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

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Authors
Takashi Kamada[1, 2], Chin-Soon Phan[1], Tatsufumi Okino[3], Charles Santhanaraju Vairappan[1]

Affiliations
1 Laboratory of Natural Products Chemistry, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88450 Kota Kinabalu, Sabah, Malaysia
2 Laboratory of Natural Products Chemistry, Department of Materials and Life Science, Faculty of Science and Technology, Shizuoka Institute of Science and Technology, Fukuroi, Shizuoka 437-8555, Japan
3 Graduate School of Environmental Science, Faculty of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan

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Correspondence
Prof. Dr. Charles Santhanaraju Vairappan
Laboratory of Natural Products Chemistry
Institute for Tropical Biology and Conservation
Universiti Malaysia Sabah
Jalan UMS
88400 Kota Kinabalu
Sabah
Malaysia
Tel.: +60/88/320 000 ext. 2384, Fax: +60 88 320 291
csv@ums.edu.my

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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, lauremantanones 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 C_{15}-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.

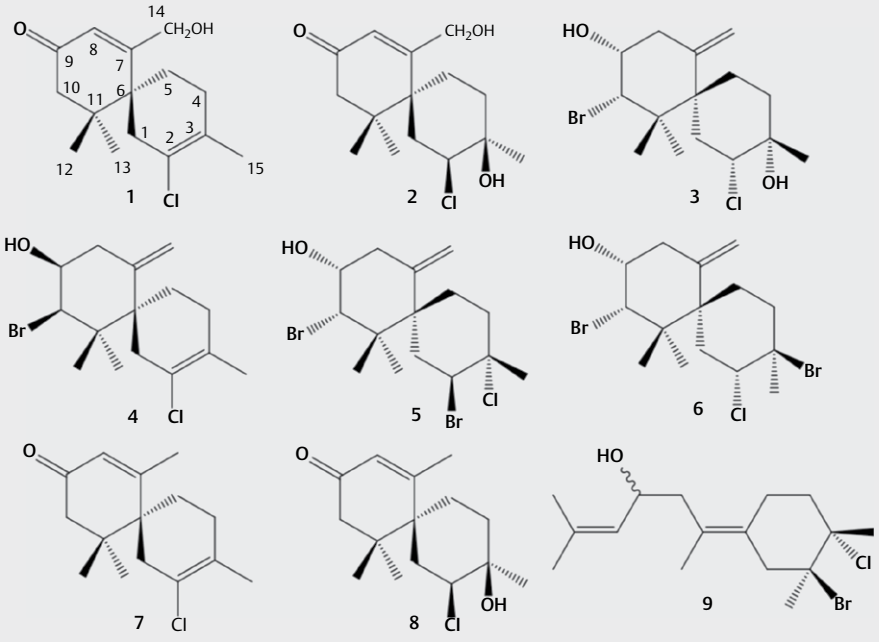
Results and Discussion

Compound **1** was isolated as colorless oil, $[\alpha]_D^{28}$ +22.3 (c 1.0, $CHCl_3$). The molecular formula was established as $C_{15}H_{21}ClO_2$ by the HRESIMS $[M + H]^+$ ion at *m/z* 269.1305 (calcd. for $C_{15}H_{22}ClO_2$, 269.1303) and it accounted for 5 degrees of unsaturation. Its IR absorption was seen at 3 420 and 1 650 cm^{-1}, indicating the presence of hydroxyl (-OH) and α,β unsaturated carbonyl (C = O) functionalities. The ^{13}C 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 NMR data (▶ **Table 1**) of **1** closely resembled that of **7** except for the replacement of a vinyl methyl at C-14 in **7** by a vinyl carbinol moiety in **1** [18, 19, 22]. The presence of a carbinol unit was further confirmed by its deshielded chemical shifts at C-14 (δ_C 64.0; δ_H 4.24) [23], IR absorption at 3 420 cm^{-1}, and HRESIMS spectrum.

