Mar. Drugs **2015**, *13*, 3103-3115; doi:10.3390/md13053103

OPEN ACCESS

marine drugs

ISSN 1660-3397

www.mdpi.com/journal/marinedrugs

Article

Capgermacrenes A and B, Bioactive Secondary Metabolites from a Bornean Soft Coral, *Capnella* sp.

Chin-Soon Phan [1], Shean-Yeaw Ng [1], Eun-A Kim [2], You-Jin Jeon [2,*], Kishneth Palaniveloo [1] and Charles Santhanaraju Vairappan [1,*]

[1] Laboratory of Natural Products Chemistry, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia;
E-Mails: yuna123@hotmail.my (C.-S.P.); sheanyeaw90@hotmail.com (S.-Y.N.); kishneth@yahoo.com (K.P.)

[2] Department of Marine Life Sciences, Jeju National University, Jeju 690-756, Korea;
E-Mail: yellow6798@hanmail.net

* Authors to whom correspondence should be addressed; E-Mails: youjinj@jejunu.ac.kr (Y.-J.J.); csv@ums.edu.my (C.S.V.); Tel.: +82-64-754-3475 (Y.-J.J.); +60-88-320-000 (ext. 2397) (C.S.V.); Fax: +82-64-756-3493 (Y.-J.J.); +60-88-320-291 (C.S.V.).

Academic Editor: Valeria Costantino

Received: 23 February 2015 / Accepted: 5 May 2015 / Published: 19 May 2015

Abstract: Two new bicyclogermacrenes, capgermacrenes A (**1**) and B (**2**), were isolated with two known compounds, palustrol (**3**) and litseagermacrane (**4**), from a population of Bornean soft coral *Capnella* sp. The structures of these metabolites were elucidated based on spectroscopic data. Compound **1** was found to inhibit the accumulation of the LPS-induced pro-inflammatory IL-1β and NO production by down-regulating the expression of iNOS protein in RAW 264.7 macrophages.

Keywords: soft coral; *Capnella* sp.; *Nephtheidae*; sesquiterpenoids; bicyclogermacrene

1. Introduction

Soft corals belonging to the genus *Capnella* (*Alcyonacea*, *Nephtheidae*) are known to produce sesquiterpenoids, such as capnellenes and capnellanes [1]. Soft coral derived secondary metabolites are reported to exhibit promising biological activities such as anti-tumor, antiviral, antifouling and anti-inflammation [2]. Our previous chemical investigation on the soft coral genus *Nephthea* led to

isolation and structural elucidation of a new cembrane diterpene [3], a new sterol [4] and a new norsesquiterpenoid [5], along with several known compounds [5]. However, there were no sesquiterpenes isolated from Bornean soft coral genus *Capnella*. Therefore, we investigated a population of *Capnella* sp. from Mantanani Island (Sabah, Malaysia) and it has led to the isolation of bicyclogermacrenes as first report in Bornean soft corals. The methanol extract gave two new bicyclogermacrenes, capgermacrenes A (**1**) and B (**2**), in addition to two known compounds, palustrol (**3**) [6] and litseagermacrane (**4**) [7,8] (Figure 1). Anti-inflammatory activity of capgermacrene A (**1**) in LPS-induced RAW 264.7 macrophages exhibited down-regulation of inflammatory mediator such as nitric oxide (NO), prostaglandin-E2 (PGE_2), tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). This paper reports the isolation, structure elucidation and anti-inflammatory activity of these four compounds.

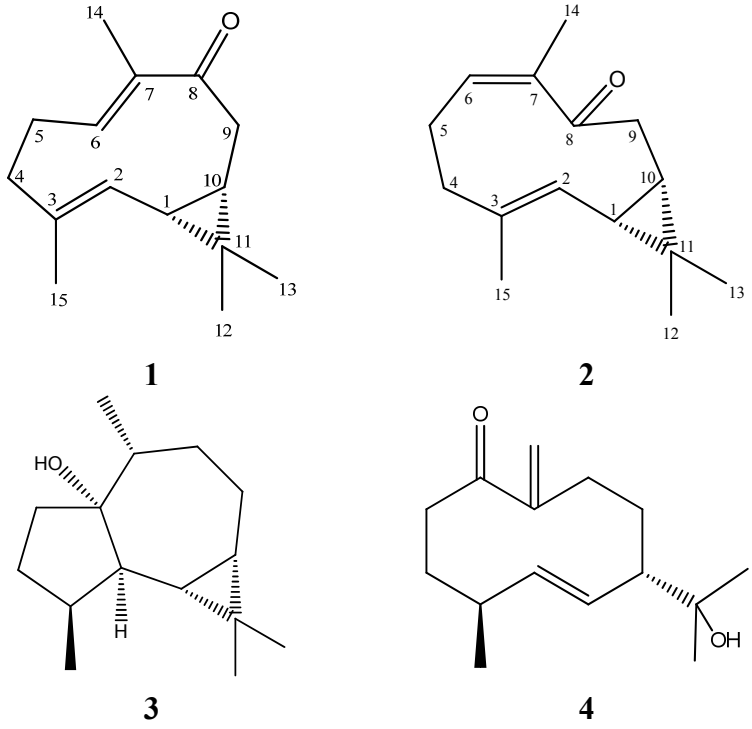


Figure 1. Structures of metabolites **1**–**4**.

