

Article

# New Cembranoids and a Biscembranoid Peroxide from the Soft Coral *Sarcophyton cherbonnieri*

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**Abstract:** Six new cembranoids, cherbonolides A–E (1–5) and bischerbolide peroxide (6), along with one known cembranoid, isosarcophine (7), were isolated from the Formosan soft coral *Sarcophyton cherbonnieri*. The structures of these compounds were elucidated by detailed spectroscopic analysis and chemical methods. Compound 6 was discovered to be the first example of a molecular skeleton formed from two cembranoids connected by a peroxide group. Compounds 1–7 were shown to have the ability of inhibiting the production of superoxide anions and elastase release in *N*-formyl-methionyl-leucyl-phenylalanine/cytochalasin B (fMLF/CB)-induced human neutrophils.

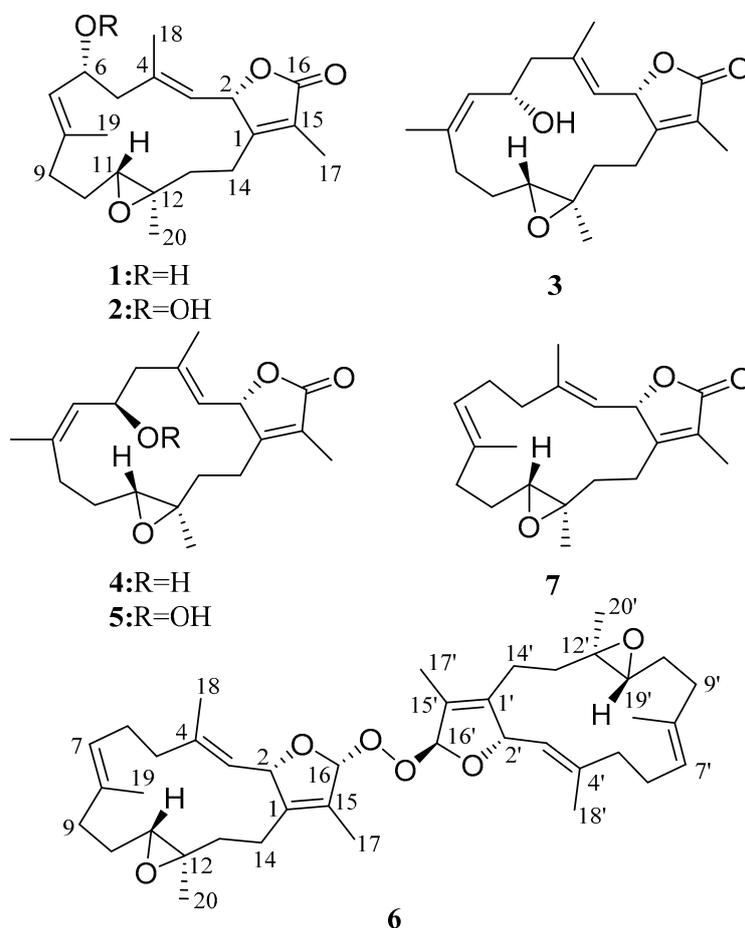
**Keywords:** *Sarcophyton cherbonnieri*; cembranoid; biscembranoid peroxide; anti-inflammatory activity; elastase release inhibition

## 1. Introduction

Many cembrane-based natural products have been shown to exhibit significant activities such as cytotoxicity [1–14] and anti-inflammatory activity [9,11,13–18]. From the experience of searching bioactive metabolites from soft corals, series of cembranoids have been unveiled from octocorals (Alcyonaceae) belonging to the genera *Sarcophyton*, [1–8,16], *Sinularia* [9–12,17,18] and *Lobophyton* [13–15]. Also, previous studies showed that two cembranoid units could form biscembranoid-type compounds by Diels-Alder reaction [19–21], radical dimerization [22,23], or connection with a sulfur atom [18], making the chemistry of cembranes more complex and interesting than monomeric form.

Our current chemical investigation on *Sarcophyton cherbonnieri* led to the discovery of six new cembranoids, cherbonolides A–E (1–5) and bischerbolide peroxide (6), and one known compound, isosarcophine (7) [24]. The structures of 1–7 (Figure 1) were elucidated by extensive spectroscopic analysis, including detailed 2D nuclear magnetic resonance (NMR) experiments and chemical methods. Compounds 2, 5, and 6 were characterized as cembranoids bearing an allylic peroxy group as those

previously discovered marine cembranoidal peroxides [25–29]. Furthermore, compound **6** is the first example of a biscembranoid with two cembranoidal units interconnected by a peroxy group. The absolute configurations of **1** and **3** were further established using a modified Mosher's reaction. Also, evaluation of the in vitro anti-inflammatory activities through the inhibition of superoxide anion ( $O_2^{\bullet-}$ ) generation and elastase release in *N*-formyl-methionyl-leucyl-phenylalanine/cytochalasin B (fMLF/CB)-induced human neutrophils was carried out.



**Figure 1.** Cembranoid isolated from *Sarcophyton cheronnieri*.

## 2. Results and Discussion

The soft coral *S. cheronnieri* (1.2 kg, wet weight) was collected using SCUBA diving from Jihui Port of Taitung, Taiwan, in March 2013, and stored in a freezer before extraction. The freeze-dried organisms (207 g) were sliced into small pieces, followed by exhaustive extraction with ethyl acetate (EtOAc). The EtOAc extract was dried with anhydrous sodium sulfate ( $Na_2SO_4$ ). After removal of EtOAc under reduced pressure, the residue yielded was separated by silica gel column chromatography and the resolved fractions were further purified by reverse-phase  $C_{18}$  high-performance liquid chromatography (HPLC) to afford compounds **1–7** (Figure 1), the structures of which were elucidated on the basis of spectroscopic analyses (Supplementary Materials, Figures S1–S46).

Cherbonolide A (**1**) was isolated as a colorless oil. The molecular formula  $C_{20}H_{28}O_4$  of **1** was determined by the high-resolution electrospray ionization mass spectrometry (HRESIMS) ( $m/z$  calcd 355.1880; found 355.1879,  $[M + Na]^+$ ), which required seven degrees of unsaturation. The IR spectrum of **1** showed the presence of a hydroxyl group ( $\nu_{max}$  3457  $cm^{-1}$ ) and a lactonic carbonyl group ( $\nu_{max}$  1746  $cm^{-1}$ ). The presence of 20 carbons in the structure of **1**, including four methyls, five  $sp^3$  methylenes, three  $sp^3$  oxygenated methines, two  $sp^2$  methines, one  $sp^3$  and five  $sp^2$  nonprotonated carbon atoms were

assigned with the aid of distortionless enhancement by polarization transfer (DEPT) spectra. The NMR peaks resonating at  $\delta_C$  174.4 (C), 160.7 (C), 123.8 (C), 77.8 (CH) and 8.8 (CH<sub>3</sub>), and  $\delta_H$  5.44 (1H, dd,  $J = 10.0, 1.6$  Hz) and  $\delta_H$  1.86 (3H, s), are characteristic signals of an  $\alpha$ -methyl- $\alpha,\beta$ -unsaturated- $\gamma$ -lactone ring by comparison of the NMR data of the  $\gamma$ -lactone ring of known compound isosarcophine (7). Signals at  $\delta_C$  60.8 (C), 61.4 (CH) and  $\delta_H$  2.42 (1H, dd,  $J = 10.8, 2.8$  Hz) showed the presence of a trisubstituted epoxide. Two trisubstituted double bonds could also be identified by NMR signals resonating at  $\delta_C$  122.2 (CH),  $\delta_C$  141.6 (C) and  $\delta_H$  4.90 (1H, d,  $J = 10.0$  Hz), and at  $\delta_C$  128.1 (CH),  $\delta_C$  139.8 (C) and  $\delta_H$  5.20 (1H, d,  $J = 10.4$  Hz), respectively. The correlations identified from the  $^1\text{H}$ - $^1\text{H}$  correlation spectroscopy ( $^1\text{H}$ - $^1\text{H}$  COSY) spectrum revealed four separate proton sequences, as shown in Figure 2, which were assembled by heteronuclear multiple bond correlation (HMBC) correlations (Figure 2). Key HMBC correlations of H-2 ( $\delta_H$  5.44, 1H, dd,  $J = 10.0, 1.6$  Hz) to C-1; H<sub>2</sub>-14 ( $\delta_H$  2.01, m)/C-1; H<sub>3</sub>-17 ( $\delta_H$  1.86, s) to C-1, C-15 and C-16; H<sub>3</sub>-18 ( $\delta_H$  1.70, s) to C-3, C-4 and C-5; H<sub>3</sub>-19 ( $\delta_H$  1.86, s) to C-7, C-8 and C-9; and H<sub>3</sub>-20 ( $\delta_H$  1.33, s) to C-11, C-12 and C-13, established the connection of the four proton sequences, and thus constructed the 14-membered ring carbon skeleton, which also indicated the presence of a hydroxyl at C-6. Thus, the planar structure of **1** was established.

