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 norditerpenoids[1-3] 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 (^{1}H-^{1}H 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 H_2O and then between EtOAc and H_2O. The EtOAc-soluble portion was concentrated under vacuum, and then fractionated by silica gel column chromatography. The eluted fractions were purified 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° (*c* 1.4, $CHCl_3$). Its HRFABMS spectrum exhibited a molecular ion peak at *m/z* 377.1967 $[M + H]^+$, consistent with a molecular formula $C_{21}H_{28}O_6$ 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 ^{1}H NMR spectrum of **1**. The ^{13}C 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 sp^2 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 (CH_2) 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 ^{1}H 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, H_3-18), and two oxy-

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

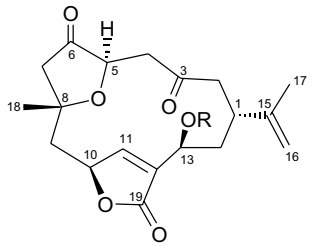
	1		**2**	
#	$^1H^a$	$^{13}C^b$	$^1H^a$	$^{13}C^b$
1	2.45 dddd (11.0, 11.0, 2.5, 2.5)[c]	37.0 (CH)[d]	2.47 dddd (11.0, 11.0, 3.0, 3.0)	37.1 (CH)
2α	2.33 t (11.0)	50.5 (CH_2)	2.36 t (11.0)	50.2 (CH_2)
2β	2.50 dd (11.0, 2.5)		2.53 dd (11.0, 3.0)	
3		207.8 (qC)		208.1 (qC)
4α	2.55 d (14.0)	44.3 (CH_2)	2.58 dd (14.5, 2.5)	44.0 (CH_2)
4β	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)
7α	2.52 d (17.5)	51.2 (CH_2)	2.53 d (17.5)	51.0 (CH_2)
7β	2.38 d (17.5)		2.38 d (17.5)	
8		79.5 (qC)		79.3 (qC)
9α	2.23 dd (15.0, 3.5)	42.1 (CH_2)	2.26 dd (15.0, 3.0)	41.6 (CH_2)
9β	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)
14α	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)
14β	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 [a]500 MHz and [b]125 MHz in $CDCl_3$ at 25 °C. [c]*J* values in Hz in parentheses. [d]Attached protons were deduced by DEPT experiments.

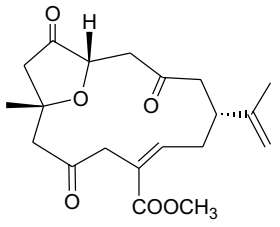
methines (δ 4.49, 1H, d, *J* = 11.0 Hz and 5.26, 1H, br t, *J* = 3.5 Hz) which are diagnostic for the C-4 norcembranoids possessing 5,8-epoxy and 12,10-carbolactone moieties.[1-8] Furthermore, it was found that the NMR data of **1** (Table 1) were nearly identical with those of scabrolide C (**6**),[3] 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 $C_{19}H_{24}O_6$. 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 $[M - H_2O + H]^+$ in the FABMS. Analysis of the NMR data assigned **2** as another C-4 norcembranoid which showed similar ^1H and ^{13}C 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



1: R = CH_2CH_3
2: R = H
6: R = CH_3



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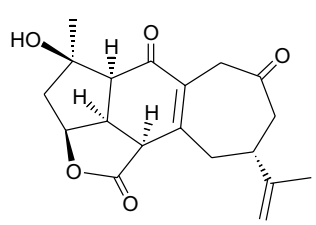

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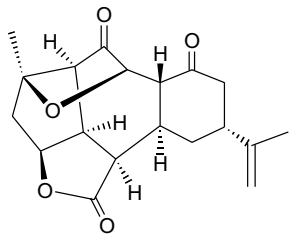

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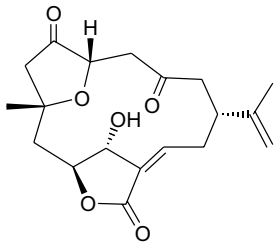

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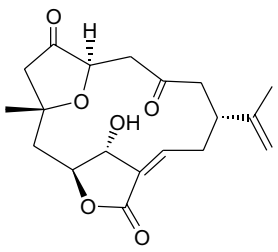

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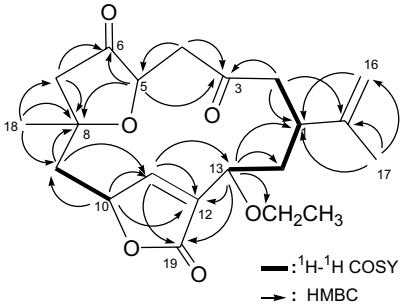
hydroxy group in **2**. This was further supported by the marked difference in the chemical shifts of the 13-oxymethine in **2** (δ_H 4.54, dd, J = 11.0, 3.0 Hz; δ_C 62.7) relative to that of **1** (δ_H 4.12, dd, J = 11.0, 3.0 Hz; δ_C 69.6). However, the identical splitting patterns and J values of H-13 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 $[\alpha]_D$) and spectral (MS, ^{1}H- and ^{13}C-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 ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard. Silica gel (Merck, 230-400 mesh) was used for column chromatography. Precoated sil-



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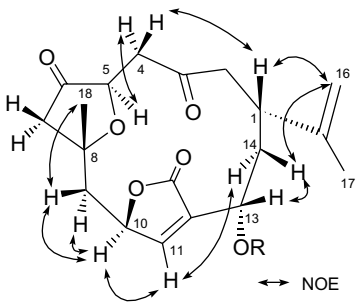

1: R = CH_2CH_3
2: R = H

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

ica gel plates (Merck, Kieselgel 60 F_{254}, 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 μm, 250 × 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 × 5). The EtOH extract (64.4 g) was partitioned between *n*-hexane and H_2O, then between EtOAc and H_2O. 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°; $[\alpha]_D^{25}$ + 17.4 (*c* 1.4, $CHCl_3$); IR (neat) ν_{max} 2973, 2934, 1755, 1709, 1645, 1381, 1267, 1198, 1088 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 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 $C_{21}H_{29}O_6$, 377.1956).

Sinulochmodin E (2)

White solid, mp 104-105°; $[\alpha]_D^{25}$ + 2.7 (*c* 1.1, $CHCl_3$); IR (neat) ν_{max} 3422, 2970, 2930, 1753, 1712, 1644, 1379, 1269, 1190, 1092 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; FABMS *m/z* 349 (1.0, $[M + H]^+$), 331 (1.7, $[M - H_2O - H]^+$), 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 $C_{19}H_{25}O_6$, 349.1644).

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