2. Results and Discussion

2.1. Structure Determination

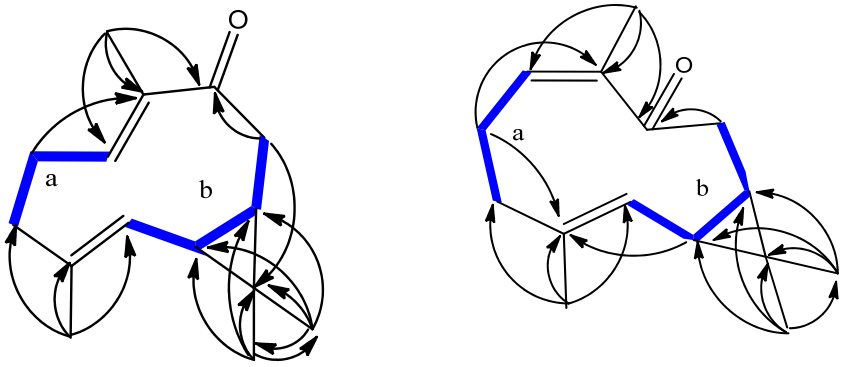
Compound **1** was isolated as colorless oil. High-resolution mass spectrometry data gave a molecular formula of $C_{15}H_{22}O$ (*m*/*z* 219.1720 $[M + H]^+$). The IR spectrum suggested the presence of α,β-unsaturated carbonyl group (1662 cm^{-1}) in the molecule. The ^{13}C-NMR spectral data of **1** (Table 1) revealed the presence of 15 carbon atoms, including four methyls, three methylenes, four methines and four quaternary carbons, based on DEPT-135 and HSQC spectra, suggesting a chemical skeleton of sesquiterpenoid. The NMR signals of **1** (Table 1) showed the presence of a ketone group at δ_C 206.6 (C), two trisubstituted double bonds at δ_C 146.5 (CH), 137.1 (C), 135.1 (C) and 125.2 (CH) (δ_H 6.09 (1H, ddq, *J* = 12.4, 3.4, 1.4 Hz) and 4.87 (1H, d, *J* = 10.3 Hz)), a cyclopropyl moiety at δ_C

26.1 (CH), 29.6 (CH) and 20.2 (C) (δ_H 1.32 (1H, dd, J = 10.3, 8.9 Hz) and 0.77 (1H, ddd, J = 11.7, 8.9, 2.8 Hz)), and four tertiary methyls at δ_C 13.3 (CH_3), 16.7 (CH_3), 15.9 (CH_3) and 29.3 (CH_3) (δ_H 1.74, 1.49, 1.12 and 1.08 (each 3H, s)) (see ^{1}H- and ^{13}C-NMR data of **1** in Supplementary Information). Five degrees of unsaturation calculated *via* HR-MS could be attributed to three double bonds and a bicyclic sesquiterpenoid framework leading to the structure of compound **1**.

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

Position	1		2	
	δC	**δH**	**δC**	**δH**
1β	26.1 (CH)	1.32 dd (10.3, 8.9)	27.9 (CH)	1.45 t (8.9)
2	125.2 (CH)	4.87 d (10.3)	122.9 (CH)	4.61 d (8.9)
3	137.1 (C)		137.6 (C)	
4α	38.9 (CH_2)	2.34 m	39.3 (CH_2)	1.58 t (11.7)
β				2.15 dd (11.7, 8.3)
5α	25.1 (CH_2)	2.47 td (12.4, 8.3)	23.7 (CH_2)	2.25 td (12.2, 8.3)
β		2.31 m		2.01 dt (12.2, 8.3)
6	146.5 (CH)	6.09 ddq (12.4, 3.4, 1.4)	130.2 (CH)	5.40 tq (8.3, 1.4)
7	135.1 (C)		140.0 (C)	
8	206.6 (C)		211.5 (C)	
9α	37.1 (CH_2)	2.77 t (11.7)	36.3 (CH_2)	2.32 d (8.9)
β		2.37 dd (11.7, 2.8)		
10β	29.6 (CH)	0.77 ddd (11.7, 8.9, 2.8)	28.3 (CH)	1.38 q (8.9)
11	20.2 (C)		21.7 (C)	
12α	15.9 (CH_3)	1.12 s	16.1 (CH_3)	1.11 s
13β	29.3 (CH_3)	1.08 s	29.2 (CH_3)	1.11 s
14	13.3 (CH_3)	1.74 s	21.9 (CH_3)	1.88 s
15	16.7 (CH_3)	1.49 s	17.4 (CH_3)	1.55 s

