Sarcophytonolides E−H, Cembranolides from the Hainan Soft Coral *Sarcophyton latum*

Rui Jia,[†] Yue-Wei Guo,*[,†] Ernesto Mollo,[‡] Margherita Gavagnin,[‡] and Guido Cimino[‡]

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Zu Chong Zi Road 555, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China, and Istituto di Chimica Biomolecolare-CNR, 80078 Pozzuoli(Na), Italy

Received November 17, 2005

Four new cembrane diterpenes, sarcophytonolides E−H (**1**−**4**), were isolated from the Hainan soft coral *Sarcophyton latum*. Their structures were elucidated by detailed spectroscopic (IR, MS, 2D NMR) analysis and by comparison with related known compounds. The absolute stereochemistry of sarcophytonolide H (**4**) was determined by applying the modified Mosher's method.

Soft corals of the genus *Sarcophyton* (family Alcyoniidae) frequently afford common 14-membered cembrane diterpenes,[1−4] and most of them exhibit various bioactivities such as ichthyotoxic,[5] cytotoxic, anti-inflammatory, antiarthritic,[6,7] Ca-antagonistic, and antimicrobial properties.[8] It has been suggested that the diterpenes were used as chemical defense compounds against predators such as other corals and fishes and against settlement of microorganisms such as fungi or bacteria.[9,10]

In our previous study[11] on the Hainan soft coral *Sarcophyton tortuosum* (where the specimen was named as *Sarcophyton* sp.), four new cembranoids, sarcophytonolides A−D (examplified by sarcophytonolide C, **5**), have been isolated and structurally characterized. Recently, during our continuing studies on bioactive substances from Hainan marine organisms,[11−14] we made a collection of *Sarcophyton latum* off the coast of Sanya, China. Chemical investigation of the Et_2O-soluble fraction from the Me_2CO extract of the animal has resulted in the isolation of four new cembranolides, named sarcophytonolides E−H (**1**−**4**). All of these new compounds are structurally related to previously reported sarcophytonolide C, containing also an α,*β*-unsaturated butenolide moiety. This paper describes the isolation and structure elucidation of these new compounds.

Specimens of *S. latum* were collected off Ximao Island, Sanya, Hainan Province, China, and kept frozen prior to extraction. The workup for the extraction and isolation of cembranolides was basically performed as previously reported.[11] This common procedure yielded four new compounds, named sarcophytonolides E−H (**1**−**4**). All new compounds showed considerable structural analogies with the known compound **5**.

