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Novel cyclic sesquiterpene peroxides from the Formosan soft coral *Sinularia* sp.

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Abstract—Four novel cyclic peroxide-containing sesquiterpenes (**1**–**4**), with a γ-alkylidene-α-methyl-α,β-unsaturated γ-lactone moiety, have been isolated from a Formosan soft coral of the genus *Sinularia*. Their structures were elucidated mainly by extensive 1D and 2D NMR experiments.

Soft coral of the genus *Sinularia* has been found to be a rich source of bioactive secondary metabolites.[1] Cyclic peroxides are of great interest because they often exhibit a wide spectrum of biological activities including antiparasitic and cytotoxic activities against cancer cells.[2–4] During the course of our investigation on the bioactive chemical constituents from marine invertebrates,[5–15] four novel sesquiterpenoids, sinularioperoxides A–D (**1**–**4**) possessing a cyclic peroxide and a γ-alkylidene-α-methyl-α,β-unsaturated γ-lactone moieties, have been isolated from a soft coral *Sinularia* sp., collected off the northeastern Taiwan coast in May 2004, at a depth of 10 m. We describe herein the isolation and structure elucidation of these compounds.

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 *n*-hexane/EtOAc (1:4) was subjected to Sephadex LH-20 column (2 × 90 cm) using acetone and followed by normal phase HPLC (*n*-hexane/acetone, 8:1) to afford compounds **1** (5.0 mg), **2** (1.0 mg), **3** (1.1 mg), and **4** (3.0 mg).

Sinularioperoxide (**1**), $[\alpha]_D^{25}$ −2 (*c* 1.64, $CHCl_3$), was isolated as a colorless oil. Its HRESIMS exhibited a pseudomolecular ion peak at *m*/*z* 303.1209 $[M+Na]^+$, corresponding to the molecular formula $C_{15}H_{20}O_5$ (calcd 303.1208). Thus, **1** possesses six degrees of unsaturation. The IR spectrum of **1** was found to exhibit absorptions of hydroxy (3366 cm^{-1}), carbon–carbon double bond (1668 cm^{-1}), and lactone carbonyl groups (1757 cm^{-1}). The characteristic NMR signals [δ_H 7.04 (1H, s, H-3), 5.66 (1H, s, H-5), and 2.01 (3H, s, H_3-13); δ_C 170.7 (C-1), 129.1 (C-2), 138.7 (C-3), 146.2 (C-4), 117.5 (C-5), and 10.5 (C-13)] and UV absorption at λ_{max} 273 nm indicated the presence of γ-alkylidene-α-methyl-α,β-unsaturated γ-lactone moiety.[16,17] Signals resonating at δ 135.4 (s) and 127.5 (d) in ^{13}C NMR spectrum of **1** suggested the presence of a trisubstituted double bond. The above functionalities account for five of the six degrees of unsaturation in the molecule of **1**, suggesting that there should be an additional ring in the molecule of sinularioperoxide A. The gross structure of metabolite **1** was further established by the 2D NMR

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studies, particularly in ^1H–^1H COSY, HMQC, and HMBC experiments. The correlations of ^1H–^1H COSY revealed two spin systems, as depicted in Figure 1. Its HMBC spectrum showed many informative correlations, such as H-5 to C-3, C-4, and C-6, H_3-13 to C-1, C-2, and C-3, H_3-14 to C-5, C-6, and C-7; and H_3-15 to C-9, C-10, and C-11 (Fig. 1). Moreover, acetylation of **1** yielded **1a**. It was found that the NMR signal of H_2-12 in **1** at δ 4.21 was downfield shifted to δ 4.61 in **1a** (Tables 1 and 2), confirming that the hydroxy group of **1** should be attached at C-12. From the above 2D NMR data, the molecular formula obtained from the HRESIMS, and the reductive cleavage of **1a** by zinc powder and acetic acid in EtOAc to afford diol **1b**[18,19] (99% yield) (Fig. 2 and Table 2) confirmed the cyclic peroxide linkage between C-6 and C-9. Combination of the above observations led to the establishment of the planar structure of **1**.

