NMR (400 MHz, CDCl\(_3\)) ppm: 8 3.91 (t, \(J = 2.3 \text{ Hz, } 2H\), 3.24 (tt, \(J = 2.2 \text{ Hz, } 2H\), 3.14 (t, \(J = 2.3 \text{ Hz, } 2H\), 2.17 (m, 2H), 1.12 (t, \(J = 7.5 \text{ Hz, } 3H\)). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) ppm: 82.32, 81.33, 75.74, 75.57, 73.19, 72.85, 14.69, 13.83, 13.26, 10.14, 9.72. IR (film) cm\(^{-1}\): \(\delta = 2977, 2930, 2918, 2888, 2879, 2246, 2233, 1719, 1321\).

2.6.7 2,5-octadiyn-1-ol (10)

Alcohol (5.5 g, 33.0 mmol) was added to a suspension of K\(_2\)CO\(_3\) (6.8 g, 49.5 mmol), C\(_6\)H\(_6\) (63.3 mmol) and NaN\(_3\) (7.4 g, 49.5 mmol) dissolved in DMF (40 ml). After addition bromide 6 (3.7 g, 25.2 mmol), the reaction mixture was stirred for 5 hours at room temperature. The reaction mixture was filtered with celite and washed with ether. The filtrate was washed with saturated NH\(_4\)Cl solution, water and brine. After drying over Na\(_2\)SO\(_4\), the reaction extract was concentrated \textit{in vacuo}. The concentrate was purified by column chromatography to give octadiynol 10 (2.7 g, 93.1%) as a colorless oil. \(^{1}\)H-NMR (400 MHz, CDCl\(_3\)) ppm: \(\delta 4.27, J = 2.2 \text{ Hz, } 2H\), 3.19 (tt, \(J = 2.3 \text{ Hz, } 2H\), 2.17 (tq, \(J = 2.3 \text{ Hz, } 7.5 \text{ Hz, } 2H\), 1.13 (t, \(J = 7.5 \text{ Hz, } 3H\)). \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)) ppm: 82.45, 80.81, 78.36, 72.67, 51.25, 13.79, 12.32, 9.79. IR (film) cm\(^{-1}\): \(\delta = 3334, 2980, 2937, 2920, 2882, 2255\).

2.6.8 2,5-octadiyn-1-bromide (11)

A solution of 1M PBBr\(_2\) (10 ml, 10.0 mmol) in ether was slowly dropped to alcohol (3.8 g, 25.0 mmol) in ether (10 ml) containing a catalytic amount of pyridine below \(-10^\circ C\). After stirring for 3 hours at room temperature, the reaction mixture was poured into an ice-water. The mixture was extracted with ether, and washed with saturated NaHCO\(_3\) solution and brine. After drying over Na\(_2\)SO\(_4\), the extract was concentrated \textit{in vacuo}. The concentrate was purified by column chromatography to give bromide 11 (2.8 g, 52.9%) as a yellow oil. \(^{1}\)H-NMR (400 MHz, CDCl\(_3\)) ppm: \(\delta 3.92, J = 2.4 \text{ Hz, } 2H\), 3.21 (tt, \(J = 2.4, 4.7 \text{ Hz, } 2H\), 2.17 (tq, \(J = 2.4, 7.5 \text{ Hz, } 2H\), 1.12 (t, \(J = 7.5 \text{ Hz, } 3H\)).
Bromide 11 (1.8 g, 9.0 mmol) was added to a suspension of CuI (1.7 g, 8.9 mmol), NaI (2.0 g, 13.5 mmol) and K₂CO₃ (1.9 g, 13.5 mmol) in DMF (10 mL). After added trimethylsilylacetylene (3; 1.1 g, 1.0 mmol), the reaction mixture was stirred for 16 hours at room temperature. The mixture was extracted with hexane, and washed with saturated NH₄Cl solution and brine. After washing over Na₂SO₄, the extract was concentrated in vacuo. The concentrate was purified by column chromatography to give triyne as a white crystal. 1H-NMR (400 MHz, CDCl₃) ppm: δ 5.44-5.30 (m, 12H), 2.85-2.80 (m, 10H), 2.11-2.04 (m, 4H), 0.98 (t, J = 7.5 Hz, 6H). 13C-NMR (100 MHz, CDCl₃) ppm: δ 132.04 (2C), 128.57 (2C), 128.25 (2C), 128.13 (2C), 127.89 (2C), 127.02 (2C), 25.63 (3C), 25.54 (2C), 20.56 (2C), 14.26 (2C). IR (film) cm⁻¹: δ = 2914, 2960, 2928, 2874, 2852, 1456, 1433, 717. TOF-MS m/z: 285.2584 (M+H)⁺ (Calcd for C₂₂H₃₃: 285.2577).