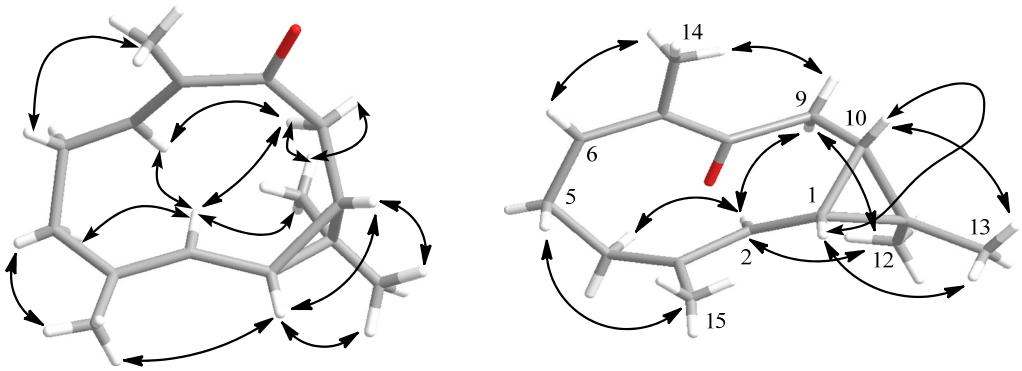
Assignment of ^{1}H-^{13}C correlations of **1** was determined by HSQC analysis. Two separate consecutive spin systems of "a" and "b" of **1** were revealed by ^{1}H-^{1}H COSY correlations. Correlations corresponding to H_2-4/H_2-5/H-6 are represented by the "a" spin system and H-1/H-2/H_2-9/H-10 by the "b" spin system, and are depicted by the bold lines in Figure 2. Both the "a" and "b" structural units were assembled to establish a bicyclic system comprised of fused 10- and 3-membered rings, suggesting a bicyclogermacrene skeleton for **1**. This was achieved using key HMBC correlations of H_3-12α to C-1, C-10, C-11 and C-13; H_3-13β to C-1, C-10, C-11 and C-12; H_3-14 to C-6, C-7 and C-8; and H_3-15 to C-2, C-3 and C-4 as depicted by arrows in Figure 2. Thus, **1** was deduced to possess a 10-membered ring with a ketone group at C-8, and a cyclopropyl moiety located at the C-1/C-10. This cyclopropyl ring was confirmed by the "b" partial structure based on the presence of HMBC correlations between H_3-12 to C-1, C-10, C-11 and C-13; and H_3-13 to C-1, C-10, C-11 and C-12. In addition, the upfield chemical shifts of H-6β (δ 1.32) and H-7β (δ 0.77), further supported the presence of this cyclopropyl moiety [9]. Based on these analyses, the planar structure of **1** was determined to be as shown in Figure 2.



1 **2**

Figure 2. Selected ^{1}H-^{1}H COSY (▬) and HMBC (→) correlations of **1** and **2**.

The relative stereochemistry of compound **1** was deduced from NOESY correlations as shown in Figure 3. In addition, the double bonds geometries were deduced from the ^{13}C-NMR chemical shifts at C-14 (δ_C 13.3) and C-15 (δ_C 16.7), which suggested *E*-configurations [10,11]. Besides, the lack of NOE correlations between H-6 with H_3-14 and H-2 with H_3-15, further supported this configuration. Other NOE analyses, H-6 showed NOE correlations to H-2, H_2-5α and H_2-9α; and H-2 showed NOE correlations with H_2-4α, H-6, H_2-9α and H_3-12α. Therefore, H_2-4α, H_2-5α, H_2-9α, and H_3-12α are suggested to be of the similar orientation, reflecting an α-orientation, hence H_2-4β, H_2-5β and H_2-9β are suggested to be of β-orientation. Furthermore, H-1β, H-10β, and H_3-13β were confirmed to be positioned on the β-orientation, due to NOE correlations observed between H-1β with H-10β, H_3-13β, and H_3-15. Besides that, the scalar coupling value between H-1β and H-10β was 8.9 Hz and this coupling value suggested a *cis*-cyclopropyl group with both methine protons as β-orientated [12]. Based on these configurations, the relative structure of **1** is reported as (1*S*,10*R*,2*E*,6*E*)-3,7,11,11-tetramethylbicyclo(8.1.0)undeca-2,6-dien-8-one. The compounds **1** and **2** were *trans* and *cis* isomers, but downfield shifts were observed at H-1β in **1** and **2**, and H-10β in **2**. The downfield shifts of both cyclopropane methines for **2** (δ_H 1.45 and 1.38) were similar to those observed in cyclocolorenone (δ_H 1.54 and 1.29) and its derivative [13,14]. It is reported that this cyclocolorenone also has one double bond beside these cyclopropane methines similar to those of **1** and **2**, where the double bond was located at C2/C3. Thus, it could be due to the presence of this double bond at C2/C3 and its proton deshielding ability towards cyclopropane methines in **1** and **2**. However, the H-10β (δ_H 0.77) in **1** was relatively more shielded as compared to those of **2**. This could be due to the presence of *trans* geometry double bond at C6/C7 and the position of oxygen atom (carbonyl group was oriented upward and outside the 10-membered ring) of **1** as compared that of **2**. The chemical shifts of H-6 and C-6 in **1** were significantly downfield compared to those observed for **2**, possibly due to the mesomeric effect [15].



1 **2**

Figure 3. Selective correlations of NOESY for **1** and **2**.

Compound **2** was obtained as colorless crystals. Its molecular formula, $C_{15}H_{22}O$ was established by HR-MS (*m*/*z* 219.1725 $[M + H]^+$), revealing five degrees of unsaturation. The IR spectrum indicated the presence of α,β-unsaturated carbonyl functionality (1683 cm^{-1}) in the molecule. Comparison of the NMR data (Table 1) of **2** with those of **1** also revealed the structure **2** to be of bicyclogermacrene type. Based on 2D NMR spectra, two separate consecutive proton spin systems "a" and "b" of **2** were revealed in ^1H-^1H COSY spectrum (Figure 2); "a" spin system for H_2-4/H_2-5/H-6 and "b" for H-1/H-2/H_2-9/H-10. Planar structure of **2** was established based on HMBC correlations. The relative configurations of the two successive chiral centers at C-1 and C-10 in **2** were determined based on NOESY data, as shown in Figure 3. It was revealed that H-6 had NOE correlation with H_3-14 and H-2 had no NOE correlation with H_3-15, implying the *Z*- and *E*-configurations for double bonds at C-6/C-7 and C-2/C-3, respectively. Therefore, by comparison of the ^{13}C-NMR of C-14 of **2** with that of **1**, it was suggested that **2** had a *cis* and **1** had a *trans* double bond at C-6/C-7 [10,11]. In addition, H-2 showed NOE interactions with H_2-4α, H_2-9α, and H_3-12α; and H_3-15 exhibited NOE correlation with H_2-5α. Therefore, H_2-4α, H_2-5α, H_2-9α, and H_3-12α reflected an α-orientation, hence indicating H_2-4β, H_2-5β and H_2-9β were β-orientated. Further analysis of other NOE correlations and scalar coupling between H-1β and H-10β, revealed **2** possessed the similar relative configurations at C-1, C-10, and C-13 as that of **1**. On the basis of above findings, the relative structure of **2** is reported as (1*S*,10*R*,2*E*,6*Z*)-3,7,11,11-tetramethylbicyclo(8.1.0)undeca-2,6-dien-8-one. The ^1H- and ^{13}C-NMR chemical shift at C-6 of **2** were more shielded as compared to those of **1** due to the absence of mesomeric effect.