Sarcophytonolide E (**1**) was obtained as a colorless oil. The HREIMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **1** as $C_{20}H_{32}O_3$. Thus, five degrees of unsaturation were determined for **1**. Compound **1** exhibited IR absorptions indicative of the presence of a hydroxyl group (ν_{max} 3315 cm^{-1}) and an ester carbonyl moiety (ν_{max} 1743 cm^{-1}). A strong UV absorption at λ_{max} 234 nm (log ϵ 4.3) suggested the presence of an α,*β*-unsaturated *γ*-lactone. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed the presence of four methyls (δ_H 0.98, 3H, d, J = 6.6 Hz, δ_C 18.4; δ_H 0.99, 3H, d, J = 6.6 Hz, δ_C 20.0; δ_H 0.90, 3H, d, J = 6.6 Hz, δ_C 20.6; δ_H 1.62, 3H, s, δ_C 16.3), one oxymethine (δ_H 4.21, δ_C 66.9), two trisubstituted double bonds (δ_H 7.07, δ_C 150.5, 130.5; δ_H 4.95, δ_C 128.1, 132.9), and one ester carbonyl (δ_C 173.4). Two double bonds and one ester carbonyl accounted for three degrees of unsaturation. The remaining two degrees of unsaturation were attributed to a bicyclic ring system in compound **1**. The NMR spectra of **1** were strongly reminiscent of those of sarcophytonolide C (**5**).[11] Comparison of the NMR data of **1** with those of **5** suggested that **1** possessed a cembrane skeleton with functionalities of an α,*β*-unsaturated butenolide moiety, a secondary hydroxyl, one methyl-bearing trisubstituted double bond, and an isopropyl group. In fact, the only difference between **1** and **5** was that the carbonyl in **5** was reduced into a secondary alcohol in **1**, in agreement with 2 mass units difference between them. Analysis of the $^1H-^1H$ COSY spectrum readily identified five spin systems (H-1 to H-3; H-1 to H_2-13; H-1 to H-15, H_3-16, H_3-17; H_2-5 to H-11; and H-8 to H_3-19). In the HMBC experiment of **1** (Figure 2), the isopropyl group attached to C-1 was confirmed by long-range correlations between H-2, H-13, H-14, H-15, H_3-16, and H_3-17 to C-1. The position of the α,*β*-unsaturated *γ*-lactone at C-4 (α), C-3 (*β*), C-2 (*γ*), and C-18 (carbonyl carbon) was deduced from HMBC correlations between H-3 and C-2, C-4, C-5, and C-18 and between H-2 and C-1. The hydroxyl at C-6 was confirmed by HMBC correlations between H_2-5 and C-6, C-4, C-3, and C-7 and between H_2-7 and C-5, C-6, and C-8. The vinyl methyl group attached at C-12 was revealed by the HMBC correlations between H_3-20 and C-11, C-12, and C-13 and between H_2-10 and C-11 and C-12. The last methyl group was assigned at C-8 mainly on the basis of biogenetic considerations and supported by HMBC correlations between H_3-19 and C-7, C-8, and C-9. With the 2D structure of **1** established, the geometry of two carbon−carbon double bonds and the configurations at four chiral centers remained to be assigned. The *Z* geometry for $\Delta^{3\,4}$ was obvious. On the basis of the ^{13}C NMR chemical shift for CH_3-20 (<20 ppm),[15] $\Delta^{11\,12}$ was defined as *E*.

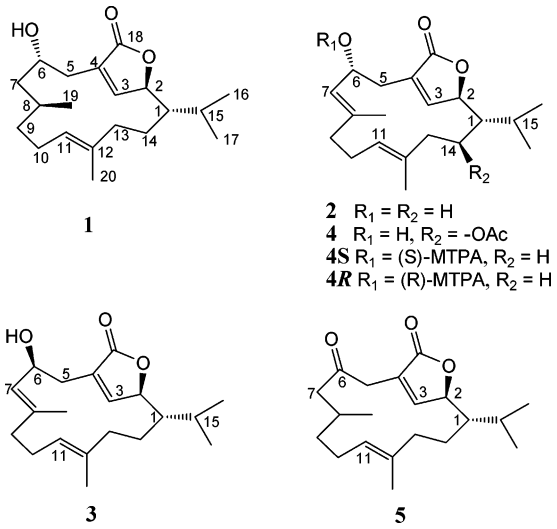

Figure 1. Structures of compounds **1**−**5**.

* Corresponding author. Tel: 86-21-50805813. Fax: 86-21-50805813. E-mail: ywguo@mail.shcnc.ac.cn.
[†] Shanghai Institute for Biological Sciences.
[‡] Istituto di Chimica Biomolecolare-CNR.

10.1021/np050477u CCC: $33.50
Published on Web 03/30/2006

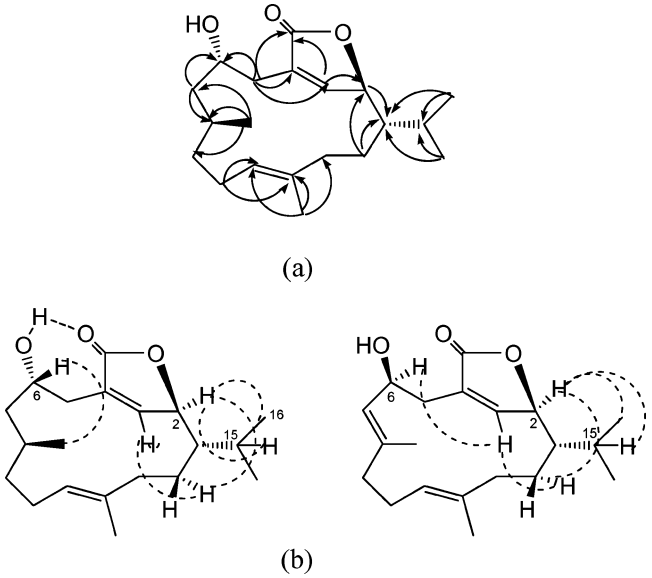

Figure 2. (a) Selected HMBC correlations (H → C) of compound **1** and (b) selected NOESY correlations of compounds **1** and **3**.

