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Sinulochmodins A–C, Three Novel Terpenoids from the Soft Coral *Sinularia lochmodes*

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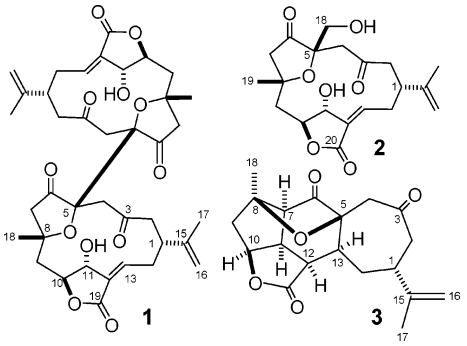
ABSTRACT



An unprecedented C,C-linked dimeric norcembranoid (sinulochmodin A, 1), a novel isocembranoid (sinulochmodin B, 2), and a novel yonarane norditerpenoid (sinulochmodin C, 3) were isolated from the soft coral *Sinularia lochmodes*. The structures of these metabolites were elucidated by extensive spectroscopic analysis and on the basis of the absolute structures of two related norditerpenoids (4 and 5), which were determined for the first time by a modified Mosher method. A plausible pathway for the biosynthesis of 1 and 3–5 from 2 was postulated.

Soft corals, particularly those of the genus *Sinularia*, have been recognized as a rich source of bioactive C-4 norcembranoids.[1−5] In a continuation of our investigation on the terpenoidal metabolites from marine invertebrates,[6−9] we have isolated a novel dimeric norcembranoid, sinulochmodin A (**1**), a novel isocembranoid, sinulochmodin B (**2**), and a new yonarane norditerpenoid, sinulochmodin B (**3**) along with 5-*epi*-sinuleptolide (**4**) and sinuleptolide (**5**) from a Formosan soft coral *Sinularia lochomdes* (Kolonko, 1926). The structure elucidations of the new metabolites were

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Table 1. ^{1}H and ^{13}C NMR Data of **1**−**3**

	1[a]		**2**[b]		**3**[c]	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.22 m	39.3 (CH)[e]	3.10 br t (5.6)	39.6 (CH)	2.66 m	37.4 (CH)
2	2.37 dd (15.0, 7.0)[d] α	47.5 (CH_2)	2.58 2H, d (5.6)	47.3 (CH_2)	2.47 m α	31.9 (CH_2)
	2.70 dd (15.0, 3.0) β				2.34 m β	
3		208.0 (qC)		206.0 (qC)		206.7 (qC)
4	3.41 d (18.5) α	47.3 (CH_2)	3.01 d (16.8) α	46.1 (CH_2)	2.56 d (7.8) α	50.2 (CH_2)
	3.26 d (18.5) β		2.91 d (16.8) β		2.66 d (7.8) β	
5		88.0 (qC)		87.1 (qC)		80.0 (qC)
6		215.6 (qC)		216.6 (qC)		213.5 (qC)
7	2.65 d (19.5) α	50.2 (CH_2)	2.86 d (18.8) α	51.0 (CH_2)	2.67 d (8.0)	57.8 (CH)
	2.33 d (19.5) β		2.68 d (18.8) β			
8		80.0 (qC)		79.2 (qC)		89.7 (qC)
9	1.69 dd (15.0, 6.0) α	42.1 (CH_2)	1.99 dd (14.4, 6.6) α	43.9 (CH_2)	2.55 dd (15.0, 7.5) α	46.6 (CH_2)
	2.07 dd (15.0, 11.0) β		2.28 dd (14.4, 11.6) β		2.22 dd (15.0, 7.5) β	
10	4.61 dd (11.0, 6.5)	80.3 (CH)	5.00 dd (10.6, 6.6)	82.1 (CH)	5.15 dd (8.0, 7.5)	83.3 (CH)
11	4.20 s	77.0 (CH)	4.60 s	77.1 (CH)	3.76 ddd (10.5, 8.0, 8.0)	51.5 (CH)
12		132.0 (qC)		134.1 (qC)	2.82 t (10.5)	38.4 (CH)
13	6.59 dd (8.5, 7.0)	143.7 (CH)	6.63 dd (9.6, 6.0)	141.9 (CH)	2.94 dd (11.0, 4.5)	51.9 (CH)
14	2.37 ddd (15.0, 7.0,7.0) α	30.8 (CH_2)	2.19 ddd (14.0, 6.0,6.0) α	30.1 (CH_2)	2.56 m α	46.5 (CH_2)
	3.47 ddd (15.0, 8.5, 5.5) β		3.85 ddd (14.0, 9.6, 4.8) β		2.99 d (14.0) β	
15		147.5 (qC)		148.2 (qC)		147.9 (qC)
16	5.01 s, 4.94 s	111.2 (CH_2)	4.91 s, 4.83 s	111.0 (CH_2)	4.83 s, 4.75 s	111.0 (CH_2)
17	1.89 3H, s	20.7 (CH_3)	1.69 3H, s	21.1 (CH_3)	1.74 3H, s	20.7 (CH_3)
18	1.72 3H, s	25.8 (CH_3)	4.22 d (11.2), 3.97 d (11.2)	66.0 (CH_2)	1.27 3H, s	22.8 (CH_3)
19		167.9 (qC)	1.72 3H, s	26.7 (CH_3)		176.1 (qC)
20				169.5 (qC)		

