Marine Natural Products. XXIII.[1] Three New Cytotoxic Dimeric Macrolides, Swinholides B and C and Isoswinholide A, Congeners of Swinholide A, from the Okinawan Marine Sponge *Theonella swinhoei*

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Following the characterization of swinholide A (1), the major cytotoxic dimeric macrolide, three new congeneric dimeric macrolides, named swinholide B (2), swinholide C (3), and isoswinholide A (10), have been isolated from the Okinawan marine sponge *Theonella swinhoei*. The structures of these dimeric macrolides have been elucidated on the basis of chemical and physicochemical evidence. These dimeric macrolides were shown to exhibit potent cytotoxicities toward KB cell lines.

Keywords swinholide A; swinholide B; swinholide C; isoswinholide A; marine sponge; *Theonella swinhoei*; dimeric macrolide; cytotoxicity

During the course of our investigations in search of new biologically active substances from marine organisms,[2] we isolated a potent cytotoxic dimeric macrolide named swinholide A (**1**) from the Okinawan marine sponge *Theonella swinhoei*. On the basis of the X-ray crystallographic analysis and chemical derivations, we elucidated the absolute stereostructure of **1**, which contains a 44-membered dilactone moiety.[1,3,4] Further investigation on the ethyl acetate-soluble portion of the acetone extract of this marine sponge has led us to the isolation of three new related dimeric macrolides. This paper deals with the structural elucidation of these congeneric macrolides.

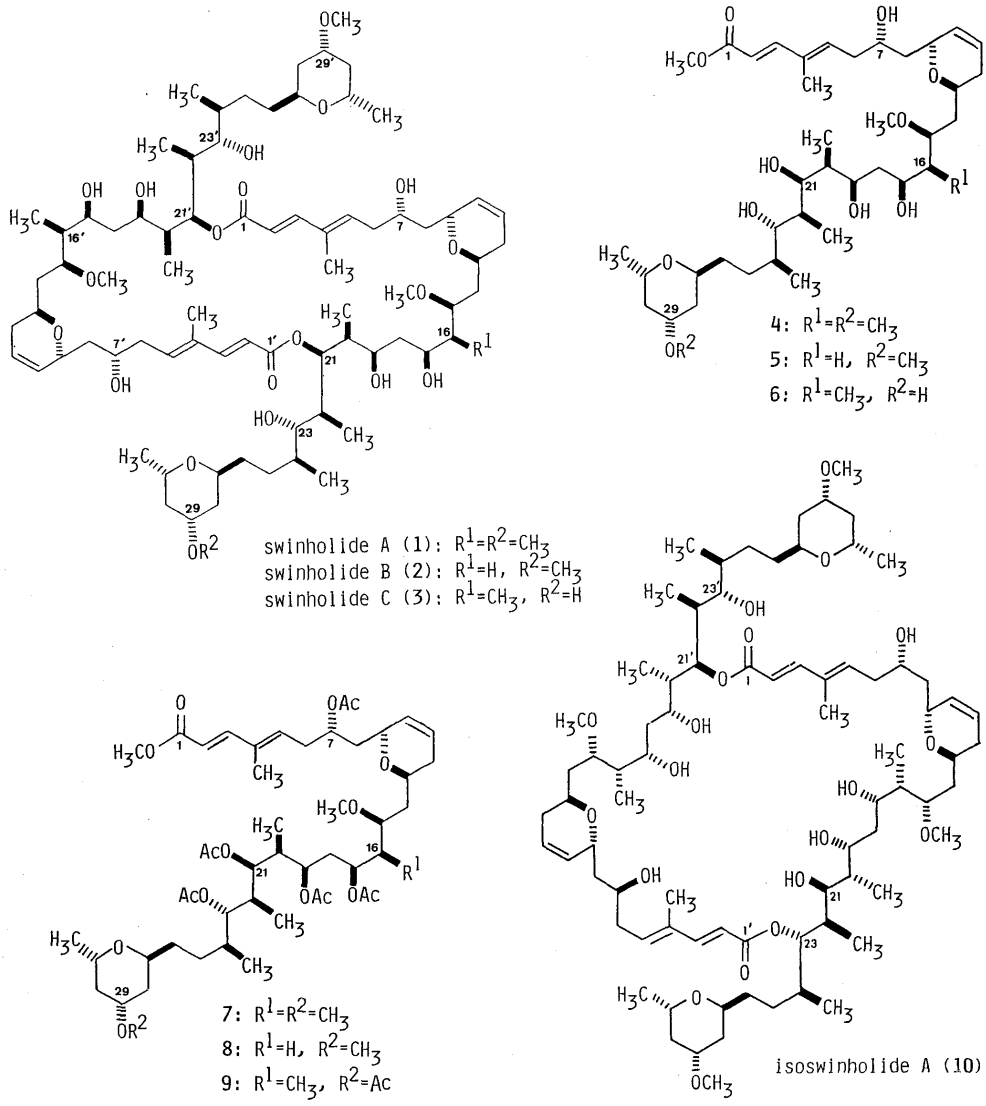

Chart 1

The marine sponge, collected in April at Kuro Island, Okinawa Prefecture, was extracted with acetone.[1] Silica gel column chromatography of the ethyl acetate-soluble portion of the acetone extract gave fractions containing swinholide A (**1**) and minor congeneric constituents. They were further subjected to reversed-phase high-performance liquid chromatography (HPLC) developed with MeOH–H_2O (10 : 1) to provide three new dimeric macrolides, named (in the order of elution) swinholide C (**3**, 0.017% from the ethyl acetate-soluble portion), swinholide B (**2**, 0.044%), and isoswinholide A (**10**, 0.0098%), together with the major macrodiolide swinholide A (**1**, 1.12%).