2.7 Syntheses of (3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15-henicosahexaene (II)

2.7.1 1, 4-decadiyne (16)

1-Heptyne (44.4 g, 39.9 mmol) was added to a solution of 3M EtMgBr (13.1 mL, 39.3 mmol) in THF at 10°C, and the mixture was stirred for 1 hour at 65°C. After added CuI (40.0 mg, 0.2 mmol), the mixture was stirred for 10 min, and then bromide (5.0 g, 41.0 mmol) was added. After stirring for 10 hours at 65°C, the reaction mixture was carefully poured into a cooled saturated NH₄Cl solution. The mixture was extracted with ether, and washed with saturated NaHCO₃ solution and brine. After washing over Na₂SO₄, the extract was distilled to give decadiyne (1.1 g, 20.6%) as a colorless oil. 1H-NMR (400 MHz, CDCl₃) ppm: δ 3.15 (dt, J = 2.5, 2.5 Hz, 2H), 2.16 (tt, J = 2.5, 7.2 Hz, 2H), 2.06 (t, J = 2.7 Hz, 1H), 1.50 (m, 2H), 1.37 (m, 4H), 0.89 (t, J = 7.1 Hz, 3H). 13C-NMR (100 MHz, CDCl₃) ppm: δ 81.32, 78.98, 72.95, 68.32, 31.04, 28.35, 22.18, 18.63, 13.93, 9.54. IR (film) cm⁻¹: δ = 3303, 2956, 2930, 2879, 2853, 2229, 1732, 1689.

2.7.2 3,6,9,12,15-henicosapentaene (17)

Diyne (485.0 mg, 3.6 mmol) was added to a suspension of CuI (685.6 mg, 3.6 mmol), NaI (539.6 mg, 3.6 mmol) and K₂CO₃ (746.3 mg, 5.4 mmol) in DMF (10 mL), the mixture was stirred at 40°C. After stirred for 5 hours, the reaction mixture was stirred for 3 hours at 45°C. The reaction mixture was diluted with ether, and washed with saturated NH₄Cl solution and brine. After drying over Na₂SO₄, the extract was concentrated in vacuo. The concentrate was purified by column chromatography to give henicosapentaene (17) (221.0 mg, 22.2%) as a colorless oil. 1H-NMR (400 MHz, CDCl₃) ppm: δ 3.13 (m, 8H), 2.16 (m, 4H), 1.49 (t, J = 7.3 Hz, 2H), 1.38 (m, 4H), 1.12 (t, J = 7.4 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H). 13C-NMR (100 MHz, CDCl₃) ppm: δ 82.19,
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80.95, 75.28, 75.22, 74.49, 74.46, 74.04, 73.99, 73.58, 73.01, 31.57, 31.07, 28.41, 22.64, 22.19, 18.67, 13.95, 13.82, 12.35, 9.80, 9.75. IR (film) cm\(^{-1}\): \(\delta = 2952, 2930, 2875, 2858, 2254, 2207, 1317\). TOF-MS \(m/z\): 277.1947 (M + H)\(^+\) (Calcd for C\(_{25}\)H\(_{36}\): 277.1951).

2.7.3 \((3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15\)-henicosapentaene (II)

Pentayne 17 (60.0 mg, 0.2 mmol) was hydrogenated in ethanol (6 mL) at 0°C over the Lindlar catalyst (30.0 mg, 5%Pd-BaSO\(_4\)) poisoned with quinoline (30.0 mg) until the theoretical amount of H\(_2\) gas (22.4 mL) had been absorbed. The catalyst was removed by celite. The filtrate was diluted with ether, and washed with brine. After drying over Na\(_2\)SO\(_4\), the extract was concentrated \textit{in vacuo}. The concentrate was purified by column chromatography to give henicosapentaene II (20.0 mg, 31.4%) as a colorless oil. \(^1\)H-NMR (400 MHz, CDCl\(_3\)) ppm: \(\delta = 5.42-5.33\) (m, 10H), 2.87-2.80 (m, 8H), 2.12-2.03 (m, 4H), 1.36-1.29 (m, 6H), 0.99 (t, \(J = 7.4\) Hz, 3H). \(^13\)C-NMR (100 MHz, CDCl\(_3\)) ppm: \(\delta = 132.03, 130.48, 128.55 (2C), 128.20, 128.18, 127.91 (2C), 127.55, 127.03, 31.59, 31.52, 29.33, 27.22, 25.64, 25.54, 22.65, 22.57, 20.55, 14.20, 14.05. IR (film)/cm\(^{-1}\): \(\delta = 3012, 2969, 2918, 2866, 2845, 1651, 1463, 719\). TOF-MS \(m/z\): 287.2729 (M + H)\(^+\) (Calcd for C\(_{22}\)H\(_{36}\): 287.2733).

