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Cytotoxic Sesquiterpenoids From Soft Soral *Capnella imbricata*

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Abstract

A new sesquiterpene, capgermacrene H (**1**) was isolated together with 7 other compounds, capgermacrenes A-G (**2-8**), from a population of soft coral *Capnella imbricata* collected from Mantanani Island, Sabah, Malaysia. The structure of this metabolite was elucidated based on spectroscopic data such as nuclear magnetic resonance and high-resolution electrospray ionization mass spectrometry. Capgermacrene A (**2**) showed potent cytotoxicity against adult T-cell leukemia, S1T cells at half-maximal inhibitory concentration of 0.79 µg/mL.

Keywords

Capnella, soft coral, sesquiterpene, capgermacrene H, cytotoxicity, adult T-cell leukemia

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Soft corals of the genus *Capnella* is known to be a rich source of sesquiterpenes.[1] Isolated terpenes often exhibited profound biological activities such as antitumor, antiviral, antifouling, and anti-inflammation.[2] In the past, we discovered and reported bicyclogermacrene-related compounds from the Bornean soft coral genus *Capnella*, which showed anti-inflammatory and antibacterial potentials.[3-5] As part of our search for cytotoxic substances, a new germacrane, capgermacrene H (**1**), together with another 7 known bicyclogermacrenes, capgermacrenes A-G (**2-8**),[3-5] was isolated from this population of *Capnella imbricata* (Figure 1). Here, we report the isolation, structure elucidation and cytotoxic activity of these compounds against adult T-cell leukemia, S1T cells.

Compound **1** was isolated as colorless oil. Its molecular formula was determined as $C_{15}H_{22}O_2$ based on high-resolution electrospray ionization mass spectrometry (HRESIMS) ions at *m/z* 257.1509 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1512). The IR spectrum suggested the presence of a hydroxy and conjugated carbonyl groups (3400 and 1683 cm^{-1}). The ^{13}C NMR data (Table 1) showed 15 distinct signals implying a sesquiterpene, while the presence of a carbonyl group at δ_C 207.3, one trisubstituted olefin at δ_C 138.1 and 136.7; δ_H 5.40, two disubstituted double bonds at δ_C 145.9, 139.5, 124.1, and 110.3; δ_H 5.33, 5.31, 4.79, and 4.78, a hydroxy-bearing carbon at δ_C 73.9, and hydroxy methyl group at δ_C 29.1; δ_H 1.27 within the molecule was indicated by both ^1H and ^{13}C NMR spectra (Table 1). The presence of two oxygen atoms was consistent with HRESIMS measurement.

Two spin systems were determined based on the correlations of H-1/H_2-2/H_2-3 and H-5/H-6/H-7/H_2-8 from ^1H-^1H correlation spectroscopy (COSY) spectrum (bold lines in Figure 2a). These substructures and key heteronuclear multiple bond correlation (HMBC) experiment (arrows in Figure 2a) of H_3-12 to C-7, C-11, and C-13; H_3-14 to **C**-1, C-9, and C-10; H_3-15 to C-3, C-4, and C-5; and H_2-8 to C-9 permitted the establishment of a 10-membered ring germacrane-type sesquiterpenoid with a hydroxy group at C-4, an isopropenyl moiety at C-7, and a ketone group at C-9. The β relative configuration was arbitrary assigned at hydroxy methyl carbon in **1** (δ_C 29.1, C-15) because of large difference in ^{13}C NMR to those of structurally related germacrene, lochmolin G with α relative configuration at hydroxy methyl

