

New Norcembranoids from the Soft Coral *Sinularia lochmodes*

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Two new C-4 norcembranoids sinulochmodins D (**1**) and E (**2**), along with three known norditerpenoids (**3-5**), have been isolated from the organic extract of a Taiwanese soft coral *Sinularia lochmodes* (Kolonko). The structures of **1** and **2** were determined on the basis of extensive spectroscopic analyses and by comparison of their spectral data with those of related metabolites.

Keywords: Norcembranoids; Soft coral; Norditerpenoids; *Sinularia lochmodes*.

INTRODUCTION

During the course of our search for bioactive metabolites from marine invertebrates of Taiwanese waters, several cytotoxic norditerpenoids¹⁻³ have been isolated from soft corals of the genus *Sinularia* (family Alcyoniidae). A previous chemical study on the EtOAc-soluble portion of the EtOH extract of *Sinularia lochmodes* led to the isolation and identification of a C-4 norcembranoid dimer, an isocembranoid and a yonarane norditerpenoid.⁴ Further chemical investigation on the same organism furnished two new C-4 norcembranoids, sinulochmodins D (**1**) and E (**2**) in addition to three known norditerpenoids (**3-5**). The structures of **1** and **2** were elucidated by spectroscopic analyses, including 2D NMR (¹H-¹H COSY, HMQC, HMBC, and NOESY), and by spectral comparisons with the related compound **6**.

RESULTS AND DISCUSSION

The tissues of the soft coral *S. lochmodes* were exhaustively extracted with EtOH. The EtOH extract was partitioned between *n*-hexane and H₂O and then between EtOAc and H₂O. The EtOAc-soluble portion was concentrated under vacuum, and then fractionated by silica gel column chromatography. The eluted fractions were puri-

fied by normal phase HPLC to afford **1-5** (see Experimental section).

Sinulochmodin D (**1**) was obtained as a white solid, $[\alpha]_D^{25} + 17.4^\circ$ (*c* 1.4, CHCl₃). Its HRFABMS spectrum exhibited a molecular ion peak at *m/z* 377.1967 [M + H]⁺, consistent with a molecular formula C₂₁H₂₈O₆ and eight degrees of unsaturation. The IR spectrum showed absorption bands due to the presence of an α,β -unsaturated- γ -lactone (1755 and 1645 cm⁻¹) and ketone carbonyl (1709 cm⁻¹) moieties. Moreover, FABMS exhibited an ion peak at *m/z* 331 [M - EtOH + H]⁺, revealing the presence of an ethoxy group in **1**. This was further supported by the proton signals appearing at δ 3.40, 3.47 (each 1H, q, *J* = 7.0 Hz), and 1.14 (3H, t, *J* = 7.0 Hz) in the ¹H NMR spectrum of **1**. The ¹³C NMR spectrum of **1** showed signals of twenty-one carbon atoms (Table 1) which were identified by DEPT spectra as three methyl, seven methylene, five methine, and six quaternary carbons. The seven sp² carbon signals appearing at δ 212.4 (qC), 207.8 (qC), 174.0 (qC), 154.6 (CH), 132.6 (qC), 145.6 (qC), and 112.8 (CH₂) were attributable to the carbons of two normal ketone carbonyls, an α,β -conjugated ester carbonyl, and a 1,1-disubstituted double bond in **1**, respectively. Therefore, compound **1** is an ethoxylated tricyclic norditerpenoid. Moreover, the ¹H NMR data of **1** revealed the presence of an isopropylene group (δ 4.77, 4.88, each 1H, s, and 1.71, 3H, s), a tertiary methyl bound to an oxygenated carbon (1.34, 3H, s, H₃-18), and two oxy-

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Table 1. NMR spectral data for compounds **1** and **2**

