Biscembranoids and Their Probable Biogenetic Precursor from the Hainan Soft Coral *Sarcophyton tortuosum*

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Five novel biscembranoids, ximaolides A−E (**2**−**6**), and their proposed biogenetic precursor, methyl tortuosoate (**1**), were isolated from the Hainan soft coral *Sarcophyton tortuosum*. The structures of compounds **1**−**6** were elucidated by spectroscopic methods, mainly NMR techniques. The relative stereochemistry of biscembranoids **2** and **6** was secured by X-ray diffraction analysis, whereas the relative configurations of chiral centers in compounds **1**, **3**, **4**, and **5** have been suggested by both biogenetic considerations and NOESY experiments.

Soft corals are well-known for their high content of diterpenes and particularly cembranoids that possess a variety of biological activities ranging from antimicrobial to cytotoxic and antitumor.[1] Biscembranoids represent an emerging group of natural products from soft corals of the genus *Sarcophyton* (family Alcyoniidae). Reports of these uncommon terpenoids from *Sarcophyton* have become numerous over recent years.[2−8] Up to now, 12 biscembranoids have been discovered from *Sarcophyton tortuosum* and *S. glaucum*. They are methyl sartortuoate,[3] methyl isosartortuoate,[2] methyl sarcophytoate,[4] methyl chlorosarcophytoate,[4] methyl neosartortuoate acetate,[5] nyalolide,[6] methyl tortuoates A and B,[7] and bisglaucumlides A−D.[8] A common structural feature among these dimeric diterpenes is that all of them could biogenetically derive from two different cembranoid units through a probable Diels−Alder addition as suggested first for methyl isosartortuoate[2] and then by many papers.[3−8] The complex and unique structures of these dimeric cembranoids have also attracted the attention of synthetic chemists for their total synthesis.[9,10]

S. tortuosum is very common on the coral reefs in the South China Sea. Recently, in the course of our ongoing research program on bioactive substances from Hainan marine invertebrates,[11] we reinvestigated *S. tortuosum*. As a result, four new cembranoids, sarcophytonolides A−D, were discovered.[12] Further chemical investigation of the Et_2O extract of the animal has now furnished an additional new cembranoid, methyl tortuosoate (**1**), along with five new biscembranoids, named ximaolides A−E (**2**−**6**). The structure of **1** is closely related to the upper portion of co-occurring biscembranoids **2**−**6**, suggesting that **1** might be the biogenetic precursor of these dimers. The details of the structure elucidation of compounds **1**−**6** are presented here.

Results and Discussion

Samples of *S. tortuosum* were collected off Ximao Island (the locality suggested the name assigned to the new biscembranoids), Sanya, Hainan, China, in 2002 and kept frozen until used. The usual workup[12] of the Et_2O-soluble fraction of the acetone extract of *S. tortuosum* yielded pure methyl tortuosoate (**1**) and ximaolides A−E (**2**−**6**).

A preliminary ^1H NMR analysis of these molecules revealed a structural similarity for all of them and indicated the presence of biscembrane frameworks in compounds **2**−**6**, according to previous chemical studies on *S. tortuosum*,[2,3,7] whereas compound **1** exhibited a common cembrane skeleton. The chemical analysis of **1**−**6** was conducted starting from the main metabolites ximaolide E (**6**) and methyl tortuosoate A (**1**), followed by the remaining biscembranes **2**−**5**. Accordingly, the structure elucidation details of these new molecules are described in this order.

Ximaolide E (**6**) was obtained as colorless crystals, mp 205−207 °C, $[\alpha]_D^{20}$ +38.4 (*c* 0.31, $CHCl_3$). HREIMS {$[M]^+$ at *m*/*z* 682.4432, Δ 1.3 mmu} revealed a molecular formula of $C_{41}H_{62}O_8$. The ^1H and ^{13}C NMR spectra of **6** (Tables 1 and 2) showed signals attributable to a methyl ester [δ_C 175.1 (q C), 51.4 (CH_3) and δ_H 3.56 (3H, s)], three ketone carbonyl groups (δ_C 212.2, 212.3, 213.6), an exo-cyclic double bond [δ_C 107.0 (CH_2), 149.3 (q C) and δ_H 4.68, 4.73 (each 1H br s)], and a tri- and a tetrasubstituted double bond [δ_C 124.5 (CH), 140.0 (q C); 130.3 (q C), 132.8 (q C)]. In addition, a tertiary hydroxyl [δ_C 74.1 (q C)], a secondary hydroxyl [δ_C 69.1 (CH) and δ_H 4.84 (1H, dd, J = 7.9 and 4.9 Hz)], and an ether linkage [δ_C 78.1 (CH), 74.1 (CH) and δ_H 4.25 (1H, d, J = 10.1 Hz), 3.65 (1H, dd, J = 11.7 and 2.4 Hz)] were revealed. The presence of seven methyl groups in the molecule was indicated by ^1H NMR signals attributed to two vinyl methyls [δ_H 1.72 (3H, s, H_3-37) and 1.62 (3H, s, H_3-38)], an isopropyl group [δ_H 0.69 (3H, d, J = 6.8 Hz, H_3-16) and 0.94 (3H, d, J = 6.8 Hz, H_3-17)], two secondary methyls [δ_H 1.10 (3H, d, J = 7.1 Hz, H_3-18) and δ_H 0.84 (3H, d, J = 7.0 Hz, H_3-19)], and a tertiary methyl [δ_H 1.10 (3H, s, H_3-40)]. These data led us to recognize that **6** should be a biscembranoid compound similar to those previously reported from soft corals of the same genus.[2−7] In particular, NMR data of **6** strongly resembled those of methyl tortuoate A (**7**).[7] The significant difference, which was observed for C-7 (δ 24.3 for **6** and 32.5 for **7**[7]) and C-25 (δ 32.5 for **6** and 24.0 for **7**[7]), disappeared by inverting these assignments in compound **7**. These data suggested that compound **6** is the $\Delta^{27(39)}$ double-bond isomer of **7**, and this was confirmed by X-ray diffraction analysis on a suitable crystal of **6** (Figure 1). This experiment also provided the relative stereochemistry (1*R**, 2*R**, 5*R**, 9*S**, 12*R**, 21*R**, 26*R**, 30*S**, 31*R**, 33*R**), which is the same as compound **7**.[7,13]

All NMR resonances of **6** were assigned by analysis of 2D NMR spectra as reported in Tables 1 and 2. Selected $^1H-^1H$ COSY, HMBC, and NOESY correlations of compound **6** are reported in Figure 2.

Attempts to determine the absolute stereochemistry of ximaolide E (**6**) using the Mosher method were unsuccessful due to the fact that no $\Delta\delta$ value was observed for the two Mosher ester derivatives. This is a case in which the Mosher method is inapplicable because of steric hindrance of the OH group,[14] and hence, the absolute stereochemistry of **6** remains undetermined.

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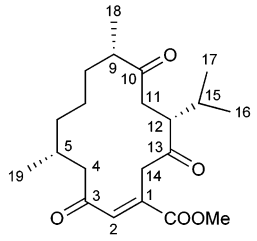
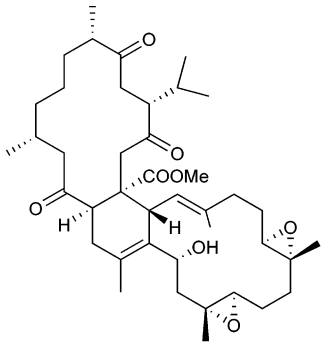
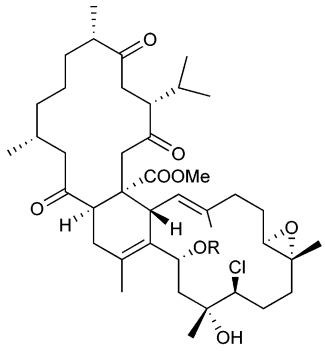
Chart 1



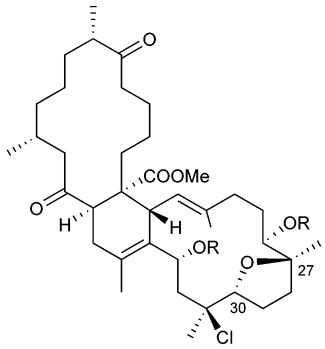
methyl tortuosoate (**1**)



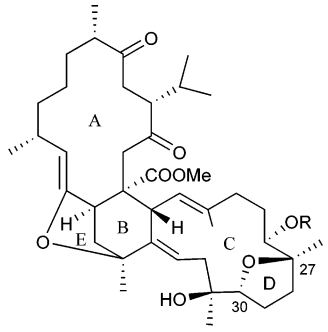
ximaolide A (**2**)