By means of positive and negative fast-atom bombardment mass spectroscopy (FABMS), which showed the largest ion peaks at *m/z* 1375 $(M+H)^+$ and *m/z* 1374 $(M)^-$, and by elemental analysis, the molecular formula of the second major dimeric macrolide swinholide B (**2**) was shown to be $C_{77}H_{130}O_{20}$, which is 14 mass units (CH_2) less than that of swinholide A (**1**). Although the infrared (IR) and ultraviolet (UV) spectra of **2** resembled those of **1**, the proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **2** were considerably complicated, which suggested a dimeric structure of **2** comprising two nonidentical units. However, the ^{1}H-NMR spectrum of **2** showed only nine methyl doublets [δ 1.21 (6H), 1.20, 0.99 (6H), 0.93, 0.86, 0.83, 0.81], thus, swinholide B (**2**) was presumed to be a desmethyl analog of swinholide A (**1**).

In order to facilitate the structural elucidation of swinholide B (**2**), methanolysis of **2** was carried out to liberate two monomeric methyl esters, **4** [*m/z* 727 $(M+H)^+$] and **5** [*m/z* 713 $(M+H)^+$]. The former was found to be identical in terms of IR, UV, ^{1}H- and ^{13}C-NMR spectra, and optical rotation, with the methyl ester (**4**), which was

TABLE I. ^{1}H-NMR Data for Methyl Esters **4**, **5**, and **6** in C_6D_6 and Acetates **7**, **8**, and **9** in $CDCl_3$

	4	5	6	7	8	9
2	5.89 d	5.89 d	5.90 d	5.82 d	5.82 d	5.82 d
3	7.62 d	7.65 d	7.64 d	7.33 d	7.32 d	7.33 d
4Me	1.53 s	1.57 s	1.59 s	1.78 s	1.79 s	1.78 s
5	5.99 dd	6.08 dd	6.08 dd	5.93 dd	5.92 dd	5.93 dd
6	2.31 ddd	2.36 ddd	2.38 ddd	2.61 ddd	2.55 m	2.61 ddd
	2.22 ddd	2.25 ddd	2.27 ddd	2.55 ddd	2.55 m	2.55 ddd
7	4.12 br ddd	4.15 br ddd	4.15 br dd	5.22 m	5.19 m	5.22 m
8	1.65 m	1.71 m	1.67 m	1.78 m	1.78 m	1.78 m
	1.38 m	1.37 m	1.44 m	1.67 m	1.65 m	1.67 m
9	4.63 d	4.67 d	4.67 d	4.22 br d	4.22 br d	4.22 br d
10	5.52 d	5.53 d	5.56 d	5.63 br d	5.61 br d	5.62 br d
11	5.63 br d	5.64 br d	5.66 m	5.81 m	5.81 m	5.81 m
12	1.85 m	1.83 m	1.84 m	1.96 m	1.98 m	1.96 m
	1.73 m	1.76 m	1.78 m	1.96 m	1.98 m	1.96 m
13	3.69 m	3.70 m	3.70 m	3.54 m	3.65 m	3.54 m
14	2.08 ddd	2.10 ddd	2.10 m	1.85 m	1.86 m	1.85 m
	1.45 m	1.40 m	1.48 m	1.52 m	1.49 m	1.52 m
15	3.91 br ddd	3.87 m	3.90 br dd	3.43 m	3.36 m	3.43 m
15OMe	3.24 s	3.30 s	3.30 s	3.26 s	3.27 s	3.28 s
16	1.70 m	1.56 m, 1.56 m	1.73 m	1.81 m	1.70 m, 1.70 m	1.81 m
16Me	0.83 d	—	0.86 d	0.89 d	—	0.89 d
17	3.97 m	4.27 m	4.02 m	4.95 m	5.12 m	4.95 m
18	1.82 m	1.75 m	1.81 m	1.91 m	1.94 m	1.91 m
	1.69 m	1.55 m	1.71 m	1.91 m	1.94 m	1.91 m
19	4.21 d	4.32 m	4.34 d	4.73 br dd	4.75 m	4.74 br dd
20	2.01 m	1.94 m	1.95 m	2.04 m	2.00 m	2.03 m
20Me	0.72 d	0.72 d	0.78 d	0.96 d	0.95 d	0.97 d
21	4.30 d	4.29 m	4.29 d	4.99 d	4.98 d	4.99 d
22	1.77 m	1.80 m	1.81 m	2.10 m	2.07 m	2.08 m
22Me	1.14 d[a]	1.12 d	1.15 d	0.92 d	0.93 d	0.92 d
23	3.39 m	3.40 dd	3.39 m	4.67 dd	4.67 dd	4.67 dd
24	1.84 m	1.83 m	1.83 m	1.93 m	1.93 m	1.91 m
24Me	0.86 d[a]	0.88 d	0.87 d	0.89 d	0.89 d	0.89 d
25	1.73 m	1.75 m	1.80 m	1.40 m	1.38 m	1.40 m
	1.40 m	1.42 m	1.30 m	1.15 m	1.17 m	1.15 m
26	1.75 m	1.78 m	1.80 m	1.84 m	1.84 m	1.85 m
	1.12 m	1.16 m	1.24 m	1.20 m	1.18 m	1.22 m
27	3.97 m	4.00 m	4.02 m	3.97 m	3.97 m	3.96 m
28	1.68 m	1.66 m	1.60 m	1.80 m	1.79 m	1.74 m
	1.65 m	1.57 m	1.60 m	1.58 m	1.59 m	1.74 m
29	3.30 dddd	3.32 m	3.82 m	3.52 dddd	3.52 dddd	5.06 dddd
29OMe	3.11 s	3.12 s	—	3.34 s	3.34 s	—
30	1.74 m	1.75 m	1.75 m	1.97 m	1.95 m	1.96 m
	1.23 m	1.22 m	1.18 m	1.18 m	1.18 m	1.20 m
31	3.58 m	3.59 m	3.59 m	3.67 ddq	3.69 m	3.75 m
31Me	1.22 d	1.21 d	1.21 d	1.20 d	1.20 d	1.19 d
EsterMe	3.48 s	3.48 s	3.50 s	3.75 s	3.74 s	3.75 s

a) Assignments were reported incorrectly in a previous paper.[3]

