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Algicidal hydroxylated C18 unsaturated fatty acids from the red alga *Tricleocarpa jejuensis:* Identification, synthesis and biological activity

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ABSTRACT

Bioassay-guided separation of a methanol extract of *Tricleocarpa jejuensis* by monitoring algicidal activity against the red tide phytoplankton *Chattonella antiqua* led to the isolation of an active fraction consisting of a mixture of four isomeric compounds. The active compounds were identified as (*E*)-9-hydroxyoctadec-10-enoic acid (**1**), (*E*)-10-hydroxyoctadec-8-enoic acid (**2**), (*E*)-11-hydroxyoctadec-12-enoic acid (**3**) and (*E*)-12-hydroxyoctadec-10-enoic acid (**4**) by NMR, IR and mass spectral data. The structures were confirmed by comparison of the NMR and MS data with those of authentic samples of **1–4** obtained by unambiguous syntheses. Synthesized hydroxy acids **1–4** and related compounds were assessed for algicidal activity against *C. antiqua* and it was found that all of **1–4** had high activity (> 80% mortality at 24 h) at a concentration of 20 μg/mL. A structure–activity relationship study using 11 related compounds revealed that the presence of the hydroxyl group is important for the activity and the double bond may be replaced with a triple bond.

1. Introduction

Harmful algal blooms (HABs), commonly known as red tides, due to eutrophication of coastal waters occur world-wide and cause serious damage to aquatic ecosystems and public health. The recent dominant species of HABs in Japan are *Chattonella antiqua* (Raphidophyceae), *Karenia mikimotoi* (Dinophyceae) and *Heterocapsa circularisquama* (Dinophyceae), which have caused mass mortality of cultivated fish and shellfish. Various physical, chemical, physico-chemical, and biological methods tocontrol HABs have been developed [1]; however, many of them are unacceptable for practical use in marine environments due to the second pollution, high cost, or difficulty of handling.

Macroalgae have been shown to produce and release allelopathic substances toxic to HAB species [2,3]. Consequently, considerable studies on the isolation and identification of the allelochemicals of macroalgae have been conducted [4] with the goal of developing an environmentally benign, natural product-based, anti-red tide agent. The algicidal (antialgal) compounds isolated so far include polyunsaturated fatty acids (PUFAs) from *Cladosiphon okamuranus* [5], *Botryococcus braunii* [6], *Ulva fasciata* [7] *Lithophyllum yessoense* [8], and *Sargassum thunbergii* [9]; glycerolipids from *Ishige sinicola* [10] and *Ulva prolifera* [11,12]; terpenoids from *Dictyota dichotoma* [13], *Gracilaria lemaneiformis* [14,15], *Dictyopteris undulata* [16], and *Ulva pertusa* [17]; and phenolics [15,17]. Many of these compounds are reported to have potent algicidal activity at concentrations of low μg/mL range against some of the raphidophytes and dinoflagellates responsible for red tides. We screened 17 species of macroalgae including 9 Rhodophyta, 6 Phaeophyta, and 2 Chlorophyta collected from the coastal region of Nagasaki Prefecture, Japan, for their algicidal activity against the red tide phytoplankton *Chattonella antiqua* and found that a methanol extract of the red alga *Tricleocarpa jejuensis* had cell lysis activity at a concentration of 0.1 mg/mL (Supplementary data). Herein, we describe the separation, structure elucidation, synthesis and structure–activity study of the algicidal principles of *T. jejuensis*.

2. Materials and methods

2.1. General experimental procedure

NMR spectra were recorded on a Varian System 500PS SN spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), a JOEL JNM AL400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) or a Varian Gemini 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) in $CDCl_3$ using tetramethylsilane and $CDCl_3$ as the internal standards for ^{1}H and ^{13}C nuclei, respectively. High resolution (HR) electron impact mass spectroscopy (EIMS) was carried out on a JEOL JMS-700 N spectrometer. Electron spray ionization (ESI) and direct analysis in real time (DART) mass spectra (MS) were obtained on a JEOL JMS-T100TD spectrometer.

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IR spectra were recorded on a ThermoFisher Scientific Nicolet Nexus 670NT spectrophotometer. Optical rotation was measured on a JASCO P-2200 polarimeter using a 10-cm microcell. GC-EIMS analysis was performed using an Agilent Technologies GC7890A-MS7000A system equipped with an HP-1MS capillary column (length 30 m, inside diameter 0.250 mm, film thickness 0.25 μm) in EI mode at 70 eV. GLC conditions: carrier gas, He; flow rate, 1.8 mL/min; oven, 120 °C, 5 min isothermal, 120 °C ~ 300 °C with 10 °C/min.

Silica gel gravity and medium pressure column chromatography separations were performed using Kanto Chem. Co. Ltd. Silica Gel N (spherical neutral) 100–210 μm and 40–60 μm, respectively. Preparative TLC was performed using Merck Silica Gel 60 F_{254} (20 × 20 cm, layer thickness 1.0 mm).

2.2. Plant material

A specimen of *T. jejuensis* was collected from Ishigaki Island of Okinawa Prefecture, Japan, in June 2016. All samples were saved in a freezer and brought to the laboratory in plastic bags. After thawing at rt. (*ca.* 25 °C), the samples were briefly washed with tap water to remove possible contaminants, and dried in air.

2.3. Cultivation of phytoplankton

Chattonella antiqua, isolated from Shimabara Bay, Japan in 2010 by Dr. Tatsuya Oda, Nagasaki University, was cultured aseptically in PES medium at 20 °C under 40 μmol/m^2/s using 40 W fluorescent lamps with a 12 h day cycle and 12 h night cycle and sub-cultured after approximately 14 days.

2.4. Algicidal assay

The algicidal assay was performed according to Kakisawa's procedure [5], with a slight modification. In brief, a methanol solution of the extract or sample at varying concentrations was added to the cell suspension (cell density *ca.* 2 × 10^4 cells/mL) of *C. antiqua* in a 48-well microplate to make the final concentrations of 5, 20, or 80 μg/mL (methanol concentration ≤ 1%). After incubation at 20 °C for 24 h, the cell mortality was calculated under microscope observation (×400). The assay was performed in triplicate. Algicidal activity (AA) was calculated using a formula: AA (%) = (1 − T/C) × 100, where T and C represent number of the living cells in the presence and absence of the compound tested, respectively. Swollen and burst cells were considered dead cells.

2.5. Extraction and isolation of algicidal compounds

T. jejuensis (240 g dry wt) was powdered using a blender, extracted twice with MeOH (2 L × 2) for 3 days, whereupon the MeOH was evaporated under reduced pressure. The crude extract was partitioned between hexane and 80% aqueous MeOH. After almost of the MeOH had been removed *in vacuo*, the aqueous layer was partitioned between water and EtOAc. The EtOAc layer (1.5 g) was separated through HP20 resin by successive elutions with 20%, 40%, 60%, 80%, and 100% MeOH, and finally with acetone. The active fraction eluted with 100% MeOH (734 mg) was separated by silica gel column chromatography followed by TLC using hexane-EtOAc (1:1) as the solvent, to give two active fractions (Fr. TC5–1 and TC5–2). Fr. TC5–2 (13.1 mg) was separated by reversed-phase HPLC (Capcell Pak C18, 10 mm × 250 mm, 90% MeOH) to give active fraction **f5** (4.4 mg).

Fraction **f5**: a colorless oil, $[\alpha]_D^{20}$–0.67° (*c* 0.1, MeOH). ESIMS *m/z* 321 $[M + Na]^+$, EIMS (bis-TMS derivative) *m/z* 442 (M^+), 427, 357, 329, 227, 199. HR-EIMS (bis-TMS derivative) calcd for $C_{24}H_{50}O_3Si_2$: 442.3298, found 442.3299. ^{1}H NMR (500 MHz) δ 0.879 and 0.881 (3H, t x 2, J = 7.0 Hz), 1.22–1.41 (14H, m), 1.42–1.45 (1H, m), 1.45–1.59 (1H, m), 1.64 (2H, m), 2.57 (2H, m), 2.35 (2H, t, J = 7.5 Hz), 3.45–3.65 (1H, br), 4.03 (1H, m), 5.443 and 5.445 (1H, dd × 2, J = 7.1, 1.0 Hz), 5.62 (1H, m). ^{13}C NMR (125 MHz) δ 14.05, 14.10, 14.10, 14.11, 22.60, 22.66, 24.58, 24.66, 25.39, 25.43, 25.49, 28.52, 28.75, 28.79, 28.96, 29.11, 29.14, 29.18, 29.26, 29.3, 29.55, 31.36, 31.82, 31.84, 31.87, 32.00, 32.18, 33.54, 37.25, 33.59, 33.59, 37.32, 73.20, 73.25, 73.26, 73.30, 131.97, 132.20, 132.28, 132.31, 132.93, 132.94, 132.97, 133.16, 177.09, 177.12, 177.16, 177.18. IR (KBr) ν_{max} 980, 1260, 1445, 1710, 2870, 2920 cm^{-1}.