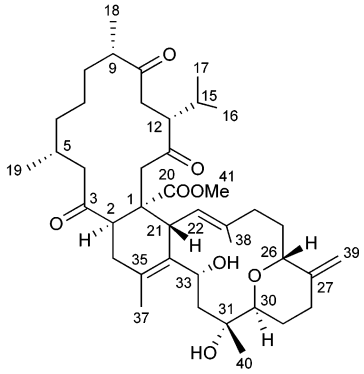
R = H ximaolide B (**3**)
R = Ac **3a**



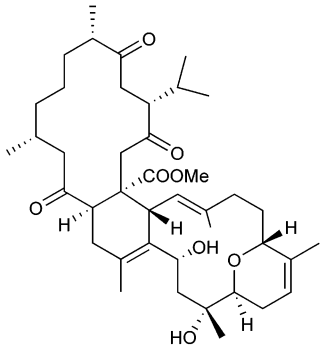
R = H ximaolide C (**4**)
R = Ac **4a**



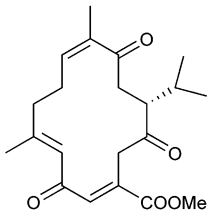
R = H ximaolide D (**5**)
R = Ac **5a**



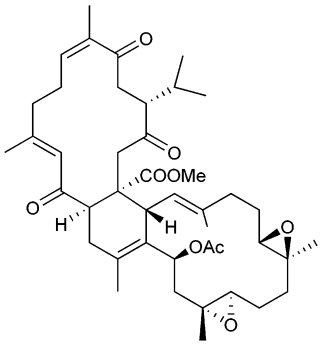
ximaolide E (**6**)



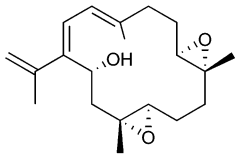
methyl tortuate A (**7**)[7,13]



methyl sarcoate (**8**)



methyl neosartortuate acetate (**9**)[5]



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Methyl tortuosoate (**1**) was isolated as a colorless oil. Its molecular formula, $C_{21}H_{32}O_5$, was deduced from HREIMS data {*m/z* 364.2246 $[M]^+$, calcd 364.2250}, indicating six degrees of unsaturation. Analysis of the ^{13}C NMR for **1** (Table 2) revealed the presence of three carbonyl functions (δ 205.8, 206.1, 213.4), a methyl ester [δ 167.4 (q C), 52.8 (CH_3)], and a trisubstituted double bond [δ 133.4 (CH), 138.8 (q C)]. These account for five degrees of unsaturation, and thus **1** must have a single ring. The ^1H NMR spectrum (Table 1) displayed signals attributable to an isopropyl group [δ 0.89 (3H, d, J = 6.9 Hz, H_3-16), 0.97 (3H, d, J = 7.0 Hz, H_3-17), and 3.18 (1H, ddd, J = 11.3, 6.3, and 2.5 Hz)], two secondary methyls [0.91 (3H, d, J = 7.0 Hz, H_3-19) and 1.13 (3H, d, J = 7.0 Hz, H_3-21)], a methoxy group [δ 3.82 (3H, s)], a vinyl proton of an α,β-conjugated system [δ 7.34 (H-2, s)], and an isolated allylic methylene [δ 4.39 (1H, d, J = 16.5 Hz, H_2-14a) and 3.57 (1H, d, J = 16.5 Hz, H_2-16b)]. These data were consistent with a functionalized cembrane skeleton exhibiting three ketone groups, a double bond, and a oxidized methyl at C-1. The *E*-geometry of the double bond was indicated by the downfield

Table 1. ^{1}H NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Compounds **1**−**6**[a]

	1	**2**	**3**	**4**	**5**	**6**
position	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)	δ mult. (*J* in Hz)
2	7.34, s	3.85, dd (8.6, 5.8)	3.66, t (7.9)	3.48 m	1.99 m	3.76 dd (8.1, 6.5)
4	2.57, dd (17.6, 9.9)	3.02 (m)	3.11, m	3.26 dd (20.0, 10.3)	3.77 d (10.0)	2.69 dd (7.9, 4.9)
	2.36, dd (17.6, 2.3)	2.40, m		2.38 dd (20.0, 2.0)		2.31 m
5	2.15, m	1.80, m	1.80, m	1.78 m	2.53 m	1.98 m
6	1.38, m	1.03, m[b]	1.07, m[b]	1.09 m	0.82 m	1.15 m
	0.98, m	0.97, m	1.02, m		1.57 m	0.95 m
7	0.93, m	1.04, m[b]	1.24, m	1.12 m	1.75 m	1.05 m
	0.60, m	1.23, m	1.08, m[b]	1.27 m		
8	1.42, m	1.53, m	1.51, m	1.50 m	1.49 m	1.61 m
	1.42, m	1.45, m				1.43 m
9	2.32, m	2.43, m	2.46, m	2.50 m	2.42 m	2.40 m
11	3.05, dd (17.3, 11.3)	1.90, m	3.05, m	2.95 m	2.87 m	2.91 m
	2.20, dd (17.3, 2.5)	1.95, m	2.96, m	1.85 m	1.59 m	2.09 m
12	3.18, ddd (11.3, 6.3, 2.5)	3.02, m	3.04, m	3.05 m	2.22 m	2.97 m
14	4.39, d (16.5)	3.05, s	2.99, d (18.7)	3.00 d (19.0)	2.98 d (18.4)	3.14 s
	3.57, d (16.5)		2.85, d (18.7)	2.78 d (19.0)	2.70 d (18.4)	
15	1.95, m	2.14, m	2.20, m	2.30 m	1.71 m	2.01 m
16	0.89, d (6.9)	0.69, d (6.8)	0.70, d (6.8)	0.68 d (6.9)	0.83 d (6.8)	0.78 d (6.8)
17	0.97, d (7.0)	0.94, d (6.8)	0.97, d (6.8)	0.98 d (6.9)	0.95 d (6.8)	0.94 d (6.8)
18	1.13, d (7.0)	1.10, d (7.1)	1.12, d (7.0)	1.11 d (7.1)	1.18 d (7.0)	1.14 d (7.1)
19	0.91, d (7.0)	0.84, d (7.0)	0.85, d (6.8)	0.85 d (7.0)	0.93 d (7.0)	0.86 d (6.9)
21	3.82, s	3.32, d (11.0)	3.55, d (10.8)	3.67 d (10.8)	2.93 d (10.1)	3.55 d (10.5)
22		5.11, d (11.0)	5.10, d (10.8)	4.95 d (10.8)	4.94 d (10.1)	5.18 d (10.5)
24		2.25, m	2.26, m	2.12 m	2.05 m	2.50 m
		2.07, m				2.03 m
25		1.55, m	1.86, m	2.01 m	1.24 m	2.17 m
		1.70, m	1.52, m	1.29 m		1.62 m
26		2.86, dd (5.2, 1.0)	2.59, m	3.18 dd (9.3)	3.24 d (8.8)	4.25 d (10.1)
28		2.07, m	1.92, m	2.41 m	2.67 m	2.36 m
			1.89, m	1.66 m	2.22 m	2.28 m
29		1.60, m	1.63, m	1.95 m	1.64 m	1.91 m
				1.57 m		1.44 m
30		2.31, m	3.62, d (8.2)	4.27 dd (10.2, 6.0)	3.83 dd (10.7, 4.6)	3.65 dd (11.7, 2.4)
32		1.86, d (6.0)	1.72, m	1.37 m	2.65 m	1.96 m
			1.52, m	2.45 m	1.77 m	1.58 m
33		4.77, t (6.0)	5.06, d (9.3)	5.04 d (10.8)	5.30 t (3.7)	4.84 dd (7.9, 4.9)
36		2.36, m	2.38, m	1.95 m	2.67 m	2.44 m
		2.25, m	2.13, m	2.25 m	1.39 m	2.09 m
37		1.72, s	1.71, s	1.61 s	1.34 s	1.89 s
38		1.62, s	1.64, s	1.73 s	1.63 s	1.77 s
39		1.23, s[b]	1.25, s	1.20 s	1.15 s	4.68, 4.73, br s
40		1.24, s[b]	1.47, s	1.54 s	1.24 s	1.07 s
41		3.49, s	3.51, s	3.51 s	3.68 s	3.56 s

[a] The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b]Interchangeable values.

chemical shift (δ 7.34) of H-2.[15] Analysis of 2D-NMR experiments allowed us to determine the carbon connectivity leading to structure **1**.