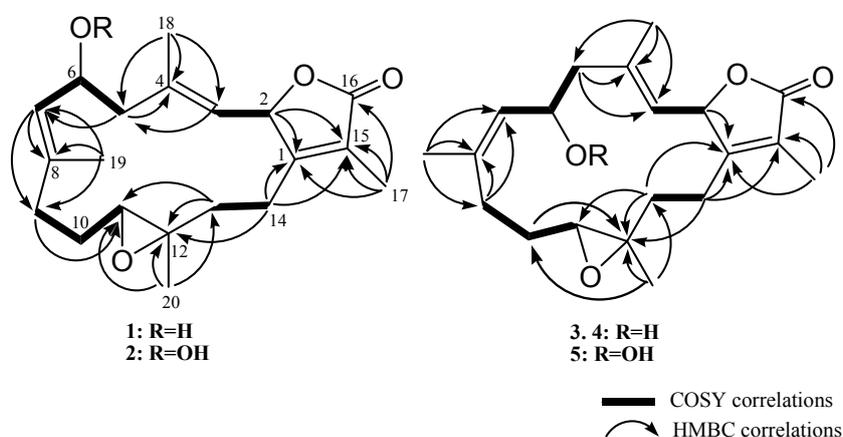


Figure 2. Selected  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations of **1**, **2** and **3–5**.

Further, careful analysis of nuclear Overhauser enhancement (NOE) correlations was applied to establish the relative stereochemistry of **1**, as shown in Figure 3. The NOE spectrum revealed that H-2 ( $\delta_H$  5.44, dd,  $J = 10.0, 1.6$  Hz) showed NOE correlation with H<sub>3</sub>-18 ( $\delta_H$  1.70, s); therefore, assuming a  $\beta$ -orientation of H-2, H<sub>3</sub>-18 should be  $\beta$ -oriented, too. Moreover, H<sub>3</sub>-18 exhibited NOE correlation with H-6 ( $\delta_H$  4.70, ddd,  $J = 10.4, 10.4, 5.2$  Hz), revealing the  $\beta$ -orientation of H-6 and the  $R^*$  configuration of C-6. One methylene proton at C-13 exhibited NOE correlation with H-2 and was characterized as H-13 $\beta$  ( $\delta_H$  1.06, m), while the other proton was assigned as H-13 $\alpha$  ( $\delta_H$  2.03, m). NOE correlations of H-13 $\beta$  with H-11 ( $\delta_H$  2.42, dd,  $J = 10.8, 2.8$  Hz) and H-13 $\alpha$  with H<sub>3</sub>-20 ( $\delta_H$  1.33, s) reflected the  $\beta$ -orientation of H-11 and the  $\alpha$ -orientation of H<sub>3</sub>-20, and hence the  $R^*$  configurations of both C-11 and C-12. The  $E$  geometries of the trisubstituted C-3/C-4 and C-7/C-8 double bonds were also assigned from the NOE correlations of H<sub>3</sub>-18 ( $\delta_H$  1.70, s) with H-2, but not with H-3 ( $\delta_H$  4.90, d,  $J = 10.0$  Hz), as well as H<sub>3</sub>-19 ( $\delta_H$  1.86, s) with H-6, but not with H-7 ( $\delta_H$  5.20, d,  $J = 10.4$  Hz), and were also confirmed by the upfield chemical shifts ( $\delta_C < 20$  ppm) observed for both C-18 ( $\delta_C$  15.9) and C-19 ( $\delta_C$  14.9) [30]. Based on the above observations and the detailed analysis of other NOE correlations, the relative configuration of this compound was established. Furthermore, the absolute configuration of **1** at C-6 was determined by the modified Mosher's esterification method [31,32]. The (*S*)- and (*R*)-MTPA esters of **1** (**1a** and **1b**, respectively, as shown in Figure 4) were afforded by the reaction of **1** with (*R*)-(-) and (*S*)-(+)-MTPA chloride, respectively. Determination of the  $\Delta\delta$  values ( $\delta_S - \delta_R$ ) for protons nearing C-6 resulted in the establishment of the  $R$  configuration at C-6 in **1** (Figure 4). The absolute configuration of **1** was

thus assigned as *2S,6R,11R,12R*, mostly the same as that of isosarcophine (7) [24], except that of C-6. Therefore, cherbonolide A (1) was identified as 6- $\alpha$ -hydroxyisosarcophine.

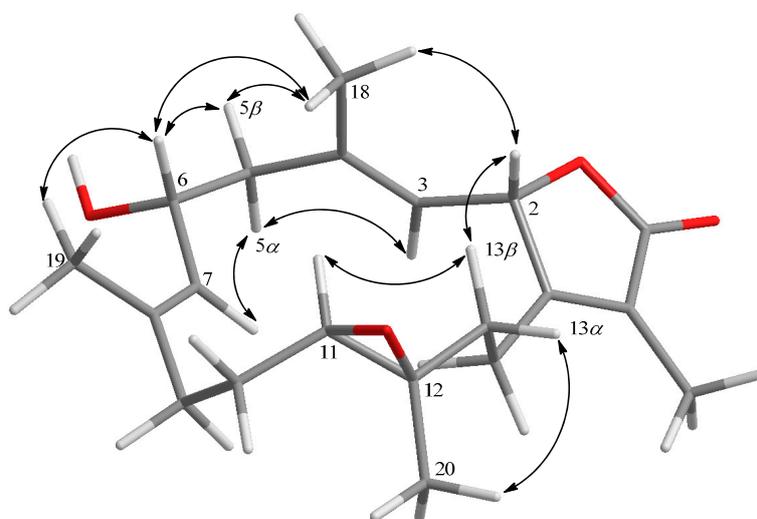


Figure 3. Key NOESY correlations of 1.

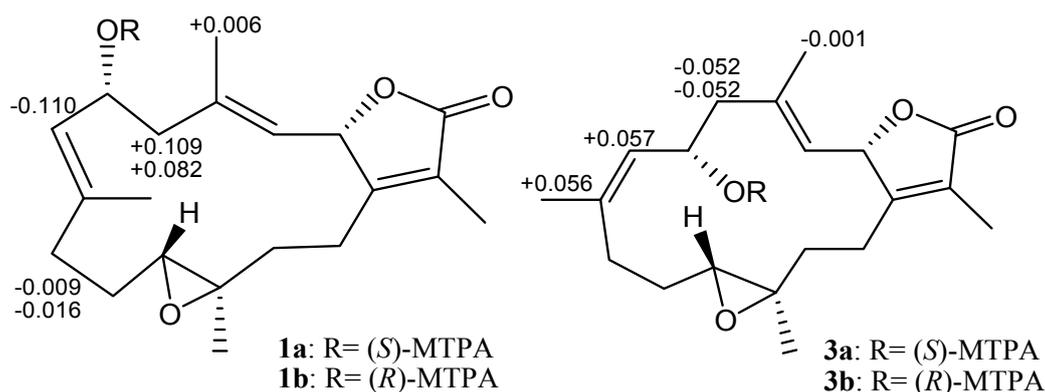


Figure 4.  $^1\text{H}$  NMR chemical shift differences  $\Delta\delta$  ( $\delta_S - \delta_R$ ) in ppm for the MTPA esters of 1 and 3.

The molecular formula of cherbonolide B (2) was determined to be  $\text{C}_{20}\text{H}_{28}\text{O}_5$  by the HRESIMS ( $m/z$  calcd 371.1830; found 371.1829,  $[\text{M} + \text{Na}]^+$ ), having one more oxygen than 1. Moreover, both 1 and 2 had almost identical  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Table 1), except for those of C-6. The allylic hydroxy group of 1 at C-6 was substituted by a hydroperoxyl in 2, with the characteristic signal of a broad singlet in the downfield region,  $\delta_{\text{H}}$  7.99 [26,33,34]. Obvious downfield shifts of C-6 ( $\delta_{\text{C}}$  65.2 in 1, 78.3 in 2) and H-6 ( $\delta_{\text{H}}$  4.70 in 1, 4.97 in 2) were also observed, indicating that 2 possesses the hydroperoxy group at C-6. Furthermore, reduction of 2 by reaction with triphenylphosphine afforded 1. On the basis of the above analyses, the planar structure and the (*2S,6R,11R,12R*)-configuration of 2 were determined.

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts for compounds 1–4.

