

New Cembranoids from the Okinawan Soft Coral *Sinularia mayi*Takenori KUSUMI, Kengo YAMADA, Midori O. ISHITSUKA, Yoshiharu FUJITA,  
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Three new cembranoids, (+)-isosarcophine (**1**), (+)-8-hydroxyisosarcophytoxide-6-ene, and (+)-methyl cembra-1,3,7,11-tetraene-16-carboxylate have been isolated from *Sinularia mayi*, and their structures including the absolute configuration of **1** have been determined spectroscopically and by means of chemical reaction.

A growing number of recent investigations on marine natural products have revealed that marine organisms are rich in biologically active compounds, many of which have unique structures never found in terrestrial organisms.<sup>1)</sup> In the course of our works on physiologically significant substances from marine resources,<sup>2)</sup> we isolated new cembranoids showing moderate cytotoxicity from the Okinawan soft coral *Sinularia mayi*.

Freshly collected (at Henoko bay in April, 1988) *S. mayi* was extracted with acetone, and the extract was partitioned between hexane and water. The hexane soluble material (50 g) was fractionated chromatographically to give isosarcophytoxide (30 g),<sup>3)</sup> together with three cembranoids **1** (10 mg), **2** (72 mg), and **3** (2 mg).

(+)-Isosarcophine (**1**),  $[\alpha]_D^{25} +235.3^\circ$  (c 0.14, CHCl<sub>3</sub>), C<sub>20</sub>H<sub>28</sub>O<sub>3</sub>,  $m/z$  316 (M<sup>+</sup>), shows the IR absorptions at 1750 and 1670 cm<sup>-1</sup> due to an unsaturated  $\gamma$ -lactone. Absence of other C=O and OH bands in the IR spectrum indicates that the residual oxygen is involved in an ether linkage. Presence of a trisubstituted oxirane is suggested by the <sup>1</sup>H NMR (500 MHz) signals at  $\delta$  2.53 (1H, dd) and 1.31 (3H, s) and <sup>13</sup>C NMR (125 MHz) signals at  $\delta$  62.0 (d) and 60.9 (s). The double-doublets nature of the oxirane proton suggested that the proton is adjacent to a methylene group. The <sup>1</sup>H NMR spectrum exhibits two olefinic proton signals at  $\delta$  4.97 and 4.84, and the <sup>13</sup>C NMR spectrum shows that three olefinic bonds [ $\delta$  161.2 (s), 145.0 (s), 133.8 (s), 125.3 (d), 123.5 (s), 120.6 (d)] are included, which necessitates one more ring in **1**. One of the olefin protons ( $\delta$  4.84) is coupled with an oxymethine proton ( $\delta$  5.41) with  $J = 10.3$  Hz. The routine analysis of COSY and H,C-COSY spectra led to the partial structures A, B, C, and D, which could be mutually

combined to form **1** (plain) by observing the correlation peaks of the COLOC spectrum: A $\leftrightarrow$ B;  $\delta$  2.21 (5-H)  $\rightarrow$   $\delta$  145.0 (4-C),  $\delta$  120.6 (3-C),  $\delta$  15.2 (18-C);  $\delta$  4.84 (3-H)  $\rightarrow$   $\delta$  38.7 (5-C): B $\leftrightarrow$ C;  $\delta$  1.98 (9-H)  $\rightarrow$   $\delta$  133.8 (8-C), 14.9 (19-C);  $\delta$  4.97 (7-H)  $\rightarrow$   $\delta$  36.8 (9-C): C $\leftrightarrow$ D;  $\delta$  1.04 (13-H)  $\rightarrow$   $\delta$  60.9 (12-C), 16.0 (20-C);  $\delta$  1.31 (20-H)  $\rightarrow$   $\delta$  37.2 (13-C): D $\leftrightarrow$ A;  $\delta$  2.08 (14-H)  $\rightarrow$   $\delta$  161.2 (1-C), 123.5 (15-C);  $\delta$  2.52 (14-H)  $\rightarrow$   $\delta$  161.2 (1-C), 78.5 (2-C). The geometry of the two olefinic bonds at C-3 and 7 was determined to be *E* from the chemical shifts of the olefinic methyls (C-18;  $\delta$  15.2, C-19;  $\delta$  14.9). Finally, the stereochemistry and conformation of **1** were elucidated as depicted in **1a** on the basis of NOEs observed in the phase-sensitive NOESY spectrum as well as the coupling patterns of the protons (Table 1).

