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SINULAFLEXIOLIDE P, A CEMBRANE-TYPE DITERPENOID FROM BORNEAN SOFT CORAL *Sinularia flexibilis*

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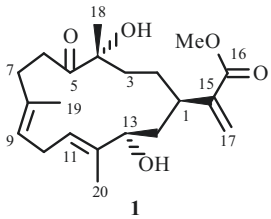
*A new cembrane-type diterpenoid, sinulaflexiolide P (**1**), along with three known derivatives: sinulaflexiolide H (**2**), 11-*epi*-sinulariolide acetate (**3**), and (1S*,3S*,4S*,7E,11E)-3,4-epoxy-13-oxo-3,7,11,15-cembratriene (**4**), was isolated from a population of Bornean soft coral* Sinularia flexibilis. *The structures of these metabolites were elucidated based on spectroscopic data including NMR and HR-ESI-MS. In addition, these compounds were tested against six strains of marine fungi.*

Keywords: *Sinularia flexibilis*, soft coral, diterpenoid, cembrane, antifungal.

Marine organisms are well known to be an essential source of bioactive natural products [1, 2]. Soft corals (Alcyoniidae) are recognized to be a rich source of sesquiterpenoids [3, 4], cembrane-based diterpenoids [5–7] including their dimers [8, 9], xenicane-type [10, 11] and to a lesser extent prenyleudesmane-derived diterpenoids [12, 13], eunicellin-based diterpenoids [14], cutibane-type diterpenoids [15], casbane-based diterpenoids [16], and prenylated germacrene-type diterpenoids [17], as well as meroditerpenoids [18], while terpenoids including sesquiterpenoids [19, 20], lobane-type diterpenoids [21, 22], cembranoids [19, 20, 23–27], and steroids [28] have been reported from soft coral belonging to the species *Sinularia flexibilis*. Some of these compounds exhibit cytotoxic [19, 20, 24, 25, 28], antibacterial [21, 23], anti-inflammatory [26, 27], and antifungal activities [20, 29]. Because of the great interest in this organism, the study of one population of Bornean soft coral *Sinularia flexibilis* collected from Mantanani Island (Sabah, Malaysia) has led to the isolation of one new cembrane-type diterpenoid, sinulaflexiolide P (**1**), along with three known derivatives, sinulaflexiolide H (**2**) [24], 11-*epi*-sinulariolide acetate (**3**) [30], and (1*S**,3*S**,4*S**,7*E*,11*E*)-3,4-epoxy-13-oxo-3,7,11,15-cembratriene (**4**) [31]. This paper reports the isolation, structure elucidation, and antifungal potentials of these compounds.

Compound **1** was isolated as a colorless oil: $[\alpha]_D^{25}$ –44.0° (*c* 0.20, $CHCl_3$). Its molecular formula was determined as $C_{21}H_{32}O_5$ based on HR-ESI-MS ions at *m/z* 387.2156 $[M + Na]^+$ (calcd for $C_{21}H_{32}O_5Na$, 387.2142), 365.2335 $[M + H]^+$ (calcd for $C_{21}H_{33}O_5$, 365.2323), and 347.2210 $[M + H - H_2O]^+$ (calcd for $C_{21}H_{31}O_4$, 347.2217). The IR (KBr) absorption at 3400, 1720, 1650, and 1010 cm^{-1} indicated the presence of hydroxyl, carbonyl, and alkoxy groups in the molecule.



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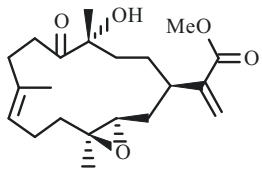

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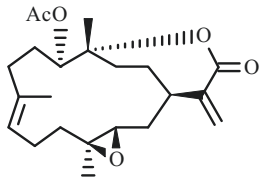

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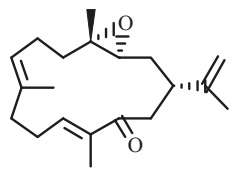

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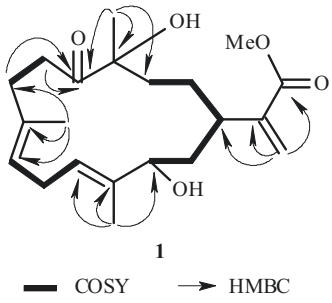
TABLE 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	2.61 (m)	37.4	11	5.44 (t, J = 8.3)	125.3
2	1.33–1.34 (m)	25.1	12	–	136.8
	1.20–1.21 (m)		13	3.98 (dd, J = 10.3, 3.4)	75.2
3	1.73–1.74 (m)	39.0	14	1.86 (ddd, J = 13.8, 6.9, 3.4)	37.7
	1.27–1.28 (m)			1.75–1.76 (m)	
4	–	79.1	15	–	141.0
5	–	212.8	16	–	167.8
6	2.86 (dd, J = 18.6, 9.6)	34.3	17	6.36 (s)	125.0
	2.53 (dd, J = 18.6, 9.6)			5.40 (s)	
7	2.46 (dd, J = 13.8, 9.6)	30.4	18	1.27 (s)	25.8
	2.22 (dd, J = 13.8, 9.6)		19	1.71 (s)	17.2
8	–	134.3	20	1.57 (s)	10.7
9	5.21 (t, J = 8.3)	122.2	16-OMe	3.76 (s)	52.1
10	2.74 (dt, J = 13.8, 8.3)	26.0			
	2.57 (dt, J = 13.8, 8.3)				



1

COSY HMBC

Fig. 1. The ^{1}H–^{1}H COSY and selective HMBC correlations of **1**.

