A Novel Diterpenoid from the Soft Coral *Sarcophyton crassocaule*

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A novel hydroperoxide cembrane-type diterpenoid was isolated from the soft coral *Sarcophyton crassocaule* collected from the Xisha Islands in the South China Sea. The structure of 3 was established by spectroscopy and X-ray diffraction analysis, named as sarcophyocrassolide A. It exhibited strong cytotoxicity against the P-388 cell line with IC_{50} value of 0.1 μg/mL.

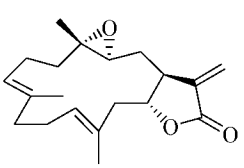
Keywords soft coral, *Sarcophyton crassocaule*, sarcophyocrassolide A

Introduction

The soft coral of the genus *Sarcophyton* is known to contain secondary metabolites with unique cembrane-type diterpenoids and remarkable activities.[1-5] Their biological activities for inhibiting tumor promotion have made them intriguing. As a part of our study, we were interested in the genus *Sarcophyton crassocale* because of the anticancer activity of its $CHCl_3$ extract against P-388 (mouse lymphocytic leukemia). In previous reports, one new compound **2** and one known compound **1**, α-methylene-γ-lactone cembranes were reported.[6] Further investigation of $CHCl_3$ extract of this species led to the isolation of a novel hydroperoxide cembrane-type diterpenoid, named as Sarcophycrassolide A. Here structure of **3** is reported. To our knowledge, it is the first example that possesses 8-hydroperoxide group in α-methylene-γ-lactone cembranolide skeleton.

Results and discussion

Compound **3**, colorless needle crystals, m.p. 172—174 ℃. The HRFABMS exhibited a molecular ion peak at *m/z* 367.2215 $(M+1)^+$, corresponding to $C_{20}H_{30}O_6$, and the unsaturation was 6. The IR spectra showed the hydroxyl group (3444 cm^{-1}), carbonyl group (1756 cm^{-1}) and double bond (1630 cm^{-1}). A strong UV absorption at λ_{max} 236 nm showed the presence of an α,β-unsaturated carbonyl group. In DEPT experiment, the 20 resonance lines were assigned to three methyls, seven methylenes, five methines and five quaternary carbons, and revealed the presence of one carbonyl carbon, two double bonds, and five oxygenated carbons. Therefore compound **3** must be three cyclic structure. Based on the analysis of ^1H-^1H COSY and ^1H-^{13}C COSY, three partial structures could be established as A—C.



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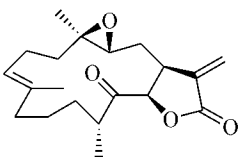

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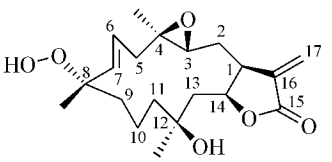

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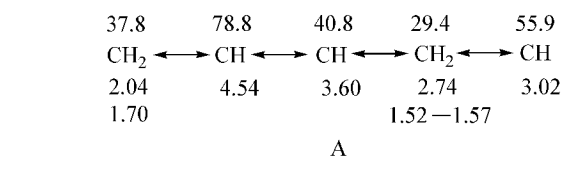

Two sharp doublets at δ_H 6.35 (d, J = 2.0 Hz) and 5.72 (d, J = 2.0 Hz) showed the presence of an exocyclic α-methylene lactone ring. This was supported by ^{13}C NMR [δ_C 169.0 (s), 138.2 (s), 123.3 (t)] and IR spectra (1756 cm^{-1}) and a secondary oxygenated carbonsignal at δ_C 78.8 hinted at a γ-lactone.[7] Carbon sig-

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nals were observed at δ_C 60.2 (s) and 55.7 (d), and a proton signal at δ_H 3.02 (t, J = 9.6 Hz, 1H) indicated the presence of a trisubstituted epoxide. Three remaining oxygenate carbons exist two carbon signals at δ_C 85.6 (s) and δ_C 77.3 (s), respectively. It hinted that compound **1** most likely possessed a hydroperoxide group. Two olefinic methine proton signals at δ_H 5.54 (d, J = 16.0 Hz, 1H) and 5.23 (ddd, J = 4.8, 9.9, 16.0 Hz, 1H) implied the presence of a *trans* ethylenic double bond. The ^{1}H NMR showed signals for three tertiary methyl groups at δ_H 1.22, 1.31 and 1.59. Based on the HMBC correlation of **1**, the partial structure A—C could be connected as a cembranolide skeleton. From the HMBC experiment, the positioning of γ-methylene lactone at C-15, C-1, C-17, C-14 and C-16 (carbonyl carbon) was confirmed by long-range correlations between H-1 and C-2, C-3, C-15, C-16 and C-17; H-14 and C-2 and C-16; and H-17 and C-1, C-15 and C-16. The methyl-bearing trisubstituted epoxide at C-3 (methane), C-4 (quaternary carbon) was deduced from HMBC correlations between H-2 and C-1, C-3, C-4, C-14 and C-15; H-3 and C-2 and C-4; H-5 and C-3, C-4, C-6, C-7 and C-18; H-18 and C-3, C-4 and C-5; H-6 and C-4, C-5, C-7 and C-8. The methyl group attached at C-8 was confirmed by HMBC correlations between H-19 and C-7, C-8 and C-9; H-7 and C-5, C-6, C-8, C-9 and C-19. The other methyl group attached at C-12 was revealed by the HMBC correlations between H-20 and C-11, C-12 and C-13; H-11 and C-20. All of ^{1}H and ^{13}C NMR signals were unambiguous assigned based on ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY and HMBC experiment (Table 1). The relative stereochemistry of compound **1** was determined by a NOESY experiment, the NOE correlations from H-14 to H-1 and 20-Me, and H-1 to H-14 and H-3 indicated that these protons were on the same face of the 14-membered ring and were assigned as the α-protons. On the other hand, H-6 show NOE responses with H-5 β and 19-Me, but not with H-7, and the β-orientation 19-Me as well as the *trans* orientation of the Δ^6 double bond were confirmed. The conformation was also implied by the strong NOE correlation between 18-Me and H-5 α. Furthermore, in order to confirm the position of —OOH group, the molecular structure and relative stereochemistry, an X-ray structure analysis was made. The result showed that the —OOH group attaches to C-8 which is close to the vinylic H-7 proton (δ_H 5.54). The bond distances and bond angles are listed in Tables 2 and 3.