This compound was closely related to methyl sarcoate (**8**), previously isolated from an Okinawan sample of *S. glaucum*,[16] being its corresponding tetrahydro derivative. Methyl tortuosoate (**1**) displays the same carbon framework as that contained in both the co-occurring ximaolide E (**6**) and methyl tortuoate A (**7**).[7] Due to this, the relative stereochemistry at chiral centers C-5, C-9, and C-12 of **1** could be suggested to be the same as **6** by biogenetic considerations. In addition, the observed NOESY correlations (Figure 3) supported the proposed structure.

Ximaolide A (**2**) was obtained as a highly viscous, colorless oil. A molecular formula of $C_{41}H_{62}O_8$ was determined by HREIMS (M^+, *m*/*z* 682.4468; calcd 682.4465). The NMR spectra (Tables 1 and 2) showed the following functionalities: one methyl ester [δ_C 174.7, C-20; 51.2, C-41; δ_H 3.49 (3H, s, H_3-41)]; three ketone carbonyl groups (δ_C 210.5, C-13; 213.6, C-3; 213.8, C-10); one tri- and one tetrasubstituted double bond [δ_C 126.2 (C-22), 133.8 (C-23), 130.4 (C-35), 132.0 (C-34)]; two vinyl methyl groups [δ_H 1.72 (3H, s, H_3-37) and 1.62 (3H, s, H_3-38)]; an isopropyl group [δ_H 0.69 (3H, d, J = 6.8 Hz, H_3-16) and 0.94 (3H, d, J = 6.8 Hz, H_3-17)]; and two secondary methyl groups [δ_H 1.10 (3H, d, J = 7.1 Hz, H_3-18) and 0.84 (3H, d, J = 7.0 Hz, H_3-19)]. In addition, compound **2** possessed two trisubstituted epoxide groups with methyl singlets at δ_H 1.23 (3H, s, H_3-39) and 1.24 (3H, s, H_3-40), epoxymethine multiplets at δ_H 2.86 (H-26) and 2.31 (H-30), and ^{13}C NMR signals at 61.5 (C-26), 59.3 (C-27), 60.7 (C-30), and 59.8 (C-31) ppm. Finally, the last oxygen atom that remained unassigned was attributed to a secondary hydroxyl group [δ_H 4.77 (H-33) and δ_C 64.8 (C-33)].

Comparison of these data with those of the main metabolite **6** indicated that ximaolide A (**2**) had the same carbon framework, differing from **6** only in the arrangement of the oxygen functionalities at chiral centers C-26, C-27, C-30, and C-31. In particular, a detailed analysis of 2D NMR data allowed the location of two epoxide moieties at these carbon atoms, as reported in structure **2**. A similar functionalization has been reported for the biscembrane methyl neosartortuate acetate (**9**) from Australian *S. tortuosum*.[5] Careful comparison of NMR resonances of carbon atoms from C-21 to C-40 of **2** with the corresponding values of neosartortuate acetate (**9**)[5] revealed strong similarities. In particular, ^{1}H and ^{13}C NMR values of carbons from C-25 to C-31 were essentially identical in **2** and **9**, strongly suggesting the same relative orientation of the two epoxide moieties, whereas differences were observed for carbons spatially close to C-33.[5] This was probably due to the different stereochemistry reported at C-33 as well as to the presence in **9** of the sterically hindered −OAc substituent in place of the −OH group.

Table 2. ^{13}C NMR (125 MHz, $CDCl_3$) Data for Compounds **1**−**6**[a]

	1	2	3	4	5	6
1	138.8, qC	50.5, qC	50.3, qC	49.5, qC	44.4, qC	49.6, qC
2	133.4, CH	44.8, CH	44.0, CH	43.4, CH	48.2, CH	46.5, CH
3	206.1, qC	213.6, qC	213.4, qC	213.4, qC	148.9, qC	212.3, qC[b]
4	50.7, CH_2	53.0, CH_2	53.6, CH_2	54.1, CH_2	107.7, CH	51.1, CH_2
5	28.3, CH	27.2, CH	27.4, CH	27.5, CH	28.9, CH	26.6, CH
6	37.9, CH_2	37.0, CH_2	37.3, CH_2	37.5, CH_2	39.0, CH_2	36.6, CH_2
7	25.7, CH_2	25.2, CH_2	25.5, CH_2	25.6, CH_2	28.8, CH_2	24.3, CH_2
8	34.7, CH_2	34.0, CH_2	34.1, CH_2	34.0, CH_2	36.2, CH_2	33.3, CH_2
9	48.2, CH	48.2, CH	48.1, CH	47.8, CH	49.4, CH	47.5, CH
10	213.4, qC	213.8, qC	213.8, qC	213.9, qC	213.5, qC	213.6, qC
11	36.2, CH_2	31.9, CH_2	31.4, CH_2	31.1, CH_2	36.0, CH_2	34.7, CH_2
12	53.4, CH	50.6, CH	51.1, CH	51.7, CH	54.7, CH	51.0, CH
13	205.8, qC	210.5, qC	209.6, qC	209.1, qC	208.4, qC	212.2, qC[b]
14	41.9, CH_2	47.5, CH_2	46.4, CH_2	45.4, CH_2	48.4, CH_2	47.1, CH_2
15	29.9, CH	28.9, CH	28.9, CH	28.9, CH	30.6, CH	29.1, CH
16	19.7, CH_3	17.7, CH_3	17.6, CH_3	17.5, CH_3	20.4, CH_3	18.4, CH_3
17	21.1, CH_3	21.2, CH_3	21.3, CH_3	21.3, CH_3	21.0, CH_3	21.0, CH_3
18	17.0, CH_3	17.5, CH_3	17.6, CH_3	17.5, CH_3	18.4, CH_3	17.2, CH_3
19	22.6, CH_3	21.9, CH_3	22.2, CH_3	22.3, CH_3	22.1, CH_3	21.8, CH_3
20	167.4, qC	174.7, qC	174.7, qC	174.9, qC	175.6, qC	175.1, qC
21	52.8, CH_3	43.5, CH	42.6, CH	43.0, CH	37.3, CH	42.4, CH
22		126.2, CH	127.9, CH	127.2, CH	124.9, CH	124.5, CH
23		133.8, qC	135.5, qC	137.4, qC	134.4, qC	140.0, qC
24		36.4, CH_2	37.1, CH_2	36.6, CH_2	35.9, CH_2	37.7, CH_2
25		26.0, CH_2	24.7, CH_2	29.5, CH_2	26.7, CH_2	32.5, CH_2
26		61.5, CH	59.0, CH	73.7, CH	72.1, CH	78.1, CH
27		59.3, qC	59.8, qC	85.9, qC	83.8, qC	149.3, qC
28		36.0, CH_2	31.7, CH_2	35.4, CH_2	36.4, CH_2	29.2, CH_2
29		23.8, CH_2	27.5, CH_2	27.9, CH_2	26.3, CH_2	25.5, CH_2
30		60.7, CH	73.5, CH	89.7, CH	87.3, CH	74.1, CH
31		59.8, qC	74.6, qC	75.0, qC	73.9, qC	74.1, qC
32		39.9, CH_2	44.5, CH_2	43.2, CH_2	33.7, CH_2	40.8, CH_2
33		64.8, CH	66.0, CH	66.4, CH	119.9, CH	69.1, CH
34		132.0, qC	132.4, qC	132.4, qC	140.8, qC	132.8, qC
35		130.4, qC	128.4, qC	125.7, qC	76.9, qC	130.3, qC
36		33.0, CH_2	32.7, CH_2	32.8, CH_2	43.3, CH_2	34.5, CH_2
37		18.9, CH_3	18.3, CH_3	18.0, CH_3	22.4, CH_3	19.8, CH_3
38		16.9, CH_3	15.2, CH_3	16.3, CH_3	16.1, CH_3	18.5, CH_3
39		16.2, CH_3	18.8, CH_3	20.5, CH_3	20.2, CH_3	107.0, CH_2
40		18.4, CH_3	25.8, CH_3	25.6, CH_3	22.6, CH_3	23.2, CH_3
41		51.2, CH_3	51.2, CH_3	51.0, CH_3	52.1, CH_3	51.4, CH_3

[a] Chemical shifts (ppm) referred to $CHCl_3$ (δ 77.0). [b]Interchangeable values.