[a] Spectra recorded at 500 MHz for ^{1}H in $CDCl_3$ and 125 MHz for ^{13}C. [b,c] Spectra recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C (b) in C_5D_5N and (c) in $CDCl_3$. [d] The *J* (Hz) are shown in parentheses. [e] Attached protons were determined by DEPT experiments.

accomplished by extensive spectroscopic analysis and on the basis of the absolute structures of **4** and **5**, which were determined for the first time by a modified Mosher method.[10,11]

The soft coral *S. lochomdes* (1.9 kg) was collected by hand using scuba off the coast of the southernmost tip of Taiwan. The EtOH extract (64.4 g) of the frozen organsim was partitioned between *n*-hexane and H_2O and then between EtOAc and H_2O. The EtOAc-soluble portion (2.1 g) was subjected to column chromatography (Si gel, EtOAc−*n*-hexane, 0:10 to 10:0, gradient). A fraction eluted with EtOAc−*n*-hexane (1:4) was purified by normal-phase HPLC (EtOAc−*n*-hexane, 1:5) to afford **3** (1 mg). Another fraction eluted with EtOAc−*n*-hexane (1:1) was further separated utilizing normal-phase HPLC (EtOAc−*n*-hexane, 1:3 to 2:1, gradient) to yield **1** (3 mg), **2** (3.7 mg), **4** (35 mg), and **5** (15 mg).

The absolute configurations of known metabolites **4**, $[\alpha]^{25}_{D}$ −119 (*c* 4.0, $CHCl_3$),[2] and **5**, $[\alpha]^{25}_{D}$ +63 (*c* 4.0, $CHCl_3$),[2] were determined by a modified Mosher method.[10] Comparison of ^{1}H NMR chemical shifts between the (*R*)- and (*S*)-MTPA esters of each compound (see Figure 1) led to the assignment of the *R*-configuration at C-11 for both compounds. Therefore, the absolute structures of **4** and **5** were unambiguously determined as shown in their formulas.

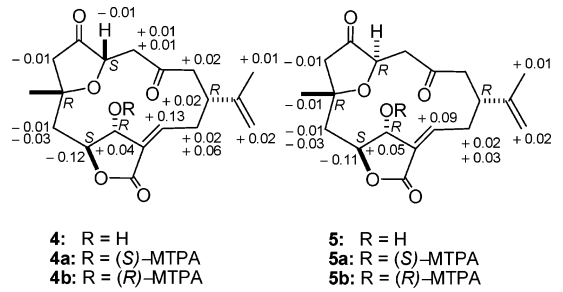

Figure 1. ^{1}H NMR chemical shift differences $\Delta\delta$ ($\delta_S - \delta_R$) in ppm for the MTPA esters of **4** and **5**.

Sinulochmodin A (**1**), $[\alpha]^{25}_{D}$ −129 (*c* 1.2, $CHCl_3$), was obtained as a white powder and had the molecular formula $C_{38}H_{46}O_{12}$ as determined by HRESIMS (*m/z* calcd 717.2887; found 717.2882, $[M + Na]^+$). The IR spectrum revealed the presence of hydroxy (3402 cm^{-1}), conjugated γ-lactone (1749 cm^{-1}), and ketone (1720 cm^{-1}) groups. The NMR spectra (Table 1) indicated the presence of 19 carbons, including one 1,1-disubstituted double bond, one trisubstituted double bond, three carbonyls, two methyls, five sp^3

