A New Cembranolide from the Soft Coral *Sinularia capillosa*

Jingyu Su, Ruolin Yang, Yunyan Kuang, and Longmei Zeng*

Department of Chemistry, Zhongshan University, Guangzhou, People's Republic of China 510275

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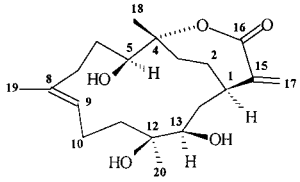
A new cembranolide, capillolide (**1**), and three known cembranolides were isolated from the soft coral *Sinularia capillosa* collected from the South China Sea. Their structures and the relative stereochemistry of **1** were deduced on the basis of spectroscopic methods.

Soft corals of the genus *Sinularia* yield a wide variety of sesquiterpenes, diterpenes and steroids.[1−4] Bowden has reported the isolation of 15 furanosesquiterpenes from *Sinularia capillosa* collected at Magnetic Island (Townsville), Australia.[5] *Sinularia* spp. are very abundant in the South China Sea. In our search for bioactive substances from soft corals, a new cembranolide, capilloloid (**1**), and three known compounds (**2**−**4**) were obtained from *Sinularia capillosa* Tix.-Dur. (Alcyoniidae) collected from the Bay of Sanya, Hainan Island, China.



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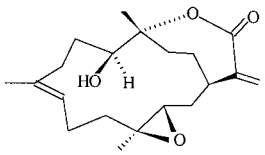

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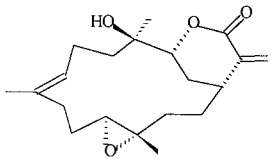

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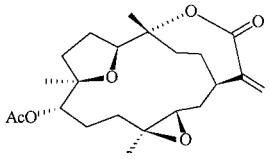

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Capillolide (**1**) was isolated as colorless crystals. Its molecular formula, $C_{20}H_{32}O_5$, was established by FABMS *m*/*z* 375 $[M + Na]^+$ and ^{13}C NMR data implying five degrees of unsaturation. Its IR spectrum showed a broad absorption between 3000 and 3400 cm^{-1} (OH stretching) and a strong absorption at 1700 cm^{-1}, consistent with the presence of an α,β-unsaturated ester function. The ^{13}C NMR and DEPT experiments revealed the presence of one carbonyl carbon, two double bonds, and four oxygenated carbons. ^1H and ^{13}C NMR spectra, combined with 2D NMR experiments, allowed the chemical structure of **1** to be established.

The presence in the ^1H NMR spectrum of two broad singlets at δ 6.37 (1H, br s) and 5.75 (1H, br s) suggested the presence of an exocyclic α-methylene lactone ring. This was supported by the ^{13}C NMR (δ 169.7, 142.2, 125.0) and IR spectra (1700 cm^{-1}). A tertiary oxygenated carbon signal at δ 87.2 suggested the presence of an ϵ-lactone moiety,[1] which was confirmed by the $^1H-^1H$ COSY and HMBC experiment (Table 1 and Figure 1). Carbon signals were observed at δ 68.4 (d), 73.3 (d), and 74.9 (s), and two proton signals at δ 4.16 and 3.38 indicated the presence of two

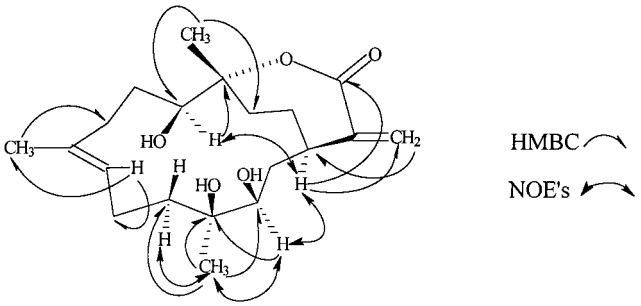

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Figure 1. Important $^1H-^{13}C$ long-range correlations and NOE interactions of **1**.