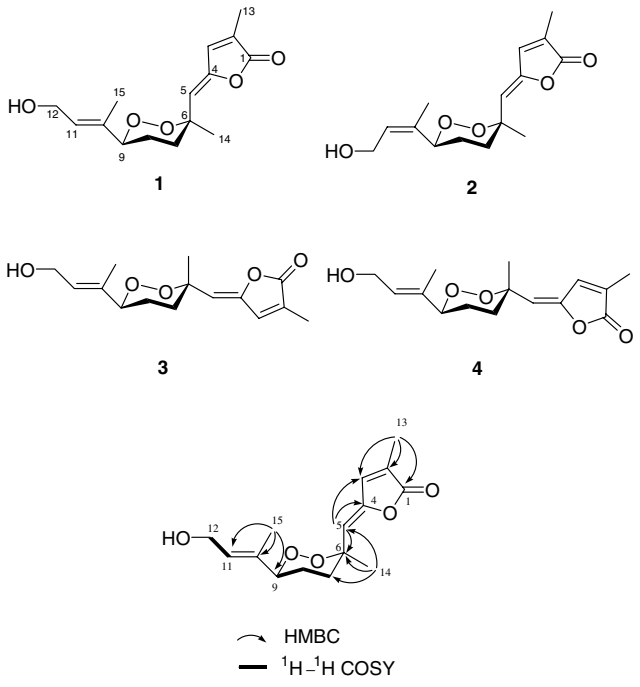

Figure 1. Selective ^1H–^1H COSY and HMBC correlations of **1**.

The relative stereochemistry of **1** was determined by the NOE correlations observed in a NOESY experiment and also by the analysis of the coupling constant of H-9. It was found that this proton (δ 4.44) displayed a large coupling constant ($J = 11.0$ Hz), revealing its axial orientation. In the NOESY spectrum of **1**, H-9 was found to show NOE interactions with both H-8α and H-7α, and H_3-14 exhibited NOE correlation with H-7α and H-7β. Thus, H_3-14 must be equatorially oriented, and positioned on the α face (Fig. 3). The *E* geometry was assigned for the 10,11-double bond on the basis of the observation of an NOE correlation between H_3-15 and H_2-12. The *Z* geometry of both 2,3- and 4,5-double bonds was established by the observation of the NOE correlations of H_3-13/H-3 and H-3/H-5. Therefore, the relative structure of **1** was established unambiguously.

Compound **2**, $[\alpha]_D^{25}$ −40 (*c* 0.80, $CHCl_3$), revealing IR absorptions at 3422, 1755, and 1668 cm^{-1}, and UV absorption (MeOH) at λ_{max} (log ε) 272 nm (4.19), was isolated as a colorless oil. Its HRESIMS exhibited a pseudomolecular ion peak at *m*/*z* 303.1206 $[M+Na]^+$ and established the same molecular formula as that of **1**. Thus, **2** is an isomer of **1**. It was found that except for H-9, the ^1H NMR spectral data of **2** are very similar to those of **1**. Inspection of the ^{13}C NMR data of compounds **1** and **2** also showed obvious differences between the carbon shifts at C-9 and C-15. The carbon resonance