3 Results and discussion

Essential oils (EOs) of the brown alga \textit{Sargassum thunbergii} prepared by a simultaneous distillation extraction (SDE) method were obtained in 2.6 \(\times\) 10\(^{-3}\)% yield. Figure 1 shows TIC of the EOs from the brown alga \textit{S. thunbergii}. Four kinds of volatile polyenes were detected in the EOs by GC-MS analysis. Among of these, the MS spectra of two major volatile polyenes I and II were very similar with those of henicosahexaene and henicosapentaene. However, \((6Z,9Z,12Z,15Z,18Z)-1,6,9,12,15,18\)-henicosahexaene and \((6Z,9Z,12Z,15Z)-1,6,9,12,15\)-henicosapentaene were determined on the other ion signals in the EOs by comparison with the GC-MS and NMR spectra of synthetics. These polyenes with a terminal double bond were found as major aroma components in the EOs from dried "wakame" \textit{Undaria pinnatifida} by the SDE method\(^{29}\). From these results, the volatile polyenes I and II appeared to be their isomers of henicosahexaene and henicosapentaene.

In order to identify the structures of related polyenes of henicosahexaene and henicosapentaene, the EOs were purified by a preparative TLC to afford polyene I and II, respectively. The compound I was characterized by a molecular ion (M\(^+\)) at \(m/z\) 284 by GC-MS (Fig. 1). The compound I was not contained in a set of vinyl protons, while twelve olefinic protons, five bisallylic methylene protons of pentadienyl structure, two adjacent methylene protons of double bonds and two methyl protons were estimated by \(^1\)H-NMR.

Fig. 1 GC-MS analysis of the essential oils from the brown alga \textit{Sargassum thunbergii}. a: \((6Z,9Z,12Z,15Z,18Z)-1,6,9,12,15,18\)-henicosahexaene b: \((6Z,9Z,12Z,15Z)-1,6,9,12,15\)-henicosapentaene c: \((3Z,6Z,9Z,12Z,15Z,18Z)-3,6,9,12,15\)-henicosapentaene (I) d: \((3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15\)-henicosapentaene (II).

The proton signals of double bonds in the compound I indicated a polyene structure with 1,4-skipped dienes. \(^{13}\)C-NMR spectrum of the compound I showed only ten carbon signals, which identified by a DEPT experiment as six olefinic carbons, three methane carbons and one methyl carbon, although the whole skeletal structure estimated as 21 carbons. The carbon signals of the compound I suggested a plane of symmetry in the polyene structure. Furthermore, the H-H COSY and HMBC spectra observed the correlation of the terminal methyl with the 1,4-skipped diene structures. Thus, the major polyene I in the EOs from the brown alga was proposed as 3,6,9,12,15,18-henicosapentaene. On the other hand, the other compound II in the EOs was characterized by a molecular ion (M\(^+\)) at \(m/z\) 286 by GC-MS (Fig. 1). The compound II was not also observed in the vinyl protons, while ten olefinic protons, four bisallylic methylene protons, two adjacent methylene protons of double bonds, three methylene protons and two methyl protons were estimated by \(^1\)H-NMR. \(^{13}\)C-NMR spectrum of the compound II showed no signals of vinyl
Scheme 1  Synthesis of (3Z,6Z,9Z,12Z,15Z,18Z)-3,6,9,12,15,18-henicosahexaene (1). Reagents: a PBr₃, pyridine; b DHP, H⁺; c EtMgBr, Cul, THF; d Cul, NaI, K₂CO₃, DMF; e PTS, MeOH; f CsF, DMI; g Lindlar catalyst, H₂, quinoline.

carbons, although the amount of sample was too low to confirm the whole skeletal carbons. From these results, the other polyene II was proposed as 3,6,9,12,15-henicosapentaene. It was proposed that these related polyenes of henicosahexaene and henicosapentaene in the EOs from the brown alga have no the terminal double bond. In the previous reports, one type of polyenes with the terminal double bond such as 1,6,9,12,15,18-henicosahexaene and 1,6,9,12,15-henicosapentaene was commonly detected in brown and red algae, while the other type of polyenes with their saturated terminal structures was found in green algae.