Upon careful comparison of NMR data (Table 1) between **1** and sinulaflexiolide G, we found a general similarity in their structure, except for the replacement of the ethoxy functionality (δ_C 60.6, 13.9; δ_H 4.10, 1.08) at C-16 in sinulaflexiolide G by a methoxy moiety (δ_C 52.1; δ_H 3.76) in **1** [24].

The planar structure was further confirmed by three consecutive ^{1}H–^{1}H spin systems, determined via an ^{1}H–^{1}H COSY experiment, which are connected through key HMBC cross peaks of H_2-17 to C-1, C-15 and C-16; H_3-18 to C-3, C-4 and C-5; H_3-19 to C-7, C-8 and C-9; H_3-20 to C-11, C-12, and C-13; and both H_2-6 and H_2-7 to C-5 (Fig. 1). The stereogenic centers at C-1, C-4, and C-13 were determined to be identical to those of sinulaflexiolide G upon examination of the chemical shifts [24].

Compounds **1**–**4** were screened against six fungal strains: *Fusarium moniliforme* (NJM 8995), *F. oxysporum* (NJM 0179), *F. solani* (NJM 8996), *Haliphthoros milfordensis* (IPMB 1603), *H. sabahensis* (IPMB 1402), and *Lagenidium thermophilum* (IPMB 1401). The MICs of compounds **1–4** were as follows: for *F. moniliforme*, *F. oxysporum*, and *F. solani*, > 50 μg/mL; for *H. sabahensis* and *L. thermophilum*, 50 μg/mL; for *H. milfordensis*, 25 μg/mL. These strains are known to cause fungal infections in aquatic organisms, especially in fishes and mangrove crabs [32]. Hence, it is imperative to search for new antifungal agents against these fungi. The results showed that the antifungal potentials of **1**–**4** against *H. sabahensis* were similar to those of other cembranoids: *ent*-sinuflexibilin D, 14-deoxycrassin, diepoxycembrene A, 5-dehydrosinulariolide, and 11-*epi*-sinulariolide acetate, except for sinularin, which displayed a lower MIC value [20]. This may be because sinularin possesses α-methylene-δ-lactone and epoxide units. In addition, *H. milfordensis* was more susceptible to **1**–**4** than other tested strains.

EXPERIMENTAL

General. The NMR spectra were recorded on a 600-MHz FT-NMR (Jeol, Tokyo, Japan) instrument using $CDCl_3$ with TMS as internal standard. The high-resolution mass spectrum was acquired via LC-ESI-IT-TOF-MS (Shimadzu, Kyoto,

Japan). An AUTOPOL IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, USA) was used to measure the optical rotation value at 25°C. Infrared spectra were recorded on a FTIR spectroscopy (Thermo Nicolet, Waltham, USA). Silica gel preparative TLC (Kieselgel 60, F_{254}) and column chromatography (Kieselgel 60, 70–230 mesh) were performed (Merck, Darmstadt, Germany).

Biological Material. The specimen of *Sinularia flexibilis* was collected from Mantanani Island, Sabah (06°42′4.19″N, 116°19′58.43″E) in May 2017. A voucher specimen (BORMI0017) was deposited in the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Extraction and Isolation. The fresh soft coral (0.8 kg wet wt) was chopped and extracted with MeOH at room temperature for 5 days. The resulting MeOH extract was concentrated and partitioned between EtOAc and H_2O. The EtOAc fraction was further partitioned with hexane and 90% MeOH. The 90% MeOH crude (1.0 g) was subjected to column chromatography eluting with a gradient of hexane–EtOAc (9:1, 8:2, 7:3, 1:1, and 1:0) to yield fractions 1–5. Repeated preparative TLC using $CHCl_3$–EtOAc (95:5) and hexane–EtOAc (9:1) yielded **4** (3.3 mg) from fraction 3 (70.0 mg). Fraction 4 (180.0 mg) was subjected to repeated preparative TLC with toluene–EtOAc (9:1) and $CHCl_3$–EtOAc (9:1) to isolate **2** (5.0 mg), and **3** (9.9 mg), while the residue was further purified by preparative TLC, again using hexane–EtOAc (8:2) to obtain **1** (2.0 mg).

Antifungal Assay. The minimum inhibitory concentration (MIC) of the fungistatic on hyphae was performed by incorporating the pure compound solutions (100, 50, 25, and 12.5 µg/mL) onto PYGS agar in a petri dish followed by inoculation of six tested fungal strains [20, 32]. The MIC was determined visually as the lowest concentration showing no hyphal growth when they were incubated at 25°C for 7 days.

Sinulaflexiolide P (1). Colorless oil; $[\alpha]_D^{25}$ –44.0° (*c* 0.20, $CHCl_3$). IR (KBr, λ_{max}, cm^{-1}): 3400, 1720, 1650, and 1010. ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data (see Table 1). HR-ESI-MS *m/z:* 387.2156 $[M + Na]^+$ (calcd for $C_{21}H_{32}O_5Na$, 387.2142), 365.2335 $[M + H]^+$ (calcd for $C_{21}H_{33}O_5$, 365.2323), and 347.2210 $[M + H - H_2O]^+$ (calcd for $C_{21}H_{31}O_4$, 347.2217).

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