The relative stereochemistry at C-1 and C-2 of **1** was established to be the same as those of **5** by extensive analysis of the NOESY spectrum (Figure 2), in which H-2 (δ 4.73) correlated with H-15 (δ 2.08) and H_3-16 (δ 0.98)/H_3-17 (δ 0.99), suggesting H-2 and the isopropyl at C-1 both to be on the same side of **1**. The stereochemical assignments of the methyl group at C-8 as well as the C-6 hydroxyl posed a significant challenge in light of the flexible nature of such a large ring,[16] although the diagnostic cross-peak was observed between H-6 (δ 4.21) and CH_3-19 (δ 0.90) in the NOESY spectrum. The configuration of 6-OH was tentatively assigned as α mainly on the basis of the analysis of coupling constants and splitting patterns of H_2-5. Thus, analysis of the Drieding model of **1** revealed that the α-oriented hydroxyl group is spatially close to the carbonyl (C-18), and hence a hydrogen bond between 6α-OH and the carbonyl (C-18) may be formed. This hypothesis was supported by a larger coupling constant observed between H-6 and H-5a (J = 10.4 Hz) and a smaller one (very close to zero) between H-6 and H-5b, implying the dihedral angles between H-6 and H-5a and H-6 and H-5b were almost 0° and 90°, respectively, consistent with the observation in the Drieding model.

Sarcophytonolide F (**2**) was obtained as a colorless oil. Its molecular formula, $C_{20}H_{30}O_3$, was established by HREIMS at *m*/*z* 318.2193 (calcd 318.2195), 2 mass units less than that of **1**. Careful comparison of ^{1}H and ^{13}C NMR data of **2** and **1** (Tables 1 and 2) revealed that the former differs from the latter only by the presence of an additional trisubstituted double bond (δ_H 5.11; δ_C 127.4, 139.6), while the rest of the molecule was the same. ^{1}H−^{1}H COSY, HMQC, and HMBC experiments allowed the unambiguous definition of the structure of **2**. Especially, ^{1}H−^{1}H COSY correlations of H-6 (δ 4.80) and H-7 (δ 5.11) attached the additional double bond to C-7 and C-8. This assignment was further confirmed by HMBC correlations between H_3-17 (δ 1.50) and C-7 (δ 127.4) and C-8 (δ 139.8). Analogously to **1**, the relative stereochemistry of three chiral centers at C-1, C-2, and C-6 was elucidated to be the same as those of **1** by the NOESY experiments, as well as by analysis of the coupling constants and splitting pattern of H-1, H-2, and H_2-5. The configuration of double bonds at $\Delta^{7(8)}$ and $\Delta^{11(12)}$ was inferred to be *E* by the ^{13}C NMR chemical shifts of CH_3-19 and CH_3-20.[15]

Sarcophytonolide G (**3**) was also obtained as a colorless oil. The molecular formula, $C_{20}H_{30}O_3$, established by HREIMS at *m*/*z* 318.2194 (calcd 318.2195), was identical to that of **2**. A detailed 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC) analysis of **3** and comparison with the NMR data of **2** (Tables 1 and 2) revealed that its structure was almost the same as that of **2**. In fact, the main differences between **3** and **2** were the ^{1}H NMR chemical shifts and splitting patterns of H-6 (δ 4.59 for **3** and δ 4.80 for **2**), H-5a (δ 2.61, dd, J = 14.7, 4.2 Hz for **3** and δ 2.39, dd, J = 12.7, 10.8 Hz for **2**), and H-5b (δ 2.72, dd, J = 14.7, 5.7 Hz for **3** and δ 2.78, d, J = 12.7 Hz for **2**). This evidence clearly suggested that the relative configuration of the hydroxyl group at C-6 of **3** was different from that of **2**. In addition, this fact, in turn, further supports the hypothesis of formation of an intramolecular hydrogen bond between 6α-OH and the carbonyl at C-18. Accordingly, the structure of **3** was thus determined to be an epimer of **2** at C-6.