The ^1H-^1H COSY (▶ **Fig. 1S**, Supporting Information) correlation between H_2–4 and H_2–5, together with the HMBC (▶ **Fig. 1S**, Supporting Information) correlations from H_3–12, 13 to C-6, 10, 11, from H_3–15 to C-2, 3, 4, from H_2–14 to C-6, 7 , 8, from H_2–1 to C-2, 5, 6, from H_2–10 to C-9, and from H-8 to C-10 were enough to establish the planar structure of an α-chamigrane-type sesquiterpenoid for **1**. The relative configuration at C-6 was assigned *S* *, identical to that of (+)-**7**, based on a chemical shift (δ_C 46.4) and the optical rotation $[\alpha]_D^{25}$ +39.4 (c 0.2, $CHCl_3$) as well as a biogenetic pathway given the fact that both compounds were isolated from the same specimen. (▶ **Fig. 2S–7S**)

Compound **2** was isolated as colorless oil, $[\alpha]_D^{28}$–35.0 (c 0.5, $CHCl_3$). The molecular formula was determined as $C_{15}H_{23}ClO_3$ through the HRESIMS $[M + H]^+$ ion at *m/z* 287.1412 (calcd. for $C_{15}H_{24}ClO_3$, 287.1409). Both hydroxyl and carbonyl functionalities were detected by IR absorption at 3 444 and 1 651 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. 1S**, Supporting Information) revealed an α-chamigrane framework for **2**. (▶ **Fig. 8S–13S**)

The relative stereochemistry of **2** was determined by NOESY experiments. The NOESY correlations between H-2/H_2–4 α, H-2/H_3–15, and H_2–4 α/H_3–15 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 H_2–1/H_3–12, chemical shifts, and the optical rotation $[\alpha]_D^{25}$–46.0 (c 0.22) [13]. The methylene H_2–14 (δ_H 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** (δ_H 4.24, H_2–14). 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



▶ **Fig. 1** Structures of sesquiterpenoids **1**–**9** from *L. majuscula*.

stereochemistry of the B ring in **2** is the same as that of **8**. Therefore, 2*S* *, 3*R* *, and 6*S* * were assigned similarly to those of (-)-**8**. The natural product designated as laurencenone B [22] was incorrectly assigned due to discrepancies that existed between published ^{1}H 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 ^{1}H and ^{13}C NMR data. The specific rotation and ^{1}H and ^{13}C 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 $IC_{50} \leq 5.0$ 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 $IC_{50} \leq 1.0$ 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 1 000–1 250 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 (IC_{50} of 9.7 and 6.2 µg/mL, respectively) and amastigotes (IC_{50} 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 (IC_{50} of < 1.0 and 7.0 µg/mL, respectively), liver carcinoma (IC_{50} of < 1.0 µg/mL), and breast carcinoma (IC_{50} of < 1.0 and 1.5 µg/mL, respectively), while **5** showed an IC_{50} 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].

▶ **Table 1** ^{13}C and ^{1}H NMR (150 and 600 MHz) data of **1** and **2** ($CDCl_3$, δ in ppm, *J* in Hz).

	1		2	
No.	δ_C	δ_H	δ_C	δ_H
1	36.2	2.55 d (17.9)	35.4	2.24 d (6.2)
		2.26 d (17.9)		2.23 d (11.0)
2	126.4		68.4	4.28 dd (11.0, 6.2)
3	130.4		70.6	
4	30.8	2.17 dd (17.2, 5.5)	36.9	1.93–1.95 m
		2.00–203 m		1.78–1.81 m
5	31.2	1.92 td (12.4, 5.5)	23.8	1.97–1.99 m
		1.76 ddt (12.4, 4.8, 2.1)		1.78–1.81 m
6	46.2		47.5	
7	171.1		168.7	
8	124.1	6.32 s	124.5	6.18 s
9	199.3		199.1	
10	49.8	2.64 d (17.2)	50.6	2.41 d (19.3)
		2.10 d (17.2)		2.36 d (19.3)
11	41.3		41.6	
12*	25.2	1.06 s	28.1	1.16 s
13*	24.6	0.96 s	27.9	1.13 s
14	64.0	4.24 s	63.9	4.46 d (16.5)
				4.42 d (16.5)
15	20.4	1.79 s	29.3	1.36 s

* Assignments may be reversed

Materials and Methods

General experimental procedures

The ^{1}H NMR (600 MHz) and ^{13}C 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 an FTIR (Thermo Nicolet). For preparative TLC (Kieselgel 60 F_{254}, 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 $CDCl_3$ was purchased from Merck.

Plant material

Specimens of *L. majuscula* (Harvey) Lucas were collected from Mantanani Island, Sabah (06°59.728'N, 116°34.830'E) in November 2017. A voucher specimen (BORH39093) 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 2** Cytotoxicity of compounds **1**–**9** against cancer cell lines HeLa, MCF-7, and P-388.

