Cytotoxic Diterpenoids from the Soft Coral *Sinularia microclavata*

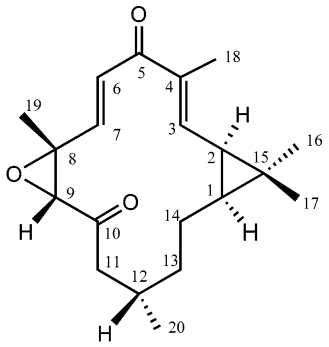
Cui-Xian Zhang,[†] Su-Jun Yan,[†] Guang-Wen Zhang,[†,§] Wei-Gang Lu,[†] Jing-Yu Su,[†] Long-Mei Zeng,*[,†] Liang-Quan Gu,[†] Xiao-Ping Yang,[‡] and Yong-Ju Lian[‡]

School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, 510275, People's Republic of China, Cancer Research Institute, Sun Yat-sen University, Guangzhou, 510060, People's Republic of China, and Institute of Traditional Chinese Medicine & Natural Products, Jinan University, 510632, People's Republic of China

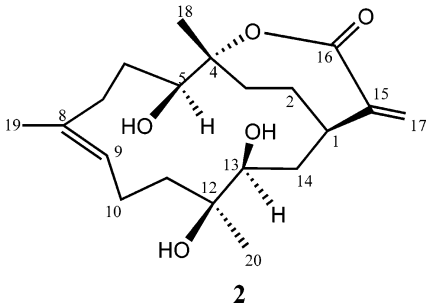
Received January 14, 2005

The soft coral *Sinularia microclavata* collected from the bay of Sanya, Hainan Island, China, yielded a new diterpenoid, microclavatin (**1**), and a known cembranolide, capillolide (**2**). The structure of compound **1** was determined on the basis of spectroscopic methods and X-ray single-crystal diffraction analysis. Microclavatin (**1**) showed cytotoxic activities against tumor cell lines KB and MCF with IC_{50} values of 5.0 and 20.0 μg/mL, respectively, and capillolide (**2**) showed potent cytotoxic activity against tumor cell lines (A-549) with an IC_{50} value of 0.5 μg/mL.

Soft corals are a rich source of secondary metabolite content with diverse structures and various biological activities, such as antitumor, antimicrobial, and HIV-inhibitory activity.[1−6] They are also believed to play an important role in the protection of soft corals from predators.[7] As a part of our continuing studies on the bioactive substances of soft corals and other marine organisms from the South China Sea, we isolated a new diterpenoid, named microclavatin (**1**), and a known cembranoid, capillolide (**2**), from the soft coral *Sinularia microclavata*. Their structures were determined on the basis of extensive spectroscopic methods and X-ray single-crystal diffraction analysis.



1



2

Compound **1** was crystallized as colorless prisms (EtOAc/petroleum ether, 1:4), mp 145−146 °C; $[\alpha]^{20}_D$ −15.7° (*c* 0.07, $CHCl_3$). The HRFABMS of **1** established its molecular formula as $C_{20}H_{28}O_3$, indicating seven sites of unsaturation. UV and IR spectra displayed characteristic absorption bands for conjugated carbon−carbon double bonds. The presence of a conjugated dienone fragment and an isolated ketone group was elucidated by UV (λ_{max} 282.2 (log ϵ 4.04), 241.6 (log ϵ 3.89) nm), IR (ν 1710 (s), 1639 (s), 1617 cm^{-1}), and ^{13}C NMR (δ_C 203.9 (C), 196.4 (C), 146.0 (CH), 137.8 (CH), 137.3 (C), and 134.8 (CH)) data. The IR (1226 cm^{-1} (s)) and ^{13}C NMR (δ_C 66.2 (d), 61.6 (s)) displayed the presence of an epoxy function. These spectral data, coupled with seven sites of unsaturation, suggested that **1** was a bicyclic compound. The ^1H NMR spectrum exhibited five distinct methyl groups [δ_H 1.89 (s, 3H), 1.63 (s, 3H), 1.14 (s, 3H), 1.08 (d, 3H, J = 7.5), 0.94 (s, 3H)] (Table 1), indicating that **1** was probably a diterpenoid.