However, the relative stereochemistry of ximaolide A was definitively established by X-ray diffraction analysis of a single crystal of **2**. The 11 chiral carbons of **2** were unambiguously determined as 1*R**, 2*R**, 5*R**, 9*S**, 12*R**, 21*R**, 26*R**, 27*R**, 30*R**, 31*R**, and 33*R**, as reported in the X-ray structure shown in Figure 4. Consequently, the relative stereochemistry of the epoxide moiety at C-26/C-27 in compound **9** should also be 26*R**, 27*R**, different from that reported by Leone et al.[5]

All NMR resonances of **2** were assigned by analysis of 2D NMR spectra as reported in Tables 1 and 2. Selected $^1H-^1H$ COSY, HMBC, and NOESY correlations of compound **2** are reported in Figure 5.

It is interesting to observe that the structures of compounds **2** and **6**, both secured by X-ray analysis, display the same relative stereochemistry at all chiral centers with the exception of C-30. This apparent anomaly can be justified if **6** is considered to be derived biogenetically from **2** by a nucleophilic attack of a hydroxyl group at C-27, with opening of the epoxide ring. This could be followed by a second nucleophilic attack at C-30 by the oxygen linked to C-26 with subsequent inversion of stereochemistry at C-30 and opening of the C-30/C-31 epoxide. This mechanism could lead to a partial structure between C-26 and C-31 similar to that possessed by methyl sartortuate[3,17] and easily could give the exomethylene group at C-27 by elimination of H_2O.

Ximaolide B (**3**), a colorless oil, was revealed to have the molecular formula $C_{41}H_{63}O_8Cl$ by HRESIMS (*m*/*z* 741.4100 [M + Na]$^+$, calcd 741.4109). The presence of one chlorine atom in the molecule was further confirmed by the dominant sodiated pseudomolecular ion [M + Na]$^+$ at *m*/*z* 741 and 743 with intensities of 1/0.33 in the LRESIMS spectrum. Interpretation of ^1H and ^{13}C NMR spectra of **3**, in comparison with those of **2**, led to the identification of three partial structures: **a** (from C-1 to C-20), **b** (from C-21 to C-29), and **c** (from C-32 to C-36/37) (Figure 6), identical to the corresponding sections of **2**. In fact, **3** differs from **2** only in the substitution pattern at C-30 and C-31, bearing a chlorine atom and a hydroxyl group in place of the epoxide moiety. Because the ^{13}C NMR chemical shifts of C-30 and C-31 were almost the same (Table 2), the position of the substituents at these carbons was evaluated by analyzing the NMR data of the corresponding acetyl derivative **3a**. The ^1H NMR spectrum of **3a** showed an acetyl methyl singlet at δ 2.11 (3H, s) and a doublet (H-33) downfield shifted from δ 5.06 ppm in **3** to 6.00 ppm in **3a**. The ^{13}C chemical shift of C-33 was also downfield shifted from δ 66.0 ppm in **3** to 69.8 ppm in **3a**, while the NMR signals at C-30 were almost unaffected. These data suggest that a secondary hydroxyl group is not present at C-30, but rather that the chlorine atom is at this position. On the other hand, the hypothesis of a sterically hindered −OH at C-30 that does not undergo standard acetylation should also be taken into consideration. Comparison of ^{13}C NMR values of compound **3** with those of compound **6** seems to support this latter hypothesis. In fact, both C-32 and C-40 resonate in **3** at downfield-shifted values (δ 44.5 and 25.8, respectively) with respect to **6** (δ 40.8 and 23.2, respectively)

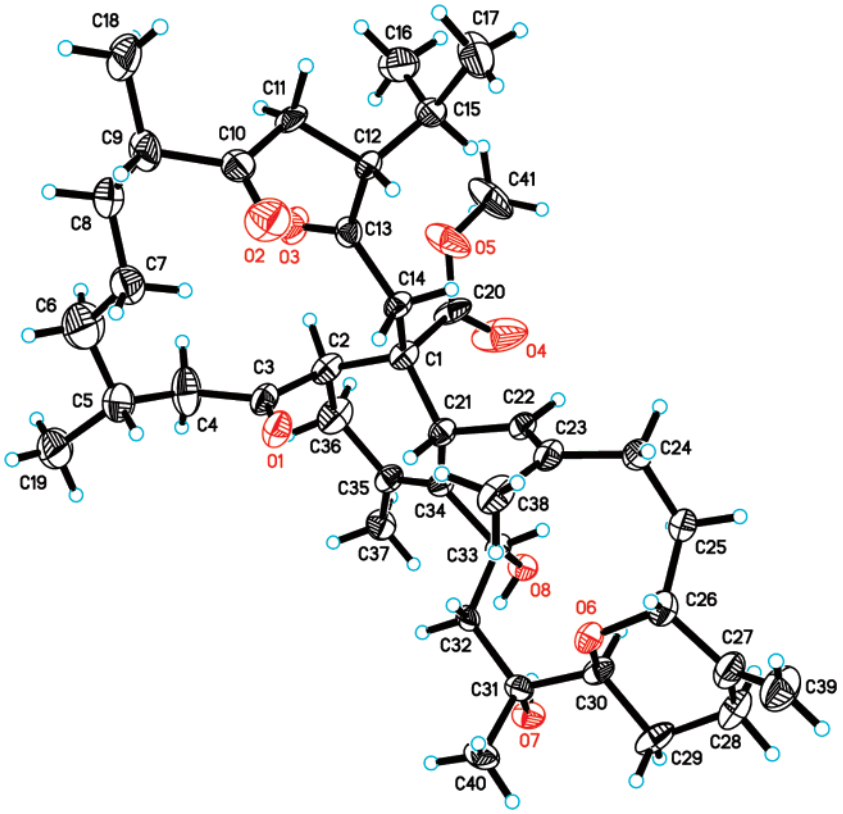


Figure 1. Perspective drawing of the X-ray structure of ximaolide E (**6**).

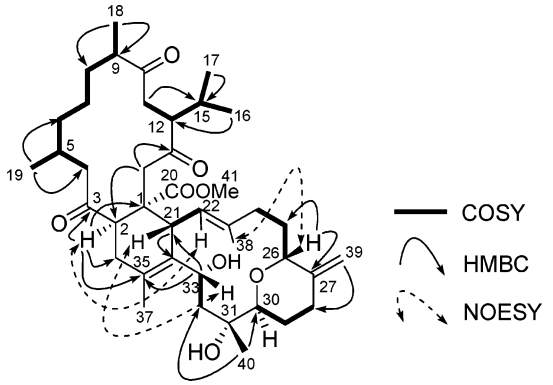
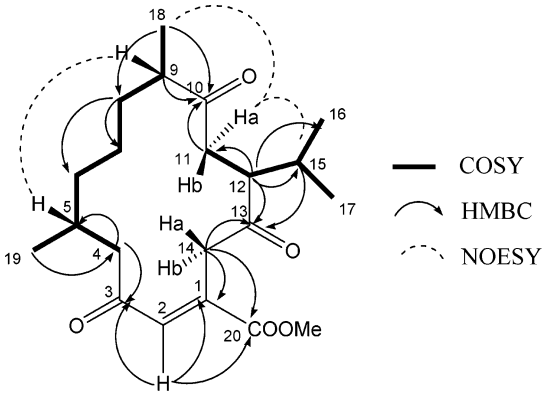


Figure 2. ^{1}H−^{1}H COSY, selected key HMBC, and NOESY correlations of **6**.



1

Figure 3. ^{1}H−^{1}H COSY, selected key HMBC, and NOESY correlations of **1**.

according to the presence of a chlorine atom in place of a hydroxyl group[15] at C-31. This leads us to propose the structure **3**.

