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Anti-inflammatory norditerpenoids from the soft coral *Sinularia maxima*

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ABSTRACT

Chemical investigation of the soft coral *Sinularia maxima* resulted in the isolation of seven norditerpenoids, including two new compounds, 12-hydroxy-scabrolide A (**2**) and 13-*epi*-scabrolide C (**6**). The structures of the isolated compounds were elucidated based on extensive spectroscopic evidence including Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and both one- and two-dimensional nuclear magnetic resonance (1D and 2D NMR, respectively), in comparison with reported data. Compound **6** potently inhibited IL-12 and IL-6 production in LPS-stimulated bone marrow derived dendritic (BMDCs) with IC_{50} values of 5.30 ± 0.21 and 13.12 ± 0.64 μM, respectively. Compound **1** exhibited moderate inhibitory activity against IL-12 and IL-6 production with IC_{50} values of 23.52 ± 1.37 and 69.85 ± 4.11 μM, respectively.

Marine organisms are considered as a gold mine with respect to the diversity of their chemical metabolites and biological activities. Many marine metabolites have unique structures that are not found in terrestrial organisms. Soft corals are a group of colonial invertebrates which form a significant set of marine organisms occurring widely in the coral reefs throughout the world.[1,2] Among the Alcyonacean soft corals, genus *Sinularia* is one of the most widely distributed soft coral genera, constituting a dominant portion of the biomass in the tropical reef environment. *Sinularia* species are rich sources of structurally unique and biologically active diterpenoids.[1] To date, several novel norditerpenoids have been isolated and structurally elucidated from *Sinularia* species[1,3] with some exhibiting interesting biological activities including anti-cytomegalovirus,[3] anti-inflammatory,[3] and cytotoxic activities.[4,5] As a part of our investigations of the chemical constituents and biological activities of Vietnamese marine soft corals, this study involved the isolation and structure elucidation of seven norditerpenoids (see Fig. 1) from the soft coral *Sinularia maxima*, as well as an evaluation of their in vitro anti-inflammatory effect.

The sample of *S. maxima* was collected at Nha Trang Bay in November 2011 and identified by Professor Do Cong Thung (Institute of Marine Environment and Resources, VAST). A voucher specimen (SM112010_01) was deposited at the Institute of Marine Biochemistry VAST. A MeOH extract (38.75 g) of the soft coral *S.*

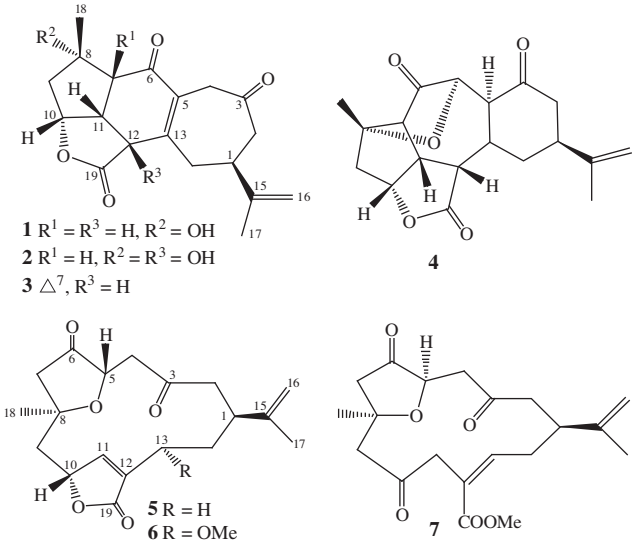

Figure 1. Structures of **1**–**7**.

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maxima was suspended in H_2O and successively extracted with CH_2Cl_2 and EtOAc. The CH_2Cl_2 and EtOAc extracts were subjected to multiple chromatographic steps over silica gel and YMC RP-18 column chromatography (CC) to provide compounds **1**–**7** (see Supplementary data). The known compounds were identified as scabrolide A (**1**),[5] yonarolide (**3**),[6] ineleganolide (**4**),[4] 5-*epi*-norcembrene (**5**),[7] and norcembrene 5 (**7**)[8] by detailed analyses of their spectroscopic data (1D-2D NMR and MS) in comparison with reported values.