The planar structure of **1** was determined by a detailed analysis of 1D and 2D NMR spectra. The HMQC experiment allowed us to assign all the protons to the corresponding carbon atoms (Table 1), and the $^1H-^1H$ COSY spectrum revealed sequences of the correlations depicted by the bold lines (Figure 1). These two sequences along with the functions mention above were assembled with the help of a HMBC experiment (Figure 1). Key HMBC correlations from H-11 to C-10 and C-9, H-9 to C-8 and C-10, H-7 to C-8, H_3-19 to C-8 and C-7, H-6 to C-5, H_3-18 to C-4 and C-5, H-3 to C-5 and C-18, H-2 to C-15 and C-16, and H-1 to C-15 and C-17 permitted the connectivity of the isolated spin systems (Figure 1).

The relative configuration of the chiral carbons in the molecule of **1** came from a 2D NOESY analysis. As shown in Figure 2, H-2 showed NOE interactions with H-1 and H_3-20, indicating that H-2, H-1, and H_3-20 were situated on the same face. Alternatively, interactions between H-12 and H-11b, H-11b and H-9, and H-9 and H_3-19 were observed; thus H-12, H-9, and H_3-19 should be positioned on the other face. As referred to the lowest carbon having *R*-chirality, the relative stereochemistry of chiral carbons was assigned as 1*R**, 7*S**, 8*S**, 11*R**, 14*R**. The *E*-configuration of the $\Delta^{6,7}$, $\Delta^{3,4}$double bonds was established by NMR data. The olefinic protons at δ_H 6.44 and 5.81 with the coupling constant 16.5 Hz and a vinyl methyl (18-Me) signal at δ_C 11.8 disclosed that two double bonds in the molecule possessed 6*E* and 3*E* configuration.[8,9] On the basis of the above analysis, the structure and stereochemistry of the new diterpenoid was established as **1**.

* Corresponding author. Tel: 86-20-84036447. Fax: 86-20-84112245. E-mail: ceszlm@zsu.edu.cn.
[†] School of Chemistry and Chemical Engineering, Sun Yat-sen University.
[‡] Cancer Research Institute, Sun Yat-sen University.
[§] Institute of Traditional Chinese Medicine & Natural Products, Jinan University.

10.1021/np058006v CCC: $30.25
Published on Web 06/15/2005

Table 1. ^{1}H and ^{13}C NMR Spectral Data for **1**

position	δ_H [mult. J (Hz)]	δ_C[a]	^{1}H−^{1}H COSY	key HMBC (H to C)
CH(1)	1.05 (m, 1H)	34.0 (CH)	H-14, H-2	C_{15}, C_{17}
CH(2)	1.45 (dd, 1H, J = 10.5, 8.0)	27.5 (CH)	H-1, H-3	C_{15}, C_{16}
CH(3)	6.00 (d, IH, J = 10.5)	146.0 (CH)	H-1	C_5, C_{15}, C_{18}
C−(4)		137.3 (C)		
C=O(5)		196.4 (C)		
CH(6)	6.44 (d, 1H, J = 16.5)	134.8 (CH)	H-7	C_5
CH(7)	5.81 (d, 1H, J = 16.5)	137.6 (CH)	H-6	C_8
C(8)		61.6 (C)		
CH(9)	3.67 (s, 1H)	66.2 (CH)		C_8, C_{10}
C=O(10)		203.9 (C)		
CH_2(11)	2.70 (dd, 1H, J = 2.5, 15.0)	49.5 (CH_2)	H-12	C_9, C_{20}
	2.38 (dd, 1H, J = 10.5, 15.0)			
CH(12)	1.85 (m, 1H)	29.8 (CH)	H-11, H-13, H-20	C_{10}
CH_2(13)	1.26 (m, 1H),	36.3 (CH_2)	H-12, H-14	
	1.16 (m,1H)			
CH_2(14)	1.82 (m, 1H)	23.8 (CH_2)	H-1, H-13	
	0.90 (m, 1H)			
C(15)		26.6 (C)		
CH_3(16)	1.14 (s, 3H)	29.1 (CH_3)		C_{14}, C_{15}, C_1, C_2
CH_3(17)	0.94 (s, 3H)	16.2 (CH_3)		C_{15}, C_{14}, C_1, C_2
CH_3(18)	1.89 (s, 3H)	11.8 (CH_3)		C_4, C_5
CH_3(19)	1.63 (s, 3H)	19.8 (CH_3)		C_7, C_8
CH_3(20)	1.08 (d, 3H, J = 7.5)	21.8 (CH_3)		C_{11}, C_{12}, C_{13}