secondary and one tertiary hydroxyl groups. Two olefinic carbons were further identified. A signal at δ 5.03 attributed to an olefinic proton, together with a methyl carbon signal at δ 15.9, indicated the *E* configuration for this double bond. Signals for a vinyl methyl at δ 1.48 (3H, s) and two tertiary oxygenated methyl groups at δ 1.31 (3H, s) and 1.22 (3H, s) were observed in the ^1H NMR spectrum. The ^1H NMR and ^{13}C NMR spectra of **1** clearly revealed its cembranolide skeleton. All the ^1H and ^{13}C NMR signals of **1** were unambiguously assigned based on $^1H-^1H$ COSY, HMQC, and HMBC NMR experiments. The $^1H-^{13}C$ HMBC NMR technique was employed to position the hydroxyl groups about the cembrane ring as illustrated in Figure 1.

The relative stereochemistry of **1** was deduced mainly by 2D NOESY and NOE difference data. In these experiments, both H-13 and H-5 showed NOEs with H-1 but not with H_3-18. Moreover, H_3-20 shared mutual NOE enhancement with H-13 and H-11α at δ 1.41. These observations indicated that all three hydroxy groups in **1** were β-oriented and H-1, H-5, H-13, and CH_3-20 were α-oriented with respect to this ring (Figure 1). An additional argument for the stereochemistry of **1** is that **1** and sinulariolide (**2**) were obtained from the same animal, and their NMR spectral data at H_3-18 and C-4 are almost identical (H_3-18 δ 1.31, C-4 δ 87.2 for **1** and H_3-18 δ 1.34, C-4 δ 87.0 for **2**). Furthermore, since H_3-18 did not exhibit a NOE response with H-5, it is reasonable to conclude that CH_3-18 of **1** has the same β-configuration as **2**.

The identities of compounds **2**−**4** were established by comparison of their spectral data with those of the known compounds reported. They are sinulariolide (**2**),[6] flexibilide (**3**),[7] and (1*R*,13*S*,12*S*,9*S*,8*R*,5*S*,4*R*)-9-acetoxy-5,8:12,13-diepoxycembr-15(17)-en-16,4-olide (**4**).[8]

Capillolide (**1**) showed moderate cytotoxic activity. ED_{50} values in a test against P-388 and L1210 cell lines are 15.0 and 18.5 μg/mL, respectively. ED_{50} values for cytotoxicity toward the P388 and L1210 cell lines are 8.5 and 10.0 μg/

* To whom correspondence should be addressed. Tel.: 86−20−84036447. Fax: 86-20-84112245. E-mail: ceszlm@zsu.edu.cn.

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Table 1. ^{1}H and ^{13}C NMR Signals of Capillolide (**1**)

position	^{13}C [a]	^{1}H (*J*) [b]	^{1}H−^{1}H COSY	HMBC
1	36.3 d	2.35, m	2.12, 1.62, 2.07, 1.21	6.37, 5.75, 2.07, 1.21
2	36.2 t	2.07, m	2.35, 1.21, 1.78, 2.06	1.78, 2.06
		1.21, m	2.35, 2.07, 1.78, 2.06	
3	31.6 t	1.78, m	2.07, 2.06, 1.21	2.07, 1.21
		2.06, m	2.07, 1.78, 1.21	
4	87.2 s			1.78, 2.06, 4.16, 1.31
5	68.4 d	4.16, dd(7.3, 1.1 Hz)	1.39, 2.18	1.78, 2.06, 1.31
6	30.2 t	1.39, m	4.16, 2.18, 1.65, 2.10	1.65, 2.10
		2.18, m	4.16, 1.39, 2.10	
7	36.6 t	1.65, m	1.39, 2.10	1.48
		2.10, m	1.39, 1.65, 2.18	
8	135.6 s			1.48, 5.03
9	127.2 d	5.03, dd(10.9, 1.2 Hz)	1.68, 2.09	1.48, 1.68, 2.09
10	22.1 t	1.68, m	5.03, 2.09, 1.90	5.03
		2.09, 1.90	1.68, 1.90, 1.41	
11	37.9 t	1.90, m	1.68, 2.09, 1.41	1.22
		1.41, m	2.09, 1.90, 1.68	
12	74.9 s			1.90, 1.41, 1.22, 3.38
13	73.3 d	3.38, dd(7.3, 1.1 Hz)	2.12, 1.62	1.22, 2.12, 1.62
14	28.6 t	2.12, m	1.62, 2.35	2.35
		1.62, m	2.12, 2.35	
15	142.2 s			2.35
16	169.2 s			2.35, 6.37, 5.75
17	125.0 t	6.37, br s		2.35
		5.75, br s		
18	22.7 q	1.31, s		4.16
19	15.9 q	1.48, s		1.65, 2.10
20	24.1 q	1.22, s		