The relative stereochemistry of all asymmetric centers of **3**, except for C-30 and C-31, was confirmed to be the same as those in **2** by analyzing 1D and 2D NMR data (Tables 1 and 2). The relative stereochemistry at C-30 and C-31 was tentatively suggested as depicted in **3** by taking into consideration that **3** could biogenetically derive from ximaolide A (**2**). In fact, the opening of the epoxide ring at C-30/C-31 in **2** by the nucleophilic attack of a chlorine ion at C-31 should lead to the chlorohydrin moiety in **3** exhibiting the same configuration at C-30 and opposite configuration at C-31 with respect to compound **2**. Ximaolide B (**3**) is only the second example of a biscembranoid possessing a chlorine atom in the molecule.[4]

Ximaolide C (**4**) was revealed to have the same molecular formula of $C_{41}H_{63}O_8Cl$ as **3** by HRESIMS. Like compound **3**, the dominant sodiated pseudomolecular ion $[M + Na]^+$ at *m*/*z* 741 and 743 with intensities of 1/0.33 in the LRESIMS spectrum supported the presence of a chlorine atom in the molecule. Comparison of its spectroscopic data with those of **3** revealed a close structural similarity between them, consistent with the presence of the same upper-portion (C-1 to C-20) partial structure (**a**) and the same segments **d** (from C-21 to C-25) and **c** (from C-32 to C-36/37) (Figure 6). In addition, the NMR spectra exhibited signals attributable to the partial structure **e** (from C-26 to C-31/40) (Figure 6). For unit **e**, the double-doublet signal at δ 3.18 (H-26) was coupled with H_2-25 (δ 2.01, H-25a; 1.29, H-25b). On the other hand, H-30 (δ 4.27) exhibited clear correlations with the adjacent methylene protons (δ 1.95, H-29a; 1.57, H-29b), which in turn were connected to H_2-28 (δ 2.41, 1.66). Finally, two methyl substituents were linked at C-27 and C-31. Consequently, one hydroxyl and one chlorine atom, bearing also an unassigned quaternary carbon (δ 75.0), had to be connected at C-26 and C-31, respectively. A series of significant ^{1}H−^{13}C long-range correlations of H_3-39/C-26, H_3-40/C-30, C-31, and H-30/C-31, C-40 confirmed the assignments for partial structure **e** according to structure **4** for ximaolide C. Once again, the nearly identical ^{13}C NMR values for C-26 (δ 73.7) and C-31 (δ 75.0) prevented unambiguous assignment for the position

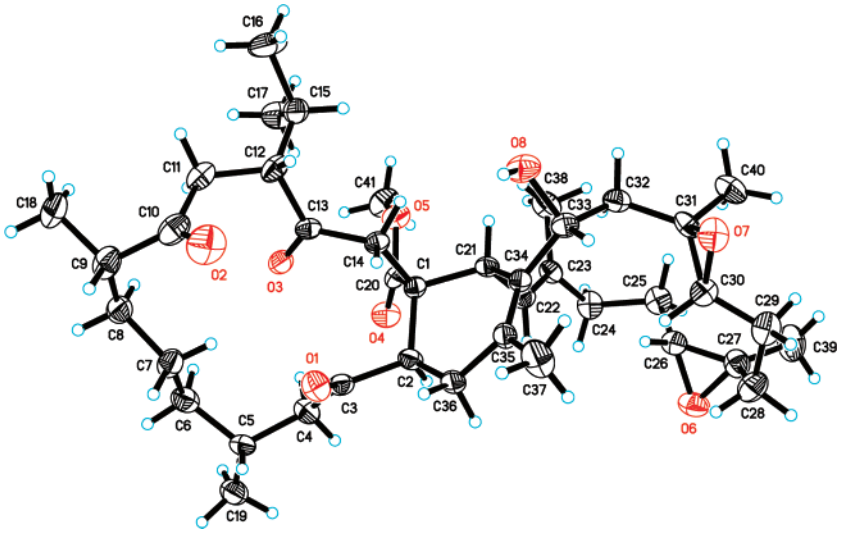


Figure 4. Perspective drawing of the X-ray structure of ximaolide A (**2**).

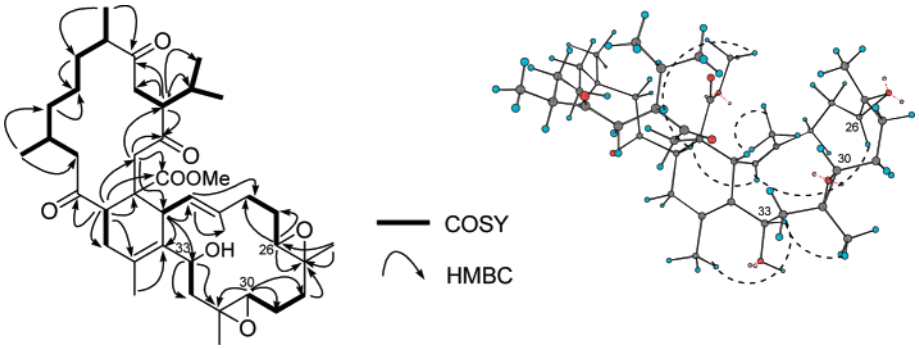


Figure 5. $^{1}H-^{1}H$ COSY, selected key HMBC, and NOESY correlations of **2**.

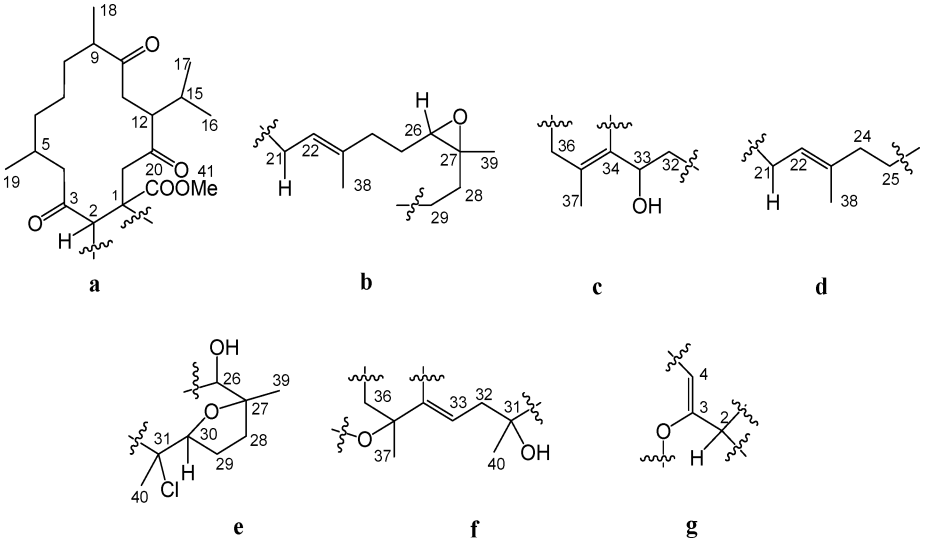


Figure 6. Partial structures **a** (**3**, **4**), **b** (**3**), **c** (**3**, **4**), **d** (**4**), **e** (**4**), **f** (**5**), and **g** (**5**).

of the chlorine atom. Analogously with **3**, compound **4** was treated with pyridine and acetic anhydride, yielding **4a**, which showed only two acetyl methyl singlets at δ 2.08 (3H, s) and 2.11 (3H, s). Careful analysis of the NMR data of **4** and **4a** revealed that the proton signals of H-26 and H-33 in **4a** were downfield shifted by about 1.6 and 1.1 ppm, respectively, with respect to those of **4**. On the basis of this consideration, the two secondary hydroxyl groups were fixed at C-26 and C-33, and consequently, the chlorine atom was placed at C-31. This assignment was further supported by the similar ^{13}C NMR values for C-32 and C-40 in compounds **3** and **4** (see Table 2). Comparison of carbon and proton chemical shifts of ximaolide C (**4**) with those of the co-occurring metabolites described above strongly supported the same relative stereochemistry for the upper-half portion as well as for C-33 (see Tables 1 and 2). The relative stereochemistry at C-26, C-27, C-30, and C-31 was suggested, analogously with **3**, by biogenetic correlation of **4** with compound **2**, from which **4** could logically derive. A concerted mechanism can be hypothesized involving the nucleophilic attack of a chlorine ion at C-31, opening of the epoxide ring (C-30/C-31), formation of the ether bridge from C-30 to C-27, and consequent opening of the second epoxide ring (C-26/C-27) with formation of the carbinol function at C-26. This implies retention

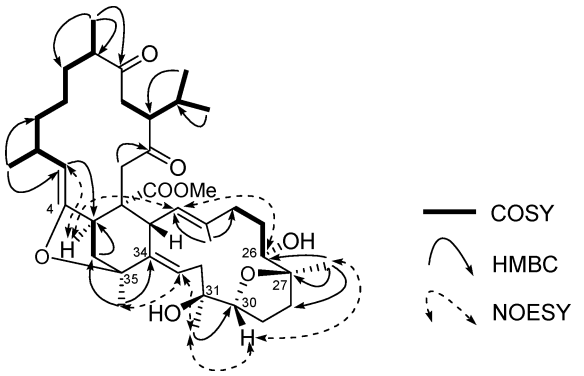


Figure 7. ^{1}H−^{1}H COSY, selected key HMBC, and NOESY correlations of **5**.

of configuration at both C-26 and C-30 and inversion at both C-27 and C-31 in compound **4** with respect to ximaolide A (**2**). Diagnostic NOE effects were observed between H-30 and H_3-39, supporting this proposed stereochemistry. This is the first report of a biscembranoid containing a tetrahydrofuran ring between C-27 and C-30.