#	1		2	
	$^1\text{H}^a$	$^{13}\text{C}^b$	$^1\text{H}^a$	$^{13}\text{C}^b$
1	2.45 dddd (11.0, 11.0, 2.5, 2.5) ^c	37.0 (CH) ^d	2.47 dddd (11.0, 11.0, 3.0, 3.0)	37.1 (CH)
2 α	2.33 t (11.0)	50.5 (CH ₂)	2.36 t (11.0)	50.2 (CH ₂)
2 β	2.50 dd (11.0, 2.5)		2.53 dd (11.0, 3.0)	
3		207.8 (qC)		208.1 (qC)
4 α	2.55 d (14.0)	44.3 (CH ₂)	2.58 dd (14.5, 2.5)	44.0 (CH ₂)
4 β	2.63 dd (14.0, 11.0)		2.64 dd (14.5, 10.5)	
5	4.49 d (11.0)	77.9 (CH)	4.45 dd (10.5, 2.5)	77.7 (CH)
6		212.4 (qC)		212.1 (qC)
7 α	2.52 d (17.5)	51.2 (CH ₂)	2.53 d (17.5)	51.0 (CH ₂)
7 β	2.38 d (17.5)		2.38 d (17.5)	
8		79.5 (qC)		79.3 (qC)
9 α	2.23 dd (15.0, 3.5)	42.1 (CH ₂)	2.26 dd (15.0, 3.0)	41.6 (CH ₂)
9 β	2.59 dd (15.0, 3.5)		2.58 dd (15.0, 3.0)	
10	5.26 br t (3.5)	79.4 (CH)	5.24 br t (3.0)	79.3 (CH)
11	7.56 s	154.6 (CH)	7.54 s	153.8 (CH)
12		132.6 (qC)		133.7 (qC)
13	4.12 dd (11.0, 3.0)	69.6 (CH)	4.54 dd (11.0, 3.0)	62.7 (CH)
14 α	1.85 ddd (11.0, 11.0, 3.0)	36.3 (CH ₂)	1.95 ddd (14.0, 11.0, 3.0)	38.2 (CH ₂)
14 β	2.00 ddd (11.0, 11.0, 3.0)		2.02 ddd (14.0, 11.0, 3.0)	
15		145.6 (qC)		145.6 (qC)
16	4.77 s, 4.88 s	112.8 (CH ₂)	4.77 s, 4.87 s	112.7 (CH ₂)
17	1.71 3H, s	18.7 (CH ₃)	1.71 3H, s	18.6 (CH ₃)
18	1.34 3H, s	28.0 (CH ₃)	1.36 3H, s	27.9 (CH ₃)
19		174.0 (qC)		173.0 (qC)
Ethyl	3.40 q (7.0)	64.6 (CH ₂)		
	3.47 q (7.0)			
	1.14 t (7.0)	15.5 (CH ₃)		

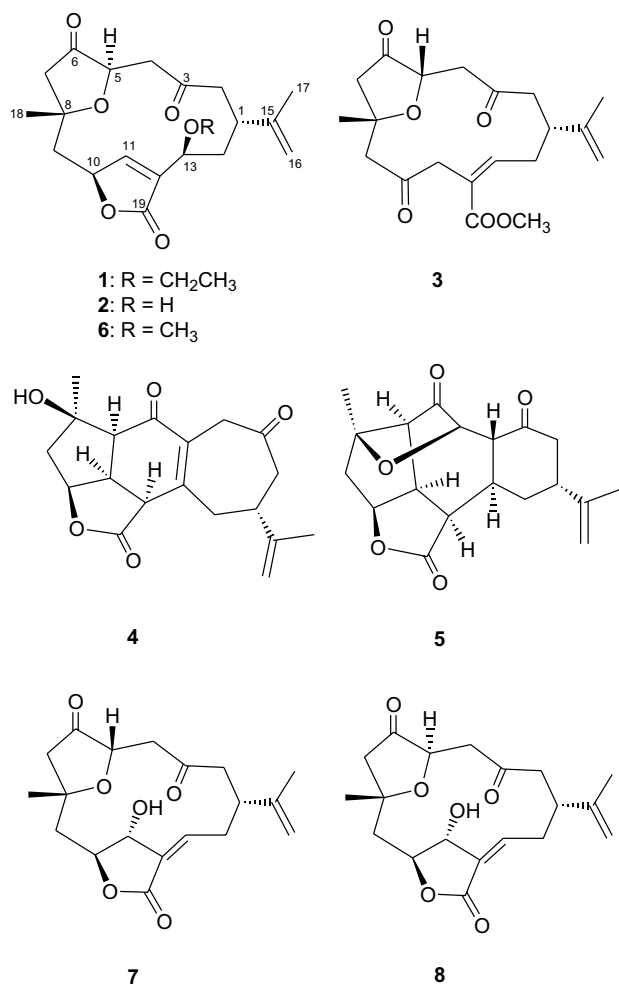
Spectra recorded at ^a500 MHz and ^b125 MHz in CDCl₃ at 25 °C. ^c*J* values in Hz in parentheses. ^dAttached protons were deduced by DEPT experiments.

methines (δ 4.49, 1H, d, *J* = 11.0 Hz and 5.26, 1H, br t, *J* = 3.5 Hz) which are diagnostic for the C-4 norcembranoids possessing 5,8-epoxy and 12,10-carbolactone moieties.¹⁻⁸ Furthermore, it was found that the NMR data of **1** (Table 1) were nearly identical with those of scabrolide C (**6**),³ isolated from *S. scabra*, except for the presence of an ethoxy group in **1** instead of the methoxy group in **6**. The gross structure of **1** together with the C-13 location of the ethoxy group were deduced from the ¹H-¹H COSY and HMBC correlations as shown in Fig. 1.