[a] ^{13}C NMR determined at 125 MHz in $CDCl_3$. Multiplicities determined by DEPT and HMQC experiments. [b] ^{1}H NMR determined at 500 MHz in $CDCl_3$.

mL for sinulariolide (**2**), 1.5 and 3.0 μg/mL for flexibilide (**3**), and 2.5 and 5.0 μg/mL for (1*R*,13*S*,12*S*,9*S*,8*R*,5*S*,4*R*)-9-acetoxy-5,8:12,13-diepoxycembr-15(17)-en-16,4-olide (**4**).

Experimental Section

General Experimental Procedures. The melting points were determined using a X_4 micro-melting point apparatus and are uncorrected. IR spectra were recorded with a EQUINOX55 (Bruker) spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity INOVA spectrophotometer at 500 and 125 MHz, respectively, in $CDCl_3$ using TMS as internal standard. MS spectra were obtained with a VG ZAB-HS mass spectrometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. Si gel (Qingdao, 200−300 mu) was used for column chromatography.

Animal Material. The soft coral *Sinularia capillosa* (Alcyoniidae) was collected off the Bay of Sanya, Hainan Island, China. A voucher specimen (No. 98-sy-21) is preserved in the Research Centre of Organic Natural Products, Zhongshan University.

Extraction. The soft coral *S. capillosa* (1.2 kg, dried wt) was extracted with EtOH. The EtOH extract was concentrated in vacuo, and the residue was extracted successively with petroleum ether, CH_2Cl_2, and n-BuOH. The CH_2Cl_2 extract was subjected to Si gel column chromatography eluting with petroleum ether containing increasing concentrations of EtOAc. Four fractions were obtained: Fraction A (fr. 26−28) gave sinulariolide (**2**) (15 mg), fraction B (fr. 29,30) gave compound (**4**) (20 mg), fraction C (fr. 50−53) yielded flexibilide (**3**) (100 mg), and fraction D (fr. 79,80) afforded capillolide (**1**) (500 mg).

Capillolide (1): colorless crystals; mp 158−160 °C; $[\alpha]^{25}_D$ +42.8° (*c* 0.05, EtOH); IR (KBr) ν_{max} 3417, 2930, 1700, 1618, 1049 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; FABMS *m/z* 375 $[M + Na]^+$; EIMS *m/z* 335 $[M + 1 - H_2O]^+$ (37), 317 (23), 299 (16), 279 (10), 154 (64), 140 (62), 121 (35), 55(100).

Sinulariolide (2): colorless crystals, mp 171−173 °C; $[\alpha]^{25}_D$ +134.4° (*c* 0.03, EtOH); IR (KBr) ν_{max} 3456, 2937, 1712, 1462, 1382, 1238, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.29 (1H, s; C_{17}−H), 5.42 (1H, s; C_{17}−H), 5.13 (1H, br d, J = 9.0 Hz; H-9), 4.08 (1H, dd, J = 10.0, 7.0 Hz; H-5), 2.93 (1H, dd, J = 11.0, 3.0 Hz; H-13), 1.61 (3H, s; H-19), 1.34 (3H, s), 1.20 (3H, s); ^{13}C NMR ($CDCl_3$) δ 168.9 (C-16), 144.4 (C-15), 134.8 (C-8), 126.6 (C-9), 124.1 (C-17), 87.0 (C-4), 68.7 (C-5), 63.9 (C-13), 60.4 (C-12), 38.1 (CH_2), 35.9 (CH_2), 35.1 (C-1), 33.1 (CH_2), 31.7 (CH_2), 31.4 (CH_2), 26.9 (CH_2), 25.0 (CH_2), 22.8 (CH_3), 16.0 (CH_3), 15.8 (CH_3).