[a] Multiplicity was obtained from DEPT experiments.

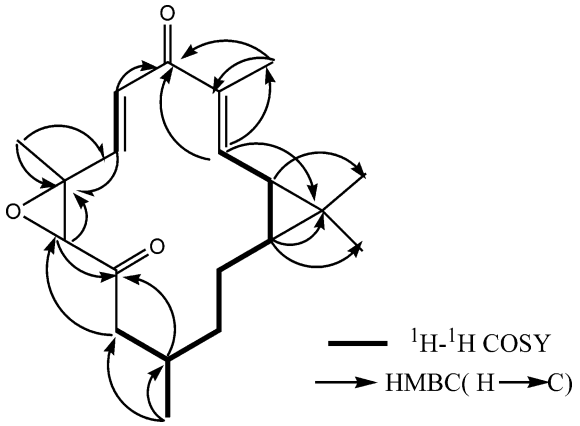


Figure 1. ^{1}H−^{1}H COSY correlations (bold lines) and key HMBC correlations (arrows) of **1**.

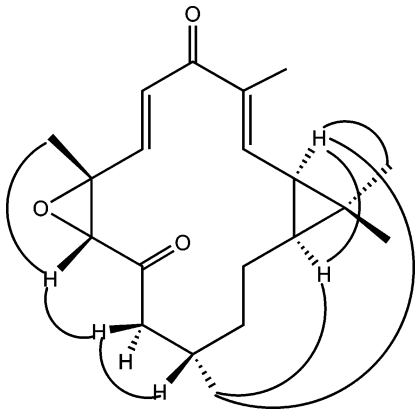


Figure 2. Key NOE interactions of **1**.

Microclavatin (**1**) readily crystallizes from a mixture of EtOAc/petroleum ether, forming good quality prismatic crystals. An X-ray crystallographic analysis was undertaken. A perspective drawing of a single molecule **1** is given in Figure 3, which confirmed the structure deduced by NMR studies (Figure 3, Table 2).

Compound **2** was isolated as colorless prisms. Its molecular formula, $C_{20}H_{32}O_5$, was established by HRFABMS m/z 353.2332 (calc 353.2327) $[M + 1]^+$. The ^{1}H and ^{13}C

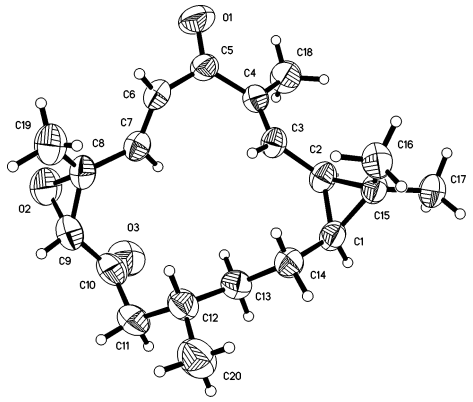


Figure 3. Perspective drawing of the X-ray structure of **1**.