2.6. p-Bromophenacyl esterification of *f5*

A mixture of **f5** (1 mg) and K_2CO_3 (spray dried, 8 mg) in dry acetone (1 mL) was stirred at rt. for 15 min. A 0.1 M acetone solution of *p*-bromophenacyl bromide (0.090 mL, 9.0 mmol) was added and the whole was stirred for 5 h. The mixture was diluted with CH_2Cl_2 (1 mL) and filtered. The filtrate was concentrated and the residue was purified by silica gel TLC (0.25 mm thickness; 10 × 20 cm; solvent, hexane-EtOAc (2:1)) to afford fraction **f5a** (Rf 0.53, 0.2 mg) and **f5b** (Rf 0.48, 0.2 mg).

Fraction **f5a**. ^{1}H NMR (500 MHz) δ 0.880 and 0.884 (3H, t × 2, J = 6.8 Hz), 1.20–1.65 (21H, m), 1.69 (1H, m), 2.00–2.05 (2H, m), 2.48 (2H, deformed-t, J = 7.5 Hz), 3.67 (1H, s), 4.03 (1H, m), 5.28 (2H, s), 5.41–5.48 (1H, m), 5.59–5.67 (1H, m), 7.64 (2H, d, J = 8.6 Hz), 7.78 (2H, d, J = 8.6 Hz).

Fraction **f5b**. ^{1}H NMR (500 MHz) δ 0.877 and 0.881 (3H, t × 2, J = 7.1 Hz), 1.20–1.65 (21H, m), 1.66–1.75 (2H, m), 1.99–2.07 (2H, m), 2.479 and 2.483 (2H, t × 2, J = 7.5 Hz), 4.03 (1H, m), 5.29 (2H, s), 5.41–5.48 (1H, m), 5.59–5.67 (1H, m), 7.64 (2H, d, J = 8.6 Hz), 7.78 (2H, d, J = 8.6 Hz).

2.7. Chemicals

(*E*)-Octadec-9-enoic acid (elaidic acid) was prepared by nitrous acid mediated isomerization of oleic acid [18]. (*R*)-(+)-Ricinoleic acid was purchased from Tokyo Kasei, Tokyo.

2.8. Synthesis

2.8.1. Octadec-10-ynoic acid (5)

To a cooled (−78 °C) solution of 10-undecynoic acid (1.00 g, 5.49 mmol) in anhydrous THF (40 mL) and HMPA (10 mL), was added dropwise *via* a syringe a 2.5 M cyclohexane solution of BuLi (5.27 mL, 13.2 mmol) over a period of 30 min. The mixture was wormed up to 0 °C and kept at this temperature for 2 h. The mixture was cooled again to −78 °C and 1-bromoheptane (0.95 mL, 6.04 mmol) was injected. The whole was stirred at rt. for 18 h before being quenched with 10% NH_4Cl and 1 M HCl solutions. The THF was removed *in vacuo*, and the residue was acidified to pH 1 with 1 M HCl and extracted twice with EtOAc. The organic layer washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified with flash chromatography on silica gel eluted with hexane-EtOAc (4:1) to give **5** (0.421 g, 1.50 mmol, 27%) as white crystals, mp 43 °C, with 50% recovery of 10-undecynoic acid. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.7 Hz), 1.25–1.40 (16H, m), 1.40–1.54 (4H, m), 1.57–1.70 (2H, m), 2.14 (4H, t, J = 7.0 Hz), 2.35 (2H, t, J = 7.62 Hz), 9.96–10.42 (1H, br.). ^{13}C NMR (100 MHz) δ 14.00, 18.65, 22.55, 24.56, 28.69, 28.74, 28.76, 28.87, 28.93, 29.02, 29.07, 29.08, 29.70, 31.70, 33.97, 80.12, 80.29, 180.28. DART-MS *m/z* (rel intensity) 282 (26), 281 (100), 215 (17), 180 (16). HR-DART-MS $[M + H]^+$ *m/z* 281.24840 (calcd for $C_{18}H_{33}O_2$: 281.24806).

2.8.2. Methyl octadec-10-ynoate (6)

To a solution of **5** (123 mg, 0.440 mmol) in a mixture of CH_2Cl_2 (6 mL) and MeOH (6 mL), was added 2 M ethereal solution of $TMSCH_2N_2$ (0.9 mL, 1.8 mmol) and the mixture was stirred at rt. until TLC revealed the disappearance of the acid. The reaction was then quenched with one drop AcOH and the solvent was removed *in vacuo* to

afford **6** (129 mg, 0.439 mmol, 100%) as a colorless oil. This was used for the next step without further purifications. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.23–1.41 (16H, m), 1.41–1.54 (4H, m), 1.58–1.70 (2H, m), 2.14 (4H, t, J = 7.0), 2.30 (2H, t, J = 7.6 Hz), 3.67 (3H, s).

2.8.3. (Z)-Methyl octadec-10-enoate (7)

The acetylenic fatty acid ester **6** (107 mg, 0.363 mmol) was hydrogenated over 5% Pd/$CaCO_3$ poisoned with Pb (67.8 mg) in EtOAc (10 mL) under H_2 (balloon pressure) for 35 min at rt. The mixture was filtered through a short column on silica gel and concentrated *in vacuo* to give olefin **7** (125 mg, 0.420 mmol, 96%) as a pale yellow oil. This was used for the next step without further purifications. ^{1}H NMR (500 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.22–1.40 (20H, m), 1.55–1.68 (2H, m), 1.96–2.07 (4H, m), 2.30 (2H, t, J = 7.6 Hz), 3.66 (3H, s), 5.32–5.37 (2H, m). ^{13}C NMR (125 MHz) δ 14.10, 22.67, 24.95, 27.18, 27.20, 29.13, 29.22 (×2), 29.27, 29.33, 29.72, 29.76, 31.86, 34.10, 51.42, 129.80. 129.94, 174.33. DART-MS *m/z* (rel intensity) 298 (20), 297 (100). HR-DART-MS $[M + H]^+$ *m/z* 297.28021 (calcd for $C_{19}H_{37}O_2$: 297.27936).

2.8.4. (E)-Methyl 9-hydroxyoctadec-10-enoate (8) and (E)-Methyl 12-hydroxyoctadec-10E-enoate (9)

A mixture of **7** (87.3 mg, 0.294 mmol), SeO_2 (28.0 mg, 0.252 mmol), and *t*-BuOOH (5 M in decane, 0.213 mL, 1.18 mmol) in dry CH_2Cl_2 (2 mL) was stirred at rt. for 50 h. The reaction was then quenched by addition of 10% $Na_2S_2O_3$ solution (5 mL) and extracted three times with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by a column chromatography on silica gel eluted with hexane-EtOAc (4:1–3:1) to give a mixture of **8** and **9** (67.6 mg, 0.216 mmol, 74%). Further elution of the column with EtOAc gave methyl (*E*)-9,12-dihydroxyoctadec-10-enoate (13.9 mg, 0.0424 mmol, 14%) as a mixture of diastereomers. The mixture of **8** and **9** was separated by MPLC on silica gel eluted with hexane-EtOAc (9:1).

Compound **8**; a colorless oil. ^{1}H NMR (500 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.15–1.38 (18H, m), 1.45–1.75 (5H, m), 1.97–2.07 (2H, m), 2.30 (2H, t, J = 7.6 Hz), 3.67 (3H, s), 4.02 (1H, q, J = 6.5H), 5.38–5.48 (1H, m), 5.63 (1H, dt, J = 15.2, 6.5 Hz). ^{13}C NMR (75 MHz) δ 14.11, 22.64, 24.89, 25.39, 29.04, 29.09, 29.13, 29.16, 29.16, 29.31, 31.82, 32.16, 34.06, 37.23, 51.44, 73.18, 132.26, 132.92, 174.30. DART-MS *m/z* (rel intensity) 311 (20), 296 (32), 295 (100), 177 (11). HR-DART-MS $[M + H\text{-}H_2O]^+$ *m/z* 295.26377 (calcd for $C_{19}H_{35}O_2$: 295.26371).

Compound **9**; a colorless oil. ^{1}H NMR (500 MHz) δ 0.88 (t, 3H, J = 7.0 Hz), 1.23–1.40 (18H, m), 1.42–1.67 (5H, m), 2.01 (2H, q, J = 6.7 Hz), 2.30 (2H, t, J = 7.0 Hz), 3.67 (3H, s), 4.03 (1H, q, J = 6.7 Hz), 5.44 (1H, ddt, J = 15.3, 7.0, 1.2 Hz), 5.62 (1H, dt, J = 15.3, 7.0 Hz). ^{13}C NMR (75 MHz) δ 14.11, 22.58, 24.89, 25.43, 29.00, 29.07, 29.11, 29.15, 29.20, 29.20, 31.80, 31.13, 34.07, 37.32, 51.43, 73.20, 132.07, 133.05, 174.32. DART-MS *m/z* (rel intensity) 312 (12), 311 (26), 295 (100), 284 (20), 282 (23), 256 (20). HR-DART-MS $[M + H\text{-}H_2O]^+$ *m/z* 295.26214 (calcd for $C_{19}H_{35}O_2$: 295.26371).