The structure and the absolute configuration of **1** were firmly established by chemical conversion: a solution of isosarcophytoxide in CH<sub>2</sub>Cl<sub>2</sub> was allowed to stand in the presence of light<sup>4)</sup> (daylight and fluorescent light) for 1 week at room temperature. After separation of the product, a lactone was obtained. The IR and NMR properties of the lactone are identical with those of (+)-isosarcophine (**1**). Also the rotation of the product,  $[\alpha]_D +192.5^\circ$  (c 0.13, CHCl<sub>3</sub>), is the same in the sign of that of **1** (+235.3°), which reinforces the absolute configuration of **1** to be as shown in the structure because the configuration of isosarcophytoxide has been absolutely determined.<sup>3)</sup>

8-Hydroxyisosarcophytoxide-6-ene (**2**),  $[\alpha]_D +90.7^\circ$  (c 0.52, CHCl<sub>3</sub>), C<sub>20</sub>H<sub>30</sub>O<sub>3</sub>, *m/z* 318 (M<sup>+</sup>), exhibits a broad IR band at 3500-3100 cm<sup>-1</sup> assignable to OH. No carbonyl band appears in the IR spectrum. The <sup>1</sup>H and <sup>13</sup>C NMR are as a whole similar to those of isosarcophytoxide except that a disubstituted *E*-olefin [ $\delta_H$  5.50 (1H, d, *J* = 16.3 Hz), 5.57 (1H, dt, *J* = 16.3, 7.1 Hz)] and a methyl carbinol [ $\delta_H$  1.13 (3H, s),  $\delta_C$  72.5 (s)] are present in **2**. One ( $\delta$  5.57) of the *E*-olefin protons is coupled with the doubly allylic methylene protons ( $\delta$  2.64, 2.40). The NMR data of **2** further suggested the presence of the dihydrofuran [ $\delta_H$  5.54 (1H, m, H-2),  $\delta_C$  85.0 (d, C-2);  $\delta_H$  4.58 (1H, dd, *J* = 11.3, 5.5 Hz, H-16), 4.51 (1H, dd, *J* = 11.3, 4.7 Hz, H-16'),  $\delta_C$  79.4 (t, C-16);  $\delta_H$  1.42 (3H, bs, H<sub>3</sub>-17),  $\delta_C$  10.6 (q, C-17)] and oxirane [ $\delta_H$  2.80 (1H, t, *J* = 6.3 Hz, H-11),  $\delta_C$  62.2 (d, C-11);  $\delta_H$  1.18 (3H, s, H<sub>3</sub>-20),  $\delta_C$  17.0 (q, C-20)] rings as seen in isosarcophytoxide. Consideration of the correlation peaks found in COLOC spectrum (see structure) led to the plain structure **2**, whose stereochemistry (**2a**) was deduced by means of the NOESY spectrum.

The structure of the minor component, (+)-methyl cembra-1,3,7,11-tetraene-16-carboxylate (**3**),<sup>5)</sup>  $[\alpha]_D +43.4^\circ$  (c 0.09, CHCl<sub>3</sub>), was determined essentially in the same manner as described for **1** and **2**: The UV maximum at 242 nm ( $\epsilon$  9,800) is compatible with the trisubstituted diene. The *E*-configurations of 3, 7, and 11-olefins are confirmed by the <sup>13</sup>C NMR chemical shifts of the olefinic methyls (either three of  $\delta$  17.2, 17.1, 16.7, 15.7). The *E*-configuration of 1-olefin was confirmed by observing NOE between H-2 and H-15 in the NOE difference spectrum.

The present cembranoid showed the following cytotoxic activities against HCT-116 cells: **1**; 64  $\mu$ g/ml; **2**, 27  $\mu$ g/ml.