TABLE II. ^{13}C-NMR Data for Methyl Esters **4**, **5**, and **6** in C_6D_6 and Acetates **7**, **8**, and **10** in $CDCl_3$

	4	5	6	7	8	9
1	168.1 s	168.1 s	168.1 s	167.9 s	167.8 s	167.9 s
2	115.9 d	115.6 d	115.7 d	116.1 d	116.1 d	116.1 d
3	150.3 d	150.3 d	150.2 d	149.3 d	149.2 d	149.3 d
4	134.7 s	134.4 s	134.5 s	135.3 s	135.4 s	135.3 s
4Me	12.6 q	12.4 q	12.4 q	12.4 q	12.4 q	12.4 q
5	139.4 d	139.5 d	139.3 d	135.9 d	135.7 d	135.9 d
6	38.1 t	38.1 t	37.9 t	33.7 t	33.9 t	33.7 t
7	67.4 d	67.1 d	67.2 d	70.1 d	70.1 d	70.1 d
8	40.8 t	40.6 t	40.8 t	37.4 t	37.5 t	37.5 t
9	68.9 d	69.0 d	68.5 d	69.2 d	69.2 d	69.2 d
10	130.7 d	130.6 d	130.6 d	129.3 d	129.2 d	129.3 d
11	124.0 d	123.9 d	123.7 d	124.4 d	124.5 d	124.5 d
12	31.1 t	31.3 t	30.9 t	31.0 t	31.0 t	31.0 t
13	65.3 d	64.7 d	65.2 d	64.7 d	64.3 d	64.6 d
14	35.4 t	38.8 t	35.2 t	36.5 t	40.1 t	36.6 t
15	78.3 d	74.9 d	78.0 d	77.4 d	74.4 d	77.3 d
15OMe	57.3 q	56.3 q	56.9 q	57.0 q	56.7 q	57.0 q
16	41.5 d	40.0 t	41.2 d	39.7 d	39.3 t	39.8 d
16Me	10.8 q	—	11.1 q	8.8 q	—	8.8 q
17	75.7 d	69.9 d	75.6 d	72.9 d	68.2 d	72.9 d
18	37.1 t	42.6 t	37.1 t	33.1 t	37.1 t	33.2 t
19	72.9 d	72.7 d	72.8 d	69.7 d	69.0 d	69.7 d
20	41.0 d	40.8 d	40.9 d	35.6 d	36.2 d	35.7 d
20Me	12.0 q	11.7 q	11.7 q	10.0 q	10.1 q	10.0 q
21	75.4 d	74.8 d	75.1 d	71.3 d	71.3 d	71.2 d
22	36.0 d	35.6 d	36.1 d	34.9 d	34.9 d	34.9 d
22Me	11.1 q[a]	10.9 q	10.6 q	10.1 q	10.1 q	10.1 q
23	80.7 d	80.4 d	81.0 d	78.3 d	78.3 d	78.3 d
24	35.6 d	35.8 d	35.4 d	33.8 d	33.8 d	33.8 d
24Me	17.0 q[a]	16.9 q	16.9 q	16.9 q	16.9 q	16.9 q
25	28.8 t	28.4 t	29.1 t	26.9 t	26.9 t	26.8 t
26	29.7 t	29.5 t	29.3 t	29.2 t	29.2 t	29.2 t
27	71.7 d	71.4 d	72.4 d	71.8 d	71.8 d	71.6 d
28	35.6 t	35.6 t	38.5 t	35.2 t	35.1 t	34.6 t
29	73.7 d	73.5 d	64.4 d	73.4 d	73.4 d	67.8 d
29OMe	55.3 q	55.0 q	—	55.4 q	55.4 q	—
30	38.9 t	38.7 t	43.0 t	38.6 t	38.6 t	38.5 t
31	65.2 d	65.1 d	65.0 d	64.8 d	64.8 d	64.7 d
31Me	22.1 q	21.9 q	22.1 q	21.7 q	21.9 q	21.9 q
EsterMe	51.4 q	51.2 q	51.2 q	51.5 q	51.6 q	51.6 q
Acetyl				170.9 s	170.9 s	170.9 s
(C=O)				170.6 s (2C)	170.7 s (2C)	170.6 s (2C)
				170.2 s	170.3 s	170.3 s
				170.1 s	170.2 s	170.2 s
						169.7 s

a) Assignments were reported incorrectly in a previous paper.[3]

obtained previously as a single product by methanolysis of swinholide A (**1**).[1,3] The ^{1}H-NMR spectrum of **5** showed four methyl doublets (δ 1.21, 1.12, 0.88, 0.72), while five methyl doublets (δ 1.22, 1.14, 0.86, 0.83, 0.72) were observed in the case of **4**. Acetylation of the methyl ester **5** with acetic anhydride and pyridine furnished the pentaacetate **8**, *m/z* 923 $(M+H)^+$; δ 2.09s, 2.02s, 2.01s, 1.99s, 1.97s. Homo and hetero correlation spectroscopy (COSY) studies of **5** and **8** revealed that the methyl ester **5** lacked a secondary methyl group at C_{16} of the methyl ester **4** (see Tables I and II). The close similarities observed in the ^{1}H- and ^{13}C-NMR spectra of **4** and **5** and in the circular dichroism (CD) spectra of **1** ($\Delta\varepsilon$ −5.2 at 280 nm) and **2** ($\Delta\varepsilon$ −5.3 at 270 nm) led us to presume that **5** has the same absolute stereostructure as **4**. Furthermore, the locations of two lactone linkages in swinholide B (**2**) have been shown to be at C_{21} and at $C_{21'}$ by detailed comparison of the ^{1}H-NMR data for **4**, **5** and **2** (Tables I and III). Thus, the signals assignable to 21-H [δ 4.30d for **4** and δ 4.29m for **5**] geminal to 21-OH in **4** and **5** were observed at higher field than those [δ 5.31d, 5.40d] for **2**. Consequently, the whole structure of swinholide B (**2**) has been elucidated as shown in Chart 1.[5]