Position	1		2		3		4	
	$\delta_{\text{H}}$ , m (J in Hz) <sup>a</sup>	$\delta_{\text{C}}$ <sup>b</sup> , type	$\delta_{\text{H}}$ , m (J in Hz) <sup>a</sup>	$\delta_{\text{C}}$ <sup>b</sup> , type	$\delta_{\text{H}}$ , m (J in Hz) <sup>c</sup>	$\delta_{\text{C}}$ <sup>d</sup> , type	$\delta_{\text{H}}$ , m (J in Hz) <sup>c</sup>	$\delta_{\text{C}}$ <sup>d</sup> , type
1		160.7, C		160.6, C		160.6, C		159.8, C
2	5.44, dd (10.0, 1.6)	77.8, CH	5.43, dd (10.0, 1.6)	77.8, CH	4.91, dd (10.0, 1.6)	78.4, CH	4.98, d (10.4)	77.8, CH
3	4.90, d (10.0)	122.2, CH	4.91, d (10.0)	122.7, CH	4.55, d (10.0)	123.9, CH	4.45, d (10.4)	123.5, CH
4		141.6, C		140.8, C		140.2, C		139.4, C
5 $\alpha$	2.20, m	47.9, CH <sub>2</sub>	2.18, dd (12.4, 10.8)	42.6, CH <sub>2</sub>	2.42, dd (12.0, 3.2)	49.1, CH <sub>2</sub>	1.98, m	49.3, CH <sub>2</sub>
5 $\beta$	2.76, dd (12.8, 5.2)		2.87, dd (12.4, 4.4)		1.89, m		2.25, dd (12.8, 3.6)	
6	4.70, ddd (10.4, 10.4, 5.2)	65.2, CH	4.97, ddd (10.8, 9.2, 4.4)	78.3, CH	3.84, dd (9.2, 9.2)	69.6, CH	4.21, ddd (11.2, 9.2, 3.6)	64.8, CH
7	5.20, d (10.4)	128.1, CH	5.05, d (9.2)	123.1, CH	5.09, d (9.2)	131.6, CH	4.84, d (9.2)	131.2, CH
8		139.8, C		144.2, C		138.4, C		139.4, C
9 $\alpha$	2.03, m	36.8, CH <sub>2</sub>	2.07, m	36.9, CH <sub>2</sub>	2.21, ddd (13.6, 13.6, 2.4)	28.2, CH <sub>2</sub>	1.58, m	28.5, CH <sub>2</sub>
9 $\beta$	2.38, m		2.42, m		1.63, m		2.30, dd (13.2, 4.8)	
10 $\alpha$	1.29, m	23.5, CH <sub>2</sub>	1.35, m	23.6, CH <sub>2</sub>	1.16, m	23.9, CH <sub>2</sub>	1.60, m	22.7, CH <sub>2</sub>
10 $\beta$	2.51, m		2.17, m		1.84, m		1.28, m	
11	2.42, dd (10.8, 2.8)	61.4, CH	2.43, m	61.4, CH	2.24, dd (10.4, 2.4)	58.9, CH	1.98, m	62.6, CH
12		60.8, C		60.8, C		59.7, CH		60.9, C
13 $\alpha$	2.03, m	36.9, CH <sub>2</sub>	2.02, m	37.0, CH <sub>2</sub>	1.49, m	35.5, CH <sub>2</sub>	1.61, m	37.1, CH <sub>2</sub>
13 $\beta$	1.06, m		1.07, m		0.98, m		0.65, m	
14 $\alpha$	2.49, m	23.7, CH <sub>2</sub>	2.52, m	23.7, CH <sub>2</sub>	1.58, m	22.2, CH <sub>2</sub>	2.08, m	23.1, CH <sub>2</sub>
14 $\beta$	2.01, m		2.03, m		1.58, m		1.65, m	
15		123.8, C		123.8, C		123.8, C		123.7, C
16		174.4, C		174.4, C		173.9, C		174.3, C
17	1.86, s	8.8, CH <sub>3</sub>	1.86, s	8.7, CH <sub>3</sub>	1.64, s	8.8, CH <sub>3</sub>	1.63, s	8.8, CH <sub>3</sub>
18	1.70, s	15.9, CH <sub>3</sub>	1.72, s	15.9, CH <sub>3</sub>	1.31, s	18.1, CH <sub>3</sub>	1.28, s	16.9, CH <sub>3</sub>
19	1.86, s	14.9, CH <sub>3</sub>	1.89, s	15.3, CH <sub>3</sub>	1.45, s	22.2, CH <sub>3</sub>	1.32, s	21.8, CH <sub>3</sub>
20	1.33, s	15.8, CH <sub>3</sub>	1.33, s	15.8, CH <sub>3</sub>	1.00, s	17.1, CH <sub>3</sub>	0.99, s	16.4, CH <sub>3</sub>
6-OOH			7.99, br s					

<sup>a</sup> Spectrum recorded at 400 MHz in CDCl<sub>3</sub>. <sup>b</sup> Spectrum recorded at 100 MHz in CDCl<sub>3</sub>. <sup>c</sup> Spectrum recorded at 400 MHz in C<sub>6</sub>D<sub>6</sub>. <sup>d</sup> Spectrum recorded at 100 MHz in C<sub>6</sub>D<sub>6</sub>.

Cherbonolide C (**3**) should have the same molecular formula as **1**, according to HRESIMS data. Also, the  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations (Figure 2) of **3** are similar to those of **1**, suggesting that these compounds possess almost the same molecular skeleton. Analysis of NOE correlations (Figure 5) showed that the relative configurations at C-2, C-11 and C-12 in **1** and **3** are the same. Assuming the  $\beta$ -orientation of H-2, as H<sub>3</sub>-18 showed NOE interaction with H-2 but not with H-3, the *E* geometry was assigned to the trisubstituted C-3/C-4 double bond. One of the methylene protons at C-5 ( $\delta_{\text{H}}$  2.42, dd,  $J = 12.0, 3.2$  Hz) displayed NOE interaction with H-3, but not with H<sub>3</sub>-18, and was hence determined as H-5 $\alpha$ . Further, H-6 ( $\delta_{\text{H}}$  3.84, dd,  $J = 9.2, 9.2$  Hz) showed NOE correlations with H-5 $\alpha$  and H-9 $\alpha$ , but not with H-9 $\beta$  and H<sub>3</sub>-19. These observations, together with NOE correlations of H-9 $\beta$ /H<sub>3</sub>-19, H<sub>3</sub>-19/H-7 and H-7/H<sub>3</sub>-18, enabled deduction of the  $\alpha$ -orientation of H-6 and led to the assignment of a 6*S*\* configuration and a *Z* geometry of the trisubstituted C-7/C-8 double bond in **3**. The olefinic methyl group attaching at C-8 showed carbon signal at  $\delta_{\text{C}}$  22.2 ppm further confirmed the *Z* geometry of C-7/C-8 double bond [30]. The absolute configuration of **3** at C-6 was also verified by using the modified Mosher's method. Determination of the  $\Delta\delta$  values ( $\delta_{\text{S}} - \delta_{\text{R}}$ , shown in Figure 4) for protons neighboring C-6 further confirmed the *S* configuration at C-6 in **3** (Figure 4). The absolute configuration of **3** was thus assigned as 2*S*,6*S*,11*R* and 12*R*. Thus, cherbonolide C (**3**) is the 7*Z* isomer of cherbonolide A (**1**).

Cherbonolide D (**4**) was found to be an isomer of **3** according to HRESIMS. Both compounds have almost the same  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations, indicating they have the same molecular skeleton. NMR data of **3** and **4** are nearly the same (Table 1), except for those of CH-6, suggesting that **4** could be the C-6 epimer of **3**. The (2*S*,6*R*,11*R*,12*R*)-configuration and the *E* and *Z* geometries of C-3/C-4 and C-7/C-8 double bonds of **4**, respectively, were also established by analysis of NOE correlations to be as those of **3** (Figure 5).

Cherbonolide E (**5**) was determined to have a molecular formula  $\text{C}_{20}\text{H}_{28}\text{O}_5$  from its HRESIMS data ( $m/z$  calcd 371.1830; found 371.1829,  $[\text{M} + \text{Na}]^+$ ), with one more oxygen than in **4**. Compounds **4** and **5** displayed almost identical  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Table 2), except for those of CH-6. It was found that the hydroxy substituent of **4** at C-6 was replaced by a hydroperoxy group in **5**, with the characteristic signal of a broad singlet at  $\delta_{\text{H}}$  7.25 [26,33,34]. The obvious downfield shifts of C-6 ( $\delta_{\text{C}}$  64.8 in **4**, 78.9 in **5**) and H-6 ( $\delta_{\text{H}}$  4.21 in **4**, 4.58 in **5**) also confirmed the substitution of a hydroperoxy group at C-6 of **5**. Furthermore, reduction of **5** with triphenylphosphine could afford **4**. Therefore, the structure of **5**, with the (2*S*,6*R*,11*R*,12*R*)-configuration, was determined.