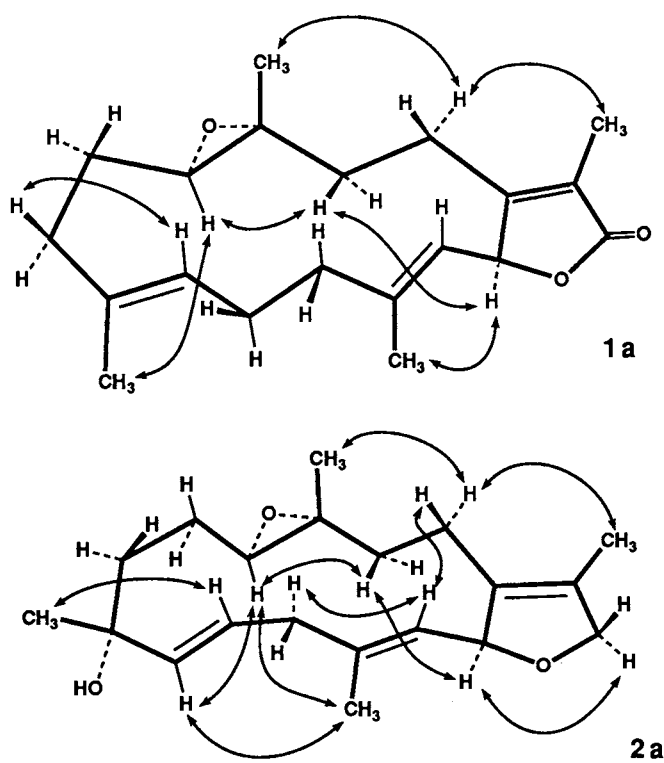
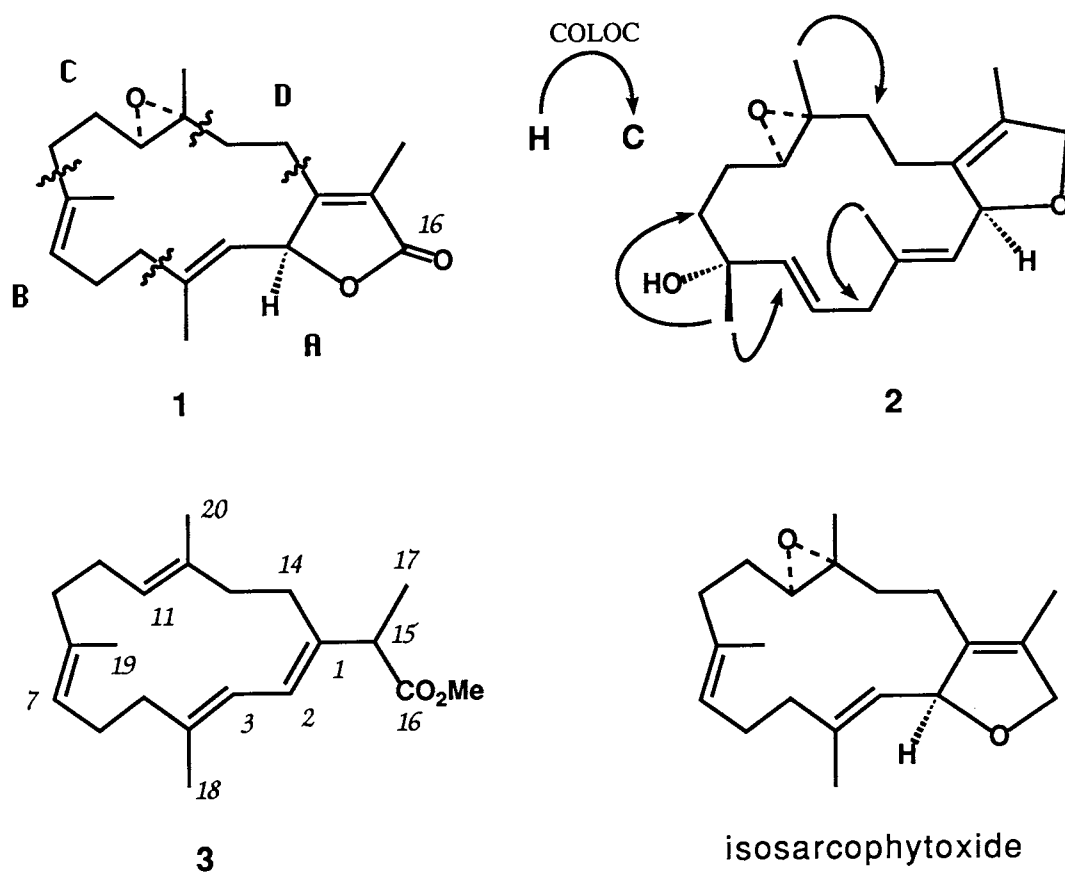