The molecular formula of swinholide C (**3**) was determined to be $C_{77}H_{130}O_{20}$ by positive and negative FABMS, which gave ion peaks at *m/z* 1397 $(M+Na)^+$ and at *m/z* 1374 $(M)^-$, and by elemental analysis. Thus, swinholide C (**3**) was shown to have the same molecular formula as swinholide B (**2**), which was also 14 mass units (CH_2) less than that of swinholide A (**1**). In the ^{1}H- and ^{13}C-NMR spectra of **3** (Tables III and IV), most signals assignable to protons and carbons from C_1 to C_{21} and from $C_{1'}$ to $C_{21'}$ were observed at similar chemical shifts to those of **1**. However, some signals [(1H, m at δ 3.99 due to H_{29}), (1H, m, δ 3.54 for $H_{29'}$), (3H, d each at δ 1.19 and δ 1.20 due to $H_{31\text{-Me}}$ and $H_{31'\text{-Me}}$)] were observed at different chemical shifts because of the asymmetrical nature of **3**. Since one (δ 3.34s, δ_C 55.1q) of the two methoxy signals was observed with a half intensity as compared to the other one (δ 3.36s, δ_C 57.3q), the structural difference between **1** and **3** was presumed to be in the number of methoxy groups. In consequence, swinholide C (**3**) was shown to have one hydroxy group instead of the methoxy group at C_{29} in swinholide A (**1**).

As in the case of swinholide B (**2**), swinholide C (**3**) was subjected to methanolysis to facilitate the NMR analysis. Two monomeric methyl esters **4** [*m/z* 727 $(M+H)^+$] and **6** [*m/z* 713 $(M+H)^+$] were obtained, and the former was shown to be identical with **4** previously obtained by methanolysis of swinholide A (**1**) (IR, UV, ^{1}H- and ^{13}C-NMR spectra, FABMS, and optical rotation). The NMR spectra of the more polar methyl ester **6** showed signals due to only one methoxy residue at δ 3.30s (an ester methyl at δ 3.50s), and at δ_C 56.9q (an ester methyl at δ_C 51.2q), while **4** showed two methoxy signals at δ 3.24s and 3.11s (an ester methyl at δ 3.48s) and at δ_C 57.3q and 55.3q (an ester methyl at δ_C 51.4q). Thus, it was suggested that one of the two methoxy groups in **4** was missing in **6**. The two-dimensional NMR (2D-NMR) study of **6** revealed that the signal due to H_{29} (δ 3.82) was shifted to lower field than that (δ 3.30) of **4** (see Tables I and II).

In order to confirm the structural difference in the terminal tetrahydropyranyl moiety of **4** and **6**, both methyl esters were acetylated with acetic anhydride and pyridine to afford the pentaacetate **7** [*m/z* 937 $(M+H)^+$; δ 2.11s, 2.02s, 2.01s, 1.98s, 1.96s] and the hexaacetate **9** [*m/z* 965 $(M+H)^+$; δ 2.11s, 2.04s, 2.02s, 2.01s, 1.98s, 1.96s], respectively. The signal due to C_{29}-H in the hexaacetate **9** was observed at lower field (δ 5.06dddd) as compared with that (δ 3.52dddd) in the pentaacetate **7**, thus indicating that **9** has an acetoxy group at C_{29} instead of a methoxy group at C_{29} of **7**. Since the coupling constants of the C_{29} proton (J=9.7, 9.7, 5.1, 5.1 Hz) in **9** are essentially the same as those (J=10.1, 10.1, 4.6, 4.6 Hz) in **7**, the absolute configuration at C_{29} in **6** has been elucidated to be *R*, the same as that in **4**.

Furthermore, the close similarities in the NMR spectra of **4** and **6** (Tables I and II) and the CD spectra of swinholide A (**1**) ($\Delta\varepsilon$ −5.2 at 280 nm) and swinholide C (**3**) ($\Delta\varepsilon$ −5.2 at 280 nm) led us to presume that both **4** and **6** have the same absolute stereostructures. Next, the locations of the lactone linkages in **3** were determined to be at C_{21} and $C_{21'}$ by detailed comparison of the ^{1}H-NMR data for **4**, **6**, and **3** (Tables I and III). Thus, the signals due to 21-H [δ 4.30d in **4**, δ 4.29d in **6**] geminal to 21-OH in **4** and **6** were observed at higher fields than those [δ 5.36d and 5.36d due to 21-H

TABLE III. ^{1}H-NMR Data for Swinholides A (**1**), B (**2**), C (**3**), and Isoswinholide A (**10**) in $CDCl_3$