2.2. Anti-Inflammatory Properties

Anti-inflammatory activity bioassay of compounds **1**–**4** against RAW 264.7 macrophages were evaluated based on the accumulation of NO production and cell viability induced by LPS (1 μg/mL) (Figures 4 and 5). The results showed compound **1** displayed potent anti-inflammatory potential by significantly reducing the NO production of LPS-induced RAW macrophages to 28.0% and 14.2% at 10 and 20 μg/mL, respectively. Cell viability of LPS-induced RAW macrophages in the presence of compound **1** was 105.1% and 74.6% at 10 and 20 μg/mL, respectively. Therefore, compound **1** was selected for further anti-inflammatory investigation against accumulation of NO, PGE_2, and

pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) production and the expression of iNOS and COX-2 proteins induced by LPS (1 μg/mL) in RAW 264.7 cells were evaluated. The effect of compound **1** on NO production in LPS-treated RAW 264.7 macrophages was repeated at lower concentrations, as shown in Figure 6. The findings showed NO production of compound **1** was 69.1%, 60.0%, and 24.6% at the concentrations of 5, 10, and 20 μg/mL, respectively. Based on the cell viability screening, compound **1** revealed the potential to significantly inhibit LPS-induced NO production in a concentration dependent manner, also suggesting absence of cytotoxic effects in RAW 264.7 macrophage cells. Dexamethasone used as positive control inhibited NO production to 16.01% at 5.0 μg/mL. At the concentration of 12.51 ± 0.16 μg/mL (57.34 μM) of compound **1**, it inhibited 50% of NO production in LPS-stimulated RAW 264.7 macrophages.

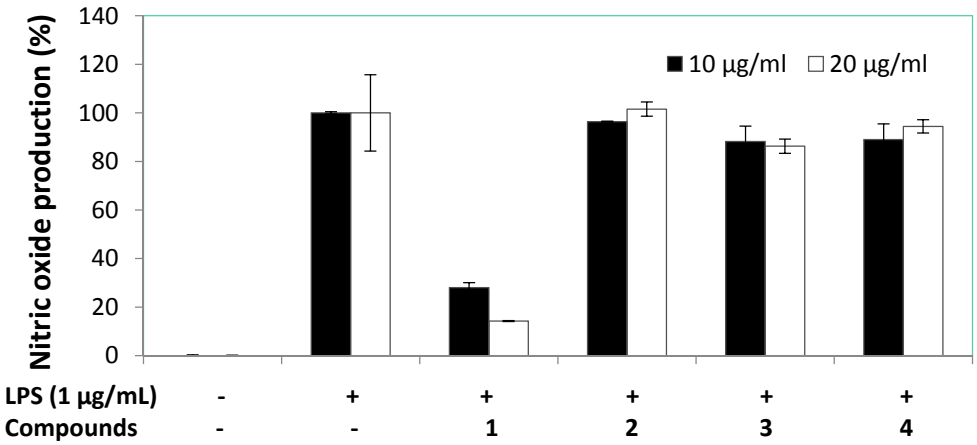


Figure 4. The effect of compounds **1**–**4** on NO production in RAW 264.7 macrophages.

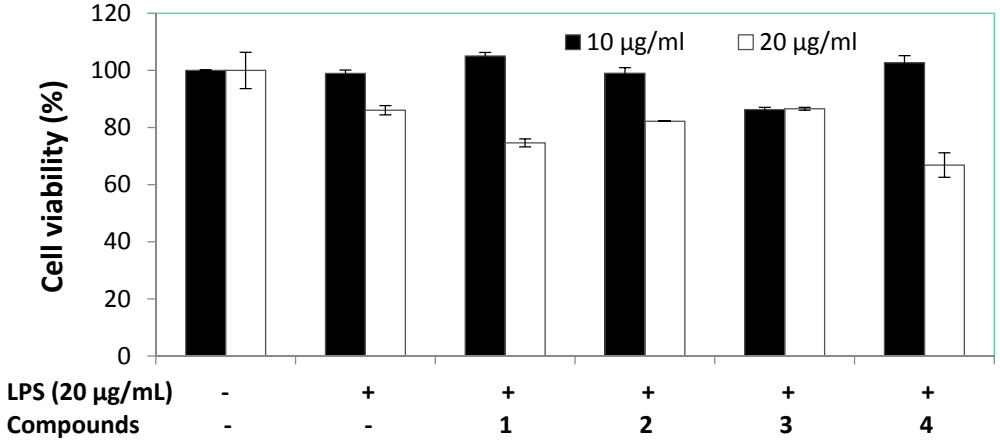


Figure 5. The effect of compounds **1**–**4** on cell viability in RAW 264.7 macrophages.