In order to confirm the positions and their geometries of double bonds in the proposed polyenes and to clarify the two types of polyenes with the terminal double bond and with the saturated structure, we attempted to synthesize the polyenes with the saturated structure such as 3,6,9,12,15,18-henicosahexaene and 3,6,9,12,15-henicosapentaene, regio- and stereo-selectively. Synthesis of 3,6,9,12,15,18-henicosahexaene I is summarized in Scheme 1. The skipped dienes can be constructed by the Grignard or Jeffery coupling. Thus, 2-pentyn-1-ol (1) was treated with PBr₃ to afford bromide 4. In addition, the hydroxyl group of 1 was protected with DHP to afford THP ether 5 in 2 steps. Coupling of THP ether 5 with bromide 4 by employing the Grignard reagent in THF gave 2,5-hexadiynylxyl-THP 7 in 3 steps. Treatment of 2-pentyn-1-ol (2) with PBr₃ afforded bromide 6. Jeffery coupling of THP ether 7 with bromide 6 in DMF and subsequently deprotection of the THP group with PTS in MeOH gave undecatriyn-1-ol 8 in 3 steps. Treatment of alcohol 8 with PBr₃ in ether yielded bromide 9 as one counter part in 6 steps. On the other hand, Jeffery coupling of alcohol 1 and bromide 6 in DMF gave 2,5-octadiyn-1-ol (10). Treatment of alcohol 10 with PBr₃ in ether afforded bromide 11. Jeffery coupling of trimethylsilylacetylene (3) with bromide 11 in DMF gave TMS ether 12. Then, deprotection of TMS group yielded 1,4,7-decatriyne (13) as the other counter part. To construct the whole C21 skeleton structure, Jeffery coupling of triyne 13 with bromide 9 in DMF gave henicosahexayne 14. Finally, catalytic hydrogenation of hexayne 14 with the Lindlar catalyst yielded (3Z,6Z,9Z,12Z,15Z,18Z)-3,6,9,12,15,18-henicosahexaene (I). The overall yield of I was 0.06% based on 1 in 12 steps. Interestingly, the synthetic polyene I showed a subtly marine aroma.

Synthesis of 3,6,9,12,15-henicosapentaene II performed in Scheme 2. Coupling of 1-heptyne (15) with bromide 4 by employing the Grignard reagent in THF gave 1,4-decadiyne (16). Jeffery coupling of dylene 16 with the counter part 9 afforded henicosapentaene 17. Finally, catalytic hydrogenation of pentayne 17 with the Lindlar catalyst yielded (3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15-henicosapentaene (II). The overall yield of II was 1.4% in 3 steps. The synthetic polyene II exhibited less marine aroma as compared with the polyene I.

It is difficult to specify the position and geometry of the double bonds in the polyenes by using the MS spectra and the libraries, because of their similar fragment patterns and retention indexes. However, these synthetics can serve as standards for identifying the polyenes in the EOs.

Then, we tried to determine the structures for the related polyenes I and II in the TIC of EOs from the brown alga S. thunbergii. The volatile polyene I obtained from the EOs was compared with the synthetic henicosahexaene by GC-MS and NMR spectra. The retention time and the NMR spectra of polyene I from the EOs were exactly coincident.
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The polyenes with the saturated terminal structure such as 15-henicosapentaene, while green and diatoms produced fatty acids such as octadecatetraenoic acid or icosapentaenoic acid in the lipids. These results suggested the elongation of fatty acids such as octadecatetraenoic acid or icosapentaenoic acid followed by decarboxylation to produce the polyenes in the dried wakame. For the biosynthesis of the polyenes, a unique enzymatic reaction seems to be required to form the terminal double bond after decarboxylation of the elongated polyunsaturated fatty acids. On the other hand, the polyenes with the saturated terminal structure were also detected in the EOs from the brown alga S. thunbergii. However, the biosynthesis of the polyenes derived by selectively reducing a carboxy group of the fatty acids remained unclear. Thus, we are currently investigating the mechanistic pathway for the biogeneration of both the types of polyenes.

In conclusion, these findings showed that the polyenes in the EOs from the brown alga S. thunbergii were determined as volatile components by GC-MS, isolation and synthesis. Synthesis of the proposed polyenes provided more detailed information about the two types of polyenes with the terminal double bond and with the saturated terminal structure. It was interesting that both the types of polyenes are existed in the EOs of the brown algae S. thunbergii. This work will focus attention on a new insight into the chemotaxonomy in marine algae.

References

5) Pohnert, G. Phospholipase A2 activity triggers the

Scheme 2  Synthesis of (3Z,6Z,9Z,12Z,15Z)-3,6,9,12,15-henicosahexaene (I). Reagents: a EtMgBr, Cul, THF; b Cul, NaI, K2CO3, DMF; c Lindlar catalyst, H2, quinoline.

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