Cells	**IC_{50} (µg/mL)**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
HeLa	2.50	2.50	0.75	0.75	1.00	1.00	-	-	-
MCF-7	2.50	5.00	1.00	0.75	0.75	1.00	-	-	-
P-388	5.00	2.50	0.75	1.00	1.00	1.00	-	-	-

▶ **Table 3** Antibacterial activities of compounds **1** and **2** against clinical strains.

Strains	MIC (MBC) in µg/mL	
	1	**2**
Bacillus cereus	250 (1000)	250 (1250)
Escherichia coli	-	-
Salmonella typhi	> 500	> 500
Vibrio cholera	> 500	> 500
Positive control = kanamycin, with an MIC and MBC of 2.0 µg/mL		

Extraction and isolation

After air drying for 3 days, the alga (110 g) was extracted with methanol (MeOH). The resulting MeOH extract was concentrated in vacuo and partitioned between ethyl acetate (EtOAc) and H_2O. The EtOAc fraction was washed with water, dried over anhydrous Na_2SO_4, and evaporated to leave a dark green oil (3.0 g). The EtOAc extract (1.5 g) was then fractionated by Si gel column chromatography with a step gradient (hexane and EtOAc). The fraction eluted with hexane-EtOAc (8:2) was subjected to preparative TLC with toluene to give compounds **4** (160 mg: 10.7 %), **5** (99 mg: 6.6 %), **6** (90 mg: 6.0 %), **7** (101 mg: 6.7 %), and **9** (41 mg: 2.7 %). The fraction eluted with hexane-EtOAc (7:3) was subjected to preparative TLC with chloroform ($CHCl_3$) to give **3** (41 mg: 2.7 %). The fraction eluted with hexane-EtOAc (1:1) was subjected to preparative TLC with $CHCl_3$ to give **8** (82 mg: 5.5 %). The fraction eluted with EtOAc (100 %) was subjected to preparative TLC with $CHCl_3$-MeOH (95:5) to give **1** (16 mg: 1.1 %) and **2** (15 mg: 1.0 %). Compounds that were isolated using the preparative TLC technique were further purified using reverse phase HPLC with an ODS-3 column under acetonitrile, MeCN-H_2O (50–100 % MeCN), at 210 nm UV detection. Yields were calculated as a percentage of the EtOAc crude extract.

Lauremantanone A (**1**): colorless oil; $[\alpha]^{28}_D$ +22.3 (c 1.0, $CHCl_3$); IR (neat) ν_{max} 3420, 2930, 1650, 1455, 1318, 1078, and 979 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) spectral data: see ▶ **Table 1**; HRESIMS m/z 269.1305 $[M + H]^+$ (calcd. for $C_{15}H_{22}ClO_2$, 269.1303).

Lauremantanone B (**2**): colorless oil; $[\alpha]^{28}_D$ -35.0 (c 0.5, $CHCl_3$); IR (neat) ν_{max} 3444, 2925, 1651, 1455, and 1118 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$); spectral data: see ▶ **Table 1**; HRESIMS m/z 287.1412 $[M + H]^+$ (calcd. for $C_{15}H_{24}ClO_3$, 287.1409).

(+)-Laurencenone B (**7**): white powder; $[\alpha]^{25}_D$ +39.4 (c 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ: 5.89 (1H, s), 2.54–2.62 (2H, br m), 2.27 (1H, d, *J* = 17.2 Hz), 2.16–2.20 (1H, br m), 2.02–2.09 (2H, br m), 1.97 (3H, s), 1.93 (1H, td, *J* = 12.7, 5.6 Hz), 1.81 (3H, s), 1.75–1.77 (1H, br m), 1.06 (3H, s), 0.97 (3H, s); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 198.1, 168.5, 129.7, 127.5, 126.3, 48.9, 46.4, 40.5, 36.3, 30.4, 30.2, 24.8, 23.8 (overlapped two signals), 19.7.

Cytotoxic assay

The *in vitro* cytotoxicity assay was conducted against the cell lines human cervical epithelioid carcinoma (HeLa), human breast carcinoma (MCF-7), and murine lymphocytic leukemia (P-388). The tested concentrations were 100, 10, 1, 0.1, 0.01, and 0.001 µg/mL. The assay was carried out using a previous procedure [25]. Positive control experiments were conducted using H_2O_2 as the test chemical.

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.

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