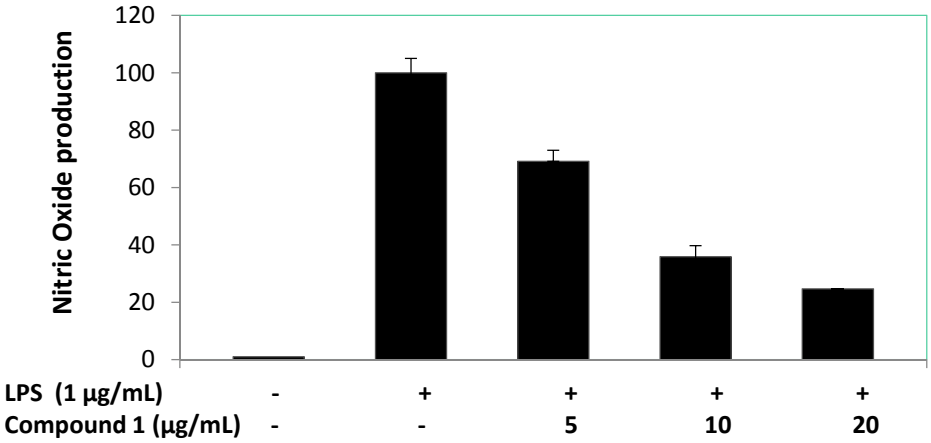


Figure 6. Concentration dependent effects of compound **1** on NO production in RAW 264.7 macrophages.

Further, we evaluated the effect of compound **1** on the LPS-induced production of PGE_2 in RAW 264.7 macrophage cells (Figure 7). However, it was apparent that compound **1** was not a good inhibitor of PGE_2 production in LPS-treated RAW 264.7 macrophages at 5 and 10 µg/mL, but compound **1** reduced the PGE_2 production to below 40% at 20 µg/mL. Dexamethasone (positive control) inhibited PGE_2 production to 11.58% at 5.0 µg/mL. Compound **1** at 18.97 ± 0.63 µg/mL (86.93 µM) inhibited 50% of PGE_2 production in LPS-stimulated RAW 264.7 macrophages.

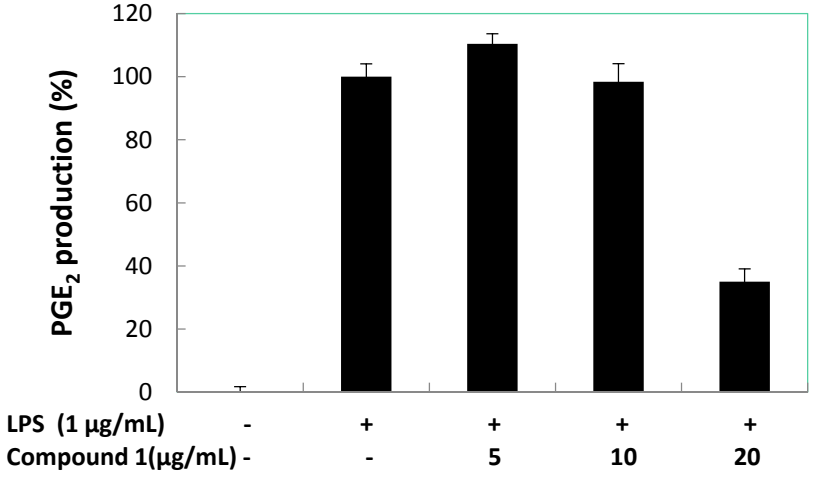


Figure 7. Concentration dependent effects of compound **1** on LPS-induced PGE_2 production in RAW 264.7 macrophages.

Subsequently, the effects of compound **1** on pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) production induced by LPS in RAW 264.7 macrophage cells were quantified (Figures 8–10). The result shows there was almost no inhibition of TNF-α and IL-6 production by compound **1** at 5, 10, and 20 µg/mL. However, compound **1** was able to inhibit the production of IL-1β with a reduction of 40% compared to the LPS-induced group at 5 and 10 µg/mL, and the IL-1β production value dropped to below 35% when 20 µg/mL of compound **1** was used. At 5.0 µg/mL of positive control (dexamethasone)

inhibition of TNF-α, IL-1β and IL-6 reduced production to 15.31, 12.23 and 13.56%, respectively. At the concentration of 12.89 ± 1.38 μg/mL (59.06 μM) compound **1**, inhibited 50% of IL-1β production in LPS-stimulated RAW 264.7 macrophages.

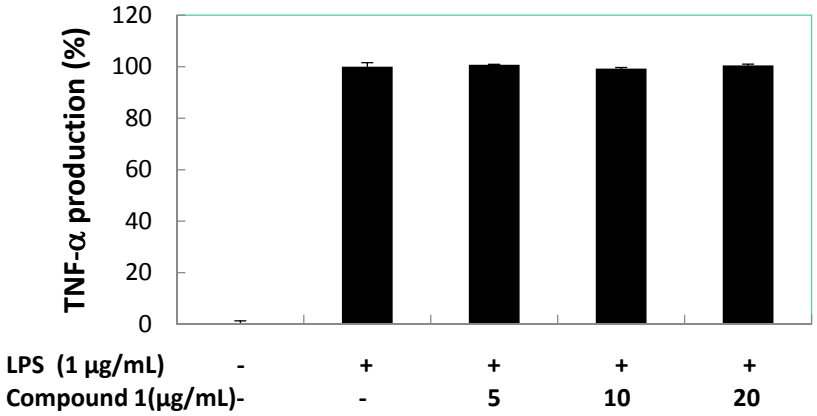


Figure 8. Concentration dependent effects of compound **1** on LPS-stimulated TNF-α release in RAW 264.7 cells.