	1	**2**		**3**		**10**	
		H_n	$H_{n'}$	H_n	$H_{n'}$	H_n	$H_{n'}$
2	5.79 d	(5.78 d	5.79 d)	5.79 d (2H)		5.85 d	5.84 d
3	7.58 d	(7.57 d	7.59 d)	7.58 d (2H)		7.45 d	7.40 d
4Me	1.83 s	1.82 s (6H)		1.82 s (6H)		1.84 s	1.82 s
5	6.08 dd	(6.12 dd	6.07 dd)	6.08 dd (2H)		6.10 dd	6.11 dd
6	2.18 ddd	(2.16 m	2.28 m)	2.18 m (2H)		2.37 m (2H)	
	2.46 br d	(2.46 m	2.48 m)	2.47 ddd (2H)		2.45 m	2.37 m
7	4.14 br dd	4.15 m (2H)		4.14 br dd (2H)		4.01 m	4.11 m
8	1.58 m	1.58 m (2H)		1.63 m (2H)		(1.45 m	1.50 m)
	1.63 m	1.61 m (2H)		1.76 m (2H)		1.65 m (2H)	
9	4.51 br d	(4.50 m	4.51 m)	4.51 d (2H)		(4.50 m	4.52 m)
10	5.69 br d	5.70 m (2H)		5.69 d (2H)		5.68 m (2H)	
11	5.78 br d	5.78 m (2H)		5.76 br d (2H)		5.80 m (2H)	
12	1.82 m	1.94 m	1.83 m	1.84 m (2H)		(1.92 m	1.93 m)
	2.27 br d	2.27 m	2.32 m	2.27 br d (2H)		(2.03 m	2.08 m)
13	3.86 m	3.73 m	3.94 m	3.87 m (2H)		(3.73 m	3.70 m)
14	1.46 ddd	1.33 m	1.43 m	1.44 m (2H)		(1.47 m	1.52 m)
	2.14 ddd	2.10 m	2.19 m	2.13 m (2H)		(1.98 m	1.96 m)
15	4.01 m	3.76 m	4.04 m	4.00 m (2H)		(3.71 m	3.73 m)
15OMe	3.35 s	3.38 s	3.36 s	3.36 s (6H)		(3.36 s	3.38 s)
16	1.68 m	1.40 m, 1.67 m	1.60 m	1.65 m (2H)		1.63 m (2H)	
16Me	0.81 d	—	0.86 d	0.81 d (6H)		(0.79 d	0.81 d)
17	3.83 dd	4.15 m	3.82 m	3.83 dd (2H)		(3.77 m	3.75 m)
18	1.62 m	1.75 m	1.62 m	1.58 m (2H)		1.50 m (2H)	
	1.69 m	1.75 m	1.67 m	1.65 m (2H)		1.50 m	1.55 m
19	3.98 m	3.99 m	3.94 m	3.97 m (2H)		4.25 br d	3.80 m
20	1.75 dq	(1.75 m	1.70 m)	1.59 m (2H)		1.66 m	1.75 m
20Me	0.97 d	(1.00 d	0.93 d)	0.97 d (6H)		0.81 d	0.90 d
21	5.36 d	(5.40 d	5.31 d)	5.36 d (2H)		3.56 br d	5.34 d
22	1.95 m	(1.97 m	1.94 m)	1.92 m (2H)		1.90 m	1.88 m
22Me	0.84 d	(0.83 d	0.81 d)	(0.83 d	0.84 d)	0.89 d	0.87 d
23	3.12 d	(3.14 d	3.12 d)	3.12 d (2H)		4.91 dd	3.07 d
24	1.65 m	1.65 m (2H)		1.64 m (2H)		1.93 m	1.66 m
24Me	0.99 d	0.99 d (6H)		0.99 d (6H)		0.93 d	0.99 d
25	1.27 m	1.28 m (2H)		1.27 m (2H)		1.33 m	1.26 m
	1.38 m	1.38 m (2H)		1.36 m (2H)		1.48 m	1.37 m
26	1.30 m	1.26 m (2H)		(1.23 m	1.25 m)	1.22 m (2H)	
	1.90 m	1.87 m (2H)		1.87 m (2H)		(1.86 m	1.87 m)
27	4.02 m	4.00 m (2H)		(4.00 m	4.03 m)	4.01 m (2H)	
28	1.60 m	1.59 m (2H)		(1.60 m	1.61 m)	1.57 m (2H)	
	1.82 m	1.82 m (2H)		(1.80 m	1.81 m)	1.78 m (2H)	
29	3.53 dddd	3.54 m (2H)		3.99 m	3.54 m	3.52 m (2H)	
29OMe	3.33 s	3.34 s (6H)		—	3.34 s	(3.35 s	3.34 s)
30	1.18 ddd	1.18 m (2H)		(1.17 m	1.18 m)	1.16 m (2H)	
	1.96 m	1.97 m (2H)		(1.92 m	1.97 m)	1.94 m (2H)	
31	3.69 ddq	3.70 m (2H)		3.69 m (2H)		(3.67 m	3.69 m)
31Me	1.20 d	(1.20 d	1.21 d)	(1.19 d	1.20 d)	1.20 d (6H)	

and 21′-H] in **3**. In addition, the 2D-NMR studies on swinholide C (**3**) allowed us to assign all ^{1}H and ^{13}C signals in **3** as given in Tables III and IV. Consequently, the whole structure of swinholide C (**3**) has been elucidated as shown in Chart 1.[5)]

The third dimeric macrolide, isoswinholide A (**10**), was shown to have the same molecular formula $C_{78}H_{132}O_{20}$ as that of swinholide A (**1**), as determined by elemental analysis and by positive and negative FABMS, which gave largest ion peaks at *m/z* 1411 $(M+Na)^+$ and at *m/z* 1388 (M^-). Although the IR and UV spectra of **10** were very similar to those of **1**, the ^{1}H- and ^{13}C-NMR spectra were very complicated, thus indicating an asymmetric dimeric nature of **10**. However, methanolysis of isoswinholide A (**10**) furnished the monomeric methyl ester **4** as a sole product, which was identical with **4** liberated above from swinholide A (**1**). Therefore, the structural difference between **1** and **10** was assumed to be in the location of their lactone linkages.

