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Sinularianins A and B, novel sesquiterpenoids from the Formosan soft coral *Sinularia* sp.

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Abstract—Two novel sesquiterpenoids, sinularianins A and B (**1** and **2**), have been isolated from the Formosan coral *Sinularia* sp. The skeleton of sinularianins A (**1**), namely sinulariolane, was found to be unprecedented. Sinularianin B (**2**), with a novel spirobutenolide moiety, possesses a valerenane skeleton, which had been reported mostly from the plant. The structures of both metabolites were established by extensive analysis of spectroscopic data.

Soft coral of the genus *Sinularia* has been found to be a rich source of bioactive secondary metabolites.[1] During the course of our investigation on the bioactive chemical constituents from marine invertebrates,[2–12] two new sesquiterpenoids, sinularianin A (**1**) and sinularianin B (**2**) (Fig. 1), have been isolated from the soft coral *Sinularia* sp., collected off the northeastern Taiwan coast, in May 2004, at a depth of 10 m. Sinularianin A possesses an unprecedented bicyclic skeleton, namely sinulariolane. Sinularianin B has a valerenane skeleton, with a spirobutenolide moiety. Metabolites with valerenane skeleton had been isolated mostly from the plant *Valeriana officinalis*,[13–16] and only a few representatives have been reported. Although, there is one letter that reported the isolation of valerenanoids from soft coral,[17] however, valerenane-related metabolites with a spiro-butenolide moiety had never been found before. We describe herein the isolation and structure elucidation of these compounds.

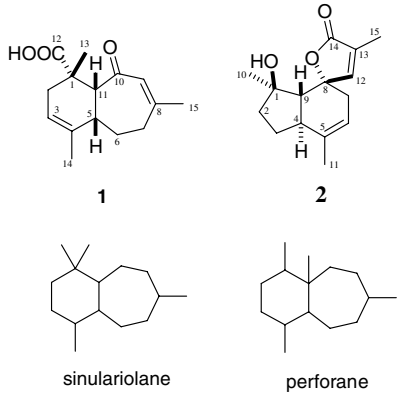

Figure 1. Structures of metabolites **1** and **2**, and skeletons of sinulariolane and perforane.

The organism (1.0 kg fresh wt) was collected and freeze-dried. The freeze-dried material was minced and extracted exhaustively with EtOH. The organic extract was concentrated to an aqueous suspension and partitioned between EtOAc and water. The EtOAc extract (9.8 g) was fractionated by open column chromatography on silica gel using *n*-hexane and *n*-hexane/EtOAc mixtures of increasing polarity. A fraction eluted with hexane/EtOAc (1:4) was subjected to Sephadex LH-20 column (2×90 cm) using acetone and followed by normal phase HPLC (hexane/acetone, 8/1) to afford compounds **1** (3.0 mg) and **2** (1.5 mg).

Keywords: Sinularianin A; Sinularianin B; *Sinularia* sp.; Novel sesquiterpene; Soft coral.
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Sinularianin A (**1**) was isolated as a white powder, $[\alpha]_D^{25}$ −5 (*c* 1.48, $CHCl_3$). The HRESIMS of **1** exhibited a pseudomolecular ion peak at *m/z* 271.1312 $[M+Na]^+$ (calcd for $C_{15}H_{20}O_3Na$, 271.1310). Thus, the molecular formula of **1** was determined as $C_{15}H_{20}O_3$, indicating six degrees of unsaturation. The ^{13}C NMR spectral data revealed the presence of three methyls, three sp^3 methylenes, two sp^3 methines, one sp^3 quaternary carbon, two sp^2 methines, two sp^2 quaternary carbons, and two carbonyl carbons. The IR and NMR spectral data indicated the presence of one trisubstituted double bond (δ_C 121.6, d, and 130.7 s), one α,β-unsaturated ketone (δ_C 202.7, s, 130.5, d, and 160.0, s; IR absorptions at ν_{max} 1658 cm^{-1}),[18,19] and one carboxylic acid (δ_C 183.4, s; IR 1695 cm^{-1}). The above functionalities account for four of the six degrees of unsaturation in the molecule of **1**, revealing a bicyclic structure for **1**. The gross structure of **1** was established by the assistance of extensive 2D NMR analysis (^1H–^1H COSY, HMQC, and HMBC). Inspection of ^1H–^1H COSY spectrum of **1** revealed two spin systems, as depicted in Figure 2. The olefinic methyls (δ_H 1.63, s and 1.94, s) attached at C-4 and C-8 were confirmed by the HMBC correlations from H_3-14 to C-3, C-4, and C-5 and H_3-15 to C-7, C-8, and C-9, respectively. The C-1 position of carboxylic acid functionality was determined by the HMBC correlations from H_3-13 to C-1, C-2, C-11, and C-12. The above data and other key HMBC correlations illustrated in Figure 2 established the planar structure of **1** (Table 1).

The relative stereochemistry of **1** was mainly established by NOESY experiment (Fig. 3). In the NOESY spectrum of **1**, H-11 showed NOE interactions with both H-5 and H_3-13, suggesting the same orientation of these protons. The large coupling constant of H-11 (J = 8.5 Hz) was due to the small dihedral angle between H-11 and H-5. As **1** was isolated in rare quantity and has been mostly consumed in the determination of MS spectrum and biological assay, it is not possible to determine the absolute structure of this metabolite at the present stage.