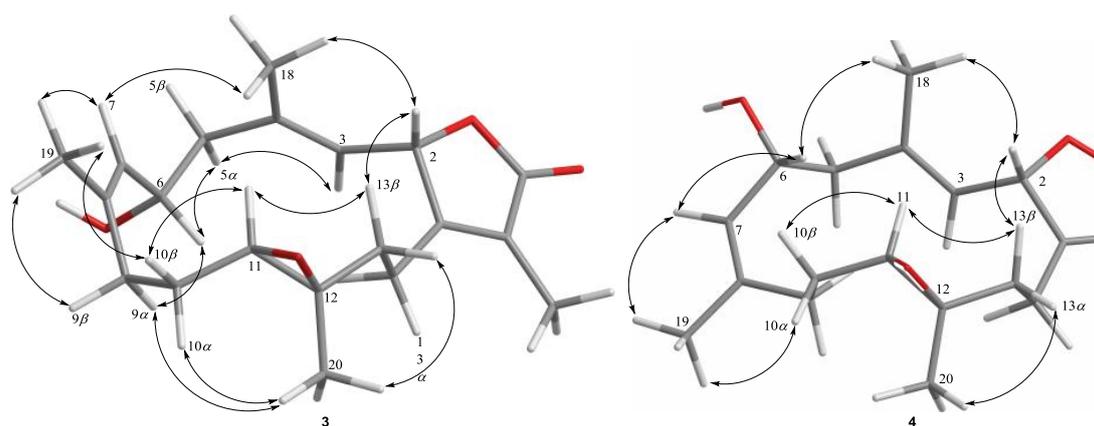


Figure 5. Key NOESY correlations for **3** and **4**.

**Table 2.** <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts for compounds 5 and 6.

Position	5		6		Position	6	
	$\delta_{\text{H}}$ , m (J in Hz) <sup>a</sup>	$\delta_{\text{C}}$ <sup>b</sup> , type	$\delta_{\text{H}}$ , m (J in Hz) <sup>c</sup>	$\delta_{\text{C}}$ <sup>d</sup> , type		$\delta_{\text{H}}$ , m (J in Hz) <sup>c</sup>	$\delta_{\text{C}}$ <sup>d</sup> , type
1		160.4, C		141.4, C	1'		141.5, C
2	4.95, d (10.0)	78.4, CH	5.28, d (10.0)	82.7, CH	2'	5.50, d (10.0)	81.9, CH
3	4.42, d (10.0)	124.6, CH	5.06, d (10.0)	126.4, CH	3'	4.92, d (10.0)	125.1, CH
4		139.2, C		140.2, C	4'		141.0, C
5 $\alpha$	1.95, m	44.6, CH <sub>2</sub>	2.21, m	38.5, CH <sub>2</sub>	5' $\alpha$	2.21, m	38.8, CH <sub>2</sub>
5 $\beta$	2.47, br d (11.0)		2.32, m		5' $\beta$	2.31, m	
6	4.58, ddd (11.0, 9.5, 2.5)	78.9, CH		24.2, CH <sub>2</sub>	6'		24.2, CH <sub>2</sub>
6 $\alpha$			2.07, m		6' $\alpha$	2.07, m	
6 $\beta$			2.42, m		6' $\beta$	2.42, m	
7	4.78, d (9.5)	126.6, CH	4.98, dd (9.2, 9.2)	125.6, CH	7'	4.95, dd (9.2, 9.2)	125.5, CH
8		143.8, C		133.1, C	8'		133.3, C
9 $\alpha$	1.65, m	29.8, CH <sub>2</sub>	1.96, m	36.6, CH <sub>2</sub>	9' $\alpha$	1.96, m	36.6, CH <sub>2</sub>
9 $\beta$	2.52, dd (14.0, 4.5)		2.27, m		9' $\beta$	2.27, m	
10 $\alpha$	1.28, m	23.4, CH <sub>2</sub>	1.22, m	23.6, CH <sub>2</sub>	10' $\alpha$	1.22, m	23.7, CH <sub>2</sub>
10 $\beta$	1.62, m		2.04, m		10' $\beta$	2.04, m	
11	1.97, m	63.3, CH	2.51, m	62.1, CH	11'	2.51, m	62.2, CH
12		61.5, C		61.2, CH	12'		61.3, C
13 $\alpha$	1.59, m	37.6, CH <sub>2</sub>	1.83, m	37.3, CH <sub>2</sub>	13' $\alpha$	1.83, m	37.4, CH <sub>2</sub>
13 $\beta$	0.64, m		0.95, m		13' $\beta$	0.95, m	
14 $\alpha$	2.07, m	23.7, CH <sub>2</sub>	2.33, m	22.6, CH <sub>2</sub>	14' $\alpha$	2.33, m	22.7, CH <sub>2</sub>
14 $\beta$	1.61, m		1.81, m		14' $\beta$	1.81, m	
15		124.3, C		124.9, C	15'		124.9, C
16		174.3, C	6.13, br s	114.3, C	16'	6.17, d (3.6)	114.4, CH
17	1.63, s	9.4, CH <sub>3</sub>	1.72, s	10.2, CH <sub>3</sub>	17'	1.73, s	10.2, CH <sub>3</sub>
18	1.29, s	17.3, CH <sub>3</sub>	1.58, s	14.6, CH <sub>3</sub>	18'	1.59, s	14.6, CH <sub>3</sub>
19	1.34, s	22.5, CH <sub>3</sub>	1.65, s	14.7, CH <sub>3</sub>	19'	1.65, s	14.7, CH <sub>3</sub>
20	0.98, s	16.9, CH <sub>3</sub>	1.27, s	15.7, CH <sub>3</sub>	20'	1.27, s	15.7, CH <sub>3</sub>
6-OOH	7.25, br s						

<sup>a</sup> Spectrum recorded at 500 MHz in C<sub>6</sub>D<sub>6</sub>. <sup>b</sup> Spectrum recorded at 125 MHz in C<sub>6</sub>D<sub>6</sub>. <sup>c</sup> Spectrum recorded at 400 MHz in CDCl<sub>3</sub>. <sup>d</sup> Spectrum recorded at 100 MHz in CDCl<sub>3</sub>.

Bischerbolide peroxide (**6**) was afforded as a white powder with the molecular formula  $C_{40}H_{58}O_6$  from HRESIMS ( $m/z$  calcd 657.4124; found 657.4125,  $[M + Na]^+$ ), appropriate for twelve degrees of unsaturation. The  $^{13}C$  NMR spectroscopic data of **6** revealed the presence of 40 carbons (Table 2). The DEPT spectra of **6** showed the presence of eight methyls, twelve  $sp^3$  methylenes, six  $sp^3$  oxygenated methines, four  $sp^2$  methines, two  $sp^3$  and eight  $sp^2$  nonprotonated carbons (including two ester carbonyls). NMR signals resonating at  $\delta_C$  114.3 (CH), 141.4 (C), 124.9 (C), 82.7 (CH) and 10.2 (CH<sub>3</sub>), and  $\delta_H$  5.28 (1H, d,  $J = 10.0$  Hz) and  $\delta_H$  1.72 (3H, s), and another group of signals observed at  $\delta_C$  114.4 (CH), 141.5 (C), 124.9 (C), 81.9 (CH) and 10.2 (CH<sub>3</sub>), and  $\delta_H$  5.50 (1H, d,  $J = 10.0$  Hz) and  $\delta_H$  1.73 (3H, s), revealed the presence of two slightly different 2,5-dihydrofuran rings with a peroxy group by comparison of the similar NMR data of five-membered rings in the literature [35]. Also, two groups of signals resonating at  $\delta_C$  61.2 (C), 62.1 (CH) and  $\delta_H$  2.51 (1H, m), and  $\delta_C$  61.3 (C), 62.2 (CH) and  $\delta_H$  2.51 (1H, m) showed the presence of two trisubstituted epoxides. Four trisubstituted olefinic bonds were revealed from NMR signals appearing at  $\delta_C$  126.4 (CH),  $\delta_C$  140.2 (C) and  $\delta_H$  5.06 (1H, d,  $J = 10.0$  Hz); at  $\delta_C$  125.6 (CH),  $\delta_C$  133.1 (C) and  $\delta_H$  4.98 (1H, dd,  $J = 9.2, 9.2$  Hz); at  $\delta_C$  125.1 (CH),  $\delta_C$  141.0 (C) and  $\delta_H$  4.92 (1H, dd,  $J = 10.0$  Hz); and at  $\delta_C$  125.5 (CH),  $\delta_C$  133.3 (C) and  $\delta_H$  4.95 (1H, dd,  $J = 9.2, 9.2$  Hz), respectively.