One of the lactone linkages in isoswinholide A (**10**) was directly assigned as being between C_1 (δ_C 169.6s) and $C_{21'}$ (δ_C 74.7d, $H_{21'}$: δ 5.34d) from the heteronuclear multiple bond correlation (HMBC) spectrum of **10**. Although the other lactone linkage was not identifiable from the HMBC spectrum of **10**, the hydroxy group at C_{23} was shown to be involved in the lactone linkage with $C_{1'}$ by the following connectivity studies around C_{23} by means of homo and hetero COSY, HMBC, and homonuclear Hartman-Hahn (HOHAHA) experiments. Thus, a low-field-shifted proton H_{23} (observed at δ 4.91dd) was coupled with protons H_{22} (δ 1.90m) and H_{24} (δ 1.93m), which were further coupled with methyl doublets at C_{22} (δ 0.89) and C_{24} (δ 0.93), respectively. The methyl group at C_{22} showed a cross peak with the oxymethine group at C_{21} (δ 3.56 br d, δ_C 70.7d), which was further connected to another methyl group at

TABLE IV. ^{13}C-NMR Data for Swinholides A (**1**), B (**2**), C (**3**), and Isoswinholide A (**10**) in $CDCl_3$

	1	**2**		**3**		**10**	
		C_n	$C_{n'}$	C_n	$C_{n'}$	C_n	$C_{n'}$
1	169.6 s	(169.7 s	169.8 s)	169.9 s (2C)		169.6 s	169.2 s
2	113.3 d	113.5 d (2C)		113.4 d (2C)		114.9 d	114.8 d
3	152.5 d	(152.5 d	152.6 d)	152.9 d (2C)		151.7 d	150.9 d
4	133.9 s	(133.9 s	134.1 s)	134.2 s (2C)		134.5 s	134.4 s
4Me	12.0 q	12.1 q (2C)		12.2 q (2C)		12.5 q (2C)	
5	141.2 d	(140.9 d	141.6 d)	141.8 d (2C)		139.8 d	139.6 d
6	37.4 t	(37.2 t	37.8 t)	37.3 t (2C)		37.7 t	37.4 t
7	66.6 d	(66.4 d	67.1 d)	66.7 d (2C)		67.3 d	67.2 d
8	40.4 t	41.0 t (2C)		40.7 t (2C)		40.6 t (2C)	
9	66.7 d	(65.5 d	67.9 d)	66.4 d (2C)		(68.0 d	68.6 d)
10	129.7 d	(129.8 d	129.9 d)	129.8 d (2C)		129.9 d (2C)	
11	123.1 d	(123.0 d	123.5 d)	123.3 d (2C)		(123.8 d	123.6 d)
12	30.2 t	31.0 t	29.9 t	30.2 t (2C)		(30.7 t	30.5 t)
13	65.1 d	65.9 d	64.7 d	65.6 d (2C)		(65.0 d	65.2 d)
14	34.6 t	38.5 t	33.9 t	34.3 t (2C)		(35.2 t	34.9 t)
15	75.6 d	74.4 d	75.3 d	75.5 d (2C)		(78.0 d	77.8 d)
15OMe	56.9 q	56.5 q	57.1 q	57.3 q (2C)		(57.3 q	57.4 q)
16	41.4 d	43.2 t	40.3 d	41.4 d (2C)		40.3 d (2C)	
16Me	9.0 q	—	9.1 q	9.0 q (2C)		(10.7 q	10.4 q)
17	73.5 d	68.5 d	73.8 d	73.8 d (2C)		(74.8 d	74.4 d)
18	38.1 t	34.7 t	38.1 t	38.4 t (2C)		(38.6 t	37.7 t)
19	70.9 d	70.7 d	71.0 d	71.2 d (2C)		73.3 d	71.1 d
20	40.7 d	40.3 d	41.0 d	40.9 d (2C)		40.9 d	40.3 d
20Me	8.9 q	9.1 q (2C)		(9.2 q	9.3 q)	9.9 q	8.8 q
21	74.1 d	74.5 d	74.1 d	74.3 d (2C)		70.7 d	74.7 d
22	37.2 d	37.1 d	37.4 d	37.5 d (2C)		36.0 d	37.1 d
22Me	8.8 q	9.1 q (2C)		9.1 q (2C)		9.4 q	9.2 q
23	75.8 d	76.1 d	75.9 d	(76.0 d	76.1 d)	80.1 d	75.9 d
24	32.9 d	33.1 d (2C)		33.2 d (2C)		32.8 d	33.3 d
24Me	17.4 q	17.5 q (2C)		(17.5 q	17.6 q)	16.9 q	17.8 q
25	23.7 t	(23.7 t	23.9 t)	(23.9 t	24.1 t)	25.7 t	24.2 t
26	29.0 t	29.1 t (2C)		(28.9 t	29.2 t)	(28.7 t	29.3 t)
27	70.9 d	71.0 d (2C)		(71.2 d	71.9 d)	71.5 d (2C)	
28	34.6 t	34.7 t (2C)		37.9 t	34.8 t	(35.0 t	35.1 t)
29	72.9 d	73.1 d (2C)		64.2 d	73.2 d	73.4 d (2C)	
29OMe	54.8 q	55.0 q (2C)		—	55.1 q	55.3 q (2C)	
30	38.3 t	38.7 t (2C)		42.7 t	38.6 t	(38.6 t	38.7 t)
31	64.3 d	64.4 d (2C)		64.4 d (2C)		(64.6 d	64.8 d)
31Me	21.4 q	21.6 q (2C)		21.7 q	21.8 q	21.8 q (2C)	

C_{20} (δ 0.81d, δ_C 9.9q). The HOHAHA spectrum further connected H_{19} (δ 4.25 br d) to H_{21}, H_{20} (δ 1.66m), and $H_{20\text{-Me}}$, $H_{18a,18b}$ (δ 1.50m, 2H). Finally, all ^{1}H and ^{13}C signals in the NMR spectra of isoswinholide A (**10**) may be assigned as shown in Tables III and IV, and the structure of isoswinholide A has been elucidated as **10** having a 46-membered dilactone moiety.

Further confirmation of the structure **10** for isoswinholide A was secured by the following experiments. Treatment of swinholide A (**1**) in $CHCl_3$ with *p*-toluenesulfonic acid monohydrate provided isoswinholide A (**10**, 12%) and two acyl-migrated products **11** (7%) and **12** (6%) with the recovered starting material (**1**, 60%). Detailed analysis of the chemical structures of these acyl-migrated products (**10**, **11**) is being undertaken. The above results have shown that acyl migration may occur easily in these dimeric macrolides, so that a part of isoswinholide A (**10**) may be produced from swinholide A (**1**) during the isolation procedure, which includes silica gel column chromatography.

