Cytotoxicity and Antibacterial Potential of Halogenated Chamigrenes from Malaysian Red Alga, Laurencia majuscula

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Key words
Laurencia majuscula, Rhodomelaceae, red alga, halogenated, chamigrane sesquiterpene

ABSTRACT
Red algae of the genus Laurencia have been known to produce a wide array of bioactive secondary metabolites. Here, we report the isolation of two new halogenated chamigrenes, lau remantanones A (1) and B (2), along with seven known compounds, dendroidiol (3), (+)-elatol (4), cartilagineol (5), obtusol (6), (+)-laurencenone B (7), 2-chloro-3-hydroxy-α-chamigren-9-one (8), and puertitol A (9), from a population of Laurencia majuscula (Harvey) Lucas from Mantanani Island (North Borneo). The structures of the two new metabolites were determined based on spectroscopic data (IR, 1D and 2D NMR, and MS). Compounds isolated from this alga exhibited potent cytotoxic (HeLa, MCF-7, P-388) and antibacterial (against antibiotic-resistant clinical bacteria) activities. The major metabolite of this population has significant importance in the geographical distribution of this species globally.

Introduction
Red algae of the genus Laurencia are known to produce an array of diverse halogenated secondary metabolites represented as terpenoid, alkaloid and C15-acetogenin chemical skeletons [1–4]. Presence and quantity of the major halogenated metabolites within Laurencia populations often vary with their species and geographical distribution [5–9]. Halogenated compounds have also been shown to exhibit various biological activities with pharmaceutical importance, such as antibacterial [7], cytotoxic [10], and insect repellent activities [11]. As part of our ongoing effort to document the diversity of halogenated secondary metabolites, we collected a population of Laurencia majuscula from the coastal waters of North Borneo (Mantanani Island), which led to the isolation of two new sesquiterpenoids, lauremantanones A (1) and B (2), along with...
seven known compounds, dendroidiol (3) [12], (+)-elatol (4) [13], cartilagineol (5) [14–16], obtusol (6) [15–17], (+)-laurencenone B (7) [18, 19], 2-chloro-3-hydroxy-α-chamigren-9-one (8) [13, 20], and puertitol A (9) [21] (Fig. 1). Herein, we describe the isolation, structural elucidation, cytotoxicity, and antibacterial activities of these compounds.

Results and Discussion

Compound 1 was isolated as colorless oil, [α]D25 +28.3 (c 1.0, CHCl3). The molecular formula was established as C15H21ClO2 by the HRESIMS [M + H]+ ion at m/z 269.1305 (calcld. for C15H22ClO2, 269.1303) and it accounted for 5 degrees of unsaturation. Its IR absorption was seen at 3420 and 1650 cm−1, indicating the presence of hydroxyl (-OH) and α,β unsaturated carbonyl (C=O) functionalities. The 13C NMR revealed the presence of 15 signals whose multiplicities were attributed by DEPT-135 and HSQC spectra to three methyls, five methylenes, including an oxygenated methylene, a trisubstituted, and a tetrasubstituted olefins, two quaternary carbons, and a carbonyl carbon. These signals accounted for three degrees of unsaturation, implying 1 possesses a bicyclic system. The relative configuration at C-6 was assigned S*, identical to that of (+)-7, based on a chemical shift (δC 46.4) and the optical rotation [α]D25 +39.4 (c 0.2, CHCl3) as well as a biogenetic pathway given the fact that both compounds were isolated from the same specimen. (Fig. 25–75)

Compound 2 was isolated as colorless oil, [α]D28 –35.0 (c 0.5, CHCl3). The molecular formula was determined as C15H22ClO3 through the HRESIMS [M + H]+ ion at m/z 287.1412 (calcld. for C15H24ClO3, 287.1409). Both hydroxyl and carbonyl functionalities were detected by IR absorption at 3444 and 1651 cm−1, respectively. Upon careful comparison, NMR data of 2 (Table 1) were almost identical to those of 8 except for the replacement of an olefinic methyl at C-14 in 8 by a vinyl carbinol unit in 2 [13]. Detailed assignment of 1H-1H COSY and HMBC correlations (Fig. 15, Supporting Information) revealed an α-chamigrane framework for 2. (Fig. 8S–135)