2.8.5. (E)-12-Hydroxyoctadec-10-enoic acid (4)

A solution of **9** (21.7 mg, 0.0694 mmol) in a mixture of 10% NaOH (1 mL) and MeOH (4 mL) was heated at reflux for 7.5 h. After cooling, the MeOH was removed *in vacuo,* the aqueous residue was diluted with water, acidified with 3 M HCl, extracted twice with ether, washed with brine and concentrated. The crude product was purified by silica gel TLC developed with hexane-EtOAc (1:1) to give **4** (18.4 mg, 0.0616 mmol, 89%) as white crystals, mp 47.5–49.5 °C. ^{1}H NMR (500 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.22–1.41 (18H, m), 1.42–1.57 (2H, m), 1.57–1.66 (2H, m), 2.02 (2H, q, J = 7.0 Hz), 2.34 (2H, t, J = 7.3 Hz), 4.04 (1H, q, J = 6.7 Hz), 4.50–6.50 (2H, br), 5.42–5.47 (1H, m), 5.62 (1H, dt, J = 15.2, 6.6 Hz). ^{13}C NMR (125 MHz) δ 14.07, 22.58, 24.58, 25.42, 28.83 (×2), 29.01, 29.04, 29.06, 29.20, 31.80, 32.10, 33.95, 37.26, 73.30, 132.19, 132.94, 179.22. DART-MS *m/z* (rel intensity) 298 (38), 297 (35), 282 (22), 281 (100), 187 (22). HR-DART-MS *m/z* $[M + H\text{-}H_2O]^+$ 281.24701 (calcd for $C_{18}H_{33}O_2$: 281.24806).

2.8.6. (E)-9-Hydroxyoctadec-10-enoic acid (1)

The title compound was obtained from **8** in 85% yield in a similar procedure used for the synthesis of **4**. Mp 49–50.5 °C. ^{1}H NMR (500 MHz) δ 0.88 (3H, t, J = 7.1 Hz), 1.22–1.41 (18H, m), 1.12–1.50 (1H, m), 1.51–1.57 (1H, m), 1.58–1.66 (2H, m), 2.02 (2H, q, J = 7.1 Hz), 2.34 (2H, t, J = 7.4 Hz), 4.03 (1H, q, J = 6.7 Hz), 4.67–5.60 (2H, br), 5.41–5.48 (1H, m), 5.62 (1H, dt, J = 15.4, 6.7 Hz). ^{13}C NMR (125 MHz) δ 14.08, 22.64, 24.65, 25.37, 28.96, 29.10, 29.12, 29.15, 29.17, 29.29, 31.82, 32.16, 33.95, 37.21, 73.23, 132.34, 132.85, 179.22. DART-MS *m/z* (rel intensity) 298 (31), 297 (22), 282 (42), 281 (100). DART-MS *m/z* 298, 287, 282, 281, 263. HR-DART-MS *m/z* $[M + H\text{-}H_2O]^+$ 281.24717 (calcd for $C_{18}H_{33}O_2$: 281.24806).

2.8.7. 12-Hydroxyoctadec-10-ynoic acid (10)

To a cooled (−78 °C) and stirred solution of 10-undecynoic acid (424 mg, 2.33 mmol) in dry THF (24 mL), was added dropwise a 2.5 M solution of BuLi in hexane (2.05 mL, 5.12 mmol). After 10 min at that temperature, the cooling bath was removed and the whole was stirred at rt. for 45 min. The mixture was cooled again to −78 °C and heptanal (293 mg, 2.56 mmol) dissolved in THF (2 mL) was injected. The cooling bath was removed and the mixture was stirred at rt. for 1.5 h. The reaction was then quenched with 2 M HCl solution and extracted twice with ether. The ethereal extracts were combined, washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel column chromatography eluted with hexane-EtOAc (2:1) to give **10** (332 mg, 1.12 mmol, 48%) as white crystals, mp 35–36.5 °C. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.5 Hz), 1.22–1.55 (18H, m), 1.58–1.72 (4H, m), 2.20 (2H, dt, J = 6.8, 1.8 Hz), 2.34 (2H, t, J = 7.6 Hz), 4.36 (1H, dt, J = 6.5, 1.8 Hz), 5.50–6.52 (2H, br). ^{13}C NMR (125 MHz) δ 14.02, 18.57, 22.53, 24.53, 25.11, 28.50, 28.53, 28.69, 28.77, 28.89, 28.92, 31.72, 33.97, 38.05, 62.68, 81.21, 85.36, 179.47.

2.8.8. (E)-12-Hydroxyoctadec-10-enoic acid (4) from compound 10

Clean cut Li (42 mg, 6.0 mmol) was added in small portions to liquid NH_3 (*ca.* 3 mL) at −78 °C. After 10 min, a solution of **10** (35.4 mg, 0.120 mmol) in dry THF/*t*-BuOH (3:1, 1.5 mL) was added as drops to the deep blue solution of Li metal in liquid NH_3 and the mixture was stirred at this temperature for 2 h. The reaction was quenched by addition of solid NH_4Cl (0.5 g) and the cooling bath was removed. After the NH_3 was evaporated, the residue was acidified with 3 M HCl solution, extracted twice with ether, washed with brine, dried over Na_2SO_4, and evaporated. The crude product was purified by reversed-phase HPLC (Capcell Pak C18, 10 mm × 250 mm) eluted with 85% CH_3CN to give **4** (13.7 mg, 0.0459 mmol, 38%) as white crystals, mp 49–51 °C.

2.8.9. (E)-Methyl 11-hydroxyoctadec-12-enoate (13)

A 1.0 M toluene solution of DIBAL (1.36 mL, 1.36 mmol) was injected *via* a syringe to a stirred solution of 1-heptyne (153 mg, 1.59 mmol) in dry hexane (4 mL) at rt. under Ar atmosphere. After the mixture had been stirred at 60 °C for 5 h, it was cooled to −78 °C (dry ice-acetone bath) and a solution of **11** (195 mg, 0.909 mmol) in toluene (2 mL) was added as drops. After 20 min, the cooling bath was replaced with an ice-salt bath and the mixture was stirred for 1 h. The reaction was then quenched by addition of a saturated solution of Rochelle's salt (0.2 mL), stirred overnight, dried over $MgSO_4$, and filtered through a pad of Celite, washed well with EtOAc, and concentrated. The crude product was purified by a column chromatography on silica gel eluted with hexane-EtOAc (5:1) to give **13** (79.3 mg, 0.254 mmol, 28%) as a pale yellow oil with 77.0 mg (40%) recovery of aldehyde **11**. ^{1}H NMR

(300 MHz) δ 0.89 (3H, deformed t, J = 7.0 Hz), 1.20–1.55 (19H, m), 1.55–1.68 (4H, m), 2.02 (2H, q, J = 7.0 Hz), 2.30 (2H, t, J = 7.6 Hz), 3.67 (3H, s), 4.02 (1H, q, J = 6.5 Hz), 5.44 (1H, dd, J = 15.4, 7.0 Hz), 5.63 (1H, dt, J = 15.4, 6.8 Hz). ^{13}C NMR (75 MHz) δ 14.02, 22.47, 24.91, 25.44, 28.83, 29.09, 29.18, 29.32, 29.47 (×2), 31.32, 32.11, 34.07, 37.27, 51.42, 73.18, 132.17, 132.97, 174.33. DART-MS *m/z* (rel intensity) 312 (15), 296 (22), 295 (100), 293 (38), 282 (21). HR-DART-MS *m/z* [M + H-H_2O]$^+$ 295.26347 (calcd for $C_{19}H_{35}O_2$: 295.26371).

*2.8.10. (E)-11-hydroxyoctadec-12-enoic acid (**3**)*

The title compound was obtained by alkaline hydrolysis of **13** at rt. in 78% yield in a similar procedure used for the synthesis of **4**. White crystals, mp 49 °C. ^{1}H NMR (500 MHz) δ 0.89 (3H, t, J = 7.1 Hz), 1.23–1.41 (19H, m), 1.41–1.57 (2H, m), 1.57–1.668 (2H, m), 2.03 (2H, q, J = 7.1 Hz), 2.34 (2H, t, J = 7.3 Hz), 4.04 (1H, q, J = 6.8 Hz), 5.44 (1H, dd, J = 15.4, 7.1 Hz), 5.63 (1H, dt, J = 15.4, 6.7 Hz), 9.75–9.77 (1H, br.). ^{13}C NMR (125 MHz) δ 14.03, 22.49, 24.65, 25.43, 28.85, 29.00, 29.16, 29.28, 29.46 (×2), 31.34, 32.13, 33.93, 37.26, 73.27, 132.29, 132.89, 179.23. DART-MS *m/z* (rel intensity) 298 (36), 297 (46), 282 (76), 281 (100). HR-DART-MS *m/z* [M + H-H_2O]$^+$ 281.24863 (calcd for $C_{18}H_{33}O_2$: 281.24806).