Ximaolide D (**5**), with a molecular formula of $C_{41}H_{62}O_8$, showed ^{1}H and ^{13}C NMR data similar to those of **4**, consistent with the presence of segments C-5 to C-20 and C-21 to C-30 as in **4**. Moreover, careful NMR comparison of compounds **4** and **5** (Tables 1 and 2) revealed the main differences from C-31 to C-36/37 (partial structure **f**) and from C-2 to C-4 (partial structure **g**) (Figure 6). In particular, in the ^{13}C NMR spectrum of **5**, the lack of both carbonyl (δ 213.4) and tetrasubstituted double-bond signals (C-34/C-35) along with the presence of two additional trisubstituted double bonds [δ 148.9 (q C, C-3), 107.7 (CH, C-4); 140.8 (q C, C-34), 119.9 (CH, C-33)] and an oxygen-bearing quaternary carbon (δ 76.9, C-35) strongly suggested an ether bridge linkage between C-3 and C-35. The assignments for the partial structures **f** and **g** were further confirmed by a series of HMBC correlations as reported in Figure 7. On this basis, structure **5** was assigned to ximaolide D. The 3*Z*, 22*E*, and 33*E* configurations of the olefinic bonds were determined by the ^{13}C NMR chemical shifts of the vinyl methyl groups and NOEs observed by the NOESY spectrum as shown in Figure 7.

The relative configurations of the chiral centers in **5** were assumed to be the same as in the co-occurring related metabolites. In particular, compound **5** exhibited the same C/D ring arrangement as ximaolide C (**4**). The *cis*-stereochemistry of the A/B ring junction and the ether linkage between C-3 and C-35 require that the C-37 methyl is on the same side as H-2 and the −COOMe. Ximaolide D (**5**) is the first example of a biscembranoid possessing a tetrahydrofuran ring between the A and B rings.

Biscembranoids containing tetrahydrofuran rings and chlorine atoms are very rare; the tetrahydrofuran rings between C-3 to C-35 in **5** and C-27 to C-30 in **4** and **5** are reported here for the first time from nature. Ximaolides A−E are further examples of a complex array of rare marine biscembranoids, which is rapidly expanding. The carbon skeleton of compounds **2**−**6** is the same as that of methyl tortuoate A (**7**)[7] and other dimers.[2−6,8] It is interesting to note that methyl tortuosoate (**1**) corresponds to the upper half of the biscembranoids **2**−**6**. The co-occurrence of **1** with **2**−**6** suggests this compound may play a key role in the biogenesis of the ximaolides. This group of bis-diterpenoids are likely derived by Diels−Alder reaction of two cembranes (e.g., **1** and **10** should lead to compound **2**), although the corresponding "monomeric" cembranes of dimers **2**−**6** have not been found yet. An isomer of **10**, epimeric at C-14, was reported in a previous paper by Bowden's group.[5]

It is interesting to note that all biscembranoids found in *S. glaucum* and *S. tortuosum* possess as their monomeric dienophilic unit either methyl sarcoate (**8**), which has been also reported from a specimen of this soft coral,[16] or methyl tortuosoate (**1**), which has been isolated for the first time in this work.

Further studies should be conducted to experimentally prove the true biogenetic origins of these complex cembranoid dimers. Additional studies are needed to understand the effective biological role that the ximaolides and related biscembranoids play in the life cycle and ecology of these soft corals. Finally, total syntheses of these structures are needed to confirm their unique structural features.

While the crude extract exhibited cytotoxic activity against murine leukemia P388 cells, compounds **1**−**6** were inactive against both A-549 and P-388 tumor cell lines at a concentration of 20 μg/mL. This contrasts with the reported cytotoxicity of many other cembranoids. We also tested these new metabolites for antifungal activity against the fungus *Cladosporium cucumerinum*, but the results were negative.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. UV spectra were recorded on a Varian Cary 300 Bio spectrophotometer. IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. NMR spectra were measured on either a Bruker DRX-500 or a Bruker DRX-400 spectrometer with the residual $CHCl_3$ (δ_H 7.26 ppm, δ_C 77.0 ppm) as an internal standard. ESIMS and HRESIMS spectra were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer. EIMS and HREIMS data were obtained on a Finnigan-MAT-95 mass spectrometer. Reversed-phase HPLC (Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semipreparative ODS-HG-5 [5 μm, 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 CC, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC. X-ray diffraction studies were carried out on a Bruker SMART APEX CCD diffractometer.

Biological Material. The specimens of *Sarcophyton tortuosum*, identified by Prof. R.-L. Zhou of South China Sea Institute of Oceanology, Chinese Academy of Sciences, were collected off the coast of Ximao Island, 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. *02*LS163).

Extraction and Isolation. The frozen animals (257 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 (5.3 g), which was fractionated by Si gel CC eluting with a step gradient (0−100% acetone in light petroleum ether) yielding three fractions showing interesting blue TLC spots after spraying with H_2SO_4 [R_f 0.4, 0.35, and 0.55 (petroleum ether/acetone, 2:1)]. These three fractions were further purified, respectively, by Sephadex LH-20 [PE/$CHCl_3$/MeOH (2:1:1)] followed by Si gel CC, yielding four pure compounds, **1** (10.2 mg), **2** (3.1 mg), **4** (2.7 mg), and **5** (3.6 mg), and a mixture that was further purified by RP-HPLC [semipreparative ODS-HG-5 (5 μm, 250 × 10 mm), MeCN/H_2O (75:25), 2.0 mL/min] into two additional pure compounds, **3** (2.2 mg) and **6** (10.5 mg).

Methyl tortuosoate (1): colorless oil; $[\alpha]_D^{20}$ +163.2 (*c* 0.75, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 265 (3.13), 220 (2.62) nm; IR (KBr) ν_{max} 2958, 1710, 1705, 1635, 1267, 1199 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); EIMS *m*/*z* 364 $[M]^+$ (100), 346 (8), 332 (45), 318 (16), 304 (36), 289 (17), 275 (8), 265 (21), 261 (18), 247 (16), 235 (50), 207 (37), 165 (37), 139 (20), 127 (16), 109 (12), 97 (21), 81 (11), 69 (29), 55 (42); HREIMS *m*/*z* 364.2246 ($C_{21}H_{32}O_5^+$, calcd 364.2250).

Ximaolide A (2): needles (petroleum ether/acetone); mp 200−202 °C, $[\alpha]_D^{20}$ +104.9 (*c* 1.33, $CHCl_3$); IR (KBr) ν_{max} 3466, 1741, 1707, 1196, 1068 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); EIMS *m*/*z* 682 $[M]^+$ (18), 664 (47), 632 (26), 605 (22), 587 (33), 444 (23), 401 (19), 384 (43), 351 (31), 281 (71), 237 (54), 211 (36), 183 (43), 151 (51), 125 (63), 97 (80), 81 (56), 69 (100), 55 (77); HREIMS *m*/*z* 682.4468 ($C_{41}H_{62}O_8^+$, calcd 682.4445).

X-ray Crystal Data for 2.[18] Crystal data were as follows: colorless crystal, $C_{41}H_{62}O_8$, fw 682.91, triclinic, crystal size 0.503 × 0.492 ×

0.330 mm, space group *P*1, $a = 9.464(3)$ Å, $b = 9.651(3)$ Å, $c = 13.054(4)$ Å, $V = 1078.1(5)$ Å^3, $Z = 1$, $D_{calcd} = 1.052$ g cm^{-3}, $F_{000} = 372$, 5729 collected reflections, 4939 unique reflections ($R_{int} = 0.1505$), final R1 = 0.0928 (wR2 = 0.2317) for 3485 reflections with $I > 2\sigma(I)$, R1 = 0.1099, wR2 = 0.2448 for all unique data. The X-ray measurements were made on a Bruker SMART APEX CCD X-ray diffractometer with graphite-monochromated Mo Kα (λ 0.71073 Å) radiation at 293(2) K. The structure was solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-97). The non-hydrogen atoms were refined anisotropically. All H atoms were located in a difference Fourier map, but they were introduced in calculated positions and treated as riding on their parent atoms [C−H = 0.93−0.97 Å, O−H = 0.82 Å, and $U_{iso}(H) = 1.2U_{eq}(C)$ and $1.51U_{eq}$-(C, O)].

