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Capgermacrene C, a New Sesquiterpenoid from a Bornean Soft Coral, *Capnella* sp.

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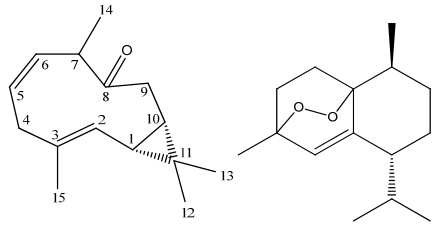
A new bicyclogermacrene, capgermacrene C (**1**), along with a known compound, 1,4-peroxy-5-muurolene (**2**), were isolated from a population of Bornean soft coral *Capnella* sp. The structures of these metabolites were determined by extensive spectroscopic analysis, including NMR, and HRESIMS. Both compounds were subjected to antibacterial activity tests against antibiotic resistant clinical bacteria, but produced only negligible inhibition.

Keywords: *Capnella* sp., Nephtheidae, Soft coral, Sesquiterpenoid, Bicyclogermacrene.

Terpenoids isolated from marine organisms have attracted great attention due to their unique structures and wide spectrum of biological potential [1]. The secondary metabolites in the soft coral genus *Capnella* are dominated by sesquiterpenes [2]. Secondary metabolites isolated from soft corals have been reported to show a variety of biological activities such as anti-tumor, antiviral, antifouling and anti-inflammatory [3]. In our previous chemical investigation of one population of a Bornean soft coral in the genus *Capnella*, we reported the isolation and structural elucidation of two new bicyclogermacrene-type sesquiterpenoids, capgermacrenes A and B [4]. In this study, we report on yet another population of *Capnella* sp. collected from a different location in Mantanani Island (Sabah, Malaysia). Chemical investigation of the methanol crude extract has led to the isolation and structure elucidation of a new bicyclogermacrene, capgermacrene C (**1**), along with a known compound, 1,4-peroxy-5-muurolene (**2**).

Compound **1** was isolated as colorless oil, $[\alpha]_D^{23}$ -32.0 (*c* 0.05, $CHCl_3$). Its molecular formula was established as $C_{15}H_{22}O$ from the HRESIMS ion at *m/z* 219.1750 $[M + H]^+$ (calcd for $C_{15}H_{23}O$, 219.1743), implying five degrees of unsaturation. The IR absorption at 1699 cm^{-1} indicated the presence of a carbonyl functional group in the molecule. The ^{13}C NMR spectral data of **1** (Table 1) revealed the presence of 15 carbon atoms, which were attributed by DEPT-135 and HSQC spectra to four methyls, two methylenes, six methines and three quaternary carbons. The NMR data of **1** (Table 1) suggested a carbonyl at δ_C 215.7 (C), a disubstituted double bond at δ_C 132.7 (CH) and 132.0 (CH); δ_H 5.87 (1H, dddd, *J* = 11.0, 6.9, 5.5, 1.8 Hz) and 5.39 (1H, ddd, *J* = 10.8, 7.6, 2.1 Hz), one trisubstituted olefin at δ_C 137.3 (C) and 126.8 (CH); δ_H 5.01 (1H, d, *J* = 9.6 Hz), and a *gem*-dimethylcyclopropane moiety at δ_C 30.1 (CH), 28.4 (CH_3), 25.0 (CH), 22.2 (C) and 15.2 (CH_3); δ_H 1.39 (1H, ddd, *J* = 9.6, 9.6, 6.1 Hz), 1.09 (3H, s), 1.44 (1H, dd, *J* = 9.6, 9.6 Hz) and 1.10 (3H, s). These signals explained three degrees of unsaturation, inferring that a bicyclic ring system was present in the structure of **1**.

The ^1H-^{13}C correlations of **1** were determined from the HSQC spectrum. In the ^1H-^1H COSY experiment, two separate consecutive spin systems were present in **1**; 'a' represents H_2-4/H-5/H-6/H-7/H_3-14 and 'b' H-2/H-1/H-10/H_2-9, depicted by the bold lines in Figure 2A.



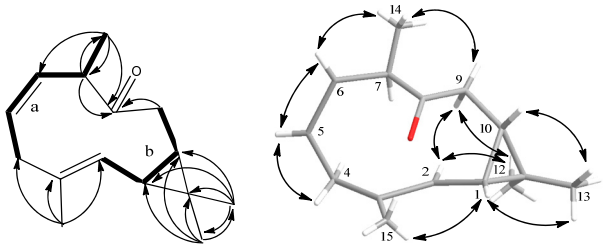
1 **2**

Figure 1: Structures of compounds **1** and **2**.