The relative stereochemistry of **1** was found to be close to that of **6** as established by the detailed analysis of NOE correlations observed in the NOESY spectrum of **1** (Fig. 1) and by comparison with those found for **6**.³ Also, the absolute structures of two related metabolites **7** and **8**, which have also been isolated previously from *S. lochmodes*,⁴ were established as shown in the representative formulas.⁴ Thus, from the biosynthetic consideration and on the basis

of the above observations, the structure of **1** was established as (1*R*,5*R*,8*R*,10*S*,13*S*)-13-ethoxy-1-isopropenyl-8-methyl-3,6-dioxo-5,8-epoxycyclotetradec-11-en-12,10-carbolactone.

Sinulochmodin E (**2**) was obtained as a white solid. Its HRFABMS spectrum exhibited a molecular ion peak at *m/z* 349.1650 [M + H]⁺, implying a molecular formula C₁₉H₂₄O₆. Its IR spectrum suggested the presence of hydroxy (3422 cm⁻¹), α,β -unsaturated- γ -lactone (1753 and 1644 cm⁻¹) and saturated ketone (1712 cm⁻¹) functionalities. The hydroxyl in **2** was further evidenced by the pseudo ion peak at *m/z* 331 [M - H₂O + H]⁺ in the FABMS. Analysis of the NMR data assigned **2** as another C-4 norcembranoid which showed similar ¹H and ¹³C NMR spectral data as those found in **1**, except for the ethoxy group in **1**. After elucidation of the gross structure of **2** utilizing the 2D NMR (¹H-¹H COSY and HMBC) spectral correlations, we found that the 13-ethoxy group in **1** was replaced by a



hydroxy group in **2**. This was further supported by the marked difference in the chemical shifts of the 13-oxy-methine in **2** (δ_{H} 4.54, dd, $J = 11.0, 3.0$ Hz; δ_{C} 62.7) relative to that of **1** (δ_{H} 4.12, dd, $J = 11.0, 3.0$ Hz; δ_{C} 69.6). However, the identical splitting patterns and J values of H-13 in

both compounds indicated the 13*S* configuration of **2**. On the basis of the above findings together with a detailed interpretation of the key NOESY correlations (Fig. 1), sinulochmodin E (**2**) was identified as (1*R*,5*R*,8*R*,10*S*,13*S*)-13-hydroxy-1-isopropenyl-8-methyl-3,6-dioxo-5,8-epoxycyclotetradec-11-en-12,10-carbolactone.

Metabolites **3-5**, which were also isolated from *S. lochmodes*, were found to be identical to the previously reported norditerpenoids: norcembrene **5** (**3**) isolated from *S. querciformis*,⁶ sacbrolide A (**4**) isolated from *S. scabra*,³ and ineleganolide (**5**) isolated from *S. inelegans*,⁹ by comparison of the physical (mp and $[\alpha]_{\text{D}}$) and spectral (MS, ¹H- and ¹³C-NMR) data. However, due to the fact that these compounds were co-isolated with **1**, **2**, **7**, and **8** from the same organism, the absolute stereochemistries of these known compounds were also assumed to be the same as shown in formulas **3-5**.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined using a Fisher-Johns melting point apparatus. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. FABMS were obtained with a VG Quattro GC/MS spectrometer. HRFABMS spectra were recorded on a JEOL-SX/SX 102A mass spectrometer. The NMR spectra were recorded on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ¹H and 125 MHz for ¹³C, respectively, in CDCl₃ using TMS as internal standard. Silica gel (Merck, 230-400 mesh) was used for column chromatography. Precoated sil-