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methylenes, three sp^3 methines (including two oxygenated), and two sp^3 quaternary oxycarbons. From the above observations and the molecular formula of **1**, it is clear that **1** is a symmetric dimer of a norcembranoidal half structure ($C_{19}H_{23}O_6$). From the NMR spectral data, and by extensive analyses on the 2D NMR ($^1H-^1H$ COSY and HMBC) correlations, it was found that the molecular framework for the monomer of **1** should be nearly identical with those of **4** and **5**. The presence of two oxygenated quaternary carbons and the absence of H-5 in **1** (cf. **4** or **5**) indicated that two monomers are linked by C−C bonds through their C-5's. The upfield-shifted C-18 (δ 25.8) in **1** and that in **4** (δ 26.6), relative to that in **5** (δ 29.6),[2] proved 5-*epi*-sinuleptolide (**4**) to be a monomeric moiety of **1**. The indicated NOE (Figure 2) correlations also revealed that **4** is the monomeric moiety

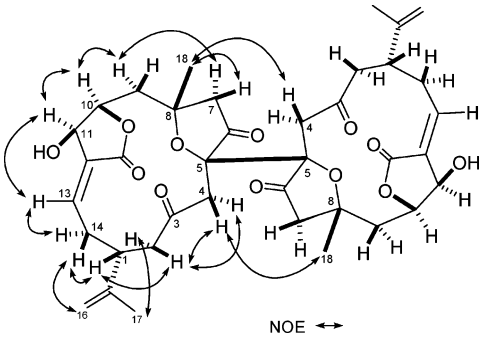


Figure 2. Key NOESY correlations of **1**.

of **1** and confirmed the 5,5-linkage of the two monomers from the significant NOE correlations found between H_3-18 and one proton of H_2-4 (δ 3.26). Therefore, the structure of sinulochmodin A (**1**) was established and was found to be the first dimeric norcembranoid via C,C-linkage.

Sinulochmodin B (**2**), obtained as colorless prisms, mp 165−166 °C, $[\alpha]^{25}_D$ −91 (*c* 1.5, C_5H_5N), was found to possess a molecular formula $C_{20}H_{26}O_7$ as established by HRESIMS (*m/z* calcd 401.1576; found 401.1577, $[M + Na]^+$). Its IR spectral absorptions (3419, 1780, 1761, and 1709 cm^{-1}), revealed functionalities similar to those found in **1**. Comparison of its NMR data (Table 1) with those of **4**[2] revealed that **2** differs from **4** only in the presence of a hydroxymethyl group (δ_H 3.97 and 4.22, each 1H, d, J = 11.2 Hz; δ_C 66.0, CH_2) instead of the presence of a proton at C-5. The HMBC correlations found from the hydroxymethyl protons to C-6 (δ 216.6, C) and from H_2-4 to the hydroxymethyl carbon C-18 (δ 66.0, CH_2) and C-5 (δ 87.1, C) suggested that the hydroxymethyl group should be placed at C-5. The $^1H-^1H$ COSY and HMBC correlations were found to be mostly identical to those observed in **1** and **4**. The upfield-shifted C-19 (δ 26.7) in **2**, which is close to that of C-18 in **4,** reflected the *cis* orientation[1,2] of both hydroxymethyl at C-5 and methyl at C-8. On the basis of the above findings, the structure of sinulochmodin B was established and was further confirmed by single-crystal X-ray diffraction analysis (Figure 3).[12] Thus, compound **2** was

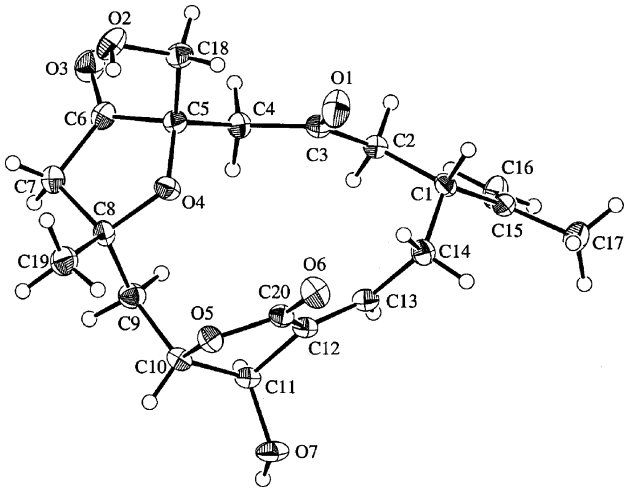


Figure 3. Molecular structure of **2** based on X-ray analysis.

considered as a novel isocembranoid in which a methyl substituent has been oxidized to hydroxymethyl group and migrated from C-4 of a cembranoid to C-5.

It is noteworthy to mention that the absolute structures of **1** and **2**, as shown in their formulas, were confirmed after determination of the absolute structure of **4** as they have the same sign of optical rotation and similar $[\alpha]_D$ values, and on the basis that they were isolated from the same organism.