Sarcophytonolide H (**4**), 14-acetoxylsarcophytonolide F, yielded an EIMS peak at *m*/*z* 376, 58 mass units more than that of **2**,

Table 1. ^{1}H NMR Data of Compounds **1**−**4**[a]

proton	**1**	**2**	**3**	**4**
1	1.49 (m)	1.27 (m)	1.43 (m)	1.45 (m)
2	4.73 (d, J = 9.0)	4.68 (d, J = 9.2)	4.81 (d, J = 9.3)	4.91 (d, J = 10.6)
3	7.07 (s)	7.20 (s)	7.14 (s)	7.47 (s)
5	2.72 (d, J = 13.1)	2.78 (d, J = 12.7)	2.72 (dd, J = 14.7, 5.7)	2.82 (d, J = 11.0)
	2.37 (dd, J = 13.1, 10.4)	2.39 (dd, J = 12.7, 10.8)	2.61 (dd, J = 14.7, 4.2)	2.38 (dd, J = 11.0, 10.9)
6	4.21 (m)	4.80 (m)	4.59 (m)	4.70 (m)
7	1.72 (m)	5.11 (d, J = 9.1)	5.09 (d, J = 8.3)	5.11 (d, J = 9.2)
	1.14 (m)			
8	1.42 (m)			
9	1.49 (m)	2.17 (m)	2.20 (m)	2.22 (m)
	1.28 (m)	2.17 (m)	2.03 (m)	2.22 (m)
10	2.20 (m)	2.16 (m)	2.17 (m)	2.20 (m)
	1.95 (m)	2.16 (m)	2.13 (m)	2.20 (m)
11	4.95 (dd, J = 6.3, 2.9)	4.91 (br s)	4.92 (t, J = 6.6)	4.90 (br s)
13	2.10 (m)	2.10 (m)	2.07 (m)	2.24 (m)
	1.90 (m)	2.10 (m)	2.07 (m)	2.24 (m)
14	1.56 (m)	1.64 (m)	1.58 (m)	5.19 (dd, J = 10.3, 4.8)
	1.08 (m)	1.14 (m)	1.11 (m)	
15	2.08 (m)	2.15 (m)	2.08 (m)	1.88 (m)
16	0.98 (d, J = 6.6)[b]	0.93 (d, J = 6.5)[b]	0.96 (d, J = 6.9)	1.07 (d, J = 7.0)
17	0.99 (d, J = 6.6)[b]	0.94 (d, J = 6.5)[b]	0.98 (d, J = 6.9)	1.11 (d, J = 7.0)
19	0.90 (d, J = 6.6)	1.50 (s)	1.65 (s)	1.50 (s)
20	1.62 (s)	1.55 (s)	1.56 (s)	1.63 (s)
21				2.08 (s)

[a] ^{1}H NMR data were measured in $CDCl_3$ on a Bruker DRX 400 MHz spectrometer; chemical shifts (ppm) are referenced to $CDCl_3$ (δ 7.26). Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b] Interchangeable values.

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a]