Flexibilide (3): colorless crystals, mp 153−155 °C; $[\alpha]^{25}_D$ −90.4° (*c* 0.03, EtOH); IR (KBr) ν_{max} 3395, 2940, 1731, 1286, 1180, 1030 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.48 (1H, d, J = 2.0 Hz; H-17), 5.70 (1H, d, J = 2.0 Hz; H-17), 5.24 (1H, br t, J = 8.0 Hz; H-7), 3.98 (1H, d, J = 11.0 Hz; H-3), 2.81 (1H, dd, J = 9.0, 4.0 Hz, H-11), 1.67 (3H, s; H-19), 1.45 (3H, s), 1.32 (3H, s); ^{13}C NMR ($CDCl_3$) δ 167.1 (C-16), 140.1 (C-15), 134.5 (C-7), 127.8 (C-17), 125.6 (C-8), 82.7 (C-3), 74.1 (C-4), 62.9 (C-11), 58.9 (C-12), 38.6 (CH_2), 35.9 (CH_2), 33.8 (C-1), 33.0 (CH_2), 27.8 (CH_2), 25.3 (CH_2), 24.9 (CH_2), 22.6 (CH_2), 15.4 (CH_3), 15.4 (CH_3).

(1*R*,13*S*,12*S*,9*S*,8*R*,5*S*,4*R*)-9-Acetoxy-5,8:12,13-diepoxycembr-15(17)-en-16,4-olide (4): colorless crystals, mp 148−149 °C; $[\alpha]^{25}_D$ +39.1° (*c* 0.03, EtOH); IR (KBr) ν_{max} 3440, 2979, 1738, 1715, 1628, 1376, 1236, 1059 cm^{-1}; ^{1}H NMR($CDCl_3$) δ 6.27 (1H, s; H-17), 5.44 (1H, s, H-17), 5.17 (1H, d, J = 9.0 Hz), 4.38 (1H, dd, J = 9.7 Hz, 3.0 Hz, H-5), 3.39 (1H, dd, J = 11.0, 4.0 Hz; H-13), 3.22 (1H, m), 2.57 (1H, dd, J = 13.0, 6.5 Hz), 2.06 (3H, s), 1.25 (3H, s), 1.24 (3H, s), 1.16 (3H, s); ^{13}C NMR-($CDCl_3$) δ 171.1, 169.5 (C-16), 145.4 (C-15), 123.5 (C-17), 88.1 (C-4), 85.4 (C-8), 83.4 (C-5), 77.9 (C-9), 60.8 (C-13), 59.9 (C-12), 35.7 (C-1), 34.9 (CH_2), 34.3 (CH_2), 33.6 (CH_2), 33.2 (CH_2), 29.9 (CH_2), 29.3 (CH_3), 27.3 (CH_2), 26.8 (CH_2), 21.0 (CH_3), 17.0 (CH_3), 16.4 (CH_3).

Cytotoxicity Testing. P-388 and L1210 tumor cells were obtained from the Cancer Institute, Sun Yat-sen University of Medical Sciences. Cytotoxicity assays of the tested compounds **1**−**4** were carried out by a modification of the MTT colorimetric method.[9] The culture cells were treated at eight concentrations of pure test compounds ranging from 0.00064 to 50 μg/mL. All assays were performed in triplicate. The results were expressed as a percentage, relative to control incubations, and the effective dose required to inhibit cell growth by 50% (ED_{50}) was determined.

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