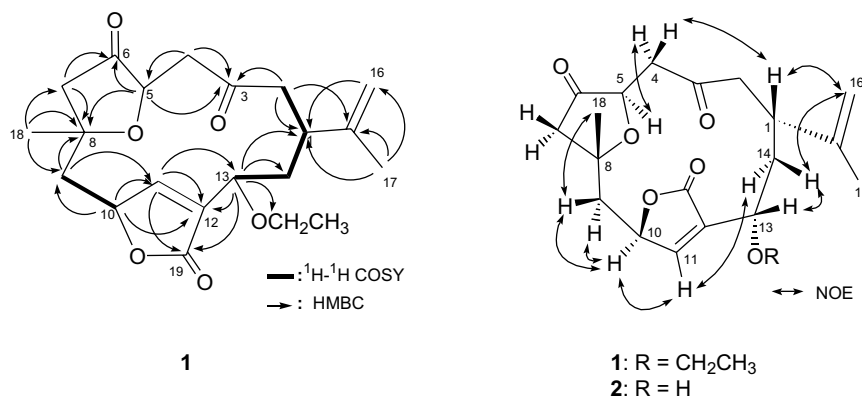


Fig. 1. ¹H-¹H COSY, HMBC for **1** and key NOE correlations for **1** and **2**.

ica gel plates (Merck, Kieselgel 60 F₂₅₄, 0.2 mm) were used for analytical TLC analyses. Isolation by HPLC was performed by a Shimadzu SPD-10A instrument equipped with a normal-phase column (Lichrosorb Si-60, 7 μ m, 250 \times 25 mm).

Animal Material

The soft coral *S. lochmodes* was collected by hand using scuba off the coast of the southernmost tip of Taiwan at a depth of 15-20 m in July 2000 and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Separation

The tissues of the soft coral (1.9 kg, wet wt) were exhaustively extracted with EtOH (2L \times 5). The EtOH extract (64.4 g) was partitioned between *n*-hexane and H₂O, then between EtOAc and H₂O. The combined EtOAc-soluble portions were evaporated under reduced pressure to yield an oily residue (2.1 g), which was subjected to CC (Si gel, EtOAc-*n*-hexane, 0:10 to 10:0, gradient). A fraction eluted with EtOAc-*n*-hexane (1:7) was purified by normal phase HPLC (EtOAc-*n*-hexane, 1:9) to afford **3** (3.5 mg). A fraction eluted with EtOAc-*n*-hexane (1:6) was isolated by normal phase HPLC (EtOAc-*n*-hexane, 1:7) to yield **1** (3.6 mg). A more polar fraction eluted with EtOAc-*n*-hexane (1:4) was separated by normal phase HPLC (EtOAc-*n*-hexane, 1:4) to afford **5** (2.5 mg). A subsequent fraction eluted with EtOAc-*n*-hexane (1:3) was further purified utilizing normal phase HPLC (EtOAc-*n*-hexane, 1:4) to give **4** (2.5 mg). Another more polar fraction eluted with EtOAc-*n*-hexane (1:1) was separated by normal phase HPLC (EtOAc-*n*-hexane, 1:3) to afford **2** (2.8 mg).

Sinulochmodin D (1)

White solid, mp 83-84 $^{\circ}$; $[\alpha]_{\text{D}}^{25} + 17.4$ (*c* 1.4, CHCl₃); IR (neat) ν_{max} 2973, 2934, 1755, 1709, 1645, 1381, 1267, 1198, 1088 cm^{-1} ; ^1H NMR (CDCl₃, 500 MHz) and ^{13}C NMR (CDCl₃, 125 MHz), see Table 1; FABMS *m/z* 399 (0.6, [M + Na]⁺), 377 (1.5, [M + H]⁺), 331 (7.3, [M - EtOH - H]⁺), 221 (1.3), 154 (10.8), 136 (51.6), 107 (29.1);

HRFABMS *m/z* 377.1967 (calcd for C₂₁H₂₉O₆, 377.1956).

Sinulochmodin E (2)

White solid, mp 104-105 $^{\circ}$; $[\alpha]_{\text{D}}^{25} + 2.7$ (*c* 1.1, CHCl₃); IR (neat) ν_{max} 3422, 2970, 2930, 1753, 1712, 1644, 1379, 1269, 1190, 1092 cm^{-1} ; ^1H NMR (CDCl₃, 500 MHz) and ^{13}C NMR (CDCl₃, 125 MHz), see Table 1; FABMS *m/z* 349 (1.0, [M + H]⁺), 331 (1.7, [M - H₂O - H]⁺), 307 (4.1), 242 (7.2), 176 (9.5), 154 (100.0), 136 (98.1), 107 (38.0); HRFABMS *m/z* 349.1650 (calcd for C₁₉H₂₅O₆, 349.1644).

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