carbon	1	2	3	4
1	45.4 (CH)	44.9 (CH)	46.5 (CH)	50.2 (CH)
2	83.5 (CH)	83.6 (CH)	84.4 (CH)	81.0 (CH)
3	150.5 (CH)	151.7 (CH)	151.7 (CH)	152.0 (CH)
4	130.5 (C)	129.3 (C)	130.8 (C)	130.5 (C)
5	36.8 (CH_2)	34.6 (CH_2)	33.9 (CH_2)	34.0 (CH_2)
6	66.9 (CH)	66.2 (CH)	67.5 (CH)	66.1 (CH)
7	43.8 (CH_2)	127.4 (CH)	127.9 (CH)	127.1 (CH)
8	28.5 (CH)	139.6 (C)	137.3 (C)	140.1 (C)
9	36.3 (CH_2)	38.6 (CH_2)	39.3 (CH_2)	38.4 (CH_2)
10	24.3 (CH_2)	23.8 (CH_2)	24.2 (CH_2)	24.1 (CH_2)
11	128.1 (CH)	125.8 (CH)	125.7 (CH)	125.5 (CH)
12	132.9 (C)	133.2 (C)	133.5 (C)	131.2 (C)
13	39.1 (CH_2)	37.2 (CH_2)	38.5 (CH_2)	39.8 (CH_2)
14	23.4 (CH_2)	22.8 (CH_2)	23.6 (CH_2)	73.2 (CH)
15	29.6 (CH)	28.8 (CH)	29.2 (CH)	25.8 (CH)
16	18.4 (CH_3)	17.7 (CH_3)	18.2 (CH_3)	25.1 (CH_3)
17	20.0 (CH_3)	20.1 (CH_3)	20.2 (CH_3)	18.8 (CH_3)
18	173.4 (C)	173.3 (C)	175.1 (C)	172.9 (C)
19	20.6 (CH_3)	16.2 (CH_3)	15.5 (CH_3)[b]	15.6 (CH_3)
20	16.3 (CH_3)	16.0 (CH_3)	15.6 (CH_3)[b]	19.0 (CH_3)
21				170.9 (C)
22				21.2 (CH_3)

[a] ^{13}C NMR data were measured in $CDCl_3$ on a Bruker DRX 400 MHz spectrometer; chemical shifts (ppm) are referenced to $CDCl_3$ (δ 77.0 ppm). The assignments were based on DEPT, $^1H-^1H$ COSY, HMQC, and HMBC experiments. [b] Interchangeable values.

indicating the presence of an acetoxyl group in **4**. A comparison of overall ^1H and ^{13}C NMR data (Tables 1 and 2) revealed strong similarities between compounds **4** and **2**. In fact, **4** differs from **2** only by the presence of an acetate functionality in the 14-membered ring, which was evident by the peaks of δ 170.9, 73.2, and 21.2 in its ^{13}C NMR spectrum. Careful analysis of the 2D NMR spectra ($H-^1H$ COSY, HMQC, HMBC) allowed the location of an acetoxyl group at C-14, which was supported by the expected downfield shifted ^{13}C NMR resonances of both C-1 and C-13 with respect to those of **2** (Tables 1 and 2). Analogously to **2**, the relative configuration of compound **4** was elucidated by the NOESY experiment. NOE correlations observed between H-2/H-14, H-2/H-15, H-2/H_3-16, H-3/H-14, H-3/H-5a, and H-6/H-5b suggested that both H-14 and H-2 were α oriented, opposite H-1 and H-6 (β). The structure of compound **4** was thus determined as an 14-acetoxyl derivative of **2**.

On the basis of the knowledge of the relative relationships of the chiral centers around the 14-membered cembrane macrocycle, advanced stereochemical studies had been performed.

Since compounds **1**−**4** all contained a secondary alcohol at C-6, the modified Mosher's method could be employed to determine their absolute stereochemistry. First, (*S*)- and (*R*)-MTPA esters of sarcophytonolide H (**4**) were prepared by treatment with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetyl (MTPA) chloride in dry pyridine at room temperature, respectively. Compound **4** was converted to the corresponding MTPA esters **4*S*** and **4*R***, respectively. All the ^1H NMR resonances of the esters were assigned by an extensive analysis of 1D and 2D NMR spectra. Significant $\Delta\delta$ values ($\Delta\delta = \delta_{S-MTPA-ester} - \delta_{R-MTPA-ester}$) for the protons near the chiral center C-6 were observed. In fact, positive $\Delta\delta$ shifts were observed for the methylene protons (H_2-5) and methine protons (H-3 and H-2), whereas negative effects were recorded for the olefinic protons (H-7 and H-11), methylene protons (H_2-9, H_2-10), and vinyl methyl protons (H_3-19, H_3-20). Inspection of the Drieding models of the MTPA esters of **4** indicated that there are no steric impediments to the MTPA group adopting the "ideal conformation" having trifluoromethyl, ester carbonyl, and carbinol methine protons coplanar. Therefore, according to the MTPA determination rule,[17−22] the *R* absolute stereochemistry at C-6 of sarcophytonolide H (**4**) was suggested. Some selected $\Delta\delta$ values are summarized in structures **4*S***/**4*R*** (Figure 3). On the basis of the above results and because the relative stereochemistry at other centers of the cembrane skeleton has been assigned by NMR techniques, we propose the 1*R*, 2*S*, and 14*S* configuration for sarcophytonolide H (**4**).