Table 1. ^1H and ^{13}C NMR spectral data of compounds **1**–**4**

C #	1		2		3		4	
	^1H[a]	^{13}C[b]	^1H[a]	^{13}C[b]	^1H[a]	^{13}C[b]	^1H[a]	^{13}C[b]
1		170.7 (s)[d]		170.7 (s)		170.6 (s)		170.2 (s)
2		129.1 (s)		129.2 (s)		129.6 (s)		131.6 (s)
3	7.04 s	138.7 (d)	7.06 s	138.6 (d)	7.00 s	138.7 (d)	7.56 s	136.8 (d)
4		146.2 (s)		146.2 (s)		146.8 (s)		148.8 (s)
5	5.66 s	117.5 (d)	5.66 s	117.3 (d)	5.27 s	116.4 (d)	5.60 s	115.8 (d)
6		81.0 (s)		81.1 (s)		80.3 (s)		79.5 (s)
7	α 1.80 m	34.0 (t)	1.80 m	33.7 (t)	2.12 m, 2H	31.6 (t)	1.92 m, 2H	34.0 (t)
	β 2.58 ddd (13.0, 3.5, 3.5)[c]		2.59 ddd (12.0, 3.5, 3.5)					
8	α 1.70 m	25.4 (t)	1.58 m	25.4 (t)	1.83 m	23.1 (t)	1.81 m	23.7 (t)
	β 1.75 m		1.83 m		1.98 m		2.02 m	
9	4.44 d (11.0)	84.8 (d)	4.87 dd (10.3, 2.5)	80.2 (d)	4.41 dd (8.0, 3.5)	83.8 (d)	4.44 br d (9.5)	84.5 (d)
10		135.4 (s)		136.0 (s)		136.0 (s)		135.6 (s)
11	5.63 t (6.5)	127.5 (d)	5.62 t (6.5)	129.7 (d)	5.70 t (6.5)	126.7 (d)	5.71 t (6.5)	127.3 (d)
12	4.21 d (6.5), 2H	59.1 (t)	4.16 dd (12.8, 6.8)	58.5 (t)	4.25 d (6.5), 2H	59.3 (t)	4.26 d (6.5), 2H	59.2 (t)
			4.21 dd (12.8, 7.3)					
13	2.01 s	10.5 (q)	2.02 s	10.5 (q)	2.01 s	10.5 (q)	2.01 s	10.8 (q)
14	1.41 s	25.2 (q)	1.41 s	25.4 (q)	1.57 s	22.6 (q)	1.56 s	24.0 (q)
15	1.68 s	14.0 (q)	1.69 s	19.5 (q)	1.75 s	13.8 (q)	1.75 s	13.7 (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.

Table 2. ^{1}H and ^{13}C NMR chemical shifts of derivatives **1a** and **1b**

C #	1a		1b	
	^{1}H[a]	^{13}C[b]	^{1}H[c]	^{13}C[d]
1		170.7 s[f]		170.3 s
2		129.1 s		129.0 s
3	7.05 s	138.7 d	6.99 s	138.6 d
4		146.2 s		146.3 s
5	5.66 s	117.4 d	5.25 s	120.0 d
6		81.0 s		72.8 s
7	1.80 m	34.0 t	1.80 m	39.1 t
	2.60 br d (10.7)[e]		1.90 m	
8	1.70 m	25.5 t	1.67 m	29.7 t
	1.75 m		1.30 m	
9	4.45 br d (7.5)	84.5 d	4.05 t (6.2)	77.3 d
10		137.7 s		143.0 s
11	5.54 t (6.2)	122.3 d	5.59 t (6.5)	119.6 d
12	4.61 d (6.8)	60.7 t	4.62 d (6.8)	60.9 t
13	2.02 s	10.4 q	2.00 s	10.5 q
14	1.41 s	25.2 q	1.48 s	29.0 q
15	1.71 s	14.0 q	1.68 s	12.3 q
OAc	2.06 s	20.9 q	2.06 s	21.0 q
		170.9 s		171.0 s

[a] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C.
[c] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[d] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[e] *J* values (in Hz) in parentheses.
[f] Multiplicity deduced by DEPT and indicated by usual symbols.

of C-9 at δ 84.8 in **1** was found to be upfield shifted to δ 80.2 in **2**, while that of C-15 at δ 14.0 in **1** was shifted to lower field (δ 19.5) in **2** (Table 1). The above differences may be due to the *E* geometry of 10,11-double bond in **1** has been converted to *Z* geometry in **2**. Those findings were further confirmed by an NOE correlation between H_3-15 and H-11 (Fig. 3). This phenomenon can be explained by the significant γ-effect[20,21] arising from the steric compression between 11-CH_2OH and C-15 in **1** and 11-CH_2OH and C-9 in **2**. After detailed examination of 1D and 2D NMR, the structure of **2** was established and named as sinularioperoxide B.