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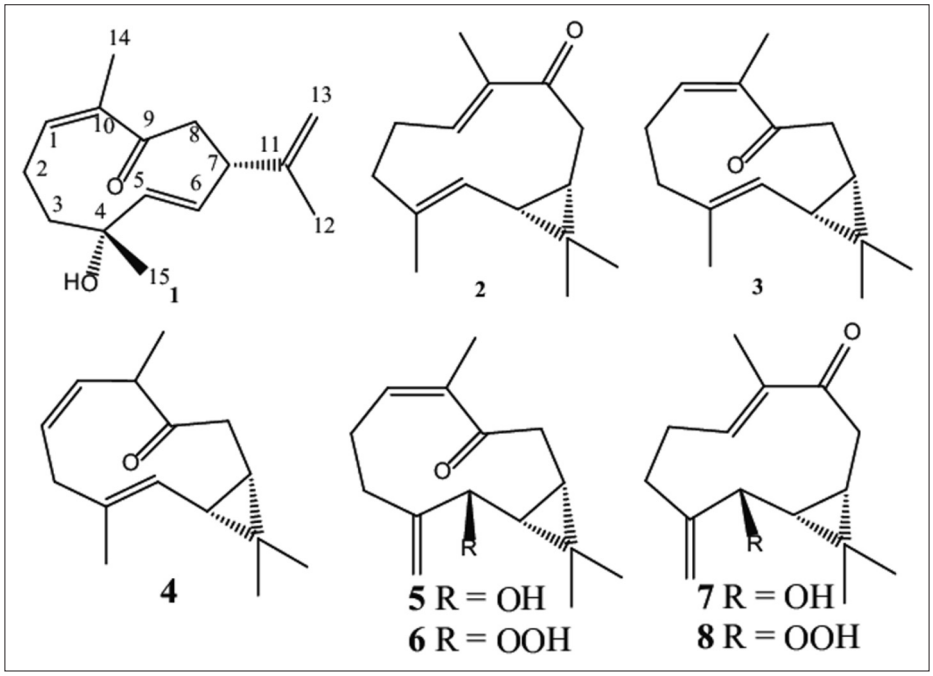

Figure 1. Structure of compounds **1** to **8**.

carbon (δ_C 23.4, C-15).[6] Based on this finding, the β relative configuration at H_3-15 has permitted the assignment of α relative configuration for an isopropenyl unit at C-7 due to nuclear Overhauser effect (NOE) correlations (double arrows in Figure 2b) between H_3-15/H-5 and H-5/H-7. Furthermore, both the coupling constant and splitting pattern of H-6 in **1** having dd (15.1, 10.3 Hz) being identical to those of lochmolin G with dd (16.0, 10.0 Hz) suggested that H-7 in **1** must be in axial-like position as identical to those of lochmolin G [6]. However, ^{1}H NMR signals of H-5 (δ_H 5.31) and H-6 (δ_H 5.33) have a poor resolution because their signals were nearly superimposed in $CDCl_3$. Therefore, different shift-inducing pulsing power (C_6D_6) was used to resolve these signals (δ_H 5.05, H-5; δ_H 5.55, H-6), thus further confirmed above NOE correlations. The geometry of the double bond at C-1/C-10 was determined to be *Z* from the ^{13}C NMR of the olefinic methyl carbon at C-14 (δ_C 20.0).[7,8] This was further confirmed by NOE correlation between H-1/H_3-14, while the *E* configuration was assigned for a pair of double bond at C-5/C-6 based on large coupling constants between H-5 and H-6 (J = 15.1 Hz).[9] Upon examination of the molecular modeling, comparison with molecular modeling of lochmolin G and the above findings, the relative configuration of **1** was established.

Cytotoxic effects of compounds **1** to **8** against adult T-cell leukemia, S1T cells were investigated by WST-8 assay except **4** due to insufficient yield. Compound **1** was inactive (half-maximal inhibitory concentration [IC_{50}] > 30.0 μg/mL) against S1T cells. The most potent compound was capgermacrene A (**2**) with IC_{50} of 0.79 μg/mL, while the positive control etoposide had IC_{50} of 0.048 μg/mL. Capgermacrenes B (**3**), D (**5**), E (**6**), F (**7**) and G (**8**) exhibited IC_{50} of 7.79, 6.19, 4.75, 2.39, and 3.97 μg/mL, respectively.