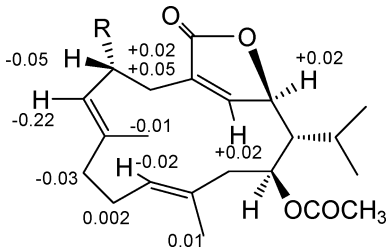

Figure 3. $\Delta\delta$ values ($\delta_S - \delta_R$) (ppm) for protons near C-6 of (*S*)- and (*R*)-MTPA esters of **4**.

The absolute stereochemistry of compounds **1**−**3** remains to be determined because of the scarcity of material. However, the consideration that these compounds are closely related to the co-occurring sarcophytonolide H (**4**), and bearing in the mind the already elaborated relative stereochemistry at all chiral centers of compounds **1**−**3**, the absolute stereochemistry of sarcophytonolides E−G could be assigned as shown in Figure 1.

It has been noted that all cembrane diterpenes of known absolute configuration at C-1 reported from the order Alcyonacea belong to the α series, while all cembrane derivatives isolated from the order Gorgonacea belong to the β series.[2] Our results presented here have added further support to this conclusion.

Compounds **1**−**4** were tested for cytotoxicity against A-549 and HL-60 tumor cell lines, but they were inactive at a concentration of 20 μg/mL. Other bioassays such as antibacterial and anti-inflammatory activities, are currently ongoing.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341MC polarimeter. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer. NMR spectra were measured on a Bruker DRX-400 spectrometer with the residual $CDCl_3$ (δ_H 7.26 ppm; δ_C 77.01 ppm) as an internal standard. EIMS and HREIMS spectra were recorded on a Finnigan-MAT-95 mass spectrometer. All solvents were of analytical grade (Shanghai Chemical Plant, Qingdao, People's Republic of China). Reversed-phase HPLC (Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm; a semipreparative ODS-HG-5 [5 m, 10 mm (i.d.) × 25 cm] column) was also employed. Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 400−600 mesh) was used for column chromatography, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC. Sephadex LH-20 gel (Amersham Biosciences) was also used for column chromatography.

Biological Material. Specimens of *Sarcophyton latum*, identified by Prof. R.-L. Zhou of South China Sea Institute of Oceanology, Chinese Academy Sciences, were collected along the coast of Ximao Island, Sanya, Hainan Province, China, in December 2002, at a depth of −20 m, and were frozen immediately after collection. A voucher specimen is available at the Institute of Materia Medica, SIBS-CAS (No. HN103).

Extraction and Isolation. The frozen animals (68 g, dry weight) were cut into pieces and extracted exhaustively with acetone at RT (3 × 1.5 L). The organic extract was evaporated to give a residue, which was partitioned between Et_2O and H_2O. The Et_2O solution was concentrated under reduced pressure to give a dark brown residue (3.2 g), which was fractionated by gradient Si gel column chromatography (0−100% acetone in light petroleum ether), yielding a mixture showing interesting blue TLC spots after spraying with H_2SO_4 [R_f 0.5−0.6 (PE/CH_3COCH_3, 2:1)]. This was further purified by RP-HPLC [semi-preparative ODS-HG-5 (5 μm, 250 × 10 mm), MeCN/H_2O (70:30), 2.0 mL/min] into four pure compounds, **1** (2.2 mg), **2** (1.9 mg), **3** (1.2 mg), and **4** (5.8 mg).