*2.8.11. 9-(Tetrahydropyran-2-yl)oxy-1-nonyne (**15**)*

A solution of **14** (1.10 g, 7.83 mmol), dihydro-2*H*-pyran (1.24 g, 14.7 mmol), and *p*-TsOH·H_2O (0.05 g) in dry CH_2Cl_2 (60 mL) was stirred at rt. for 18 h. The mixture was then washed with 5% $NaHCO_3$ solution (30 mL) and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. The oily residue was chromatographed on silica gel eluted with hexane-Et_2O (19:1) to give **15** (1.52 g, 6.76 mmol, 69%) as a colorless oil. ^{1}H NMR (300 MHz) δ 1.21–1.46 (6H, m), 1.46–1.67 (8H, m), 1.67–1.90 (2H, m), 1.94 (1H, t, J = 2.6 Hz), 2.18 (2H, dt, J = 6.9, 2.6 Hz), 3.38 (1H, dt, J = 9.5, 6.7 Hz), 3.45–3.56 (1H, m), 3.73 (1H, dt, J = 9.5, 7.0 Hz), 3.81 3.94 (1H, m), 4.58 (1H, dd, J = 4.1, 2.8 Hz). ^{13}C NMR (75 MHz) δ 18.33, 19.66, 25.44, 26.07, 28.36, 28.63, 28.89, 29.64, 30.72, 62.30, 67.55, 68.06, 84.66, 98.80. DART-MS *m/z* (rel intensity) 225 (9), 169 (12), 102 (19), 85 (100). HR-DART-MS *m/ z* [M + H]$^+$ 225.18602 (calcd for $C_{14}H_{25}O_2$: 225.18546).

*2.8.12. (E)-18-(Tetrahydropyran-2-yl)oxyoctadec-10-en-9-ol (**16**)*

To a solution of **15** (0.758 g, 3.38 mmol) in dry hexane (5 mL), a 1 M toluene solution of DIBAL (3.71 mL, 3.71 mmol) was added dropwise at rt. under Ar atmosphere, and the mixture was stirred at 60 °C for 2 h. The mixture was then cooled to −78 °C and nonanal (0.577 mg, 4.06 mmol) dissolved in toluene (4 mL) was added dropwise. After 2 h at −60 °C, the reaction mixture was warmed up to rt., quenched with water, and acidified with 1 M HCl. The whole was extracted twice with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated. The oily residue was chromatographed on silica gel eluted with hexane-EtOAc (7:1 to 2:1) gave crude **16** (0.548 g, 1.49 mmol, 44%) as a mixture of diastereomers. DART-MS *m/z* (rel intensity) 367 (5), 352 (33), 351 (100), 333 (37), 283 (37), 281 (33), 85 (94).

*2.8.13. (E)-10-(Acetoxy)octadec-8-en-1-ol (**18**)*

A solution of the crude **16** (0.548 mg, 1.49 mmol) in Ac_2O (0.5 mL) and pyridine (1 mL) was stirred at rt. for 40 h. The reaction was quenched with water, acidified with 2 M HCl, and extracted twice with ether. The ethereal extracts were combined, washed with 5% $NaHCO_3$, dried over Na_2SO_4, and concentrated. The crude product **17** was dissolved EtOH (12 mL) and a catalytic amount of *p*-TsOH·H_2O (0.05 g) was added. After the mixture had been stirred at rt. for 25 min, it was concentrated and purified by a column chromatography on silica gel eluted with hexane-EtOAc (3:1) to give **18** (160 mg, 0.491 mmol, 33%) as a pale yellow oil. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.7 Hz), 1.19–1.44 (20H, m), 1.46–1.68 (4H, m), 1.61 (1H, br. s), 1.96–2.09 (2H, m), 2.04 (3H, s), 3.64 (2H, t, J = 6.7 Hz), 5.17 (1H, q, J = 7.0 Hz), 5.36 (1H, m), 5.68 (1H, dt, J = 15.5, 6.8 Hz). ^{13}C NMR (75 MHz) δ 14.07, 21.38, 22.62, 25.15, 25.60, 28.78, 28.95, 29.14, 29.18, 29.32, 29.44, 31.81, 32.11, 32.66, 34.46, 62.93, 75.12, 128.33, 134.31, 170.50. DART-MS *m/z* (rel intensity) 326 (22), 267 (100), 429 (61), 177 (56). HR-DART-MS [M + H-AcOH]$^+$ *m/z* 267.26766 (calcd for $C_{18}H_{35}O$: 267.26879).

*2.8.14. (E)-10-(Acetoxy)octadec-8-enoic acid (**19**)*

A mixture of **18** (66.5 mg, 0.204 mmol) and PDC (268 mg, 0.713 mmol) dry DMF (2 mL) was stirred at rt. for 17 h. The mixture was poured into water, extracted twice with ether, washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by a column chromatography on silica gel eluted with hexane-EtOAc (3:1) to give **19** (31.6 mg, 0.0928 mmol, 45%) as a pale oil. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.16–1.41 (18H, m), 1.48–1.69 (4H, m), 1.95–2.11 (2H, m), 2.04 (3H, s), 2.34 (2H, t, J = 7.3 Hz), 5.17 (1H, q, J = 7.0 Hz), 5.36 (1H, m), 5.67 (1H, dt, J = 15.7, 7.0 Hz), 9.75–9.77 (1H, br.). ^{13}C NMR (75 MHz) δ 14.09, 21.42, 22.63, 24.56, 25.18, 28.65, 28.80, 29.07, 29.21, 29.33, 29.46, 31.82, 32.07, 33.99, 34.48, 75.14, 128.45, 134.16, 170.58, 179.93. DART-MS *m/z* (rel intensity) 281 [M + H-AcOH]$^+$ (42), 89 (100), 61 (38). HR-DART-MS [M + H-AcOH]$^+$ *m/z* 281.24687 (calcd for $C_{18}H_{33}O_2$: 281.24805).

*2.8.15. (E)-10-Hydroxyoctadec-8-enoic acid (**2**)*

The title compound was obtained by alkaline hydrolysis of **19** at rt. in 36% yield in a similar procedure used for the synthesis of **1**. Pale yellow crystals, mp 49 °C. ^{1}H NMR (500 MHz) δ 0.88 (3H, t, J = 6.9 Hz), 1.18–1.58 (19H, m), 1.59–1.72 (3H, m), 2.04 (2H, t, J = 7.3 Hz), 2.34 (2H, t, J = 7.3 Hz), 3.45–3.73 (2H, br.), 4.04 (1H, q, J = 6.7 Hz), 5.41–5.48 (1H, m), 5.61 (1H, dt, J = 15.4, 6.9 Hz). ^{13}C NMR (125 MHz) δ 14.09, 22.65, 24.57, 25.47, 28.26, 28.57, 28.76, 28.82, 29.25, 29.54, 31.86, 32.01, 33.88, 37.28, 73.27, 131.97, 133.12, 179.08. DART-MS *m/z* (rel intensity) 299 (31), 298 (34), 282 (38), 281 (100), 279 (58). HR-DART-MS [M + H-H_2O]$^+$ *m/z* 281.24670 (calcd for $C_{18}H_{34}O_3$: 281.24805).

*2.8.16. 1,10-Dihydroxyoctadec-8-ene (**20**)*

A solution of **16** (167.7 mg, 0.455 mmol) in MeOH (3 mL) containing a catalytic amount of PPTS was allowed to stand at rt. for 20 h. After the MeOH had been removed *in vacuo*, the residue was chromatographed on silica gel eluted with hexane-EtOAc (2:1) to give **20** (59.5 mg, 0.209 mmol, 46%) as a colorless oil. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.22–1.62 (25H, m), 1.64–1.71 (1H, m), 2.02 (1H, q, J = 7.0 Hz), 2.21 (1H, dt, J = 7.0, 1.8 Hz), 3.64 (2H, t, J = 6.7 Hz), 4.03 (1H, q, J = 6.8 Hz), 5.44 (1H, m), 5.62 (1H, dt, J = 15.2, 6.7 Hz). DART-MS *m/z* (rel intensity) 284 (18), 283 (33), 281 (25), 267 (71), 265 (100), 249 (38), 247 (31). HR-DART-MS [M + H-H_2O]$^+$ *m/z* 267.26888 (calcd for $C_{18}H_{35}O$: 267.26879).

*2.8.17. p-Bromophenacyl ester of oleic acid (**25**)*

A mixture of oleic acid (2.82 g, 10.0 mmol) and K_2CO_3 (2.28 g, 16.0 mmol) in dry acetone (25 mL) was stirred at rt. for 30 min. *p*-Bromophenacy bromide (3.06 g, 11.0 mmol) was then added and the whole was stirred overnight. The reaction mixture was filtrated and the filtrate was evaporated. The residue was then extracted with diethyl ether, washed with 5% $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated. The crystalline product was recrystallized from methanol, washed with hexane to remove unreacted oleic acid, giving **25** as a pale yellow powder. ^{1}H NMR (400 MHz) δ 0.88 (3H, t, J = 7.3 Hz), 1.17–1.45 (20H, m), 1.70 (2H, m), 1.96–2.08 (4H, m), 2.48 (2H, t, J = 7.3 Hz), 5.28 (2H, s), 5.32–5.38 (2H, m), 7.64 (2H, d, J = 8.8 Hz), 7.78 (2H, d, J = 8.8 Hz). ^{13}C NMR (100 MHz) δ 14.09, 22.67, 24.85, 27.14, 27.18, 29.03, 29.06, 29.14, 29.29 (×2), 29.49, 29.67, 29.74, 31.87, 33.84, 65.61, 129.05, 129.21 (×2), 129.73, 129.95, 132.18 (×2), 132.94, 173.15, 191.44.