Table 2. Crystal Data for **1** and **2**

	1	**2**
empirical formula	$C_{20}H_{28}O_3$	$C_{20}H_{32}O_5 \cdot H_2O$
fw	316.42	370.47
temperature (K)	293(2)	293(2)
wavelength Å	0.71073	0.71073
cryst syst, space group	monoclinic, $P(2)$	monoclinic, $C2$
unit cell dimens		
a (Å)	5.9969(7)	15.6359(17)
b (Å)	13.5643(16)	12.1299(17)
c (Å)	11.2749(14)	11.5236(14)
α (deg)	90	90
β (deg)	90.294	104.111(3)
γ (deg)	90	90
Z, calcd density (Mg/m^3)	2, 1.146	4, 1.161
final R indices [$I > 2\sigma(I)$]	R1 = 0.0469, wR2 = 0.0949	R1 = 0.0393, wR2 = 0.1014
R indices (all data)	R1 = 0.0469, wR2 = 0.0949	R1 = 0.0483, wR2 = 0.1109

NMR spectral data of **2** were identical with those of capillolide, which we had reported in a previous paper.[4]

To confirm the stereochemistry of capillolide, a X-ray single-crystal diffraction analysis was carried out (Table 2, Figure 4).

Microclavatin (**1**) exhibited cytotoxic activities against KB and MCF cell lines with IC_{50} values of 5.0 and 20.0 μg/mL, respectively, and capillolide (**2**) showed potent cytotoxic activity against tumor cell lines (A-549) with an IC_{50} value of 0.5 μg/mL.

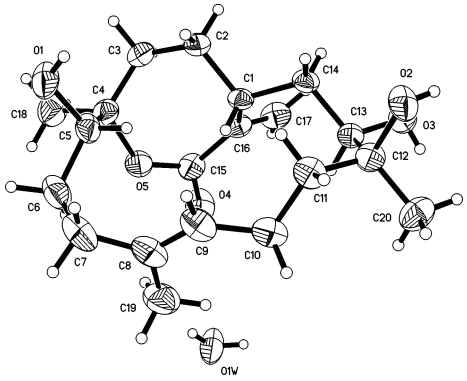


Figure 4. Perspective drawing of the X-ray structure of **2**.

Experimental Section

General Experimental Procedures. The melting points were determined using a X_6 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter, and UV spectra were obtained with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with an EQUINOX55 (Bruker) spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity INOVA spectrometer at 500 and 125 MHz, respectively. HRMS spectra were obtained with a VG ZABHS mass spectrometer. The X-ray diffraction data were collected on a Bruker SMART 1000CCD X-ray diffractometer.

Animal Material. The soft coral *Sinularia microclavata* was collected from the Bay of Sanya, Hainan Island, China, in 2002. A voucher specimen (No. 98-SY-18) is deposited in the Research Centre of Organic Natural Products, Sun Yat-Sen University.

Cytotoxicity Bioassays. The tetrazolium-based colorimetric assay (MTT assay) was used for the in vitro assay of cytotoxicity to KB, MCF, and A-549 tumor cell lines separately.

Extraction and Isolation. The soft coral (1.4 kg, dried wt) was extracted with EtOH at room temperature. The resulting extract was partitioned between EtOAc and water. The EtOAc phase (20 g) was subjected to column chromatography over Si gel eluted with petroleum ether (PE) containing increasing concentrations of EtOAc to yield 12 fractions. The fourth fraction (EtOAc/PE, 25:75) was submitted to flash chromatography on Si gel eluted with EtOAC/PE (15:85) to afford microclavatin (**1**) (7 mg). The eighth fraction (EtOAc/PE, 65:35) afforded a white powder, which was recrystallized from EtOAc to afford pure capillolide (**2**) (30 mg).

Microclavatin (1): colorless prisms (EtOAc/petroleum ether, 1:4); mp 145−146 °C; $[\alpha]^{20}_D$ −15.7° (*c* 0.07, $CHCl_3$); UV ($CHCl_3$) λ_{max} 282.2 (log ϵ 4.04), 241.6 (log ϵ 3.89) nm; IR (KBr) ν 3054, 3021, 1710, 1639, 1617, 1269, 1226, 1044, 983 cm^{-1}; ESIMS *m/z* 317 $[M + H]^+$; HRFABMS *m/z* 317.2115 $[M + H]^+$ (calc for $C_{20}H_{28}O_3$, 317.2116); ^{1}H NMR and ^{13}C NMR, see Table 1; Crystal data, see Table 2.