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

Position	Compound 1	
	δ_C	δ_H (mult., *J* in Hz)
1	25.0 (d)	1.44 dd (9.6, 9.6)
2	126.8 (d)	5.01 d (9.6)
3	137.3 (s)	
4	36.7 (t)	2.92 dd (17.0, 6.9, β-H)
		2.46 ddd (17.2, 5.5, 2.1, α-H)
5	132.7 (d)	5.87 dddd (11.0, 6.9, 5.5, 1.8)
6	132.0 (d)	5.39 ddd (10.8, 7.6, 2.1)
7	47.0 (d)	3.13 dq (7.6, 7.3)
8	215.7 (s)	
9	34.6 (t)	2.30 dd (15.0, 6.1, β-H)
		2.14 dd (15.1, 9.6, α-H)
10	30.1 (d)	1.39 ddd (9.6, 9.6, 6.1)
11	22.2 (s)	
12	15.2 (q)	1.10 s
13	28.4 (q)	1.09 s
14	19.1 (q)	1.16 d (7.3)
15	18.5 (q)	1.66 s

The combination of both 'a' and 'b' partial structures with the key HMBC correlations allowed connection of a bicyclic system comprised of fused 10- and 3-membered rings, suggesting a bicyclogermacrene skeleton for **1**: H_3-12 to C-1, C-10, C-11 and C-13; H_3-13 to C-1, C-10, C-11 and C-12; H_3-14 to C-6, C-7 and C-8; and H_2-15 to C-2, C-3 and C-4, as depicted by arrows in Figure 2A. Therefore, it was found that **1** has a 10-membered ring with one ketone group at C-8 and a *gem*-dimethylcyclopropane unit fused at C1/C10. The *gem*-dimethylcyclopropane moiety was confirmed by the 'b' structural unit and HMBC correlations of H_3-12 and H_3-13, as mentioned above. This deduction was supported by near similar chemical shifts of C-1 (δ_C 25.0; δ_H 1.44), C-10 (δ_C 30.1; δ_H 1.39) and C-11(δ_C 22.2) in **1** compared with those of cyclocolorenone (δ_C 32.8, 28.9 and 26.1; δ_H 1.54 and 1.29) [5]. The planar structure of **1** was established to be as shown in Figure 2A.



(A) (B)

Figure 2: (A) ^{1}H-^{1}H COSY and key HMBC and (B) Selected NOE correlations of **1**.

The relative configurations of the three chiral carbons at C-1, C-7 and C-10 of **1** were determined by NOE correlations (Figure 2B). It was found that H-2 showed NOE correlations with H_2-9α and H_3-12; and H_3-14 exhibited NOE interactions with H_2-9β. Based on these findings, H_2-9α and H_3-12 were assumed to have an α-orientation, and H_2-9β was a β-orientation. The β-orientation of H-1, H-10 and H_3-13 were assigned due to NOE correlations of H-1/H_3-13 and H-10/H_3-13. In addition, the scalar coupling value between H-1 and H-10 was 9.6 Hz, indicating a *syn* configuration of the cyclopropane moiety [6]. Other than that, a double bond at C-2/C-3 was found to have *E*-configuration due to lack of NOE correlation between H-2 and H_3-15. Moreover, it was further supported by the $\delta_{C\text{-}15}$ 19.2 [7,8]. Besides that, a *Z*-configuration double bond was found at C-5/C-6 due to a NOE cross-peak of H-5/H-6. This configuration was further supported by the scalar coupling value (10.8-11.0 Hz) between these two olefinic methines at C-5/C-6 [7]. Based on these findings, the relative structure of **1** was as shown in Figure 2B. Compound **2** was isolated and identified as 1,4-peroxy-5-muurolene ($[\alpha]_D^{25}$ +47.6, *c* 0.09, $CHCl_3$) based on comparison of its spectroscopic and optical rotation data ($[\alpha]_D$ +41.9) with those reported in the literature [9].

In conclusion, as a part of our chemical investigation of Bornean soft corals, a new sesquiterpenoid, capgermacrene C (**1**) and the known 1,4-peroxy-5-muurolene (**2**) were isolated from a *Capnella* sp. specimen collected from Mantanani Island, Sabah. Both the compounds were screened against two strains of clinical bacteria (*Escherichia coli* and *Staphylococcus aureus*), but showed negligible inhibition with MIC > 500 μg/mL.

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 LCMS-IT-TOF (Shimadzu). An AUTOPOL IV automatic polarimeter (Rudolph Research Analytic) was used to measure the optical rotation at 23°C. Infrared spectra were recorded on a Fourier transform infrared spectrophotometer (Thermo Nicolet). Preparative TLC was performed with silica gel glass plates (Merck, Kieselgel 60 F_{254}), and column chromatography (CC) with silica gel (Merck, Kieselgel 60, 70-230 mesh).

Biological material: A specimen of *Capnella* sp. was collected from Mantanani Island, Sabah (6°43.105”N, 116°19.143”E), in June 2013. The voucher specimen (BORMI118) was deposited in the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Extraction and isolation: Fresh soft coral (1 kg wet wt) was homogenized and extracted in MeOH at room temperature for 7 days. The resulting MeOH extract was concentrated *in vacuo* and partitioned between EtOAc / H_2O. The EtOAc fraction was further partitioned with *n*-hexane / 90% MeOH. The 90% MeOH fraction (1.20 g) was subjected to CC eluting with a gradient of *n*-hexane and EtOAc with increasing polarity. Fraction 1 (280.0 mg) was subjected to repeated preparative TLC with *n*-hexane-EtOAc (9:1) and benzene to yield compound **1** (1.5 mg) and the residue was further purified with toluene to obtain compound **2** (1.8 mg).

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