Sinulochmodin C (**3**), isolated as a white powder, $[\alpha]^{25}_D$ −12.5 (*c* 0.4, $CHCl_3$), has a molecular formula $C_{19}H_{22}O_5$ from its HRESIMS (*m/z* calcd 331.1559; found 331.1550, $[M + H]^+$), implying nine degrees of unsaturation. Its IR spectrum revealed the presence of carbonyls (1761, 1705 cm^{-1}) and absence of hydroxyls. Its ^{13}C NMR data (Table 1) also led to the assignment of one lactone carbonyl, two ketone carbonyls, and one isopropenyl group, suggesting that the structure of **3** should possess two additional rings compared to those found in **4** or **5** as the signals of (12,-13)-double bond of **4** and **5** disappeared. This was further confirmed by the HMBC correlations (Figure 4) observed from H-12 (δ 2.82) to C-13 (δ 51.9) and C-5 (δ 80.0), H-13 (δ 2.94) to C-5, and from H-7 (δ 2.67) to C-12 (δ 38.4). These correlations revealed the carbon−carbon bond forma-

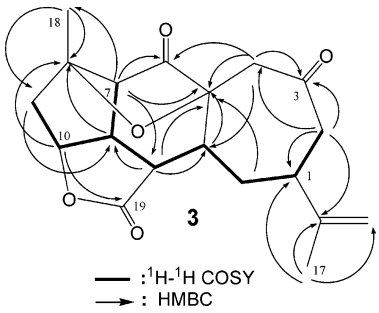


Figure 4. COSY and HMBC correlations in **3**.

tion between C-5 and C-13, and C-7 and C-11. By comparison of NMR data of **3** with its molecular formula, it was found that an oxygen has to be placed between C-5 and C-8 to form an oxo-THF ring. The NOESY spectrum of **3** displayed correlations (Figure 5) between the α-oriented

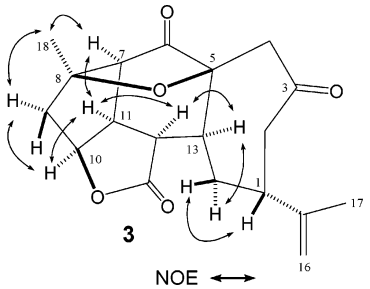
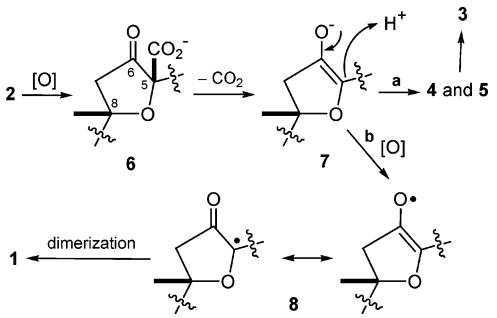


Figure 5. Key NOESY correlations of **3**.

H-10 and H-11, H-11 and H-12, H-11 and H-7, H-7 and H_3-18, reflecting the *β*-orientation of the 5,8-ether bridge. One of the methylene protons at C-14 (*δ* 2.56) was found to correlate with H-13 and assigned as H-14α. The other C-14 proton showed NOE interaction with H-1, revealing the α-orientation of the isopropenyl group. On the basis of the above observations and by biosynthetic consideration (see Scheme 1), the structure of **3** was fully established and was found to possess the 1*R*,5*S*,7*S*,8*R*,10*S*,11*S*,12*R*,13*S*-configuation. Sinulochmodin C (**3**) was found to be the first yonarane type norditerpenoid[3] with an oxo-THF ring.

(12) Crystallographic data for **2** have been deposited with the Cambridge Crystallographic Data Center as supplementry publication number CCDC 273177. Copies of the data can be obtained, free of charage, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Scheme 1. Proposed Biosynthetic Pathway for **1** and **3**−**5** from **2**



A plausible biosynthetic pathway for **1**, and **3**−**5** from **2** was proposed as illustrated in Scheme 1. Oxidation of the primary alcohol moiety of **2** would yield the carboxylate **6** which could be decarboxylated to the enolate **7**. Ketolization and the following protonization of **7** would afford **4** and **5**. The subsequent carbon−carbon bond formation in **4** and/or **5** by nucleophilic condensation between C-5 and C-13, and C-7 and C-11 further afforded **3**. Moreover, the oxidation of **7** by losing an electron would give radical **8**, which could be dimerized to **1**. To the best of our knowledge, the oxidative radical coupling to form dimeric norcembranoids of this type has not found previously.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**−**3**; ^{1}H NMR spectra of **4a**,**b** and **5a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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