12-Hydroxy-scabrolide A (**2**)[9] was obtained as a white amorphous powder. Its basic ion peaks at *m*/*z* 347 $[M+H]^+$ (on positive electron spray ionization mass spectroscopy—ESIMS) and 347.14946 $[M+H]^+$ (on high resolution electron spray ionization mass spectroscopy—HRESIMS, calcd for $C_{19}H_{23}O_6$, 347.14947) confirmed the molecular formula of $C_{19}H_{22}O_6$. The IR spectrum of **2** showed absorption due to hydroxyl (3444 cm^{-1}), carbonyl (1770 cm^{-1}), and conjugated carbonyl (1699, 1652 cm^{-1}) groups. The ^{13}C NMR spectrum of **2** in $CDCl_3$ illustrated signals of 19 carbon atoms including two methyls, five methylenes, four methines, and eight quaternary carbons, which were identified by distortionless enhancement by polarization transfer (DEPT) experiments (see Table 1). Furthermore, normal ketone (δ_C 207.89, s), α,β-conjugated ketone (δ_C 193.10, s), lactone carbonyl (δ 174.90, s), tetrasubstituted (δ_C 135.94, s and 153.38, s), and 1,1-disubstituted (δ_C 111.48, t and 148.35, s) carbon–carbon double bonds were identified. Signals of an oxymethine (δ_C 82.40, d) and two oxygenated quaternary (δ_C 73.99, s and 83.37, s) carbons were also observed. Therefore, with four remaining degrees of unsaturation, **2** was suggested to be a tetracyclic norditerpene. The ^1H NMR spectral data of **2** (in $CDCl_3$, see Table 1) showed typical signals of two tertiary methyls (δ_H 1.46 and 1.82, each 3H, s), one olefinic methylene (δ_H 4.85 and 4.86, each 1H, br s), and a lactonic methine (δ_H 5.23, 1H, dd, *J* = 5.5, 7.0 Hz). The ^1H and ^{13}C NMR data of **2** were identical to those of the known tetracyclic norditerpene, scabrolide A (**1**),[5] except for the replacement of a methine in **1** by an oxygenated quaternary carbon (δ_C 73.99, s) in **2**. The ^1H and ^{13}C NMR data of **2** were assigned by comparison with those of **1**[5] and further confirmed by heteronuclear multiple-bond correlation (HMBC) experiment. The placement of the additional oxygenated quaternary carbon at C-12 was elucidated by the HMBC correlations between H-7 (δ_H 2.72) and C-6 (δ_C 193.10)/C-12 (δ_C 73.99), H-11 (δ_H 3.43) and C-7 (δ_C 56.30)/C-8 (δ_C 83.37)/C-12 (δ_C 73.99)/C-19 (δ_C 174.90), and H-14 (δ_H 2.77 and 3.09) and C-1 (δ_C 42.99)/C-2 (δ_C 47.02)/C-5 (δ_C 135.94)/C-12 (δ_C 73.99)/C-13 (δ_C 153.38)/C-15 (δ_C 148.35). Detailed analyses of the other HMBC correlations (see Fig. 2) clearly identified the planar structure of **2**.

The relative stereochemistries of the six chiral centers C-1, C-7, C-8, C-10, C-11, and C-12 in **2** were assigned to be the same as those of **1** based on the agreement of ^1H and ^{13}C NMR data between these two compounds and further supported by the NOESY spectrum. The NOE interactions between H-11 (δ 3.43, 1H, dd, *J* = 7.0, 11.0 Hz) and H-7 (δ 2.72, 1H, d, *J* = 11.0 Hz)/H-10 (δ 5.23, 1H, dd, *J* = 5.5, 7.0 Hz)/H_β-14 (δ 2.77, 1H, dd, *J* = 10.0, 14.0 Hz) indicated that the protons positioned at C-7, C-10, C-11, and C-14 should be *syn* to each other and were assigned arbitrarily to be β-orientation, and hence indicating the *R** configuration at C-7, C-10, and C-11. The significant NOE interactions between H_3-18 (δ 1.46, 3H, s) and H-7 (δ 2.72, 1H, d, *J* = 11.0 Hz) indicated that the methyl substituent at C-8 should be located on the β-face. Compounds **1**–**3** have structures based on the fusion of five-, six-, and seven-membered carbocyclic rings linked to a 5-ring lactone. With this ring-fused framework, it might be difficult to have different configurations at only C-12 whereas the configurations at C-7, C-10, and C-11 were fixed. Thus configuration of the isopropenyl and 12-OH groups was suggested to be identical with that of scabrolide A (**1**)[5] and yonarolide (**3**),[6] on the basis of the similarity of NMR data, the same sign of optical rotations ($[\alpha]_D$), and the co-occurring of these three compounds in *S. maxima*. Consequently, the structure of **2** was elucidated as 12-hydroxy-scabrolide A.