As the  $^{13}C$  NMR spectrum of **6** was constituted by twenty sets of signals with each set containing two peaks of very similar chemical shifts, **6** was thus identified as a compound formed from the connection of two quite similar diterpenoid subunits. The entire planar structure was established by examination of  $^1H$  and  $^{13}C$  NMR data and  $^1H$ - $^1H$  COSY and HMBC correlations (Figure 6). Two methines resonating at  $\delta_C$  114.3 and  $\delta_C$  114.4 were considered to be the positions at which the two cembranoidal units were connected by insertion of a peroxy group. Based on the above analyses, the molecular skeleton of **6** was elucidated as the biscembranoid formed by the connection of two molecules of isosarcophytoxide [36] via a peroxy group at C-16 and C-16'. The fragmentation pattern of ESIMS (Figure 7) could further prove the dimeric nature of **6** and the peroxy linkage at C-16/C-16'. One ion peak displayed at  $m/z$  339 can be explained by the cleavage of O-O bond and the following elimination of H-16 from a monocembranoidal unit in **6** to form a sodiated cembranoid lactone molecular ion A (pathway a). The other ion peaks can be interpreted by the cleavage of the single bond between C-16 and peroxy oxygen to afford ion B ( $m/z$  301), and a peroxycembranoidal radical which could further abstract an hydrogen atom and form the sodium adduct C ( $m/z$  357) (pathway b). Moreover, compound **6** was found to be the first example of a biscembranoid with a molecular skeleton formed by two cembranoid units connected by a peroxy group.

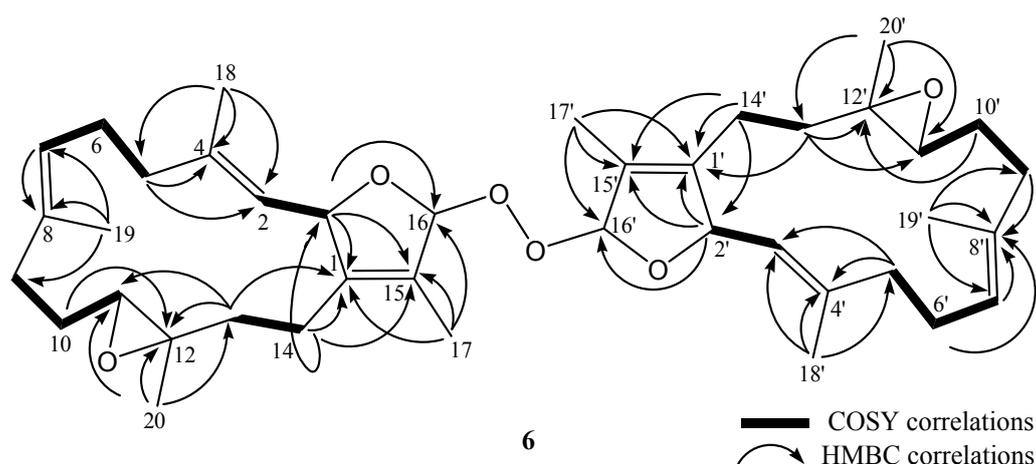


Figure 6. Selected  $^1H$ - $^1H$  COSY and HMBC correlations of **6**.

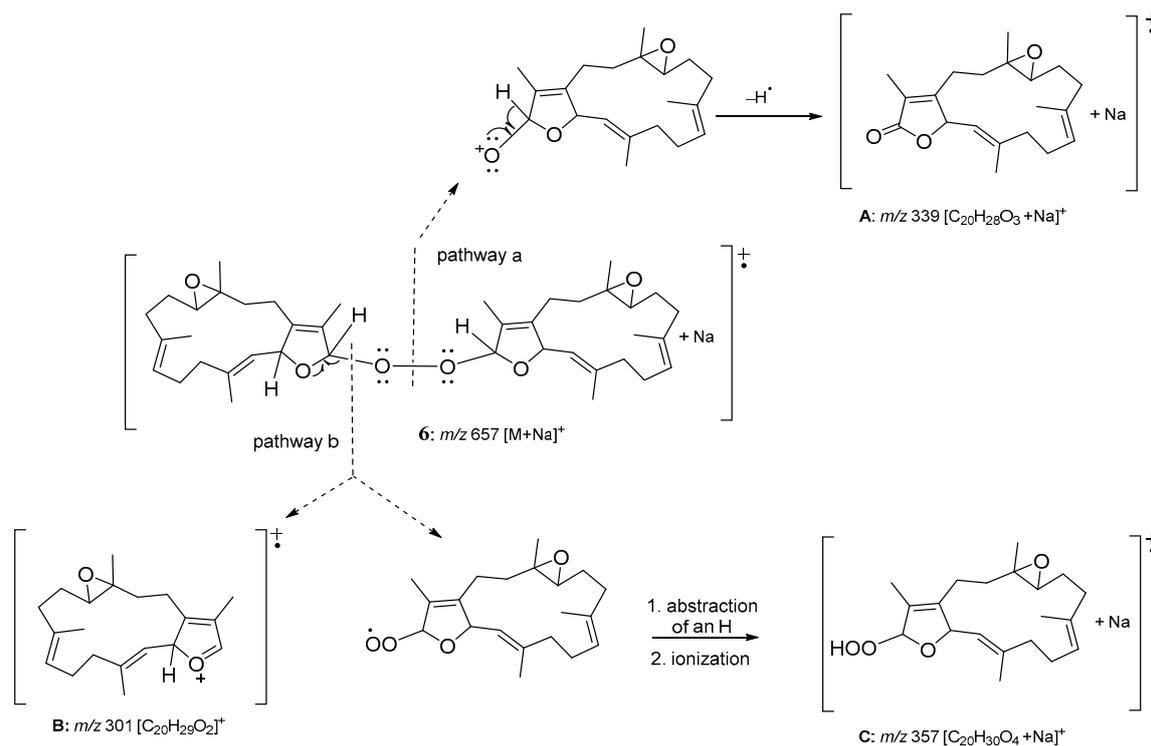
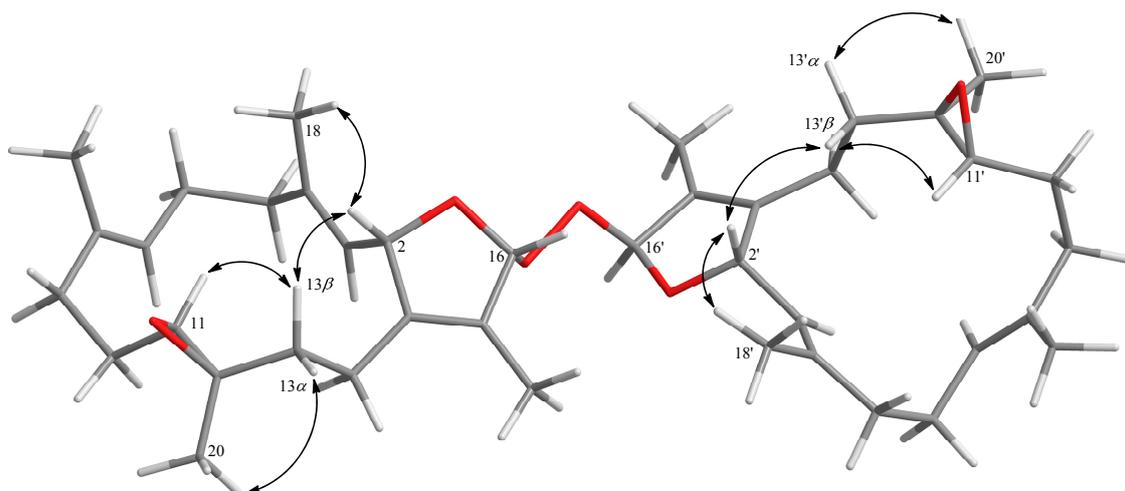


Figure 7. ESIMS fragmentation of 6.

The relative configuration of **6** was determined from a literature survey [36,37] and NOE correlations (Figure 8). The  $^{13}C$  NMR spectrum of **6** displayed 40 signals of two sets signals with nearly identical chemical shifts, representing the very similar stereochemical environments of the two structural units. In addition, compound **6** was found to have nearly identical chemical shifts for H-11 (11'), H<sub>3</sub>-18 (18'), H<sub>3</sub>-20 (20') and C-20 (20') to those of (2*S*,11*R*,12*R*)-isosarcophytoxide (**8**), and were in turn found to exhibit distinguishable differences to the corresponding chemical shifts of (2*R*,11*R*,12*R*)-isosarcophytoxide (**9**) (Table 3 and Figure 9). Thus, **6** possessed the cembranoidal structural unit derived from **8**, as also proven by observed NOE correlations (Figure 8). Different proton values were observed for H-2 ( $\delta_H$  5.28) and H-2' ( $\delta_H$  5.50), indicating that H-2' was on the same planar face as the peroxide group and was deshielded, and H-2 was on the same planar face as H-16 and was shielded. As compounds **1**–**7** were isolated from the same organism in this study, they are likely to possess the same absolute *S,R,R*-configurations at the chiral centers C-2, C-11 and C-12, respectively, as those of **1** and **3**. A previous report also showed that different absolute configurations at C-2 of the related diastomeric dihydrofuran ring-containing cembranoids could significantly influence the sign of the specific optical rotation [36,38]. For cembranoids with 2*S* configuration a significant positive and for those with 2*R* configuration a negative optical rotation were found. The  $[\alpha]_D^{25}$  of **6** was +41; thus, the absolute configuration of **6** was deduced to be 2*S*,11*R*,12*R*,16*R*,2'*S*,11'*R*,12'*R*,16'*S*.