In the process of isolation, the extract was avoided to contact any solvents containing peroxy compounds. It is impossible that the hydroperoxide group is formed artificially. Compound **1** was named as sarcocrassolide A.

Sarcocrassolide A exhibited strong cytotoxic activity against P-388 cell line with IC_{50} value of 0.1 μg/mL.

Table 1 NMR data of compound **1** (AM-500 MHz) in $CDCl_3$

Number	δ_H	δ_C	Key HMBc
1	3.58—3.60 (m, 1 H)	40.8 (d)	
2α	2.74 (ddd, J = 2.0, 4.1, 14.8 Hz)	29.4 (t)	
2β	1.52—1.57 (m)		
3	3.02 (t, J = 9.6 Hz)	55.7 (d)	
4		60.2 (s)	C-4, H-2, 3, 5, 6, 18
5α	2.52 (dd, J = 4.8, 14.7 Hz)	38.9 (t)	
5	2.32 (dd, J = 9.9, 14.7 Hz)		
6	5.23 (ddd, J = 4.8, 9.9, 16.0 Hz)	125.3 (d)	
7	5.54 (d, J = 16.0 Hz)	137.8 (d)	
8		85.6 (s)	C-8, H-6, 7, 9, 10, 19
9	1.48—1.52 (m, 2H)	38.3 (t)	
10α	1.29—1.31 (m)	22.5 (t)	
10β	1.13—1.17 (m)		
11α	1.95—1.98 (m)	31.0 (t)	
11β	1.12—1.14 (m)		
12		77.3 (s)	C-12, 10, 11, 13, 14, 20
13α	2.04 (dd, J = 6.0, 13.8 Hz)	37.8 (s)	
13β	1.70 (dd, J = 3.6, 13.8 Hz)		
14	4.54 (ddd, J = 3.6, 6.0, 9.8 Hz)	78.8 (d)	
15		138.2 (s)	C-15, H-1, 2, 14, 17
16		169.0 (s)	C-16, H-1, 14, 17
17α	6.35 (d, J = 2.0 Hz)	123.3 (d)	
17β	5.72 (d, J = 2.0 Hz)		
18	1.22 (s)	18.8 (q)	
19	1.31 (s)	22.7 (q)	
20	1.59 (s)	24.2 (q)	

Table 2 Selected bond lengths (nm)

O(1)—C(16)	0.1365(3)	O(1)—C(14)	0.1459(2)
O(2)—C(16)	0.1200(3)	O(3)—C(3)	0.14529(2)
O(4)—O(6)	0.1322(3)	O(4)—C(8)	0.1454(3)
C(5)—O(6)	0.1486(4)	C(6)—C(7)	0.1328(3)
O(5)—C(12)	0.1456(3)	C(1)—C(15)	0.1510(3)
C(1)—C(2)	0.1532(3)	C(2)—C(3)	0.1502(3)
C(3)—C(21)	0.1453(3)	C(21)—C(5)	0.1508(4)
C(8)—C(9)	0.1525(4)	C(7)—C(8)	0.1507(3)
C(9)—C(10)	0.1525(3)	C(10)—C(11)	0.1531(3)
C(11)—C(12)	0.1536(3)	C(12)—C(13)	0.1521(3)
C(13)—C(14)	0.1522(3)	C(12)—C(20)	0.1534(3)
C(15)—C(17)	0.1317(3)	C(15)—C(16)	0.1484(3)

Table 3 Selected bond angles (°)