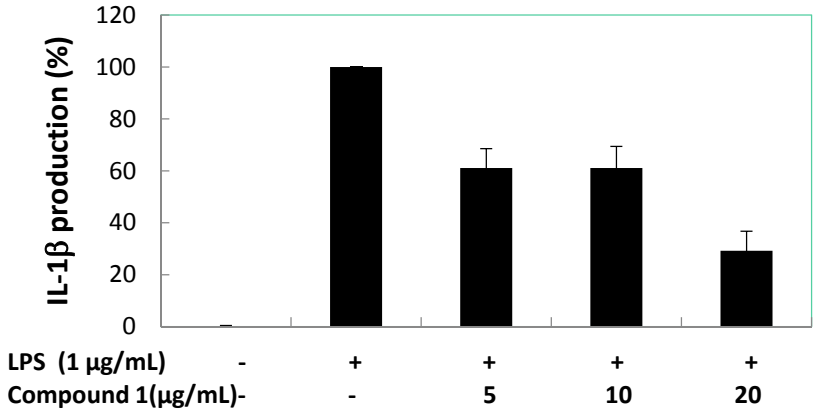


Figure 9. Concentration dependent effects of compound **1** on LPS-treated IL-1β release in RAW 264.7 macrophage cells.

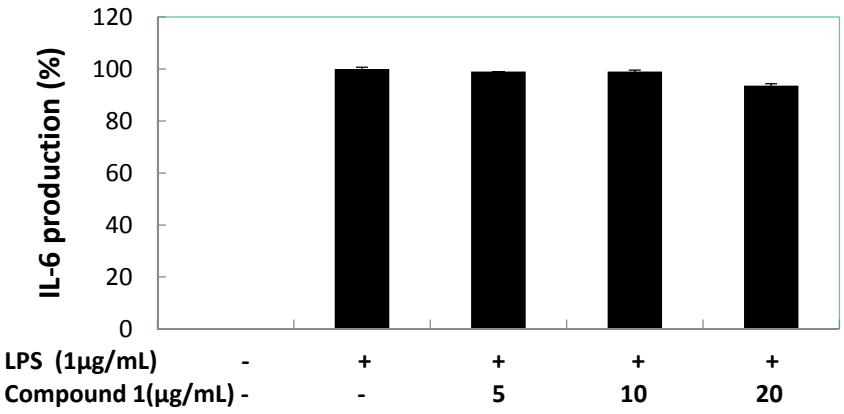


Figure 10. Concentration dependent effects of compound **1** on LPS-induced IL-6 release in RAW 264.7 macrophage cells.

Further investigation pertaining to the mechanism of anti-inflammatory activity of compound **1** was evaluated by observing the expression of iNOS and COX-2 proteins via Western blot, as shown in Figure 11. The result showed pretreatment with compound **1** significantly inhibited iNOS protein expression in a concentration dependent manner. However, compound **1** showed little inhibition of COX-2 protein expression at first concentration at 5 μg/mL, followed by 10 and 20 μg/mL by visual observation of bands densities, indicating little inhibition of PGE_2 production in LPS-treated RAW 264.7 macrophages. In this anti-inflammatory assay, we confirmed that LPS had significantly increased TNF-α, IL-1β, IL-6, PGE_2 and NO production, which resulted in the over expression of iNOS and COX-2. Pretreatment of LPS-induced RAW 264.7 macrophages with compound **1** exhibited the ability to inhibit NO and IL-1β production by down-regulating expression of iNOS. In addition, compound **1** also showed little inhibition of PGE_2 by little suppression of COX-2 expression.

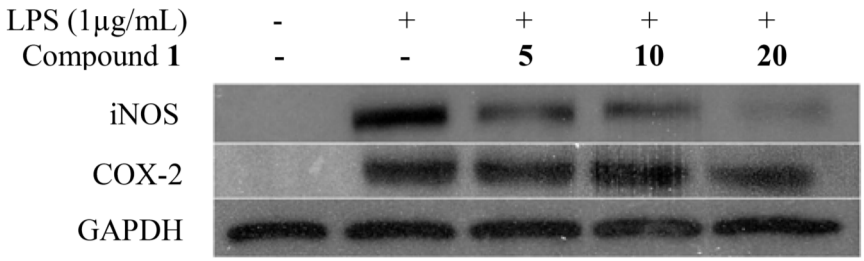


Figure 11. Concentration dependent effects of compound **1** on protein and mRNA expressions of iNOS and COX-2 in LPS-induced RAW 264.7 macrophages.

Compound **1** and **2** were *trans* and *cis* isomers, but only compound **1** showed anti-inflammatory activity. It is our assumption that the differences in activity between these two compounds are due to their stereo differences. Compound **1** has a larger space of 10-membered ring as compared those of **2**. The oxygen atom of **2** was positioned inside of the 10-membered ring, but oxygen atom of **1** was orientated outside of the 10-membered ring. Thus, the anti-inflammatory activity of compound **1** could be attributed to the larger space within the 10-membered ring and the position of oxygen atom. However, further SAR (Structure Activity Relation) analysis would be required to confirm this phenomenon.

3. Experimental Section

3.1. 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 LCMS-IT-TOF (Shimadzu, Nakagyo-ku, Kyoto 604-8511, Japan). AUTOPOL IV automatic polarimeter (Rudolph Research Analytic, 55 Newburgh Road, Hackettstown, USA) was used to measure the optical rotation. Infrared spectra were recorded on a fourier transform infrared spectroscopy (Thermo Nicolet, 81 Wyman Street, Waltham, MA, USA). Preparative TLC was performed with silica gel glass plates (Merck, Kieselgel 60 F_{254}, Menara Sunway Annexe Jalan Lagoon Timur (PJS9/1), Bandar Sunway, Malaysia). Column chromatography was performed with silica gel (Merck, Kieselgel 60, 70–230 mesh). Precoated silica gel plates (Merck Kieselgel 60 F_{254})

were used for analytical TLC. Both the UV light (254 and 365 nm) and spraying 5% phosphomolybdic acid-ethanol solution were used for spots visualization. High-performance liquid chromatography was performed on the Shimadzu prominence components using UV detector with a phenomenex C18 column (250 × 10 mm, 5 μm).