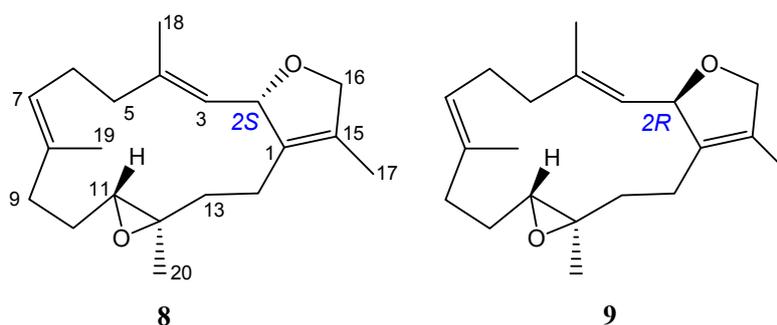


**Figure 8.** Selected NOESY correlations for **6**.

**Table 3.** Selected  $^1\text{H}$  and  $^{13}\text{C}$  NMR data comparison with **6**, (2*S*,11*R*,12*R*)-isoscaphytoxide (**8**) and (2*R*,11*R*,12*R*)-isoscaphytoxide (**9**).

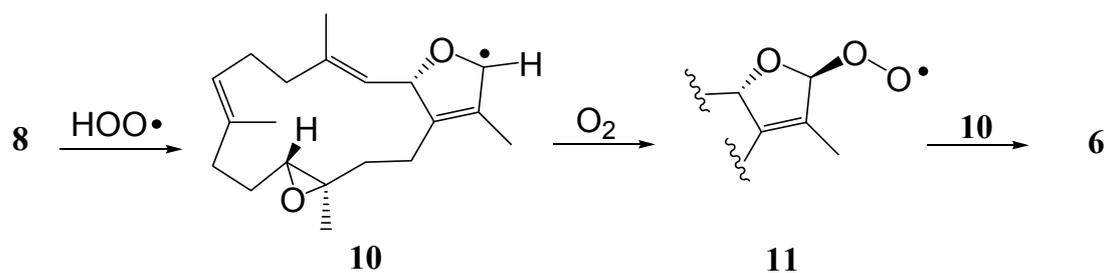
Position	<b>6</b>	<b>8</b> <sup>a</sup>	<b>9</b> <sup>a</sup>
H-11	$\delta_{\text{H}}$ 2.51 (H-11, H-11')	$\delta_{\text{H}}$ 2.50	$\delta_{\text{H}}$ 2.75
C-11	$\delta_{\text{C}}$ 62.1 (C-11)	$\delta_{\text{C}}$ 62.3	$\delta_{\text{C}}$ 61.2
	$\delta_{\text{C}}$ 62.2 (C-11')		
C-12	$\delta_{\text{C}}$ 61.2 (C-12)	$\delta_{\text{C}}$ 61.4	$\delta_{\text{C}}$ 60.7
	$\delta_{\text{C}}$ 61.3 (C-12')		
C-13	$\delta_{\text{C}}$ 37.3 (C-13)	$\delta_{\text{C}}$ 37.4	$\delta_{\text{C}}$ 35.4
	$\delta_{\text{C}}$ 37.4 (C-13')		
C-14	$\delta_{\text{C}}$ 22.6 (C-14)	$\delta_{\text{C}}$ 22.5	$\delta_{\text{C}}$ 20.4
	$\delta_{\text{C}}$ 22.7 (C-14')		
H <sub>3</sub> -18	$\delta_{\text{H}}$ 1.58 (H <sub>3</sub> -18)	$\delta_{\text{H}}$ 1.58	$\delta_{\text{H}}$ 1.70
	$\delta_{\text{H}}$ 1.59 (H <sub>3</sub> -18')		
H <sub>3</sub> -20	$\delta_{\text{H}}$ 1.27 (H <sub>3</sub> -20, H <sub>3</sub> -20')	$\delta_{\text{H}}$ 1.28	$\delta_{\text{H}}$ 1.18
C-20	$\delta_{\text{C}}$ 15.7 (C-20, C-20')	$\delta_{\text{C}}$ 15.7	$\delta_{\text{C}}$ 17.7

<sup>a</sup> The selected  $^1\text{H}$  and  $^{13}\text{C}$  data were cited from ref. [36,37].



**Figure 9.** Structures of (2*S*,11*R*,12*R*)-isoscaphytoxide (**8**) and (2*R*,11*R*,12*R*)-isoscaphytoxide (**9**) [36].

The plausible biosynthesis of **6** might arise from the proton abstraction at C-16 of **8** by hydrogen peroxide radical  $\text{HOO}\bullet$  to form a radical intermediate **10**, which could react with  $\text{O}_2$  from one plane side of radical center C-16 to afford cembranoidal peroxide radical **11**. Further reaction of **11** with **10** from another side could lead to the formation of **6** (Scheme 1). However, the possibility that **6** might be generated by autooxidation of **8** could not be neglected.



**Scheme 1.** Proposed biosynthetic pathway for **6**.

It is known that the proteolytic enzymes and toxic reactive oxygen species produced by stimulated neutrophils might play a critical role in the pathogenesis of many inflammatory diseases [39,40]. By measuring the capability to inhibit *N*-formyl-methionyl-leucyl-phenylalanine/cytochalasin B (fMLF/CB)-induced superoxide anion generation and elastase release in human neutrophils, the in vitro anti-inflammatory effects for metabolites **1–7** were evaluated [41,42]. According to the results (shown in Table 4), compound **6** had a significant inhibitory effect ( $64.6 \pm 0.8\%$ ), with an  $IC_{50}$  value of  $26.2 \pm 1.0 \mu\text{M}$ , on the generation of superoxide anions, and compounds **1** and **3** had moderate inhibitory effects ( $32.1 \pm 4.3\%$  and  $44.5 \pm 4.6\%$ , respectively) at  $30 \mu\text{M}$ . Compounds **1**, **3** and **6** revealed moderate inhibitory effects ( $37.6 \pm 5.0\%$ ,  $35.6 \pm 6.2\%$  and  $42.4 \pm 5.1\%$ , respectively) on elastase release at the same concentration. These results, obtained after stimulating the neutrophils with fMLF/CB, may suggest that **1**, **3** and **6** have potential merits against inflammatory disorders.

**Table 4.** Inhibitory effects of compounds **1–7** on superoxide anion generation and elastase release in fMLF/CB-induced human neutrophils.

Compounds	Superoxide Anion		Elastase Release
	$IC_{50}$ ( $\mu\text{M}$ ) <sup>a</sup>	Inh <sup>b</sup> %	Inh <sup>b</sup> %
<b>1</b>	>30	$32.1 \pm 4.3^{**}$	$37.6 \pm 5.0^{**}$
<b>2</b>	>30	$4.0 \pm 6.7$	$23.5 \pm 6.6^*$
<b>3</b>	>30	$44.5 \pm 4.6^{***}$	$35.6 \pm 6.2^{**}$
<b>4</b>	>30	$6.4 \pm 4.2$	$27.6 \pm 6.4^{**}$
<b>5</b>	>30	$2.6 \pm 6.2$	$30.5 \pm 4.6^{**}$
<b>6</b>	$26.2 \pm 1.0$	$64.6 \pm 0.8^{***}$	$42.4 \pm 5.1^{**}$
<b>7</b>	>30	$3.5 \pm 5.3$	$20.7 \pm 4.1^{**}$
Idelalisib	$0.07 \pm 0.01$	$102.8 \pm 2.2^{***}$	$99.6 \pm 4.2$

<sup>a</sup> Concentration necessary for 50% inhibition ( $IC_{50}$ ). <sup>b</sup> Percentage of inhibition (Inh %) at  $30 \mu\text{M}$ . Data are presented as mean  $\pm$  S.E.M. ( $n = 3-4$ ); \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared with the control value.

In summary, examination of the chemical constituents of the soft coral *Sarcophyton cherbonnieri* led to the discovery of six new compounds **1–6**, along with one known compound **7**. Although a number of natural compounds possessing a peroxy group, such as artemisinin [43], neovibsanin C [44], cardamom peroxide [45], plakortin [46] and chondrillin [47], have been discovered, compound **6** was discovered to be the first compound with a molecular skeleton consisting of two cembranoid units connected by a peroxide group. Similar to the results of previous studies indicating that natural peroxides could possess promising biological activity [48], compound **6** was found to possess anti-inflammatory activity by exhibiting stronger ability on inhibition on the generation of superoxide anions and release of elastase in fMLF/CB-induced human neutrophils.