Ximaolide B (3): colorless oil: $[\alpha]_D^{20}$ +110.7 (*c* 0.48, $CHCl_3$); IR (KBr) ν_{max} 3473, 2926, 1741, 1707, 1205, 1068 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); ESIMS *m*/*z* 741.4 $[M + Na]^+$; HRESIMS *m*/*z* 741.4100 ($C_{41}H_{63}O_8NaCl^+$, calcd 741.4109).

Acetylation of Ximaolide B (3). Compound **3** (2.0 mg) was dissolved in dry pyridine (0.5 mL) and treated with acetic anhydride (0.5 mL) overnight at RT. Standard workup followed by Si gel CC in petroleum ether/acetone (12:1) gave the acetate **3a** (2.2 mg) as a colorless oil: ^{1}H NMR($CDCl_3$, 500 MHz) δ 6.00 (1H, d, $J = 7.7$ Hz, H-33), 5.11 (1H, d, $J = 10.6$ Hz, H-22), 3.55 (1H, m, H-2), 3.54 (3H, s, H-41), 3.52 (1H, d, $J = 8.2$ Hz, H-30), 3.19 (1H, d, $J = 10.6$ Hz, H-21), 3.15 (1H, dd, $J = 20.4$, 10.5 Hz, H-4a), 3.00 (1H, m, H-12), 2.96 (1H, m, H-11a), 2.95 (1H, d, $J = 20.0$ Hz, H-14a), 2.60 (1H, m, H-9), 2.59 (1H, m, H-26), 2.42 (1H, d, $J = 20.0$ Hz, H-14b), 2.41 (1H, m, H-36a), 2.35 (1H, m, H-4b), 2.29 (2H, m, H-24), 2.26 (1H, m, H-15), 2.13 (1H, m, H-36b), 2.11 (3H, s, H_{33}-OAc), 1.95 (1H, m, H-11b), 1.91 (2H, m, H-28), 1.86 (1H, m, H-25a), 1.81 (3H, s, H-37), 1.78 (1H, m, H-5), 1.77 (1H, m, H-32a), 1.65 (3H, s, H-38), 1.63 (1H, m, H-32b), 1.57 (2H, m, H-29), 1.52 (1H, m, H-25b), 1.51 (2H, m, H-8), 1.29 (3H, s, H-40), 1.27 (3H, s, H-39), 1.12 (2H, m, H-7), 1.10 (3H, d, $J = 7.0$ Hz, H-18), 1.02 (2H, m, H-6), 1.00 (3H, d, $J = 7.0$ Hz, H-17), 0.85 (3H, d, $J = 7.0$ Hz, H-19), 0.71 (3H, d, $J = 6.7$ Hz, H-16); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 214.0 (C, C-3), 213.6 (C, C-10), 208.7 (C, C-13), 174.5 (C, C-20), 169.4 (C, C_{33}-OAc), 135.7 (C, C-23), 131.1 (C, C-34), 127.9 (C, C-35), 127.7 (CH, C-22), 74.2 (C, C-31), 73.2 (CH, C-30), 69.8 (CH, C-33), 59.6 (C, C-27), 58.7 (CH, C-26), 53.9 (CH_2, C-4), 52.2 (CH, C-12), 51.3 (CH_3, C-41), 50.0 (C, C-1), 46.5 (CH, C-9), 45.7 (CH_2, C-14), 43.7 (CH, C-21), 43.4 (CH, C-2), 42.9 (CH_2, C-32), 37.1 (CH_2, C-24), 36.7 (CH_2, C-6), 33.4 (CH_2, C-8), 33.0 (CH_2, C-36), 32.1 (CH_2, C-11), 31.7 (CH_2, C-28), 28.8 (CH, C-15), 27.3 (CH_2, C-29), 27.2 (CH, C-5), 25.0 (CH_2, C-7), 25.0 (CH_3, C-40), 24.8 (CH_2, C-25), 21.7 (CH_3, C-19), 21.3 (CH_3, C-17), 21.3 (CH_3, C_{33}-OAc), 18.9 (CH_3, C-39), 18.4 (CH_3, C-37), 17.8 (CH_3, C-16), 17.7 (CH_3, C-18), 15.2 (CH_3, C-38).

Ximaolide C (4): colorless oil; $[\alpha]_D^{20}$ +85.5 (*c* 0.48, $CHCl_3$); IR (KBr) ν_{max} 3473, 2928, 1741, 1707, 1211, 1063 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); ESIMS *m*/*z* 741.4 $[M + Na]^+$; HRESIMS *m*/*z* 741.4110 ($C_{41}H_{63}O_8NaCl^+$, calcd 741.4109).

Acetylation of Ximaolide C (4). Compound **4** (2.0 mg) was acetylated with acetic anhydride and pyridine (1:1, each 0.5 mL) and after usual workup furnished **4a** (2.1 mg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.14 (1H, d, $J = 10.5$ Hz, H-33), 5.02 (1H, d, $J = 10.6$ Hz, H-22), 4.71 (1H, d, $J = 9.2$ Hz, H-26), 4.24 (1H, dd, $J = 9.8$, 6.0 Hz, H-30), 3.50 (3H, s, H-41), 3.49 (1H, m, H-2), 3.28 (1H, d, $J = 10.5$ Hz, H-21), 3.20 (1H, m, H-4a), 3.02 (1H, m, H-12), 3.00 (1H, d, $J = 18.7$ Hz, H-14a), 2.85 (1H, m, H-11a), 2.58 (1H, m, H-9), 2.53 (1H, m, H-32a), 2.48 (1H, m, H-36a), 2.43 (1H, d, $J = 18.7$ Hz, H-14b), 2.38 (1H, m, H-4b), 2.23 (1H, m, H-15), 2.11 (3H, s, H_{33}-OAc), 2.08 (3H, s, H_{26}-OAc), 2.06 (1H, m, H-36b), 2.05 (2H, m, H-24), 1.92 (1H, m, H-11b), 1.92 (1H, m, H-29a), 1.85 (2H, m, H-28), 1.82 (1H, m, H-5), 1.78 (1H, m, H-29b), 1.77 (3H, s, H-37), 1.75 (3H, s, H-38), 1.69 (1H, m, H-32b), 1.48 (2H, m, H-8), 1.54 (2H, m, H-25), 1.31 (3H, s, H-40), 1.28 (1H, m, H-7a), 1.24 (3H, s, H-39), 1.13 (1H, m, H-7b), 1.10 (3H, d, $J = 6.8$ Hz, H-18), 1.09 (2H, m, H-6), 1.00 (3H, d, $J = 6.8$ Hz, H-17), 0.85 (3H, d, $J = 7.0$ Hz, H-19), 0.73 (3H, d, $J = 6.8$ Hz, H-16); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.7 (C, C-10), 213.6 (C, C-3), 208.7 (C, C-13), 174.6 (C, C-20), 171.1 (C, C_{27}−OAc), 169.9 (C, C_{33}−OAc), 137.1 (C, C-23), 128.7 (C, C-34), 128.6 (C, C-35), 126.9 (CH, C-22), 90.3 (CH, C-30), 84.4 (C, C-27), 74.4 (CH, C-26), 71.3 (C, C-31), 68.5 (CH, C-33), 53.8 (CH_2, C-4), 52.1 (CH, C-12), 51.1 (CH_3, C-41), 49.6 (C, C-1), 46.8 (CH, C-9), 45.7 (CH_2, C-14), 44.4 (CH, C-21), 43.4 (CH, C-2), 41.5 (CH_2, C-32), 36.9 (CH_2, C-6), 36.1 (CH_2, C-24), 35.8 (CH_2, C-28), 33.6 (CH_2, C-8), 32.8 (CH_2, C-36), 32.3 (CH_2, C-11), 29.0 (CH, C-15), 27.7 (CH_2, C-29), 27.3 (CH, C-5), 26.5 (CH_2, C-25), 25.3 (CH_3, C-40), 25.1 (CH_2, C-7), 22.0 (CH_3, C-19), 21.4 (CH_3, C-39), 21.4 (CH_3, C_{33}−OAc), 21.2 (CH_3, C-17), 21.2 (CH_3, C_{27}-OAc), 18.3 (CH_3, C-37), 17.9 (CH_3, C-16), 17.6 (CH_3, C-18), 16.2 (CH_3, C-38).