*2.8.18. Selenium dioxide oxidation of oleate **25** (**26**, **27**, and **28**)*

Compounds **26**, **27**, and **28** were synthesized in a similar procedure to that of **8** and **9** in 22%, 20%, and 13% yields, respectively.

Compound **26**: ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.9 Hz),1.15–1.18 (23H, m), 1.97–2.13 (2H, m), 2.49 (2H, t, J = 7.7 Hz), 4.04 (1H, q, J = 6.6 Hz), 5.29 (2H, s), 5.45 (1H, dt, J = 15.4, 7.2 Hz), 5.58–5.69 (1H, m), 7.64 (2H, J = 8.8 Hz), 7.78 (2H, d, J = 8.8 Hz).

Compound **27**: ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.6 Hz), 1.18–1.79 (23H, m), 2.02 (2H, q, J = 6.8 Hz), 2.48 (2H, t, J = 7.7 Hz), 4.04 (1H, q, J = 6.7 Hz), 5.29 (2H, s), 5.45 (1H, J = 15.4, 7.3 Hz), 5.57–5.68 (1H, m), 7.64 (2H, dt, J = 8.8, 1.9 Hz), 7.78 (2H, dt, J = 8.8, 1.9 Hz).

Compound **28**: ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 6.6 Hz), 1.15–1.85 (24H, m), 2.49 (2H, t, J = 7.4 Hz), 4.09–4.16 (2H, m), 5.29 (2H, s), 5.66–5.74 (2H, m), 7.64 (2H, d, J = 8.8 Hz), 7.78 (2H, d, J = 8.8 Hz).

*2.8.19. (E)-8-Hydroxyoctadec-9-enoic acid (**21**)*

The title compound was obtained by an alkaline hydrolysis of **26** at 50 °C in 38% yield in a similar procedure used for the synthesis of **4**. White crystals, mp 52–54 °C (lit. mp 54–55 °C) [19]. ^{1}H NMR (500 MHz) δ 0.88 (3H, m, J = 7.0 Hz) 1.18–1.40 (18H, m), 1.43–1.68 (4H, m), 2.02 (2H, q, J = 6.9 Hz), 2.33 (2H, t, J = 7.5 Hz), 4.04 (1H, q, J = 6.7 Hz), 5.44 (1H, dd, J = 15.28, 7.21Hz, 1H), 5.63 (1 H, dt, J = 15.2, 6.6 Hz), 6.20 (2H, br. s). ^{13}C NMR (125 MHz) δ 14.07, 22.63, 24.62, 25.45, 28.81, 28.93, 28.97, 29.02, 29.24, 29.48, 31.79, 32.07, 34.05, 37.23, 73.28, 132.11, 132.93, 179.56. EIMS (bis TMS derivative) m/z (rel intensity) 442 (M^+, 6), 427 (9), 274 (13), 242 (21), 241 (100).

*2.8.20. (E)-11-Hydroxyoctadec-9-enoic acid (**22**)*

The tile compound was obtained from **27** in 34% yield in the same procedure used for the synthesis of **4**. Mp 43–46 °C (lit. mp 43–44 °C) [19]. ^{1}H NMR (500 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.18–1.42 (18H, m), 1.42–1.68 (4H, m), 2.02 (2H, q, J = 7.1 Hz), 2.32 (2H, t, J = 7.6 Hz), 4.04 (1H, q, J = 6.9 Hz), 5.43 (1H, m), 5.46 (1H, dt, J = 15.2, 6.9 Hz), 5.80–6.97 (2H, br. s). ^{13}C NMR (125 MHz) δ 14.09, 22.65, 24.64, 25.48, 28.83, 28.95, 28.99, 29.04, 29.26, 29.50, 31.81, 32.09, 34.07, 37.25, 73.30, 132.13, 132.95, 179.58. HR-EI MS [M-H_2O + H]$^+$ m/z 281.24672 (calcd for $C_{18}H_{33}O_2$: 281.24806). EIMS (bis TMS derivative) m/z (rel intensity) 442 (M^+, 5), 427 (9), 345 (14), 344 (40), 343 (100), 227 (7).

*2.8.21. 8,11-Dihydroxyoctadec-9-enoic acid (**23**)*

The tile compound was obtained from **28** in 19% yield in the same procedure used for the synthesis of **4**. A colorless oil. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 7.2 Hz), 1.20–1.43 (17H, m), 1.43–1.70 (6H, m), 2.35 (2H, t, J = 7.5 Hz), 3.38–3.57 (1H, m), 3.65–3.83 (1H, m), 4.05–4.18 (2H, m), 5.63–5.73(2H, m).

*2.8.22. (E)-11-Oxooctadec-9-enoic acid (**24**)*

To a stirred solution of **22** (57.3 mg, 0.192 mmol) in CH_2Cl_2 (1 mL), Dess-Martin periodinane (169.7 mg, 0.400 mmol) was slowly added at rt. After stirring overnight, the reaction was quenched by adding 10% $Na_2S_2O_3$ solution. The CH_2Cl_2 layer was separated and the aqueous layer was extracted with CH_2Cl_2. Organic layers were combined and dried over anhydrous Na_2SO_4. Purification by a column chromatography on silica gel eluted with hexane-EtOAc (2:1) and then reversed phase HPLC (COSMOSIL 5C18-MS-II, 90% methanol) gave **24** (36.0 mg, 0.121 mmol, 63%) as white crystals, mp 52–54 °C. ^{1}H NMR (300 MHz) δ 0.88 (3H, t, J = 7.0 Hz), 1.18–1.72 (21H, m), 2.20 (2H, q, J = 7.1 Hz), 2.36 (2H, t, J = 7.0 Hz), 2.53 (2H, t, J = 7.3 Hz), 6.09 (1H, d, J = 15.8 Hz), 6.82 (1H, dt, J = 15.8, 7.0 Hz). ^{13}C NMR (75 MHz) δ 14.04, 22.57, 24.31, 24.57, 27.98, 28.86, 28.91, 28.94, 29.06, 29.24, 31.65, 32.36, 33.97, 40.06, 130.30, 147.27, 172.16, 201.21. HR-DART-MS [M + H]$^+$ m/z 297.24295 (calcd for $C_{18}H_{33}O_3$: 297.24297).

3. Results and discussion

3.1. Structure elucidation of the algicidal compounds of T. jejuensis

Separation of the methanol extract of *T. jejuensis* by monitoring the algicidal activity against *C. antiqua* afforded an inseparable mixture of compounds with 100% mortality to the phytoplankton at 20 μg/mL. The mixture fraction, named **f5**, showed a single molecular ion peak at m/z 321 [M + Na]$^+$ by ESI-MS, indicating that the active compounds were isomeric to each other. A molecular formula of $C_{18}H_{34}O_3$ was established by HR-EI-MS of the bistrimethylsilyl derivative of the mixture. ^{13}C NMR spectrum showed signals for carboxylic carbons at δ 177.09, 177.12, 177.16, and 177.18, olefinic carbons at δ 131.97, 132.20, 132.28, 132.31, 132.93, 132.94, 132.97, and 133.16, hydroxymethine carbons at δ 73.20, 73.25, 73.26, and 73.30, many methylene carbons, and overlapping methyl carbons in the sp^3 carbon region. The ^{1}H NMR spectrum showed overlapping signals of multiplets at δ 5.59–5.66 (1H), two doublet of doublets at δ 5.443 (J = 15.4, 7.1 Hz) and δ 5.445 (J = 15.3, 7.2 Hz), and quartets at δ 4.036 (J = 6.7 Hz) and 4.031 (J = 6.7 Hz), indicating the presence of substructure –CH = CH-CH(OH)- having *E*-configuration. At this stage, the active compounds were assumed to be four isomeric hydroxylated C18 *trans*-monounsaturated fatty acids. The positions of the double bonds and hydroxyl groups were determined by the EI-MS fragmentation pattern. The EI-MS of the bistrimethylsilyl derivatives of the mixture showed four distinct fragment ion peaks at m/z 227, 329, 199 and 357, which corresponded to the fragment ions of $CH_3(CH_2)_6CH$ = CHCHOTMS, TMSOCHCH = $CH(CH_2)_6$COOTMS, $CH_3(CH_2)_4CH$ = CHCHOTMS, and TMSOCHCH = $CH(CH_2)_8$COOTMS, respectively, from cleavage at the allylic positions adjacent to the hydroxyl groups [20,21]. From these spectroscopic data, the active compounds were assigned to be (*E*)-9-hydroxyoctadec-10-enoic acid (**1**), (*E*)-10-hydroxyoctadec-8-enoic acid (**2**), (*E*)-11-hydroxyoctadec-12-enoic acid (**3**), and (*E*)-12-hydroxyoctadec-10-enoic acid (**4**) (Fig. 1).

3.2. Synthesis of the hydroxy monounsaturated fatty acids **1**– **4**

To confirm the structure elucidation as well as to obtain pure samples for evaluation of the algicidal activity of each acid and its related compounds, we synthesized each of the acids **1**–**4** by unambiguous routes.