The relative stereochemistry of 2 was determined by NOESY experiments. The NOESY correlations between H-2/H2–4 α, H-2/H2–15, and H2–4 α/H2–15 demonstrated that these protons were located on the same orientation, while both 2-Cl and 3-OH were located on the opposite orientation of the ring B. The relative configuration at C-6 was identical to that of (-)-8 based the NOE correlation between H2–1/H2–12, chemical shifts, and the optical rotation [α]D25 +46.0 (c 0.22) [13]. The methylene H2–14 (δH 4.46 and 4.42) of 2 experienced a downfield shift of 0.2 ppm and was presented as an individual signal instead of superimposed as compared to that of 1 (δH 4.24, H2–14). This could be due to the additional hydroxyl moiety in 2 at C-3, which results in a restricted rotation of the sigma bond between C-7 and C-14. Finally, judging from the co-occurrence of 2 and 8 in the same alga, the relative
stereochemistry of the B ring in 2 is the same as that of 8. Therefore, 25S, 3R, and 65S were assigned similarly to those of (-)-8.

The natural product designated as laurencenone B [22] was incor-
rectly assigned due to discrepancies that existed between published 1H NMR data of the natural product and that of a synthetic compound [19]. Since then, no report of complete NMR and specific rotation measurements for the natural product laurencenone B (7) was found, therefore, herein, we reported its specific rotation and 1H and 13C NMR data. The specific rotation and 1H and 13C NMR data of 7 were consistent to semisynthetic and synthetic materials [13, 19]. (Fig. 14S–15S)

The α-chamigrane-type sesquiterpenoids 1 and 2 showed strong cytotoxic activities against HeLa and P-388 cell lines with an IC50 ≤ 5.0 μg/mL (= Table 2), whereas β-chamigrane-type sesquiterpenoids 3–6 displayed a much stronger cytotoxicity against cell lines HeLa, MCF-7, and P-388 with an IC50 ≤ 1.0 μg/mL. On the other hand, compounds 7–9 were found inactive against all cell lines. Compounds 1 and 2 exhibited weak activity against Bacillus cereus with MIC and MBC values of 250 and 1000–1250 μg/mL, respectively (Table 3). In a previous literature, compound 3 was reported as having no activity against NO and TNF-α production in LPS-induced RAW 264.7 macrophages, and no antibacterial property on Mycobacterium bovis [12]. Compounds 4 and 6 were reported to show antileishmanial activity against Leishmania amazonensis on promastigotes (IC50 of 9.7 and 6.2 μg/mL, respectively) and amastigotes (IC50 of 4.5 and 3.9 μg/mL, respectively), but were not active against NO production by macrophages [15]. In addition, compounds 4 and 6 were also reported to exhibit cytotoxic activities against gastric carcinoma (IC50 of < 1.0 and 7.0 μg/mL, respectively), liver carcinoma (IC50 of < 1.0 μg/mL), and breast carcinoma (IC50 of < 1.0 and 1.5 μg/mL, respectively), while 5 showed an IC50 of 1.0, 0.25, and 1.0 μg/mL on non-small lung cancer, human colon carcinoma, and melanoma cells, respectively. Compounds 4–6, however, showed negligible antibacterial activity [24].

Materials and Methods

General experimental procedures

The 1H NMR (600 MHz) and 13C NMR (150 MHz) spectra were recorded on an NMR spectrometer (Jeol). The HRESIMS was acquired via LCMS-ESI-IT-TOF (Shimadzu). An AUTOPOL IV automatic polarimeter was used to measure the optical rotation (Rudolph Research Analytical). Infrared spectra were recorded on a FTIR (Thermo Nicolet). For preparative TLC (Kieselgel 60 F254, Merck), the spots were visualized by UV light (254 and 365 nm) and spraying with a 5% phosphomolybdic acid-ethanol solution. Column chromatography was performed with silica gel (Kieselgel 60, 70–230 mesh; Merck). All organic solvents for extraction and isolation were of analytical grade (Fisher Scientific). The HPLC solvent was HPLC grade (Fisher Scientific). The deuterated CDCl3 was purchased from Merck.