The structural relation of swinholide A (**1**) and swinholide B (**2**) is just like that of misakinolide A (=bistheonellide A) and bistheonellide B, which were previously isolated from another Okinawan marine sponge of *Theonella* sp.[6,7] Thus, the occurrence of similar biosynthetic pathways for swinholides and misakinolides in both sponges is presumed.

Both swinholide B (**2**) and swinholide C (**3**) exhibited potent cytotoxicity almost equivalent to that of swinholide A (**1**) toward KB cell lines (IC_{50} 0.041 and 0.052 μg/ml, respectively) while isoswinholide A (**10**) showed weaker cytotoxicity (IC_{50} 1.1 μg/ml). These results suggest that the lactone-ring size and its conformation are critical factors for exhibiting cytotoxic activity.

Experimental

The instruments to obtain the physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1] The ^{1}H-NMR spectra were measured at 500 MHz and the ^{13}C-NMR spectra at 125 MHz.

Isolation of Swinholides A (1), B (2), and C (3), and Isoswinholide A (10) The marine sponge *Theonella swinhoei* (160 kg, wet, collected in April at Kuro Island, Okinawa) was cut and extracted with acetone (250 l) at room temperature three times. Removal of the solvent from the combined extract under reduced pressure gave an aqueous suspension (110 l) which was extracted with ethyl acetate (110 l) twice. The ethyl acetate layer was taken and the solvent was removed under reduced pressure to give 2.66 kg of the ethyl acetate-soluble portion. A part of the ethyl acetate-soluble portion (600 g) was suspended in a mixture of *n*-hexane–ethyl acetate (2 : 1), and the supernatant portion was subjected to column chromatography

(Kieselgel 60, 200 g). Fractions eluted with ethyl acetate–methanol (20 : 1) were separated into two fractions, of which the earlier eluted one (6.35 g) was rich in swinholide A (**1**), while the later one (1.08 g) contained minor swinholides. A part of the latter fraction (535 mg) was separated by reversed-phase HPLC [Cosmosil $5C_{18}$, MeOH–H_2O (10 : 1)] to give successively swinholide C (**3**) (50 mg), swinholide B (**2**) (130 mg), swinholide A (**1**) (174 mg), and isoswinholide A (**10**) (29 mg). Swinholide C (**3**): A white amorphous powder, $[\alpha]_D^{24}$ +2.8° (c=5.4, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470, 3000, 2960, 1685, 1630, 1465, 1385. UV λ_{max}^{MeOH} nm (ε): 268 (42600). CD (MeOH, c=0.02) $\Delta\varepsilon$: −5.2 at 280 nm. ^{1}H-NMR ($CDCl_3$) δ: Table III, 7.58 (2H, d, J=15.6 Hz), 6.08 (2H, dd, J=9.5, 5.2 Hz), 5.79 (2H, d, J=15.6 Hz), 5.76 (2H, br d, J=11.6 Hz), 5.69 (2H, d, J=11.6 Hz), 5.36 (2H, d, J=11.0 Hz), 4.51 (2H, d, J=8.9 Hz), 4.14 (2H, br dd, J=9.5, 9.5 Hz), 3.83 (2H, dd, J=10.3, 10.3 Hz), 3.12 (2H, d, J=10.1 Hz), 2.47 (2H, ddd, J=15.4, 9.5, 9.5 Hz), 2.27 (2H, br d, J=17.0 Hz), 1.20 (3H, d, J=6.4 Hz), 1.19 (3H, d, J=6.4 Hz), 0.99 (6H, d, J=6.4 Hz), 0.97 (6H, d, J=6.7 Hz), 0.84 (3H, d, J=7.0 Hz), 0.83 (3H, d, J=6.7 Hz), 0.81 (6H, d, J=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: Table IV. FABMS [matrix: glycerol(G)+thioglycerol(TG)]: m/z 1397 $(M+Na)^+$; m/z 1374 $(M)^-$. *Anal.* Calcd for $C_{77}H_{130}O_{20}\cdot 2H_2O$: C, 65.52; H, 9.56. Found: C, 65.89; H, 9.52. Swinholide B (**2**): A white amorphous powder, $[\alpha]_D^{22}$ +2.5° (c=6.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3445, 3000, 2945, 1680, 1615, 1455, 1385, 985. UV λ_{max}^{MeOH} nm (ε): 272 (46800). CD (MeOH, c=0.001) $\Delta\varepsilon$: −5.3 at 270 nm. ^{1}H-NMR ($CDCl_3$) δ: Table III, 7.59 (1H, d, J=15.6 Hz), 7.57 (1H, d, J=15.6 Hz), 6.12 (1H, dd, J=7.3, 7.3 Hz), 6.07 (1H, dd, J=10.0, 5.0 Hz), 5.79 (1H, d, J=15.6 Hz), 5.78 (1H, d, J=15.6 Hz), 5.40 (1H, d, J=10.7 Hz), 5.31 (1H, d, J=10.7 Hz), 3.14 (1H, d, J=9.8 Hz), 3.12 (1H, d, J=9.8 Hz), 1.21 (3H, d, J=6.1 Hz), 1.20 (3H, d, J=6.4 Hz), 1.00 (3H, d, J=6.4 Hz), 0.99 (6H, d, J=6.4 Hz), 0.93 (3H, d, J=6.7 Hz), 0.86 (3H, d, J=6.7 Hz), 0.83 (3H, d, J=7.0 Hz), 0.81 (3H, d, J=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: Table IV. FABMS (G+TG): m/z 1375 $(M+H)^+$; m/z 1374 $(M)^-$. *Anal.* Calcd for $C_{77}H_{130}O_{20}\cdot H_2O$: C, 66.35; H, 9.55. Found: C, 66.05; H, 9.56. Isoswinholide A (**10**): A white amorphous powder, $[\alpha]_D^{29}$ −42° (c=0.51, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 3000, 2970, 2940, 1680, 1615, 1455, 1380, 985. UV λ_{max}^{MeOH} nm (ε): 269 (38000). ^{1}H-NMR ($CDCl_3$) δ: Table III, 7.45 (1H, d, J=15.7 Hz), 7.40 (1H, d, J=15.7 Hz), 6.11 (1H, dd, J=6.7, 6.7 Hz), 6.10 (1H, dd, J=6.7, 6.7 Hz), 5.85 (1H, d, J=15.7 Hz), 5.84 (1H, d, J=15.7 Hz), 5.34 (1H, d, J=11.0 Hz), 4.91 (1H, dd, J=8.5, 4.0 Hz), 4.25 (1H, br d, J=10.0 Hz), 3.56 (1H, br d, J=10.0 Hz), 3.07 (1H, d, J=9.8 Hz), 1.20 (6H, d, J=6.1 Hz), 0.99 (3H, d, J=6.7 Hz), 0.93 (3H, d, J=6.7 Hz), 0.90 (3H, d, J=6.1 Hz), 0.89 (3H, d, J=7.0 Hz), 0.87 (3H, d, J=7.3 Hz), 0.81 (6H, d, J=7.0 Hz), 0.79 (3H, d, J=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: Table IV. FABMS (G+TG): m/z 1411 $(M+Na)^+$; m/z 1388 $(M)^-$. *Anal.* Calcd for $C_{78}H_{132}O_{20}\cdot H_2O$: C, 66.54; H, 9.59. Found: C, 66.36; H, 9.51.