(*E*)-9-Hydroxyoctadec-10-enoic acid (**1**) and (*E*)-12-hydroxyoctadec-10-enoic acid (**4**) are regioisomeric in the hydroxyl group position; thus both could be obtained from the same intermediate, (*Z*)-octadec-10-enoate (**7**), using a selenium dioxide allylic oxidation (Scheme 1). Alkylation of the lithium acetylide of 10-undecynoic acid with 1-bromoheptane followed by methyl esterification gave C18 acetylenic acid ester **6** in 27% yield. Partial hydrogenation of the triple bond of **6** with Lindlar's catalyst followed by selenium dioxide oxidation [19] of the resulting (*Z*)-olefin **7** afforded an equimolar mixture of alcohols **8** and **9** in 74% combined yield along with a trace of the 9,12-dihydroxylated compound (14% yield). After separation of the regioisomeric monoalcohols by silica gel chromatography, each methyl ester was hydrolyzed to obtain (*E*)-9-hydroxyoctadec-10-enoic acid (**1**) and (*E*)-12-hydroxyoctadec-10-enoic acid (**4**). Compound **4** was also synthesized by a Birch reduction of alkynol **10**, which was obtained by acetylenic addition of 10-undecynoic acid to heptanal.

Syntheses of 11- and 10-hydroxyoctadecenoic acids (**3** and **2**) were achieved *via* addition reactions of alkenyl aluminum reagents (Schemes 2 and 3). Aldehyde **11**, prepared by a Kornblum oxidation of methyl 11-bromoundecanoate using a reported procedure [22], was reacted with alkenyl aluminum **12** prepared *in situ* from heptyne and DIBAL to give (*E*)-11-hydroxy-12-octadecanoate (**13**) in 28% yield. One of the target compounds (*E*)-11-hydroxyoctadec-12-enoic acid (**3**) was obtained by

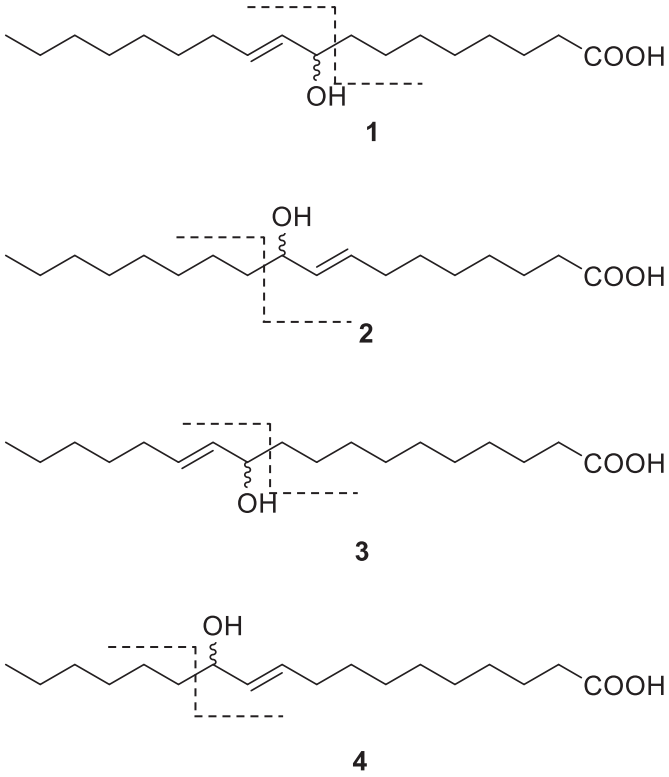


Fig. 1. Structures of compound **1**– **4.**

alkaline hydrolysis of **13** (Scheme 2). In this strategy, synthesis of another hydroxyoctadecenoic acid, **2**, required alkyne **15** as the source of alkenyl aluminum, which was prepared through an acetylene zipper reaction of commercially available 2-nonyn-1-ol according to a reported procedure [23]. After THP protection of the hydroxyl group of **14**, alkyne **15** was reacted with DIBAL to generate alkenyl aluminum, which was then trapped with nonanal to afford the *trans*-allylic alcohol **16** in 44% yield. The secondary hydroxyl group was protected as the acetate, and then the primary hydroxyl group was oxidized to furnish (*E*)-10-hydroxyoctadec-8-enoic acid (**2**) after hydrolytic removal of the acetyl group (Scheme 3).

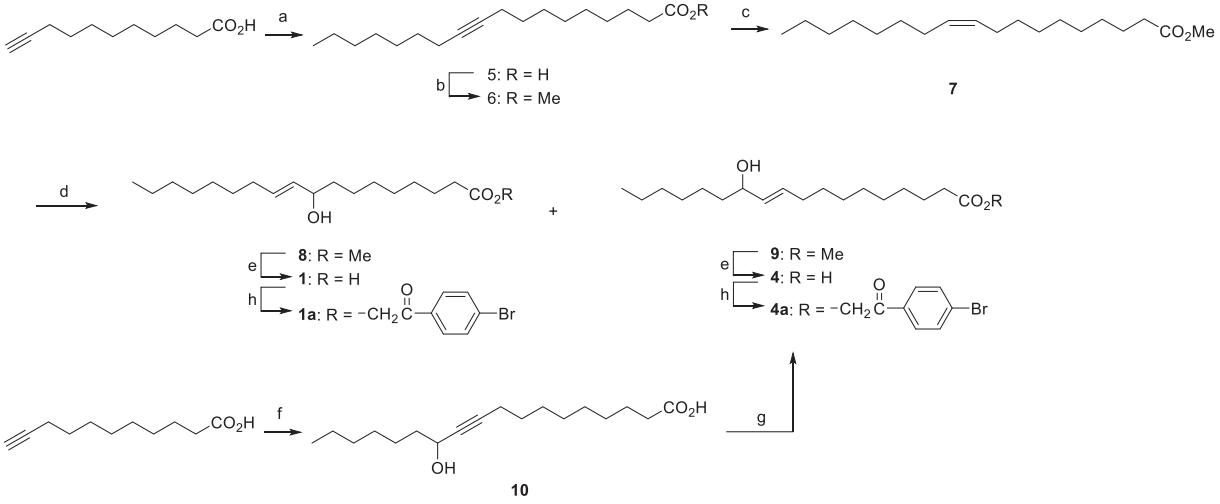
3.3. Verification of the proposed structures of 1– 4 and stereochemistry

Chemical shift values of selected carbons of the natural products **f5** and synthesized compounds **1**–**4** are listed with chemical shift difference values (Δ) in Table 1. The chemical shift values of all the carbons of **f5** exactly matched those of the corresponding carbons of synthesized compounds **1**–**4** within a 0.36-ppm difference with the exception of the carboxyl carbons. The slight change in the chemical shift values of the carboxyl carbons between the natural products and synthesized compounds might be due to a considerable difference in the concentration of the sample solutions prepared for NMR measurements. Indeed, 10-fold dilution of the NMR sample solution of **3** from 30 mg/mL to 3 mg/mL resulted in a 1.59-ppm upfield shift in the carboxyl carbon signal.

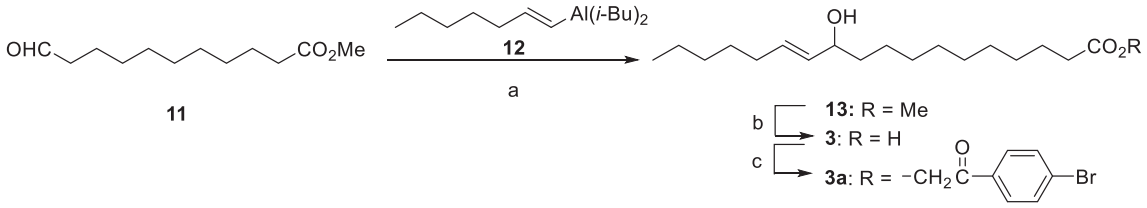
Recorded specific rotation value of **f5** was close to zero (-0.67^{o}). Esterification of **f5** with *p*-bromophenacyl bromide (K_2CO_3, acetone, rt) gave two separable fractions on silica gel TLC (hexane:EtOAc = 2:1), named as **f5a** (Rf 0.50) and **f5b** (Rf 0.43), the former being a mixture of the *p*-bromophenacyl esters of **3** (**3a**, Rf 0.49) and **4** (**4a**,Rf 0.49), and the latter being the esters of **1** (**1a**, Rf 0.46) and **2** (**2a**, Rf 0.43). HPLC analysis of **f5b** using a chiral column, Chiralpak AD-H (solvent, 2-propanol:hexane = 15:85; flow rate, 0.5 mL/min) showed two pairs of peaks of almost equal intensities corresponding to the respective enantiomers of **1a** (t_R 35.7 and 37.7 min) and **2a** (t_R 40.7 and 45.0 min), indicating **1** and **2** were isolated as racemates (Supplementary data, Fig. 23). On the other hand, **f5a** showed two peaks at t_R 41.6 min and 45.0 min in an area ratio of 1:3. In the same HPLC conditions, synthesized (±)-**3a** was separated into two peaks at t_R 41.6 min and 44.8 min, suggesting the isolated **3** was a racemate (Supplementary data, Fig. 24). However, (±)-**4a** was unable to separate by this chiral column and appeared as a single peak at nearly 45.0 min (46.1 min). Finally, separation of (±)-**4a** was achieved by using Chiralpak IA (solvent, MeOH, flow rate 0.5 mL/min) and analysis of **f5a** revealed that the isolated compound **4** was a racemate (Supplementary data, Fig. 25).