### 3. Materials and Methods

#### 3.1. General Procedures

The values of optical rotation of the metabolites were determined with a JASCO P-1020 polarimeter (JASCO Corporation, Tokyo, Japan). Infrared absorptions were recorded using a JASCO FT/IR-4100 infrared spectrophotometer (JASCO Corporation, Tokyo, Japan).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were obtained on a Varian 400MR FT-NMR (or Varian Unity INOVA500 FT-NMR) instrument (Varian Inc., Palo Alto, CA, USA) at 400 MHz (or 500 MHz) and 100 MHz (or 125 MHz), respectively, in  $\text{CDCl}_3$  or  $\text{C}_6\text{D}_6$ . The data of LRESIMS and HRESIMS were measured using a Bruker APEX II (Bruker, Bremen, Germany) mass spectrometer. Silica gel (230–400 mesh) was used as adsorbent for column chromatography. TLC analyses were performed using precoated silica gel plates (Kieselgel 60 F-254, 0.2 mm) (Merck, Darmstadt, Germany). Further purification of impure fractions or compounds were performed by high-performance liquid chromatography on a Hitachi L-7100 HPLC instrument (Hitachi Ltd., Tokyo, Japan) with a Merck Hibar Si-60 column (250 mm  $\times$  21 mm, 7  $\mu\text{m}$ ; Merck, Darmstadt, Germany) and on a Hitachi L-2455 HPLC apparatus (Hitachi, Tokyo, Japan) with a Supelco C18 column (250 mm  $\times$  21.2 mm, 5  $\mu\text{m}$ ; Supelco, Bellefonte, PA, USA).

#### 3.2. Animal Material

The soft coral *S. cherbonnieri* was collected by hand using scuba diving from Jihui Fish Port, Taiwan, in March 2013, at a depth of 10–15 m. Organisms of the marine animal were stored in a freezer until extraction.

#### 3.3. Extraction and Isolation

The frozen marine organisms, *S. cherbonnieri* (1.2 kg, wet wt), were freeze-dried (yield: 207 g), minced to small pieces and then extracted thoroughly with EtOAc (1 L  $\times$  5). The combined EtOAc extract (10.2 g) was concentrated under reduced pressure to yield a residue, which was chromatographed over a silica gel column by eluting with acetone in *n*-hexane (0–100%, stepwise), and then with MeOH in acetone (0–100%, stepwise) to yield 19 fractions. Fraction 9, eluting with *n*-hexane–acetone (6:1), was repeatedly purified by column chromatography over silica gel to yield a solid which was immersed in cold MeOH (0  $^\circ\text{C}$ ) to afford a white powder **6** (24.3 mg). Fraction 10, eluting with *n*-hexane–acetone (4:1), was further purified over silica gel using *n*-hexane–acetone (6:1) to afford seven subfractions (A1–A7) and afford **7** (320.4 mg). Subfraction A2 was further separated by reverse-phase HPLC using acetonitrile– $\text{H}_2\text{O}$  (1:1.3) to afford **1** (11.0 mg). Subfraction A3 was purified by reverse-phase HPLC using acetonitrile– $\text{H}_2\text{O}$  (1:1.1) to afford **2** (13.3 mg) and **5** (10.1 mg). Fraction 13, eluting with *n*-hexane–acetone (1:1), eluting with acetone by sephadex LH-20 to afford five subfractions (B1–B5). Subfraction B3 was purified by reverse-phase HPLC using acetonitrile– $\text{H}_2\text{O}$  (1:1.4) to afford **3** (10.6 mg) and **4** (9.4 mg).

##### 3.3.1. Cherbonnolide A (**1**)

Colorless oil;  $[\alpha]_{\text{D}}^{25} +43$  (*c* 1.00,  $\text{CHCl}_3$ ); IR (neat)  $\nu_{\text{max}}$  3444, 1746, and 1003  $\text{cm}^{-1}$ ;  $^{13}\text{C}$  and  $^1\text{H}$  NMR data see Table 1; HRMS (ESI-TOF) *m/z*:  $[\text{M} + \text{Na}]^+$  Calcd for  $\text{C}_{20}\text{H}_{28}\text{O}_4\text{Na}$  355.1880; Found 355.1879.

##### 3.3.2. Cherbonnolide B (**2**)

Colorless oil;  $[\alpha]_{\text{D}}^{25} +59$  (*c* 1.00,  $\text{CHCl}_3$ ); IR (neat)  $\nu_{\text{max}}$  3363, 1741, 1678, and 1093  $\text{cm}^{-1}$ ;  $^{13}\text{C}$  and  $^1\text{H}$  NMR data see Table 1; HRMS (ESI-TOF) *m/z*:  $[\text{M} + \text{Na}]^+$  Calcd for  $\text{C}_{20}\text{H}_{28}\text{O}_5\text{Na}$  371.1829; Found 371.1830.

### 3.3.3. Cherbonnolide C (3)

Colorless oil;  $[\alpha]_D^{25} +26$  (*c* 1.00, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3445, 1749, 1678, and 1094 cm<sup>-1</sup>; <sup>13</sup>C and <sup>1</sup>H NMR data see Table 1; HRMS (ESI-TOF) *m/z*: [M + Na]<sup>+</sup> Calcd for C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>Na 355.1880; Found 355.1882.

### 3.3.4. Cherbonnolide D (4)

White powder;  $[\alpha]_D^{25} +3$  (*c* 1.00, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3445, 1748, and 1096 cm<sup>-1</sup>; <sup>13</sup>C and <sup>1</sup>H NMR data see Table 1; HRMS (ESI-TOF) *m/z*: [M + Na]<sup>+</sup> Calcd for C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>Na 355.1880; Found 355.1883.

### 3.3.5. Cherbonnolide E (5)

Colorless oil;  $[\alpha]_D^{25} +8$  (*c* 1.00, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3389, 1748, 1678 and 1096 cm<sup>-1</sup>; <sup>13</sup>C and <sup>1</sup>H NMR data see Table 2; HRMS (ESI-TOF) *m/z*: [M + Na]<sup>+</sup> Calcd for C<sub>20</sub>H<sub>28</sub>O<sub>5</sub>Na 371.1829; Found 371.1830.

### 3.3.6. Bischerbolide Peroxide (6)

White powder;  $[\alpha]_D^{25} +41$  (*c* 1.00, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3420, 1733, 1232, 1166 and 1040 cm<sup>-1</sup>; <sup>13</sup>C and <sup>1</sup>H NMR data see Table 2; HRMS (ESI-TOF) *m/z*: [M + Na]<sup>+</sup> Calcd for C<sub>40</sub>H<sub>58</sub>O<sub>6</sub>Na 657.4125; Found 657.4124.

### 3.3.7. Reduction of Cherbonolides B and E (2 and 5)

In diethyl ether (5.0 mL), compound **2** (3.2 mg) was added followed by addition of excess amount of triphenylphosphine (2.9 mg) and the mixture was stirred at room temperature for 4 h. The solvent of the solution was evaporated under reduced pressure to afford a residue, which was purified by silica gel column chromatography using *n*-hexane–acetone (3:1) as an eluent to yield **1** (2.9 mg, 95%). Similarly, **5** (2.1 mg) was converted to **4** (1.7 mg) in 85% yield.

### 3.3.8. Preparation of (S)- and (R)- MTPA Esters of 1 and 3

Compound **1** (1.3 mg) was dissolved in pyridine 100  $\mu$ L and added (R)-(-)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl) phenylacetyl chloride (MTPA chloride) 10  $\mu$ L. The mixture was permitted to stand at room temperature overnight and the reaction was found to complete by monitoring with normal-phase TLC plate. The solution was dried completely under the vacuum of an oil pump and the residue was purified by a short silica gel column using acetone to *n*-hexane (1:3) to yield the (S)-MTPA ester **1a** (0.9 mg, 62.9%). The same procedure was applied to obtain the (R)-MTPA ester **1b** (1.0 mg, 69.9%) from the reaction of (S)-(+)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl) phenylacetyl chloride with **1** in pyridine. Selective <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) data of **1a**:  $\delta_H$  4.925 (1H, d, *J* = 10.0 Hz, H-3), 1.769 (3H, s, H<sub>3</sub>-18), 2.821 (1H, dd, *J* = 12.8, 4.8 Hz, H-5a), 2.376 (1H, m, H-5b), 5.107 (1H, d, *J* = 9.6 Hz, H-7), 1.880 (3H, s, H<sub>3</sub>-19), 2.460 (1H, m, H-9a), 1.989 (1H, m, H-9b); selective <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) data of **1b**:  $\delta_H$  4.905 (1H, d, *J* = 10.0 Hz, H-3), 1.763 (3H, s, H<sub>3</sub>-18), 2.739 (1H, dd, *J* = 12.8, 5.6 Hz, H-5a), 2.267 (1H, m, H-5b), 5.217 (1H, d, *J* = 9.6 Hz, H-7), 1.905 (3H, s, H<sub>3</sub>-19), 2.476 (1H, m, H-9a), 1.998 (1H, m, H-9b).