Table 1
NMR ($CDCl_3$, 600 MHz) spectrosopic data of **2** and **6**

C	2		6	
	δ_C	δ_H, Mult. (*J* = Hz)	δ_C	δ_H, mult. (*J* = Hz)
1	42.99 d	2.98 m	38.99 d	2.63 m
2	47.02 t	2.57 dd (7.5, 12.0)	47.40 t	2.44[a]
		2.63 dd (7.0, 12.0)		2.59[a]
3	207.89 s	–	209.38 s	–
4	40.41 t	3.35 d (17.0)	45.13 t	2.46[a]
		3.78 d (17.0)		2.54[a]
5	135.94 s	–	77.70 d	4.04 d (11.0)
6	193.10 s	–	211.87 s	–
7	56.30 d	2.72 d (11.0)	51.79 t	2.40 d (16.5)/2.48 d (16.5)
8	83.37 s	–	80.56 s	–
9	47.31 t	2.28 d (15.0)	42.38 t	2.61[a]
		1.91 dd (5.5, 15.0)		2.23 dd (3.0, 15.0)
10	82.40 d	5.23 dd (5.5, 7.0)	79.14 d	5.28 m
11	51.52 d	3.43 dd (7.0, 11.0)	157.64 d	7.51 d (1.4)
12	73.99 s	–	129.52 s	–
13	153.38 s	–	75.56 d	4.11 dd (4.8, 12.0)
14	32.88 t	3.09 dd (5.0, 14.0)	37.77 t	1.86 ddd (13.0, 12.0, 5.5)
		2.77 dd (10.0, 14.0)		2.52 m
15	148.35 s	–	147.60 s	–
16	111.48 t	4.85 br s/4.86 br s	112.69 t	4.64 br s/4.72 t (1.5)
17	21.64 q	1.82 s	20.21 q	1.68 s
18	25.91 q	1.46 s	28.83 q	1.47 s
19	174.90 s	–	171.58 s	–
OMe			57.60 q	3.27 s

[a] Overlapped signals. Assignments were confirmed by HSQC, HMBC, and NOESY spectra.

13-*epi*-Scabrolide C (**6**)[9] was obtained as a white solid. Its HRESIMS spectrum exhibited a pseudo-molecular ion peak at *m*/*z* 363.18076 $[M+H]^+$ (calcd 363.18077 for $C_{20}H_{27}O_6$), consistent with a molecular formula $C_{20}H_{26}O_6$, indicating seven degrees of unsaturation. The IR spectrum exhibited the presence of ketone (1697 cm^{-1}) and α,β-unsaturated γ-lactone (1758 cm^{-1}). The ^{13}C NMR spectrum of **6** showed signals of twenty carbon atoms (see Table 1), which were identified by DEPT spectra as three methyls (including one methoxy group), six methylenes, five methines, and six quaternary carbons. The carbon signals appearing at δ_C 211.87 (s), 209.38 (s), 171.58 (s), 157.64 (d)/129.52 (s), and 147.60 (s)/112.69 (t) were attributable to the carbons of two normal ketone carbonyls, an α,β-conjugated lactone carbonyl, a trisubstituted, and a 1,1-disubstituted double bonds, respectively. A methoxy group was identified by the carbon signal at δ_C 57.60 (q). From the above observations, compound **6** was concluded to be a methoxylated tricyclic norcembranoid. In the ^1H NMR spectrum of **6** (in $CDCl_3$), the three signals at δ_H 1.47, 1.68, and 3.27 (each 3H, s) were assigned as the signals of a tertiary methyl linked to an oxygenated carbon, an olefinic methyl, and a methoxy group, respectively. Moreover, the ^1H NMR data of **6** revealed the

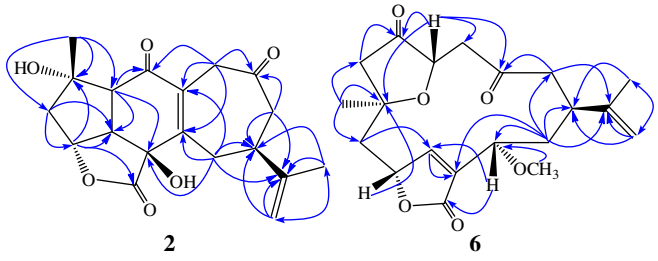

Figure 2. Key HMBC correlations of **2** and **6**.