Sarcophytonolide E (1): colorless oil, $[\alpha]_D^{20}$ +30 (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 234 (4.3) nm; IR (film) ν_{max} 3315, 2921, 2850, 1743, 1209, 1072, 1038 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1

and 2; EIMS *m*/*z* 320 $[M]^+$ (16), 302 (20), 287 (19), 277 (11), 259 (47), 249 (18), 231 (15), 215 (24), 191 (18), 149 (38), 135 (36), 123 (60), 109 (60), 95 (76), 81 (88), 69 (100), 55 (84); HREIMS *m*/*z* 320.2353 ($C_{20}H_{32}O_3^+$; calcd 320.2351)

Sarcophytonolide F (2): colorless oil, $[\alpha]_D^{20}$ +115.0 (*c* 0.54, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 236 (4.2) nm; IR (film) ν_{max} 3439, 2923, 2850, 1732, 1215, 1072, 1043 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 318 $[M]^+$ (22), 300 (21), 285 (18), 257 (38), 191 (17), 145 (24), 121 (58), 107 (42), 95 (54), 81 (100), 69 (71), 55 (70); HREIMS *m*/*z* 318.2193 ($C_{20}H_{30}O_3^+$; calcd 318.2195).

Sarcophytonolide G (3): colorless oil, $[\alpha]_D^{20}$ −1.6 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 233 (3.8) nm; IR (film) ν_{max} 3253, 2933, 2843, 1735, 1211, 1099, 1035 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 318 $[M]^+$ (16), 300 (22), 285 (10), 257 (46), 191 (31), 147 (40), 137 (42), 121 (76), 95 (61), 81 (100), 69 (71), 55 (63); HREIMS *m*/*z* 318.2194 ($C_{20}H_{30}O_3^+$; calcd 318.2195).

Sarcophytonolide H (4): colorless oil, $[\alpha]_D^{20}$ +74.7 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 237 (3.6) nm; IR (film) ν_{max} 3286, 2921, 2850, 1761, 1731, 1252, 1078, 1024 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 376 $[M]^+$ (50), 316 (11), 298 (17), 273 (21), 255 (24), 232 (28), 202 (29), 215 (24), 181 (55), 163 (37), 135 (92), 121 (48), 107 (62), 93 (100), 81 (83), 69 (67), 55 (58); HREIMS *m*/*z* 376.2265 ($C_{22}H_{32}O_5^+$; calcd 376.2250).

Preparation of (*S*)- and (*R*)-MTPA Esters. The **4*S*** derivative was obtained by treating **4** (2.0 mg) with (*R*)-MTPA-Cl in dry pyridine for ca. 16 h under stirring at RT. The reaction mixture was purified by CC (silica gel) to afford pure **4*S*** (1.8 mg). In a similar manner, **4*R*** (1.6 mg) was prepared from (*S*)-MTPA-Cl.

4*S*: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.53 (2H, m), 7.50 (1H, s, H-3), 7.39 (3H, m), 5.89 (1H, ddd, J = 11.2, 9.8, 4.0 Hz, H-6), 5.16 (1H, dd, J = 9.5, 5.1 Hz, H-14), 4.93 (1H, d, J = 11.0 Hz, H-2), 4.91 (1H, d, J = 9.8 Hz, H-7), 4.87 (1H, overlapped, H-11), 2.88 (1H, d, J = 12.6 Hz, H-5b), 2.60 (1H, dd, J = 12.6, 11.6 Hz, H-5a), 2.25 (2H, m, H_2-10), 2.23 (2H, m, H_2-13), 2.20 (1H, m, H-15), 2.15 (2H, m, H_2-9), 2.08 (3H, s, H_3-$COOCH_3$), 1.63 (3H, s, H_3-19), 1.62 (3H, s, H_3-20), 1.45 (1H, d, J = 11.0 Hz, H-1), 1.11 (3H, d, J = 6.9 Hz, H_3-16), 1.08 (3H, d, J = 7.2 Hz, H_3-17).