3.4. Algicidal activity of hydroxylated trans-monounsaturated fatty acids 1– 4 and their derivatives

Each of the synthesized hydroxy acids **1**–**4** as well as their synthetic intermediates **10** and **20** were evaluated for algicidal activity against *C. antiqua* (Fig. 2). For comparison, autoxidation products of oleic acid, (*E*)-8-hydroxyoctadec-9-enoic acid (**21**) and (*E*)-11-hydroxyoctadec-9-



Scheme 1. Synthesis of (*E*)-hydroxyoctadec-10-enoic acid (**1**) and (*E*)-12-hydroxyoctadec-10-enoic acid (**4**).
Reagents: (a) BuLi, HMPA-THF, 0 °C, 2 h, then 1-bromoheptane, r.t., 18 h (27%); (b) $TMSCHN_2$, DCM-MeOH, rt. (100%); (c) H_2, 5% Pd/Ca-Pb, EtOAc, rt. (96%); (d) SeO_2, TBHP, DCM, rt., 50 h (74%); (e) NaOH, MeOH, reflux, **1** (89%), **2** (85%); (f) BuLi, THF, rt., 20 min, then heptanal, −78 °C to rt., 1.5 h (48%); (g) Li, NH_3, *t*-BuOH, THF, −78 °C, 2 h (38%); (h) 4-bromophenacyl bromide, K_2CO_3, acetone, rt., **1a** (66%), **4a** (43%).



Scheme 2. Synthesis of (*E*)-11-hydroxyoctadec-12-enoic acid (**3**).
Reagents; (a) hexane-toluene, −10 °C, 1 h (28%); (b) NaOH, MeOH, rt., **3** (72%); (c) 4-bromophenacyl bromide, K_2CO_3, acetone (52%).

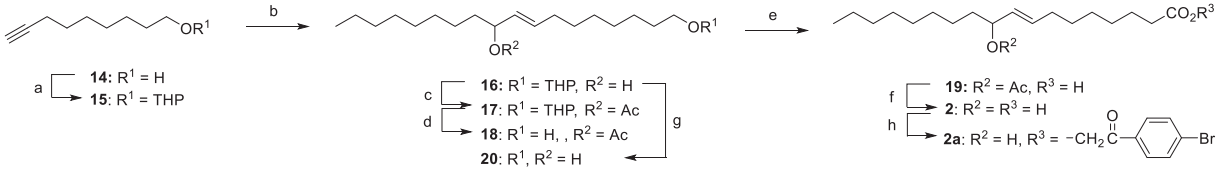
enoic acid (**22**) [24], their oxidized derivatives, diol **23** and ketone **24** (Scheme 4), (*Z*)-12-hydroxyoctadec-9-enoic acid (ricinoleic adcid), and (*E*)-octadec-9-enoic acid (elaidic acid) were tested for algicidal activity. All the compounds isolated from *T. jejuensis* except for compound **1** showed complete toxicity to the phytoplankton at a concentration of 20 μg/mL. Among the compounds tested, compound **2** had the highest activity. The autoxidation products of oleic acid **(21** and **22**) and 8,11-dihydroxy derivative **23** also showed high activity. Oxidation of the hydroxyl group of **22** as ketone **24** maintained the activity, whereas elaidic acid, which lacks the 11-OH of **22**, had no activity at concentrations less than 80 μg/mL. Ricinoleic acid having *cis*-double bond with a hydroxyl group at the homoallylic position displayed the same level of the activity as the *trans*-allylic alcohols. Taken together, presence of oxygen functional group(s) such as hydroxyl and carbonyl group is necessary for the activity, but the positions of the hydroxyl group and the geometry of the double bond are less important. Reduction of the carboxyl group to alcohol **20** caused somewhat decrease in activity compared with carboxylic acid **2**, but still maintained a moderate level of activity, indicating that the carboxyl group may be replaced with other polar functional groups. Compound **10** having triple bond had the same level of activity as **4**. Fig. 3. shows the cell of *C. antiqua* treated with 5 μg/mL of compound **10** (A) and compound **2** (B) after 0.5- and 4-h incubations. Interestingly, this propargylic alcohol **10** caused acute lysis of planktonic cells within 30 min (Fig. 3, A), at which period no other allylic alcohols affected the planktonic cells (Fig. 3, B). (See Fig. 3.)

(*E*)-9-Hydroxyoctadec-10-enoic acid (**1**) and (*E*)-10-hydroxyoctadec-8-enoic acid (**2**) have previously been isolated as the biotransformation products of oleic acid by *Pseudomonas* sp. [25,26,27,28,29]. The oxidation of unsaturated fatty acids proceeds *via* three different pathways; autoxidation, photo-oxidation and enzymatic oxidation such as that of lipoxygenases. Autoxidation of oleic acid involves allylic oxidation and allylic rearrangement of the resulting hydroperoxide, and is characterized by the formation of both cis and trans isomers of 8-hydroxyoctadec-9-enoic acid (8-OH$\Delta_{9,10}$) and 11-hydroxyoctadec-9-enoic acid (11-OH$\Delta_{9,10}$), and the trans isomers of 9-OH$\Delta_{10,11}$ (**1**) and 10-OH$\Delta_{8,9}$ (**2**) [24]. Photo-oxidation of oleic acid involves concerted ene reactions with a singlet oxygen, in which the oxidation proceeds at one end of the double bond to predominantly produce *trans*-9-OH$\Delta_{10,11}$ (**1**) and *trans*-10-OH$\Delta_{8,9}$ (**2**) [30]. (*E*)-11-hydroxyoctadec-12-enoic acid (**3**) and (*E*)-12-Hydroxyoctadec-10-enoic acid (**4**) may arise from *cis*-vaccenic acid by the same mechanism as that for **1** and **2**. Since oleic acid is widely distributed in nature, hydroxy acids **1** and **2** have been isolated from several plants and microorganisms; in some cases, both compounds were co-isolated from the same natural source. Compounds **1** and **2** isolated from stroma of the timothy plant *Epichloe typhina* showed antifungal activity against plant-pathogenic *Cladosporium herbarum* [31], and those isolated from the medicinal plant *Alternanthera brasiliana* and its endophytic bacteria had antimicrobial activity against some human pathogenic bacteria [32]. These hydroxy acids have also been found in macroalgae. Compound **2** isolated from the red alga *Gracilaria verrucosa* is reported to have moderate anti-inflammatory activity [33] and compound **1** isolated from the green alga *Caulerpa racemosa* exhibited potent protein tyrosine phosphatase 1B (PTP1B) inhibitory activity [34]. In contrast, (*E*)-11-hydroxyoctadec-12-enoic acid (**3**) and (*E*)-12-hydroxyoctadec-10-enoic acid (**4**) derived from *cis*-vaccenic acid have rarely been found in nature. Compound **3** was isolated from the green alga *Ulva fasciata* Delile and shown to have moderate and weak antibacterial activity against *Streptomyces aureus* and *Escherichia coli*, respectively [35]. Compounds **1**–**4** have been detected in particulate matter and sediment samples collected in the northwestern Mediterranean Sea in GC/EIMS [36]. Nevertheless, to our knowledge, this is the first isolation of (*E*)-12-hydroxyoctadec-10-enoic acid (**4**) from living organisms. It has also been reported that the hydroxy lipids are the photo-oxidation products of oleic and *cis*-vaccenic acids generated in senescent phytoplanktonic cells [36]. Thereafter, Rontani et al. [37] investigated the origin of the *cis*-vaccenic acid photo-oxidation products in marine environment and concluded that heterotrophic bacteria that are attached to senescent phytoplanktonic cells most likely constitute the source of *cis*-vaccenic acid oxidation products **3** and **4** detected in the particulate matter samples.

Although the exact ratio of the four compounds was not determined, a GC/EI-MS spectrum of the mixture fraction **f5** displayed two peaks at t_R 18.14 min and t_R 18.21 min in a ratio of 59:41, the former being attributed to a mixture of compounds **3** and **4** and the latter to a mixture of compounds **1** and **2** (Supplementary data). It is interesting that the hydroxy fatty acids derived from *cis*-vaccenic acid are dominant over those from oleic acid in this alga.

4. Conclusions

We isolated a highly algicidal fraction **f5** comprising four C18 hydroxy unsaturated fatty acids, (*E*)-9-hydroxyoctadec-10-enoic acid (**1**), (*E*)-10-hydroxyoctadec-8-enoic acid (**2**), (*E*)-11-hydroxyoctadec-12-enoic acid (**3**) and (*E*)-12-hydroxyoctadec-10-enoic acid (**4**), from a



Scheme 3. (*E*)-10-hydroxyoctadec-8-enoic acid (**2**) and (*E*)-1,10-dihydroxyoctadec8-ene (**20**).
Reagents; (a) DHP, *p*-TsOH, DCM, rt., 18 h (69%); (b) DIBAL, hexane-toluene, 60 °C, 2 h then nonanal, −60 °C, 2 h (44%); (c) Ac_2O, pyridine, rt. 40 h; (d) *p*-TsOH, EtOH, rt., 25 min (33%, 2 steps); (e) PDC, DMF, rt. 17 h (45%); (f) NaOH, MeOH, rt., (36%); (g) PPTS, MeOH, rt., 20 h (46%); (h) 4-bromophenacyl bromide, K_2CO_3, acetone (59%).

