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A Novel Cytotoxic Biscembranoid from the Formosan Soft Coral *Sinularia flexibilis*

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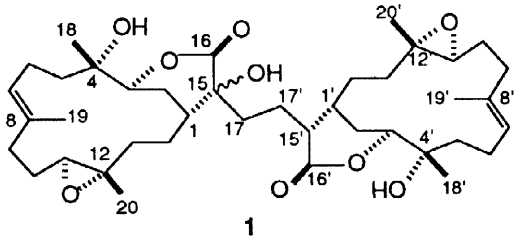
Abstract: A novel cytotoxic biscembranoid diterpene, sinuflexlin (**1**), have been isolated from the soft coral *Sinularia flexibilis*. The structure of **1** (novel carbon skeleton) was determined by 1D and 2D NMR spectral analysis.

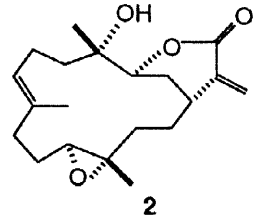
In a search for bioactive substances from marine organisms, the Formosan soft coral *Sinularia flexibilis* (Quoy and Gaimard) (Alcyoniidae) was selected for study since its CH_2Cl_2 extracts showed significant cytotoxicity in several tumor cell lines as determined by standard procedures.[1-2] Bioassay-guided fractionation resulted in the isolation of a novel cytotoxic biscembranoid diterpenes, sinuflexlin (**1**). There are several reports of biscembranoids from *Sarcophyton tortuosum*,[3-4] *Sarcophyton glaucum*[5] and *Sinularia gardineri*.[6]

Sinuflexlin (**1**) was obtained as colorless prisms, mp 248-249° C; $[\alpha]_D$ -25° (c 0.11, $CHCl_3$). Analysis of HRFABMS revealed a molecular formula of $C_{20}H_{62}O_9$ [$(M+H)^+$ *m/z* 687.4443 (Δ -1.5 mmu)]. Its IR spectrum (KBr) suggested the presence of hydroxy groups (3460 cm^{-1}) and ester carbonyl groups (1724 cm^{-1}). Its NMR spectrum showed the signals of two olefinic methyls [δ_H 1.65 (s), 1.66 (s); δ_C 16.3 (q), 16.6 (q)], four methyls attached to quaternary oxygen bearing carbons [δ_H 1.22 (s), 1.24 (s), 1.43 (s), 1.45 (s); δ_C 15.4 (q), 15.6 (q), 24.9 (q), 25.0 (q)], two trisubstituted double bonds [δ_H 5.25 (m), 5.26 (m); δ_C 127.1 (d), 134.3 (s), 126.0 (d), 133.7 (s)], two lactonic carbonyl carbons [δ_C 173.9 (s), 177.0 (s)], two lactonic methines [δ_H 3.96 (dd, J = 10.9, 1.2 Hz), 4.07 (dd, J = 10.5, 3.6 Hz); δ_C 83.7 (d), 86.7 (d)], two epoxymethine [δ_H 2.80 (m), 2.84 (t, J = 5.8 Hz); δ_C 63.0 (d), 62.9 (d)], two epoxydic quaternary carbons [δ_C 58.8 (s), 58.9 (s)], three hydroxy quaternary carbon [δ_C 73.5 (s), 74.2 (s), 74.7 (s)], three methines, and sixteen methylenes. These evidences suggested **1** may be a dimeric cembranoid.

Analysis of 1D and 2D NMR spectra (Table 1), including COSY, NOESY, HMQC, and HMBC, led to the conclusion that **1** was a biscembranoid of **2**.[7] ^1H-^1H COSY connectivities between H_2-17 and H_2-17' and HMBC correlations of H-17β to C-15' and H_2-17' to C-15 revealed that **1** was a biscembranoid connected through C17-C17' single bond formation plus a hydroxylation at C-15. The NOESY data of **1** confirmed the relative stereochemistry of **1** except the tertiary hydroxyl group at C-15.



1



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Sinuflexlin (**1**) exhibited cytotoxicity against P-388 cell culture system with ED_{50} of 1.32 μg/ml. Hetero Diels-Alder coupling of two **2** and subsequent hydrolysis (Scheme 1) may be the route for dimerization.

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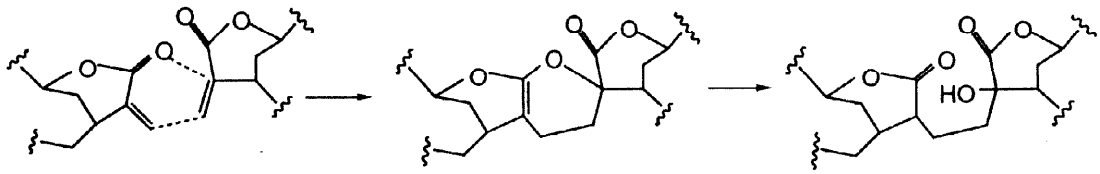
Table 1. ^{1}H- and ^{13}C-NMR data of sinuflexlin (**1**) (400 MHz and 100.6 MHz, respectively). The chemical shifts are given in ppm relative to TMS, and the coupling constants (*J*) in Hz.

Pos.	δH; mult.*J*	δC; mult.	HMBC	NOESY
1	1.89; m	40.4; d	15	20
2	1.80; m	31.1; t	3,15	
3	4.07; dd; 3.5,3.6	86.7; d		7,18,19
4		74.3; s		
5	1.78; m	38.1; t	3,4,6,18	18
6α	1.92; m	23.0; t		
6β	1.45; m		4	
7	5.25; m	127.1; d	5,9,19	3,11,19
8		134.3; s		
9α	2.17; m	36.1; t	7,8,11,19	19
9β	2.28; m		7,8,11,19	
10	1.60; m	25.5; t	11	
11	2.84; t; 5.8	63.0; d	9,10	6β,20
12		58.9; s		
13	2.10; m	34.6; t	12	
14	1.77; m	24.1; t		
15		74.7; s		
16		177.0; s		
17α	2.03; m	27.6; t	15'	
17β	1.40; m		15	20
18	1.45; s	25.0; q	3,5	3,6β
19	1.66; s	16.6; q	7,8,9	3,9β
20	1.24; s	15.6; q	11,12,13	1,11,17β
1'	1.47; m	33.5; d	2',13'	20'
2'	1.75; m	29.0; t	3',15'	
3'	3.96; dd; 10.9,1.2	83.7; d		7',18',19'
4'		74.2; s		
5'	1.78;m	37.9; t	3',4',6',18'	
6'α	1.38; m	22.9; t	4'	
6'β	2.02; m		4'	18'
7'	5.26; m	126.0; d	5',9',19'	3',11'
8'		133.7; s		
9'α	2.17; m	35.7; t	7',8',11',19'	19'
9'β	2.28; m		7',8',11',19'	
10'	1.60; m	25.4; t	11'	
11'	2.80; m	62.9; d	9',10'	7',20'
12'		58.8; s		
13'	2.10; m	34.6; t	1',12'	
14'	2.22; m	23.2; t		
15'	2.19;m	46.8; d	17'	
16'		173.9; s		
17'α	1.93; m	25.2; t	15	
17'β	1.47; m		15	3',20'
18'	1.43; s	24.9; q	3',4',5'	3',6'β
19'	1.65; s	16.3; q	7',8',9'	3,9β
20'	1.22; s	15.4; q	11',12',13'	1',11',17'β



Scheme 1. Proposed biosynthetic pathway for **2** to **1**

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