C(16)-O(1)-C(14)	109.94(16)	C(3)-O(3)-C(21)	61.11(14)
O(6)-O(4)-C(8)	114.2(2)	C(15)-C(1)-C(2)	111.28(17)
C(15)-C(1)-C(14)	100.61(17)	C(2)-C(1)-C(14)	116.93(18)
C(3)-C(2)-C(1)	114.93(19)	O(3)-C(3)-C(21)	59.50(14)
O(3)-C(3)-C(2)	118.22(18)	C(21)-C(3)-C(2)	124.9(2)
O(3)-C(21)-C(3)	59.39(14)	O(3)-C(21)-C(5)	120.6(2)
C(6)-C(5)-C(21)	115.58(19)	C(7)-C(6)-C(5)	125.9(2)
C(6)-C(7)-C(8)	124.9(2)	O(4)-C(8)-C(7)	102.3(2)
O(4)-C(8)-C(9)	109.11(19)	C(7)-C(8)-C(9)	113.06(19)
O(4)-C(8)-C(19)	110.7(2)	C(7)-C(8)-C(9)	111.6(2)
C(10)-C(9)-C(8)	117.2(2)	C(10)-C(11)-C(12)	114.41(17)
C(13)-C(120-C(11)	112.61(19)	C(20)-C(12)-C(11)	112.13(18)
O(5)-C(12)-C(13)	123.46(18)	O(1)-C(14)-C(1)	105.99(16)
O(1)-C(14)-C(13)	111.33(18)	C(17)-C(15)-C(16)	122.2(2)
O(2)-C(16)-O(1)	121.4(2)	O(2)-C(16)-C(15)	129.8(2)

Experimental

General procedures

Melting point was taken on an X-4 micro-melting point apparatus. IR spectra were recorded on a Nicolet 5DX FT-IR spectrophotometer. UV spectra were determined on a Shimadzu UV-160A spectrophotometer. NMR spectra were recorded on an AM-500 MHz spectrometer. Mass spectra on a Krator MS 50 instrument. X-Ray data were collected on a SMART CCD diffractmeter.

Soft coral material

The soft coral *Sarcophyton crassocaule* was collected from the Xisha Islands of the South Sea of China in 1998.

Extraction and isolation

The chopped soft coral (wet weight 5 kg) was extracted with EtOH at room temperature, the combined extracts were concentrated *in vacuo*, and the residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ soluble portion was subjected to silica gel column chromatography with $CHCl_3$ containing increasing of MeOH. Elution by $CHCl_3$-MeOH (9:1, *V*:*V*) afforded fractions containing **1**, **2** and **3** which was purified by a silica gel column with *n*-hexane-EtOAc (2:1, *V*/*V*) as eluting solvent. The fractions afforded compounds **1** (40 mg) and **2** (23 mg), named as scracophyocrassolide B, and was further purified by preparing PTLC and afforded compound **3** (35 mg), named as scracophyocrassolide A.

Scracophypcrassolide A, needle crystals, m.p. 172—174 ℃ (MeOH); IR (KBr) ν: 3444 (OH), 1756 (C = O), 1630, 1463, 1394, 1370, 1189 cm^{-1}; UV λ_{max}: 236 nm; HRFABMS *m*/*z*: 367.2215 $(M+1)^+$; NMR data see Table 1.

Single-crystal X-ray crystallography

Suitble colorless Prisms of **1** were obtained from a mixture of $CHCl_3$-acetone, the crystal (0.36 mm × 0.15

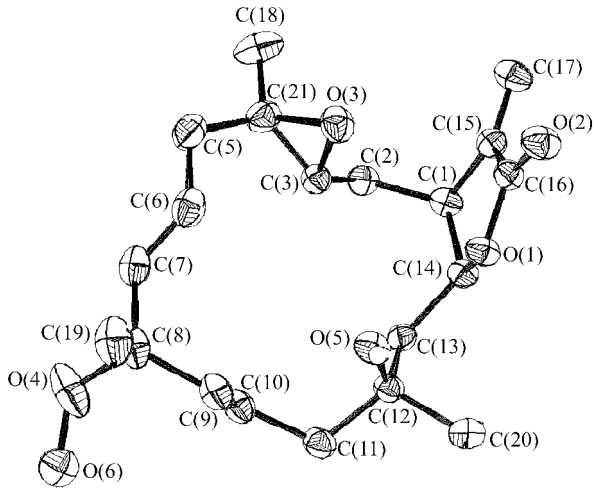

Fig. 1 Molecular structure of scarcophycrassolide A.

mm × 0.10 mm) was selected for single-crystal X-ray analysis. X-Ray data were collected at room temperature on a SMART CCD diffractmeter equipped with a normal-focus up to $2.02° \leqslant \theta \leqslant 25.11°$. A total of 8652 reflections were collected, which of 5677 were unique $[I > 2\sigma(I)]$. The structure was solved by direct method and refined by full-matrix least-squares procedure. The nonhydrogen atoms were given anisotropic thermal parameters. The crystal belongs to the monoclinic system, space group $P2_1$ with $a = 0.8688(16)$ nm, $b = 0.59847(10)$ nm, $c = 1.79351(3)$ nm, $\beta = 0°$, $V = 0.9276(3)$ nm^3, $Z = 2$, $D_c = 1.305$ g/cm^3, λ (Mo Kα) = 0.071073 nm. The refinement converged to a final $R = 0.0452$, $Rw = 0.1123$.

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