Table 1
Chemical shift values (δ_C, ppm) of the selected carbons of fraction **f5** and compounds **1– 4** (125 MHz in $CDCl_3$).

^{13}C atom	Fraction/compound					Chemical shift difference
	f5	**1**	**2**	**3**	**4**	
$\underline{C}H_3$	14.05			14.03		0.02
	14.10				14.07	0.03
	14.10	14.08				0.02
	14.11		14.09			0.02
$\underline{C}H_2$ – CH = CH -CH(OH)	31.36			31.34		0.02
	31.82				31.80	0.02
	31.84	31.82				0.02
	31.87		31.86			0.01
CH_2 – $\underline{C}H$ = CH-CH(OH)	131.97		131.97			0.00
	132.20				132.19	0.01
	132.28			132.29		0.01
	132.31	132.34				0.03
CH_2-CH = $\underline{C}H$-CH(OH)	132.93	132.85				0.05
	132.94			132.89		0.05
	132.97				132.94	0.03
	133.16		133.12			0.04
CH_2-CH = CH-$\underline{C}H$(OH)	73.20	73.23				0.03
	73.25			73.27		0.02
	73.26		73.27			0.01
	73.30				73.30	0.00
$\underline{C}H_2$ – COOH	33.54		33.88			0.34
	33.57			33.93		0.36
	33.59				33.95	0.36
	33.59	33.95				0.36
CH_2-$\underline{C}OOH$	177.09		179.08			1.99
	177.12				179.22	2.11
	177.16	179.22				2.06
	177.18			179.23		2.05

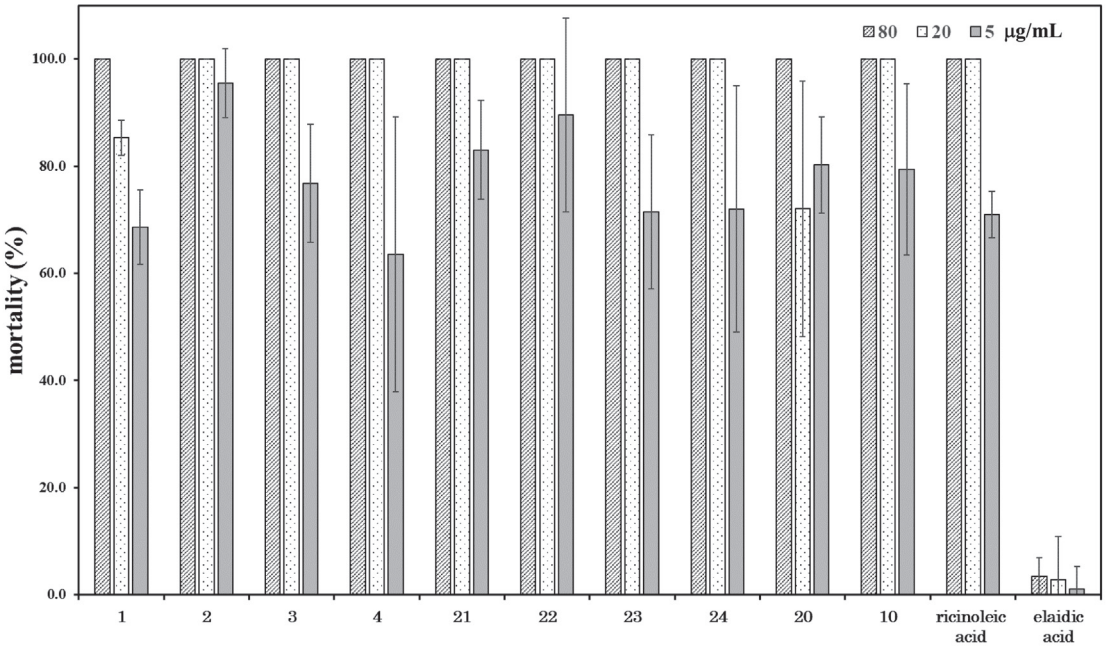
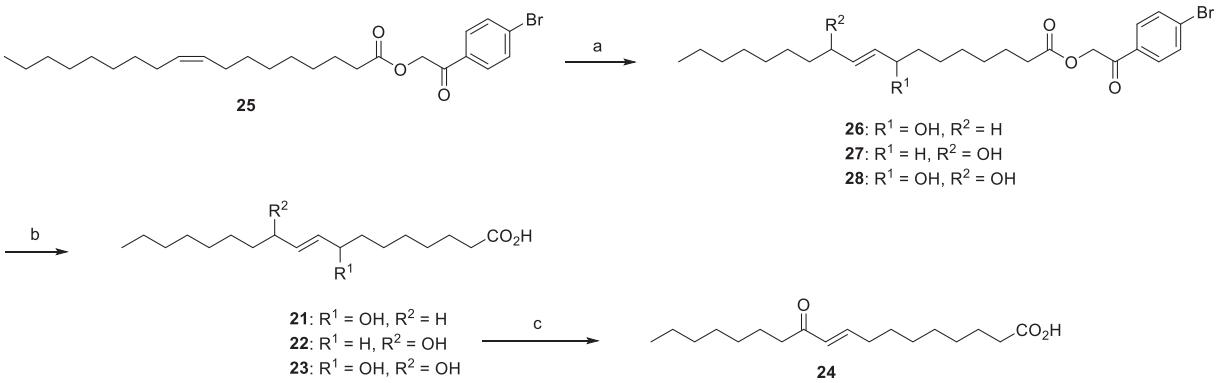


Fig. 2. Algicidal activity [mortality (%)] of compound **1– 4** and its related compounds at concentrations of 80, 20 and 5 μg/mL for 24 h against *C. antiqua*. Values are the mean ± SD from three independent experiments.

methanol extract of *T. jejuensis*. Their structures were confirmed by comparison of their spectral data with those of synthesized compounds. Among them, compound **2** was found to have the highest algicidal activity, showing > 95% mortality against *C. antiqua* at a concentration of 5 μg/mL after 24 h. We also found that propargylic derivative **10** had high acute toxicity to the phytoplankton. Further detailed biological activity study to evaluate the effectiveness of these hydroxy lipids as anti-red tide agents and to obtain an insight on the mode of action are in progress.

Declaration of Competing Interest

The authors declare no conflict of interest.



Scheme 4. Syntheses of (*E*)-8-hydroxyoctadec-9-enoic acid (**21**), (*E*)-11-hydroxyoctadec-9-enoic acid (**22**), (*E*)-8,11-dihydroxyocatdec-9-enoic acid (**23**), and (*E*)-11-oxooctadec-9-enoic acid (**24**).
Reagents: (a) SeO_2, t-BuOOH, CH_2Cl_2, rt., 72 h; (b) NaOH, MeOH; (c) Dess-Martin periodinane, CH_2Cl_2, rt., 18 h (63%).

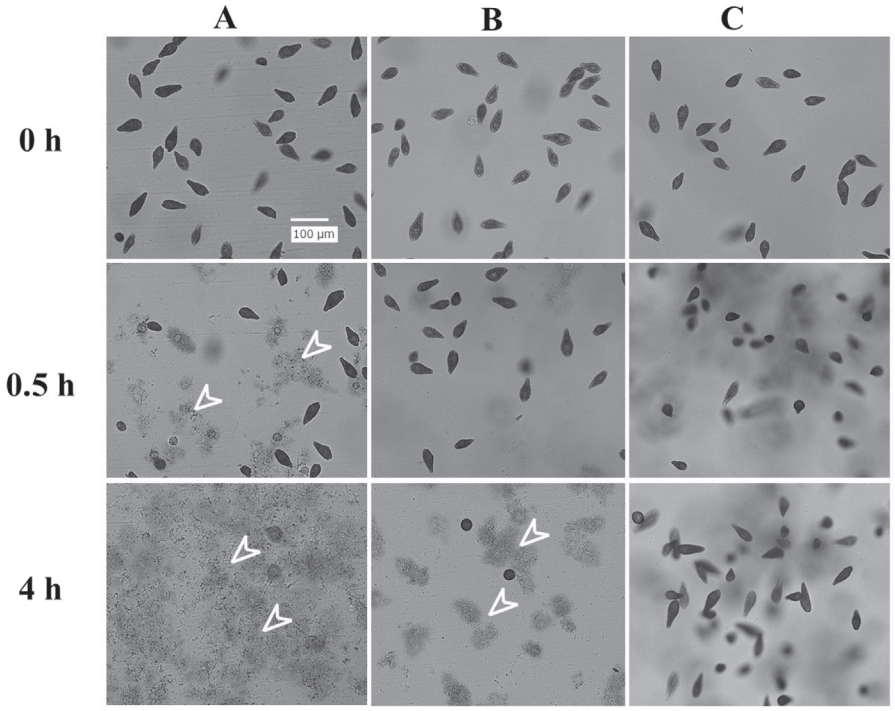


Fig. 3. The cell of *Chattonella antiqua* treated with compound **10** (A) and compound **2** (B) at a concentration of 5 µg/mL each, and untreated cells (C) just after treatment (0 h) and after 0.5- and 4-h incubations. Arrowheads indicate debris of dead cells of *C. antiqua* cells. Bar indicates 100 µm.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2020.104639.

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