Ximaolide D (5): colorless oil; $[\alpha]_D^{20}$ +4.1 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3448, 2929, 1745, 1709, 1221, 1061 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); ESIMS *m*/*z* 683.4 $[M + H]^+$; HRESIMS *m*/*z* 705.4375 ($C_{41}H_{62}O_8Na^+$, calcd 705.4342).

Acetylation of Ximaolide D (5). In a similar manner to the acetylation of compounds **3** and **4**, compound **5a** (3.0 mg) was prepared from **5** (3.0 mg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.32 (1H, d, $J = 9.5$ Hz, H-22), 5.32 (1H, d, $J = 9.5$ Hz, H-33), 4.80 (1H, d, $J = 9.1$ Hz, H-26), 3.82 (1H, dd, $J = 11.1$, 4.5 Hz, H-30), 3.73 (1H, d, $J = 10.0$ Hz, H-4), 3.69 (3H, s, H-41), 2.99 (1H, d, $J = 18.6$ Hz, H-14a), 2.95 (1H, d, $J = 9.5$ Hz, H-21), 2.87 (1H, m, H-28a), 2.70 (1H, d, $J = 18.6$ Hz, H-14b), 2.70 (1H, m, H-28b), 2.68 (1H, m, H-36a), 2.67 (1H, m, H-32a), 2.53 (1H, m, H-5), 2.41 (1H, m, H-9), 2.20 (1H, m, H-12), 1.99 (3H, s, H_{26}-OAc), 1.97 (1H, m, H-2), 1.92 (1H, m, H-24a), 1.83 (1H, m, H-32b), 1.77 (1H, m, H-11a), 1.77 (1H, m, H-24b), 1.72 (1H, m, H-15), 1.66 (2H, m, H-29), 1.64 (1H, m, H-8a), 1.63 (3H, s, H-38), 1.55 (1H, m, H-6a), 1.49 (1H, m, H-11b), 1.47 (2H, m, H-25), 1.43 (1H, m, H-8b), 1.40 (1H, m, H-36b), 1.36 (3H, s, H-37), 1.25 (1H, m, H-7a), 1.24 (3H, s, H-40), 1.18 (3H, d, $J = 7.0$ Hz, H-18), 1.17 (3H, s, H-39), 1.14 (1H, m, H-7b), 0.93 (3H, d, $J = 6.8$ Hz, H-17), 0.88 (3H, d, $J = 6.8$ Hz, H-19), 0.85 (3H, d, $J = 6.8$ Hz, H-16), 0.83 (1H, m, H-6b); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.6 (C, C-10), 208.7 (C, C-13), 175.8 (C, C-20), 170.4 (C, C_{27}−OAc), 149.0 (C, C-3), 140.8 (C, C-34), 132.8 (C, C-23), 127.9 (C, C-35), 126.0 (CH, C-22), 119.5 (CH, C-33), 107.3 (CH, C-4), 87.6 (CH, C-30), 82.7 (C, C-27), 76.5 (C, C-35), 73.9 (C, C-31), 73.5 (CH, C-26), 54.5 (CH, C-12), 52.1 (CH_3, C-41), 49.4 (CH, C-9), 48.8 (CH_2, C-14), 48.4 (CH, C-2), 44.3 (C, C-1), 43.0 (CH_2, C-36), 39.0 (CH_2, C-6), 37.1 (CH, C-21), 36.5 (CH_2, C-28), 36.5 (CH_2, C-11), 36.0 (CH_2, C-8), 35.0 (CH_2, C-24), 33.6 (CH_2, C-32), 30.6 (CH, C-15), 28.9 (CH, C-5), 26.7 (CH_2, C-7), 26.5 (CH_2, C-25), 26.3 (CH_2, C-29), 22.7 (CH_3, C-40), 22.5 (CH_3, C-37), 21.9 (CH_3, C-19), 21.1 (CH_3, C-17), 21.1 (CH_3, C-39), 21.0 (CH_3, C_{33}-OAc), 20.5 (CH_3, C-16), 18.3 (CH_3, C-18), 16.2 (CH_3, C-38).

Ximaolide E (6): colorless needles (petroleum ether/acetone); mp 205−207 °C; $[\alpha]_D^{20}$ +38.4 (*c* 0.31, $CHCl_3$); IR (KBr) ν_{max} 3419, 1743, 1711, 1203, 1063 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); EIMS *m*/*z* 682 $[M]^+$ (18), 664 (23), 646 (27), 632 (16), 605 (18), 587 (43), 669 (28), 419 (15), 384 (18), 365 (27), 333 (41), 281 (78), 237 (45), 211 (34), 183 (51), 151 (46), 121 (40), 97 (72), 69 (100), 55 (93); HREIMS *m*/*z* 682.4432 ($C_{41}H_{62}O_8^+$, calcd 682.4445).

X-ray Crystal Data for 6.[17] Crystal data were as follows: colorless crystal, $C_{41}H_{62}O_8$, fw 682.91, monoclinic, crystal size 0.516 × 0.209 × 0.056 mm, space group *P*2 (1), $a = 12.1062(15)$ Å, $b = 9.9760(13)$ Å, $c = 16.148(2)$ Å, $V = 1950.0(4)$ Å^3, $Z = 2$, $D_{calcd} = 1.163$ g cm^{-3}, $F_{000} = 744$, 11 135 collected reflections, 7342 unique reflections ($R_{int} = 0.1189$), final R1 = 0.0808 (wR2 = 0.1953) for 4285 reflections with $I > 2\sigma(I)$, R1 = 0.1178, wR2 = 0.2296 for all unique data. The X-ray measurements were made on a Bruker SMART APEX CCD X-ray diffractometer with graphite-monochromated Mo Kα (λ 0.71073 Å) radiation at 293(2) K. The structure was solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-97). The non-hydrogen atoms were refined anisotropically. All H atoms were located in a difference Fourier map, but they were introduced in calculated positions and treated as riding on their parent atoms [C−H = 0.93−0.97 Å, O−H = 0.82 Å, and $U_{iso}(H) = 1.2U_{eq}(C)$ and $1.51U_{eq}$-(C, O)].

Preparation of (*S*)-and (*R*)-MTPA Esters. MTPA derivatives were obtained by treating **6** with (*R*)- and (*S*)-MTPA-Cl in dry pyridine for ca. 16 h under stirring at RT. The reaction mixtures were purified by Si gel CC (petroleum ether/Et_2O in gradient) to afford the two derivatives. Selected ^{1}H NMR ($CDCl_3$, 400 MHz) of (*S*)-ester: δ 7.50 (2H, m), 7.38 (3H, m), 5.37 (1H, d, $J = 9.0$ Hz, H-33), 4.81 (1H, d, $J = 10.5$ Hz, H-22), 4.68 (1H, br s, H-39b), 4.63 (1H, br s, H-39a), 4.24 (1H, d, $J = 10.4$ Hz, H-26), 3.58 (3H, s, H_3-41), 3.35 (1H, dd, $J = 11.6$, 2.5 Hz, H-30), 3.35 (1H, d, $J = 10.5$ Hz, H-21), 1.85 (3H,

s, H_3-37), 1.80 (3H, s, H_3-38), 1.51 (3H, s, H_3-40), 1.09 (3H, d, J = 7.0 Hz, H_3-18), 1.00 (3H, d, J = 6.7 Hz, H_3-17), 0.86 (3H, d, J = 6.9 Hz, H_3-19), 0.70 (3H, d, J = 6.8 Hz, H_3-16); LRESIMS *m/z* = 898. Selected ^{1}H NMR ($CDCl_3$, 400 MHz) of (*R*)-ester: δ 7.50 (2H, m), 7.38 (3H, m), 5.37 (1H, d, J = 9.0 Hz, H-33), 4.81 (1H, d, J = 10.5 Hz, H-22), 4.68 (1H, br s, H-39b), 4.63 (1H, br s, H-39a), 4.24 (1H, d, J = 10.4 Hz, H-26), 3.58 (3H, s, H_3-41), 3.35 (1H, dd, J = 11.6, 2.5 Hz, H-30), 3.35 (1H, d, J = 10.5 Hz, H-21), 1.85 (3H, s, H_3-37), 1.80 (3H, s, H_3-38), 1.51 (3H, s, H_3-40), 1.09 (3H, d, J = 7.0 Hz, H_3-18), 1.00 (3H, d, J = 6.7 Hz, H_3-17), 0.86 (3H, d, J = 6.9 Hz, H_3-19), 0.70 (3H, d, J = 6.8 Hz, H_3-16); LRESIMS *m/z* = 898.

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(18) Crystallographic data for structures **2** and **6** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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