Sinularianin B (**2**), was isolated as a colorless oil, $[\alpha]_D^{25}$ −27 (*c* 1.04, $CHCl_3$). The pseudomolecular ion peak observed in HRESIMS at *m/z* 271.1308 $[M+Na]^+$ (calcd for $C_{15}H_{20}O_3Na$, 271.1310) suggested a molecular formula of $C_{15}H_{20}O_3$, indicating six degrees of unsaturation. The IR absorption at ν_{max} 3463 cm^{-1} suggested

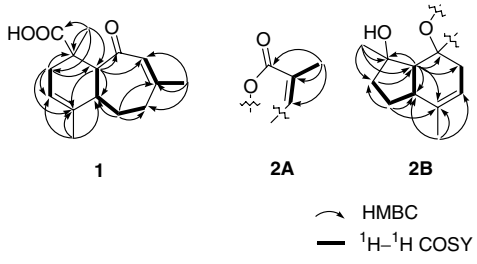

Figure 2. Selective ^1H–^1H COSY and HMBC correlations of **1**, partial structures 2A and 2B.

Table 1. ^1H and ^{13}C NMR spectral data of compounds **1** and **2**

C #	1		2	
	^1H[a]	^{13}C[b]	^1H[a]	^{13}C[b]
1		42.7 (s)[d]		78.5 (s)[d]
2	α 3.20 br d (17.0)[c]	31.6 (t)	α 1.79 m	41.8 (t)
	β 2.04 dd (18.0, 4.0)		β 1.85 m	
3	5.42 br s	121.6 (d)	α 1.93 m	25.3 (t)
			β 1.43 m	
4		130.7 (s)	2.57 m	40.9 (d)
5	2.58 m	37.1 (d)		137.3 (s)
6	α 1.30 m	31.8 (t)	5.25 dd (2.5, 1.5)	117.2 (d)
	β 2.36 m			
7	α 2.40 m	33.3 (t)	α 1.87 m	39.7 (t)
	β 2.50 m		β 2.53 m	
8		160.0 (s)		85.5 (s)
9	5.86 s	130.5 (d)	1.99 d (12.5)[c]	56.6 (d)
10		202.7 (s)	1.12 s	26.0 (q)
11	3.32 d (8.5)	54.2 (d)	1.71 s	20.2 (q)
12		183.4 (s)	7.17 d (1.5)	152.2 (d)
13	1.23 s	24.9 (q)		129.1 (s)
14	1.63 s	20.9 (q)		173.8 (s)
15	1.94 s	26.6 (q)	1.94 d (1.5)	10.6 (q)

[a] Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C.
[c] J values (in Hz) in parentheses.
[d] Multiplicity deduced by DEPT and indicated by usual symbols.

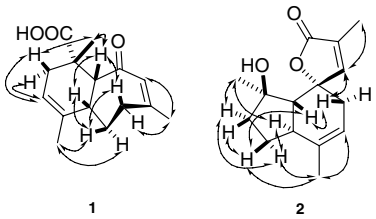

Figure 3. Selective NOE correlations of **1** and **2**.

the presence of hydroxy group. The ^{13}C NMR spectral data revealed the presence of three methyls, three sp^3 methylenes, two sp^3 methines, two sp^3 oxygenated quaternary carbons, two sp^2 methines, two sp^2 quaternary carbons, and one carbonyl carbon. The NMR signals [δ_H 7.17 (1H, d, J = 1.5 Hz, H-12), and 1.94 (3H, d, J = 1.5 Hz, H_3-15); δ_C 173.8 (C-14, qC), 152.2 (C-12, CH), 129.1 (C-13, qC), 10.6 (C-15, CH_3)], together with the IR absorption at ν_{max} 1738 cm^{-1}, indicated the presence of partial structure 2A, which was confirmed by the ^1H–^1H COSY and HMBC correlations as shown in Figure 2. Furthermore, the methyl protons resonating at δ1.12 (3H, s, H_3-10) and the quaternary carbon resonating at δ 78.5 (C-1) indicated that this methyl and a hydroxy group should be positioned at C-1. The ^1H–^1H COSY correlations between H_2-2 and H_2-3, H_2-3 and H-4, H-4 and H-9, and H-6 and H_2-7, and informative HMBC correlations from H-9 to C-4, C-5, C-7, and C-8, H_3-10 to C-1, C-2, and C-9; H_3-11 to C-4, C-5, and C-6 established the partial structure 2B (Fig. 2). It was further found that no useful HMBC correlations could be used to connect these two partial

structures, however, the C-8 spiro-butenolide functionality was deduced according to the molecular formula of **2**. The IR absorption at ν_{max} 1738 cm^{-1} also confirmed the butenolide moiety of **2**. Thus, the planar structure of **2** was established.

The relative stereochemistry of **2** was determined by the NOE correlations observed in a NOESY experiment (Fig. 3). In the NOESY spectrum of **2**, H-9 showed an NOE correlation with H-7β (2.53, m), which in turn correlated with H-12, suggesting the β orientations of H-9 and H-12. Furthermore, H_3-10 showed an NOE correlation with H-4, while both protons did not show correlations with H-9, suggesting the α orientations of H_3-10 and H-4. Therefore, the relative structure of **2** was established unambiguously. The determination of absolute structure of **2** could not be carried out due to the paucity and consumption of this metabolite in the measurement of MS spectrum.

It has to be noted here that although the biosynthesis of **1** follows the isoprene rule, however, the skeleton of **1** was discovered for the first time. The closely related skeleton could be traced to perforenones A and B (perforane skeleton) isolated from marine alga in 1975.[20] The skeleton of **1** is an unrearranged one as compared to perforane (Fig. 1), in which the methyl group at C-1 was shifted to C-11.

Preliminary biological activity screening revealed that compound **1** is not active against the growth of a limited panel of cancer cells, including A549 (human lung carcinoma), HepG2 and Hep3B (both human hepatocellular carcinoma), MCF7 and MAD-MB-231 (both human breast carcinoma) cells. Due to the paucity of compound **2**, the cytotoxicity of this metabolite toward the above cell lines has not been determined.

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