Capillolide (2): colorless prisms (EtOAc); mp 146−148 °C; $[\alpha]^{20}_D$ +40.0° (*c* 0.3, $CHCl_3$); UV ($CHCl_3$) λ_{max} 238.6 (log ϵ 4.09) nm; ^{1}H NMR δ_H 2.36 (m, 1H, H-1), 2.07 (m, 1H, H-2), 1.26 (m, 1H, H-2), 2.04 (m,1H, H-3), 1.78 (m, 1H, H-3), 4.15 (br d, 1H, J = 7.0, H-5), 2.18 (br d, 1H, J = 7.0, H-6), 1.36 (m, 1H, H-6), 2.10 (m, 1H, H-7), 1.62 (m, 1H, H-7), 5.05 (br d, 1H, J = 8.0, H-9), 2.08 (m, 1H, H-10), 1.69 (m, 1H, H-10), 1.90 (m, 1H, H-11), 1.42 (m, 1H, H-11), 3.38 (br d, 1H, J = 7.0, H-13), 2.12 (m, 1H, H-14), 1.62 (m, 1H, H-14), 6.38 (br s, 1H, H-17), 5.73 (br s, 1H, H-17), 1.30 (s, 3H, 18-Me), 1.49 (s, 3H, 19-Me), 1.22 (s, 3H, 20-Me); ^{13}C NMR δ_C 169.7 (C, C-16), 142.2 (C, C-15), 135.7 (C, C-8), 127.2 (CH, C-9), 125.0 (CH_2, C-17), 87.3 (C, C-4), 75.0 (C, C-12), 73.3 (CH, C-13), 68.4 (CH, C-5), 38.1 (CH_2, C-11), 36.7 (CH_2, C-7), 36.5 (CH, C-1), 36.4 (CH_2, C-2), 31.8 (CH_2, C-3,), 30.4 (CH_2, C-6), 28.7 (CH_2, C-14), 24.3 (CH_3, C-20), 22.8 (CH_3, C-18), 22.2 (CH_3,C-10), 15.7 (CH_3, C-19); HRFABMS *m/z* 353.2332 $[M + H]^+$ (calc for $C_{20}H_{32}O_5$, 353.2327); crystal data, see Table 2.

Acknowledgment. This work was supported by a grant from the National Natural Science Foundation (No. 29932030) and a grant from the National High Technology Development Project (863 Project, No. 2001AA620403) of China. We thank Dr. C. Li of the South China Sea Institute of Oceanology, Academia Sinica, for identification of the soft coral species.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Coll, J. C.; Bowden, D. M.; Tapiolas, R. H.; et al. *Tetrahedron* **1985**, *41*, 1085−1092.
(2) Anjaneyulu, A. S. R.; Gowrl, P. M.; Murthy, M. V. R. K. *J. Nat. Prod.* **1999**, *62*, 1600−1604.
(3) Duh, C. Y.; Wang, S. K.; Tseng, H. K.; et al. *J. Nat. Prod.* **1998**, *61*, 844−847.
(4) Su, J. Y.; Yang, R. L.; Kuang, Y.Y.; Zeng, L. M. *J. Nat. Prod.* **2000**, *63*, 1543−1545.
(5) Rashid, M. A.; Gustafson, K. R.; Boyd, M. R. *J. Nat. Prod*. **2000**, *63*, 531−533.
(6) Duh, C. Y.; Wang, S. K.; Chu, M. J.; Sheu, J. H. *J. Nat. Prod.* **1998**, *61*, 1022−1024.
(7) Coll, J. C. *Chem. Rev.* **1992**, *92*, 613−631.
(8) Kagan, H. B. *Stereochemistry, Foundamentals and Methods,* Vol. 1, *Determination of Configurations by NMR Spectroscopy*; Academic Press: New York, 1977; pp 56−57.
(9) Capon, R. J.; Miller, M.; Rooney, F. *J. Nat. Prod*. **2000**, *63*, 821−824.

NP058006V