4*R*: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.51 (2H, m), 7.49 (1H, s, H-3), 7.38 (3H, m), 5.92 (1H, dt, J = 11.0, 4.1 Hz, H-6), 5.16 (1H, dd, J = 9.3, 5.7 Hz, H-14), 5.08 (1H, d, J = 9.6 Hz, H-7), 4.92 (1H, d, J = 10.8 Hz, H-2), 4.89 (1H, overlapped, H-11), 2.81 (1H, d, J = 11.5 Hz, H-5b), 2.56 (1H, dd, J = 11.5, 11.0 Hz, H-5a), 2.25 (2H, m, H_2-10), 2.24 (2H, m, H_2-13), 2.20 (1H, m, H-15), 2.17 (2H, m, H_2-9), 2.08 (3H, s, H_3-$COOCH_3$), 1.64 (3H, s, H_3-19), 1.63 (3H, s, H_3-20), 1.46 (1H, d, J = 10.8 Hz, H-1), 1.11 (3H, d, J = 6.9 Hz, H_3-16), 1.08 (3H, d, J = 7.0 Hz, H_3-17).

Acknowledgment. This research work was financially supported by the National Marine 863 Project (No. 2005AA624040), the Natural Science Foundation of China (No. 20572116), STCSM Projects (Nos. 04ZR14156 and 054307062), and CNR-Italy/CAS-China Joint Projects 2004/2007 and partly funded by State Key Program of Basic Research of China (No. 2004CB518905). We are indebted to R.-L. Zhou of South China Sea Institute of Oceanology, Chinese Academy of Sciences, for identification of the specimen.

References and Notes

(1) König G. M.; Wright, A. D. *J. Nat. Prod.* **1998**, *61*, 494−496.
(2) Pham, N. B.; Butler, M. S.; Quinn, R. J. *J. Nat. Prod.* **2002**, *65*, 1147−1150.
(3) Duh, C.-Y.; Wang S.-G.; Chung, S.-G.; Dai, C.-F. *J. Nat. Prod.* **2000**, *63*, 1634−1637.
(4) Longeon, A.; Bourguet-Kondracki; M.-L.; Guyot, M. *Tetrahedron Lett.* **2002**, *43*, 5937−5939.
(5) Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *Tetrahedron Lett.* **1988**, *29*, 4731−4734.
(6) Schmitz, F. J.; Bowden, B. F.; Toth, S. I. In *Marine Biotechnology*; Attaway, D. H., Zaborsky, O. R., Eds.; Plenum Press: New York, 1993; Vol. 1, pp 197−308.
(7) Norton, R. S.; Kazlauskas, R. *Experientia* **1980**, *36*, 276−278.
(8) Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Yamakado, T.; Matsuzaki, T.; Hirata, Y. *Experientia* **1983**, *39*, 67−69.
(9) Coll, J. C.; Price, I. R.; Koenig, G. M.; Bowden, B. F. *Mar. Biol.* **1987**, *96*, 129−135.
(10) Coll, J. C.; Bowden, B. F.; Alino, P. M.; Heaton, A.; Koenig, G., M.; Nys, R.; Willis, R. H.; Sammarco, P. W.; Clayton, M. N. *Chem. Scr.* **1989**, *29*, 383−388.
(11) Jia, R.; Guo, Y.-W.; Mollo, E.; Cimino, G. *Helv. Chim. Acta* **2005**, *88*, 1028−1033.
(12) Sun, Y.-Q.; Guo, Y.-W. *Tetrahedron Lett.* **2004**, *45*, 5533−5525.
(13) Huang, X.-C.; Zhao D.; Guo, Y.-W.; Wu, H.-M.; Trivellone, E.; Cimino, G. *Tetrahedron Lett.* **2004**, *45*, 5501−5504.
(14) Zhang, W.; Guo, Y.-W.; Mollo, E.; Cimino, G. *Helv. Chim. Acta* **2004**, *87*, 2341−2345.
(15) Iwagawa, T.; Nakashima, R.; Takayama, K.; Okamura, H.; Nakatani, M.; Doe, M.; Shibata, K. *J. Nat. Prod.* **1999**, *62*, 1046−1049.
(16) Collins, D. O.; Reynolds, W. F.; Reese, P. B. *J. Nat. Prod.* **2004**, *67*, 179−183.
(17) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−515.
(18) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143−2148.
(19) Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *Tetrahedron Lett.* **1988**, *29*, 4731−4734.
(20) Ohtani, I.; Kusumi, T.; Ishtsuka, O. M.; Kakisawa, H. *Tetrahedron Lett.* **1989**, *30*, 3147−3150.
(21) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296−1298.
(22) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4097.

NP050477U