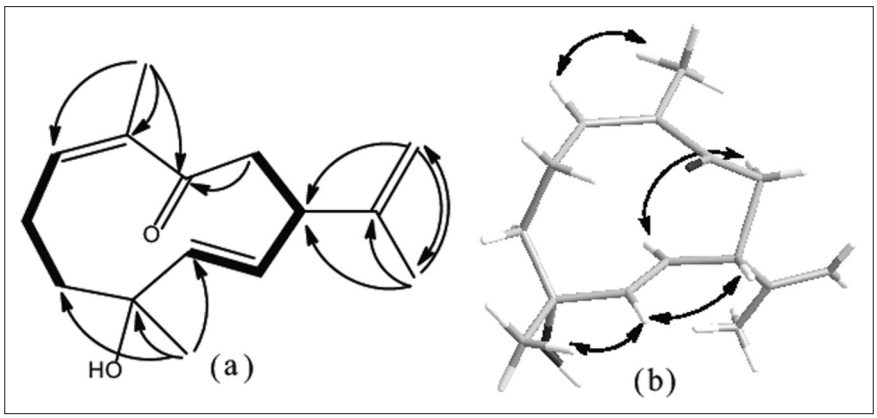

Figure 2. (a) ^{1}H-^{1}H COSY and key HMBC and (b) selected NOE correlations of **1**.

Experimental

General

^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a JEOL ECA 600 FT-NMR using $CDCl_3$ with TMS as an internal standard. The high-resolution mass spectrum was acquired via Liquid Chromatography-Mass Spectrometry Ion Trap Time Of Flight (Shimadzu). The AUTOPOL IV automatic polarimeter (Rudolph Research Analytic) and Fourier transform infrared spectrophotometer (Thermo Nicolet) were used.

Biological Material

A specimen of *Capnella imbricata* was collected from Mantanani Island, Sabah, Malaysia (6°43.059″N, 116°20.189″E), in June 2013. The voucher specimen (BORMI0006) was deposited in the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Extraction and Isolation

Soft coral (0.9 kg wet weight) was extracted in MeOH at room temperature. The MeOH extract was concentrated and partitioned between EtOAc/H_2O. The EtOAc fraction was further partitioned with hexane/90% MeOH. The hexane crude (600.0 mg) was subjected to column chromatography eluted in hexane-EtOAc (increasing polarity) to obtain 6 fractions. A total of 175.0 mg of fraction 4 (hexane-EtOAc (1:1)) was purified by HPLC to yield compound **1** (2.0 mg). HPLC conditions: C18 column, 40°C of oven temperature, 1 mL/min for flow rate, 220 nm for UV detector wavelength, isocratic mode with H_2O-MeCN (35:65 (v/v)).

Compound *1*

Colorless oil.
$[\alpha]_D^{25}$: -70.0 (*c* 0.02, $CHCl_3$).
IR (KBr) λmax: 3400 and 1683 cm^{-1}.
^{1}H and ^{13}C NMR: Table 1.
HRESIMS *m/z* 257.1509 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1512) and 217.1591 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{21}O$, 217.1587).

Cytotoxic Assay

The adult T-cell leukemia cell line S1T was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamate. The cytotoxic assay was carried out as known procedure.[10]

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Table 1. ^{1}H and ^{13}C NMR Data (600 and 150 MHz) of **1** (δ in ppm).

No	$^{a}\delta_C$	$^{a}\delta_H$ (multiple, *J* in Hz)	$^{b}\delta_C$	$^{b}\delta_H$ (multiple, *J* in Hz)
1	136.7	5.40 dd (11.0, 5.1)	136.4	5.08 dd (10.3, 4.8)
2	23.7	2.28 dt (14.6, 11.0)	24.1	2.61 m
		1.98 m		1.94 m
3	41.9	1.75 m	42.1	1.49 dd (14.4, 8.9)
		1.51 dd (14.6, 11.0)		1.16 dd (14.4, 11.0)
4	73.9		73.4	
5	139.5	5.31 d (15.1)	139.7	5.05 d (15.1)
6	124.1	5.33 dd (15.1, 10.3)	124.5	5.55 dd (15.1, 10.3)
7	47.4	3.02 m	47.8	2.94 m
8	44.5	2.84 dd (12.4, 5.5)	44.7	2.69 dd (12.4, 5.5)
		2.38 t (12.4)		2.51 t (12.4)
9	207.3		205.0	
10	138.1		138.2	
11	145.9		146.3	
12	20.9	1.76 s	20.9	1.69 s
13	110.3	4.79 s	110.2	4.89 s
		4.78 s		4.85 s
14	20.0	1.95 s	19.9	1.65 s
15	29.1	1.27 s	29.3	1.12 s

[a]Spectra recorded in $CDCl_3$.
[b]Spectra recorded in C_6D_6.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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