Plant material

Specimens of L. majuscula (Harvey) Lucas were collected from Mantanani Island, Sabah (06º59.728’N, 116º34.830’E) in November 2017. A voucher specimen (BORH939093) was deposited in the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, University of Malaysia, Sabah. Field identifications were done by Prof. Dr. Charles S. Vairappan, who is the corresponding author of this paper. Voucher specimens were examined by Assoc. Prof. Dr. Abe Tsuyoshi, Hokkaido University Museum, Sapporo, Japan.

Table 1 13C and 1H NMR (150 and 600 MHz) data of 1 and 2 (CDCl3, δ in ppm, J in Hz).

<table>
<thead>
<tr>
<th>No.</th>
<th>δC</th>
<th>δH</th>
<th>δC</th>
<th>δH</th>
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<tr>
<td>1</td>
<td>36.2</td>
<td>2.55 d (17.9)</td>
<td>35.4</td>
<td>2.24 d (6.2)</td>
</tr>
<tr>
<td>2</td>
<td>126.4</td>
<td>68.4</td>
<td>4.28 dd (11.0, 6.2)</td>
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<tr>
<td>3</td>
<td>130.4</td>
<td>70.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>30.8</td>
<td>2.17 dd (17.2, 5.5)</td>
<td>36.9</td>
<td>1.93–1.95 m</td>
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<tr>
<td>5</td>
<td>31.2</td>
<td>1.92 td (12.4, 5.5)</td>
<td>23.8</td>
<td>1.97–1.99 m</td>
</tr>
<tr>
<td>6</td>
<td>46.2</td>
<td>47.5</td>
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</tr>
<tr>
<td>7</td>
<td>171.1</td>
<td>168.7</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>124.1</td>
<td>6.32 s</td>
<td>124.5</td>
<td>6.18 s</td>
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<tr>
<td>9</td>
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<td>199.1</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>49.8</td>
<td>2.64 d (17.2)</td>
<td>50.6</td>
<td>2.41 d (19.3)</td>
</tr>
<tr>
<td>11</td>
<td>41.3</td>
<td>41.6</td>
<td></td>
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<tr>
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<td>25.2</td>
<td>1.06 s</td>
<td>28.1</td>
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<tr>
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<td>0.96 s</td>
<td>27.9</td>
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<tr>
<td>14</td>
<td>64.0</td>
<td>4.24 s</td>
<td>63.9</td>
<td>4.46 d (16.5)</td>
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<td>15</td>
<td>20.4</td>
<td>1.79 s</td>
<td>29.3</td>
<td>1.36 s</td>
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* Assignments may be reversed

Table 2 Cytotoxicity of compounds 1–9 against cancer cell lines HeLa, MCF-7, and P-388.

<table>
<thead>
<tr>
<th>Cells</th>
<th>IC50 (μg/mL)</th>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>HeLa</td>
<td>2.50</td>
</tr>
<tr>
<td>MCF-7</td>
<td>2.50</td>
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<tr>
<td>P-388</td>
<td>5.00</td>
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Antibacterial assay

The antibacterial assay was carried out using 96-well plates with a known microdilution method against the strains *Bacillus cereus* (QEB2018–01), *Escherichia coli* (QEB2018–02), *Salmonella typhi* (QEB2018–03), and *Vibrio cholera* (QEB2018–04) obtained from Queen Elizabeth General Hospital, Kota Kinabalu, Sabah, Malaysia. The tested concentrations were 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 2.0 μg/mL. The assay was carried out using a previous procedure [6]. The positive control kanamycin (contained ≥ 98 % kanamycin A) was purchased from Merck.

Supporting Information

NMR spectra of the new compounds are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

References