Preparation of (S)- and (R)- MTPA esters of **3** used the same reaction and purification procedures as the reduction of **1**, the solution of **3** (1.1 mg) was converted to the (S)-MTPA ester **3a** (0.8 mg) in 74% yield and (R)-MTPA ester **3b** (0.9 mg) in 80% yield, respectively. Selective <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 400 MHz) data of **3a**:  $\delta_H$  1.243 (3H, s, H<sub>3</sub>-18), 2.311 (1H, dd, *J* = 11.2, 2.4 Hz, H-5a), 1.930 (1H, dd, *J* = 11.2, 11.2 Hz, H-5b), 5.065 (1H, d, *J* = 9.2 Hz, H-7), 1.416 (3H, s, H<sub>3</sub>-19); selective <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 400 MHz) data of **3b**:  $\delta_H$  1.244 (3H, s, H<sub>3</sub>-18), 2.363 (1H, dd, *J* = 12.0, 3.2 Hz, H-5a), 1.982 (1H, dd, *J* = 12.0, 11.6 Hz, H-5b), 4.9515 (1H, d, *J* = 10.0 Hz, H-7), 1.360 (3H, s, H<sub>3</sub>-19).

### 3.4. In Vitro Anti-Inflammatory Testing

#### 3.4.1. Human Neutrophils

Blood was obtained from elbow vein of healthy adult volunteers (years 20–30). Neutrophils were enriched by dextran sedimentation, Ficoll-Hypaque centrifugation, and hypotonic lysis. Neutrophils were incubated in an ice-cold  $\text{Ca}^{2+}$ -free HBSS buffer (pH 7.4) [42].

#### 3.4.2. Superoxide Anion Generation

Neutrophils ( $6 \times 10^5$  cells  $\text{mL}^{-1}$ ) incubated in HBSS with ferricytochrome *c* (0.5 mg  $\text{mL}^{-1}$ ) and  $\text{Ca}^{2+}$  (1 mM) at 37 °C were treated with DMSO (as control) or compound for 5 min. Neutrophils were primed by cytochalasin B (CB, 1  $\mu\text{g mL}^{-1}$ ) for 3 min before activating fMLF (100 nM) for 10 min (fMLF/CB) [40,49].

#### 3.4.3. Elastase Release

Neutrophils ( $6 \times 10^5$  cells  $\text{mL}^{-1}$ ) incubated in HBSS with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100  $\mu\text{M}$ ) and  $\text{Ca}^{2+}$  (1 mM) at 37 °C were treated with DMSO or compound for 5 min. Neutrophils were activated with fMLF (100 nM)/CB (0.5  $\mu\text{g mL}^{-1}$ ) for 10 min [40].

#### 3.4.4. Statistical Analysis

Data are displayed as the mean  $\pm$  SEM and comparisons were performed by Student's *t*-test. A probability value of 0.05 or less was considered to be significant. The software Sigma Plot (version 8.0, Systat Software, San Jose, CA, USA) was used for the statistical analysis.

## 4. Conclusions

Six new cembranoids, cherbonolides A–E (1–5) and a cembrane dimer (bischerbolide peroxide, 6), along with isosarcophine (7) were isolated from the Formosan soft coral *Sarcophyton cherbonnieri*. Bischerbolide peroxide (6) was discovered as the first example of cembranoid dimers possessing a peroxide group as a linking group. Compounds 1, 3 and 6 showed an anti-inflammatory activity through their inhibitory effects on the generation of superoxide anion in fMLF/CB-induced human neutrophils. Moreover, peroxide 6 was also shown to exhibit stronger activity in inhibiting the elastase release which supported its anti-inflammatory activity.

**Supplementary Materials:** HRESIMS,  $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT, HMQC, COSY, HMBC and NOESY spectra of new compounds 1–6, and  $^1\text{H}$  NMR spectra of (+)-sarcophytoxide and sarcophytonin after different treatments are available online at <http://www.mdpi.com/1660-3397/16/8/276/s1>. Figure S1: HRESIMS spectrum of 1, Figure S2:  $^1\text{H}$  NMR spectrum of 1 in  $\text{CDCl}_3$ , Figure S3:  $^{13}\text{C}$  NMR spectrum of 1 in  $\text{CDCl}_3$ , Figure S4: HSQC spectrum of 1 in  $\text{CDCl}_3$ , Figure S5:  $^1\text{H}$ - $^{13}\text{C}$  HCOZY spectrum of 1 in  $\text{CDCl}_3$ , Figure S6: HMBC spectrum of 1 in  $\text{CDCl}_3$ , Figure S7: NOESY spectrum of 1 in  $\text{CDCl}_3$ , Figure S8: HRESIMS spectrum of 2, Figure S9:  $^1\text{H}$  NMR spectrum of 2 in  $\text{CDCl}_3$ , Figure S10:  $^{13}\text{C}$  NMR spectrum of 2 in  $\text{CDCl}_3$ , Figure S11: HSQC spectrum of 2 in  $\text{CDCl}_3$ , Figure S12:  $^1\text{H}$ - $^{13}\text{C}$  HCOZY spectrum of 2 in  $\text{CDCl}_3$ , Figure S13: HMBC spectrum of 2 in  $\text{CDCl}_3$ , Figure S14: NOESY spectrum of 2 in  $\text{CDCl}_3$ , Figure S15: HRESIMS spectrum of 3, Figure S16:  $^1\text{H}$  NMR spectrum of 3 in  $\text{C}_6\text{D}_6$ , Figure S17:  $^{13}\text{C}$  NMR spectrum of 3 in  $\text{C}_6\text{D}_6$ , Figure S18: HSQC spectrum of 3 in  $\text{C}_6\text{D}_6$ , Figure S19:  $^1\text{H}$ - $^{13}\text{C}$  HCOZY spectrum of 3 in  $\text{C}_6\text{D}_6$ , Figure S20: HMBC spectrum of 3 in  $\text{C}_6\text{D}_6$ , Figure S21: NOESY spectrum of 3 in  $\text{C}_6\text{D}_6$ , Figure S22: HRESIMS spectrum of 4, Figure S23:  $^1\text{H}$  NMR spectrum of 4 in  $\text{C}_6\text{D}_6$ , Figure S24:  $^{13}\text{C}$  NMR spectrum of 4 in  $\text{C}_6\text{D}_6$ , Figure S25: HSQC spectrum of 4 in  $\text{C}_6\text{D}_6$ , Figure S26:  $^1\text{H}$ - $^{13}\text{C}$  HCOZY spectrum of 4 in  $\text{C}_6\text{D}_6$ , Figure S27: HMBC spectrum of 4 in  $\text{C}_6\text{D}_6$ , Figure S28: NOESY spectrum of 4 in  $\text{C}_6\text{D}_6$ , Figure S29: HRESIMS spectrum of 5, Figure S30:  $^1\text{H}$  NMR spectrum of 5 in  $\text{C}_6\text{D}_6$ , Figure S31:  $^{13}\text{C}$  NMR spectrum of 5 in  $\text{C}_6\text{D}_6$ , Figure S32: HSQC spectrum of 5 in  $\text{C}_6\text{D}_6$ , Figure S33:  $^1\text{H}$ - $^{13}\text{C}$  HCOZY spectrum of 5 in  $\text{C}_6\text{D}_6$ , Figure S34: HMBC spectrum of 5 in  $\text{C}_6\text{D}_6$ , Figure S35: NOESY spectrum of 5 in  $\text{C}_6\text{D}_6$ , Figure S36: HRESIMS spectrum of 6, Figure S37: ESIMS spectrum of 6, Figure S38:  $^1\text{H}$  NMR spectrum of 6 in  $\text{CDCl}_3$ , Figure S39:  $^{13}\text{C}$  NMR spectrum of 6 in  $\text{CDCl}_3$ , Figure S40: HSQC spectrum of 6 in  $\text{CDCl}_3$ , Figure S41:  $^1\text{H}$ - $^{13}\text{C}$  HCOZY spectrum of 6 in  $\text{CDCl}_3$ , Figure S42: HMBC spectrum of 6 in  $\text{CDCl}_3$ , Figure S43: NOESY spectrum of 6 in  $\text{CDCl}_3$ , Figure S44:  $^1\text{H}$  NMR spectrum of (+)-sarcophytoxide in  $\text{CDCl}_3$  before treatment with acetone and silica gel under air, Figure S45:  $^1\text{H}$  NMR spectrum of (+)-sarcophytoxide

in CDCl<sub>3</sub> after treatment with acetone and silica gel under air, Figure S46. <sup>1</sup>H NMR spectrum of sarcophytonin A in CDCl<sub>3</sub> before treatment with acetone and silica gel under air, Figure S47. <sup>1</sup>H NMR spectrum of sarcophytonin A in CDCl<sub>3</sub> after treatment with acetone and silica gel under air.

**Author Contributions:** J.-H.S. conceived and guided the whole experiment. C.-C.P. isolated the compounds, and performed spectroscopic data measurement and analysis, and structure interpretation. C.-Y.H. and A.F.A. performed spectroscopic data analysis, confirmation of structures and preparation of the manuscript. T.-L.H. performed the anti-inflammatory assay. C.-F.D. contributed to species identification of the soft coral.

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