3.2. Biological Material

The specimen of *Capnella* sp. was collected from Mantanani Island, Sabah (6°43.059″N, 116°20.189″E), in June 2013. The voucher specimen (JUN117BOR) was deposited in the BORNEENSIS Collection of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

3.3. Extraction and Isolation

The fresh soft coral (1 kg wet wt) was chopped and extracted with MeOH at room temperature for 7 days. The resulting MeOH was concentrated under reduced pressure and the residue was partitioned between EtOAc and H_2O. The EtOAc fraction was further partitioned with hexane and 90% MeOH. The 90% MeOH fraction (1.20 g) was subjected to column chromatography eluting with a gradient of hexane and EtOAc in an increasing polarity to obtain six fractions. During column chromatography separation step, fractions 1, 2 and 3 were obtained by elution with hexane-EtOAc (9:1), (8:2) and (7:3), respectively. A portion of fraction 2 (80.0 mg) was subjected to preparative TLC with toluene-EtOAc (95:5) to yield compound **1** (11.8 mg) and compound **2** (8.5 mg),and was purified by reverse-phase HPLC (gradient mode; MeCN-H_2O (1:1) as solvent A, 100% MeCN as solvent B, 0.1–15.0 min with 50% of solvent B, 15.1–45.0 min from 50% to 100% of solvent B, and compound **2** peak retention time was at 25 min). A portion of fraction 1 (150.0 mg) was submitted to repeated preparative TLC with toluene, hexane–EtOAc (9:1) and toluene to afford compound **3** (6.0 mg). Fraction 3 (64.3 mg) was submitted to repeated preparative TLC with hexane–EtOAc (3:1) and $CHCl_3$-EtOAc (9:1) to yield compound **4** (1.2 mg).

*3.4. Capgermacrene A (**1**)*

Colorless oil; $[\alpha]_D^{25}$: +73.6 (*c* 0.47, $CHCl_3$); IR (KBr) λ_{max} 1662cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data: see Table 1; HR-TOFMS *m*/*z* 219.1720 $[M + H]^+$ (calcd. for $C_{15}H_{23}O$, 219.1743), and *m*/*z* 437.3359 $[2M + H]^+$ (calcd. for $C_{30}H_{45}O_2$, 437.3407).

*3.5. Capgermacrene B (**2**)*

Colorless crystals; $[\alpha]_D^{25}$: −21.7 (*c* 0.81, $CHCl_3$); IR (KBr) λ_{max} 1683 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR spectral data: see Table 1; HR-TOFMS *m*/*z* 219.1725 $[M + H]^+$ (calcd. for $C_{15}H_{23}O$, 219.1743).

3.6. In Vitro Anti-Inflammatory Assay

RAW 264.7 cells were purchased from the Korean Cell Line Bank (KCLB; Seoul, Korea). The enzyme-linked immunosorbent assay (ELISA) kit for PGE_2, TNF-α, IL-1β, and IL-6 were obtained from R&D Systems Inc. (Minneapolis, MN, USA). The antibodies against iNOS and COX-2

were purchased from Calbiochem (La Jolla, CA, USA) and BD Biosciences Pharmingen (San Jose, CA, USA), respectively. The anti-inflammatory assay was from known procedure [16,17].

The cytotoxicity assay, determination of nitric oxide (NO), and pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) productions, was carried out by cultured RAW 264.7 cell line and seeded in 96-well plate at concentration of 1.0×10^5 cells mL^{-1}. Upon incubation, the cells were treated with compound **1** or compounds **1**–**4** followed by LPS (1 μg/mL). In cytotoxicity assay, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) stock solution was added in each well. Subsequently, the absorbance was measured using ELISA. In evaluation of NO production, griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) was used. The optical density was measured using ELISA. Besides that, determination of pro-inflammatory cytokines was carried out using ELISA to report absorbance. Western blot analysis was carried out with compound **1** with a series of chemical reagents, incubation and centrifugation. Subsequently, the membrane was incubated with anti-mouse iNOS (1:1000; Calbiochem, San Diego, CA, USA) and anti-mouse COX-2 (1:1000; BD Biosciences Pharmingen, San Diego, CA, USA) overnight at room temperature. Then, the bands were visualized on X-ray film using ECL detection reagent (Amersham Biosciences, Piscataway, NJ, USA).

4. Conclusions

As part of our ongoing interest in chemical investigation of Bornean soft corals, two new bicyclogermacrenes, capgermacrene A (**1**) and B (**2**), were isolated together with two known compounds, palustrol (**3**) and litseagermacrane (**4**), from a *Capnella* sp. population collected from Mantanani Island, Sabah. This investigation has enriched our knowledge pertaining to the diversity of secondary metabolites in Bornean soft corals. Compound **1** and **2** could be regarded as relatively rare bicyclogermacrene structures isolated from *Capnella* sp. The anti-inflammatory activity and structure related activity of compound **1** revealed to be very interesting due to the mere differences in their stereochemistry. Upon detailed SAR and pharmacokinetic investigation, compound **1** could be a promising iNOS inhibiting agent.