Sinularioperoxide C (**3**), $[\alpha]_D^{25}$ −2 (*c* 1.68, $CHCl_3$), was isolated as a colorless oil. The molecular formula **3** was determined by HRESIMS and was found to be identical to that of **1**. The IR (ν_{max} 3366, 1755, and 1672 cm^{-1}) and UV (λ_{max} 272 nm) spectral data of **3** are almost the same as those of **1**. Thus, **3** is the geometric isomer of both **1** and **2**. By comparison of the NMR data of **3** with those of **1**, the obvious differences were observed for chemical shifts of C-14 (δ 22.6 in **3** and 25.2 in **1**), H-5 (δ 5.27 in **3** and 5.66 in **1**), and H-7 (δ 2.12, 2H, in **3** and 1.80 and 2.58 in **1**) (Table 1). These findings suggested that the equatorial methyl H_3-14 in **1** might be converted to the axial orientation in **3**. The above findings were further confirmed by the detailed inspection of the NOESY spectrum, which showed NOE correlations of H-9/H-8α and H-8β/H_3-14 (Fig. 3). Thus, the molecular structure of **3**, including the relative configuration, was fully determined.

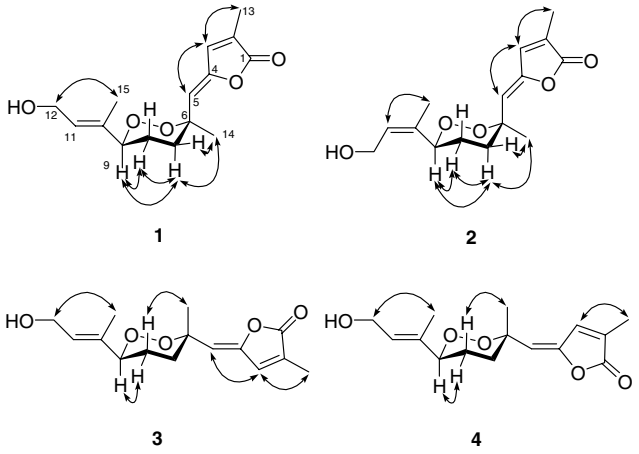

Figure 3. Selective NOESY correlations of **1**–**4**.

HRESIMS of sinularioperoxide D (**4**), appeared as a colorless oil with an $[\alpha]_D^{25}$ value of −2 (*c* 1.68, $CHCl_3$), exhibited a pseudomolecular ion peak at *m*/*z* 303.1206 $[M+Na]^+$ and established the same molecular formula as those of **1**–**3**. The IR and UV spectra of **4** showed absorptions at 3418, 1766, and 1666 cm^{-1} and 273 nm, respectively. By comparison of the ^{1}H and ^{13}C NMR spectra of **3** and **4**, these two compounds have very similar spectral data except for the deviation between proton signals of H-5 in **3** (δ 5.27) and **4** (δ 5.60) (Table 1). The NOESY spectra of **3** and **4** were also similar except for the lack of an NOE correlation between H-3 and H-5 in **4** (Fig. 3). Therefore, **4** should contain an *E* double bond between C-4 and C-5 and the structure of this metabolite was determined unambiguously.

Preliminary biological activity screening revealed that these four compounds are not active against the growth of a limited panel of cancer cell lines, including A549 (human lung carcinoma), HepG2 (human hepatocellular carcinoma), MCF7 and MAD-MB-231 (both human breast carcinoma) cells. The results of further biological activity screening will be reported elsewhere in the future.

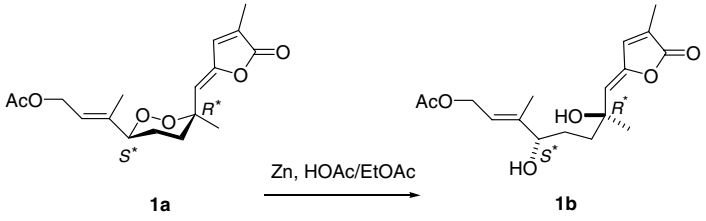

Figure 2. Conversion of **1a** to diol **1b**.

It has to be noted here that cyclic peroxides **1**–**4** are the brand-new type of cyclic peroxy terpenoids with a γ-alkylidene-α-methyl-α,β-unsaturated γ-lactone moiety. To the best of our knowledge, this type of terpenoids was discovered for the first time.

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