Table 1. NMR data of **1** and **2**

<b>1</b> (CDCl <sub>3</sub> )						<b>2</b> (C <sub>6</sub> D <sub>6</sub> )					
No.	δ <sub>C</sub>		δ <sub>H</sub>		<i>J</i> (Hz)	δ <sub>C</sub>		δ <sub>H</sub>		<i>J</i> (Hz)	
1	161.2	s	-			133.7*	s	-			
2	78.5	d	5.41	dq	10.3,2.0	85.0	d	5.54	m		
3	120.6	d	4.84	dquin	10.3,2.0	127.5	d	5.26	d	10.5	
4	145.0	s	-			138.0	s	-			
5	38.7	t	2.35	br.dt	12.8,4.4	42.3	t	2.64	dd	14.2,7.1	
			2.21	td	12.8,4.4			2.40	dd	14.2,7.1	
6	24.3	t	2.45	dddd	14.1,12.8, 9.7,4.4	126.0	d	5.57	dt	16.3,7.1	
			2.11	dt	14.1,4.4						
7	125.3	d	4.97	dq	9.7,1.6	140.5	d	5.50	d	16.3	
8	133.8	s	-			72.5	s	-			
9	36.8	t	2.31	ddd	13.2,4.8,3.2	40.4	t	1.69	td	15.5,2.9	
			1.98	td	13.2,3.2			1.64	td	15.5,2.1	
10	23.7	t	2.09	tt	13.2,3.2	24.8	t	1.81	m		
			1.26	dddd	13.2,10.5, 4.8,3.2			1.56	m		
11	62.0	d	2.53	dd	10.5,3.2	62.2	d	2.80	t	6.3	
12	60.9	s	-			61.0	s	-			
13	37.2	t	1.99	td	13.0,5.5	37.3	t	1.81	m		
			1.04	td	13.0,2.2			1.23	td	12.2,2.0	
14	24.0	t	2.52	td	13.0,5.5	23.0	t	2.09	td	12.2,5.0	
			2.08	td	13.0,2.2			1.81	m		
15	123.5	s	-			129.0*	s	-			
16	174.7	s	-			79.4	t	4.58	dd	11.3,5.5	
								4.51	dd	11.3,4.7	
17	8.6	q	1.84	br.s		10.6	q	1.42	br.s		
18	15.2	q	1.67	br.s		17.1	q	1.59	br.s		
19	14.9	q	1.67	br.s		29.1	q	1.13	s		
20	16.0	q	1.31	s		17.0	q	1.18	s		

The authors are grateful to Tokyo Bristol Myers Research Laboratory for the bioassay.

#### References

- 1) D. J. Faulkner, *Natural Prod. Reports*, **1**, 251, 551(1984); **3**, 1(1986); **4**, 539 (1987); **5**, 631 (1988).
- 2) M. O. Ishitsuka, T. Kusumi, and H. Kakisawa, *J. Org. Chem.*, **53**, 5010 (1988).
- 3) B. F. Bowden, J. C. Coll, A. Heaton, and G. König, *J. Nat. Prod.*, **50**, 650 (1987).
- 4) J. M. Frincke, D. E. McIntyre, and D. J. Faulkner, *Tetrahedron Lett.*, **21**, 735 (1980).
- 5) IR (CHCl<sub>3</sub>) 1720, 1420, 1150 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.28 (3H, d, *J* = 7.5 Hz), 1.50, 1.58, 1.73 (each 3H, s), 2.0-2.2 (10H, m), 2.3 (1H, m), 2.4 (1H, m), 3.16 (1H, q, *J* = 7.5 Hz), 3.65 (3H, s), 4.95-5.01 (2H, m), 5.90 (1H, d, *J* = 11.3 Hz), 6.13 (1H, d, *J* = 11.3 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 15.7 (q), 16.7 (q), 17.1 (q), 17.2 (q), 24.5 (t), 25.3 (t), 28.6 (t), 37.7 (t), 38.9 (t), 39.3 (t), 45.5 (d), 51.8 (q), 121.5 (d), 122.9 (d), 124.5 (d), 124.9 (d), 134.3 (s), 134.5 (s), 136.6 (s), 137.0 (s), 175.6 (s).

(Received May 28, 1990)