Acknowledgments

The author (CSV) would like to thank the Sabah Biodiversity Centre (SaBC) for granting a permit and research grant for this investigation. The authors would also like to acknowledge Kishneth Palaniveloo and Tulasiramanan Ramachandram for assistance during sample collection.

Author Contributions

The contributions of the respective authors are as follows: Chin-Soon Phan performed isolation, structure elucidation of the compounds, and prepared the manuscript. Kishneth Palaniveloo, Shean-Yeaw Ng, Eun-A Kim and You-Jin Jeon contributed to the bioassay. Charles Santhanaraju Vairappan contributed to supervising, checking and confirming all of the structural identification and revision of this manuscript. This study was performed based on the planning of Charles Santhanaraju Vairappan, the corresponding author.

Supplementary Information

Structures of new compounds, 1D and 2D NMR spectra, and MS spectra of the new compounds (**1** and **2**).

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Wright, A.D.; Goclik, E.; Konig, G.M. Oxygenated analogues of gorgosterol and ergosterol from the soft coral Capnella lacertiliensis. *J. Nat. Prod*. **2003**, *66*, 157–160.
2. Wei, W.-C.; Sung, P.-J.; Duh, C.-Y.; Chen, B.-W.; Sheu, J.-H.; Yang, N.S. Anti-inflammatory activities of natural products isolated from soft corals of Taiwan between 2008 and 2012. *Mar. Drugs* **2013**, *11*, 4083–4126.
3. Ishii, T.; Zhaoqi, Z.; Vairappan, C.S. A new cembrane diterpene from the Bornean soft coral *Nephthea* sp. *Molecules* **2010**, *15*, 3857–3862.
4. Ishii, T.; Matsuura, H.; Zhaoqi, Z.; Vairappan, C.S. A new 4α-methylated sterol from a *Nephthea* sp. (Nephtheidae) Bornean soft coral. *Molecules* **2009**, *14*, 3360–3366.
5. Ishii, T.; Matsuura, H.; Zhaoqi, Z.; Vairappan, C.S. A new norsesquiterpenoid from a Bornean soft coral genus *Nephthea*. *Molecules* **2009**, *14*, 4591–4596.
6. Al-Footy, K.O.; Alarif, W.M.; Asiri, F.; Aly, M.M.; Ayyad, S.N. Rare pyrane-based cembranoids from the Red Sea soft coral *Sarcophyton troheliophorum* as potential antimicrobial-antitumor agents. *Med. Chem. Res*. **2015**, *24*, 505–512.
7. Zhang, H.-J.; Tan, G.T.; Santarsiero, B.D.; Mesecar, A.D.; Hung, N.V.; Cuong, N.M.; Soejarto, D.D.; Pezzuto, J.M.; Fong, H.H.S. New sesquiterpenes from *Litsea verticillata*. *J. Nat. Prod*. **2003**, *66*, 609–615.
8. Fraga, B.M. Natural sesquiterpenoids. *Nat. Prod. Rep*. **2012**, *29*, 1334–1366.
9. Tseng, Y.-J.; Shen, K.-P.; Lin, H.-L.; Huang, C.-Y.; Dai, C.-F.; Sheu, J.-H. Lochmolins A–G, new sesquiterpenoids from the soft coral *Sinularia lochmodes*. *Mar. Drugs* **2012**, *10*, 1572–1581.
10. Blackman, A.J.; Bowden, B.F.; Coll, J.C.; Frick, B.; Mahendran, M.; Mitchell, S.J. Several new cembranoid diterpenes from *Nephtheabrassica* and related diterpenes from a *Sarcophyton* species. *Aust. J. Chem*. **1982**, *35*, 1873–1880.
11. Duh, C.-Y.; Wang, S.-K.; Chung, S.-G.; Chou, G.-C.; Dai, C.-F. Cytotoxic cembrenolides and steroids from the Formosan soft coral *Sarcophyton crassocaule*. *J. Nat. Prod*. **2000**, *63*, 1634–1637.
12. Lampman, G.M.; Puvia, D.L.; Kriz, G.S.; Vyvyan, J.R. *Spectroscopy*, 4th ed.; Brooks/Cole: Pacific Grove, CA, USA, 2010; pp. 128–130.
13. Rao, C.B.; Sekhar, V.C.; Sarvani, B.; Rao, D.V. A new oxygenated tricyclic sesquiterpene from a soft coral *Nephthea* species of Andaman and Nicobar coasts of Indian Ocean. *Indian J. Chem*. **2004**, *43B*, 1329–1331.

14. Handayani, D.; Edrada, R.A.; Proksch, P.; Wray, V.; Witte, L.; Ofwegen, L.; Kunzmann, A. New oxygenated sesquiterpenes from the Indonesian soft coral *Nephthea chabrolii*. *J. Nat. Prod.* **1997**, *60*, 716–718.
15. Holzgrabe, U.; Wawer, I.; Diehl, B. *NMR Spectroscopy in Pharmaceutical Analysis*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2008; p. 13.
16. Yang, X.; Kang, M.-C.; Li, Y.; Kim, E.-A.; Kang, S.-M.; Jeon, Y.-J. Anti-inflammatory activity of questinol isolated from marine-derived fungus *Eurotium amstelodami* in lipopolysaccharide-stimulated RAW 264.7 macrophages. *J. Microbiol. Biotechnol.* **2014**, *24*, 1346–1353.
17. Vairappan, C.S.; Kamada, T.; Lee, W.-W.; Jeon, Y.-J. Anti-inflammatory activity of halogenated secondary metabolites of *Laurencia snackeyi* (Weber-van Bosse) Masuda in LPS-stimulated RAW 264.7 macrophages. *J. Appl. Phycol.* **2013**, *25*, 1805–1813.