Methanolysis of Swinholide B (2) A 28% solution of sodium methoxide in methanol (50 μl) was added dropwise to a stirred solution of swinholide B (**2**) (100 mg) in methanol (2 ml). The reaction mixture was stirred for 5 h at room temperature under a nitrogen atmosphere, then partitioned into a mixture of ethyl acetate and water. The ethyl acetate layer was taken, washed with brine, and dried over $MgSO_4$, and the solvent was evaporated off under reduced pressure to give the crude products (104 mg). The product was separated by HPLC [Cosmosil $5C_{18}$, MeOH–H_2O (10 : 1)] to give **5** (46 mg) and **4** (40 mg). Methyl ester **5**: A white amorphous powder, $[\alpha]_D^{26}$ −31° (c=3.8, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 3000, 2940, 1700, 1615, 1460, 1380, 980. UV λ_{max}^{MeOH} nm (ε): 266 (15000). ^{1}H-NMR (C_6D_6) δ: Table I, 7.65 (1H, d, J=15.7 Hz), 6.08 (1H, dd, J=7.0, 7.0 Hz), 5.89 (1H, d, J=15.7 Hz), 5.64 (1H, br d, J=10.4 Hz), 5.53 (1H, d, J=10.4 Hz), 4.67 (1H, d, J=9.5 Hz), 4.15 (1H, br ddd, J=7.0, 7.0, 7.0 Hz), 3.40 (1H, dd, J=11.9, 4.8 Hz), 2.36 (1H, ddd, J=14.8, 7.0, 7.0 Hz), 2.25 (1H, ddd, J=14.8, 7.0, 7.0 Hz), 2.10 (1H, ddd, J=13.7, 9.8, 4.0 Hz), 1.21 (3H, d, J=6.4 Hz), 1.12 (3H, d, J=7.0 Hz), 0.88 (3H, d, J=6.7 Hz), 0.72 (3H, d, J=7.0 Hz). ^{13}C-NMR (C_6D_6) δ_C: Table II. FABMS (G): m/z 713 $(M+H)^+$. Methyl ester **4**: The spectral data (IR, UV, ^{1}H- and ^{13}C-NMR, FABMS, and optical rotation) were identical with those reported earlier.[3]

Acetylation of Methyl Ester 5 to Give the Pentaacetate 8 **5** (18 mg) was dissolved in a mixture of pyridine (0.5 ml) and acetic anhydride (0.5 ml). The mixture was stirred at room temperature for 16 h under a nitrogen atmosphere. The reaction mixture was then partitioned into a mixture of ethyl acetate and water, and the ethyl acetate layer was dried over $MgSO_4$, and evaporated under reduced pressure. The crude product (28 mg) was purified by HPLC [Hibar RP-18, MeOH–H_2O (10 : 1)] to give the pentaacetate **8** (15 mg, 67%). The pentaacetate **8**: a glassy solid, $[\alpha]_D^{25}$ −25° (c=0.73, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2940, 1730, 1620, 1430, 1370. UV λ_{max}^{MeOH} nm (ε): 266 (24000). ^{1}H-NMR ($CDCl_3$) δ: Table I, 7.32 (1H, d, J=15.8 Hz), 5.92 (1H, dd, J=7.0, 7.0 Hz), 5.82 (1H, d, J=15.8 Hz), 5.61 (1H, br d, J=9.8 Hz), 4.98 (1H, d, J=10.3 Hz), 4.67 (1H, dd, J=8.1, 4.7 Hz), 4.22 (1H, br d, J=9.8 Hz), 3.52 (1H, dddd, J=10.0, 10.0, 4.7, 4.7 Hz), 1.20 (3H, d, J=6.0 Hz), 0.95 (3H, d, J=6.8 Hz), 0.93 (3H, d, J=6.8 Hz), 0.89 (3H, d, J=6.4 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: Table II. FABMS (G): m/z 923 $(M+H)^+$.

Acetylation of Methyl Ester 4 to Give the Pentaacetate 7 **4** (13 mg) was dissolved in pyridine (0.3 ml) and acetic anhydride (0.3 ml), and the reaction mixture was stirred at room temperature for 12 h, then partitioned into a mixture of ethyl acetate and water. The ethyl acetate layer was dried over $MgSO_4$, then the solvent was evaporated off under reduced pressure to give the crude product (16 mg), which was purified by HPLC [Hibar RP-18, MeOH–H_2O (10 : 1)] to afford the pentaacetate **7** (12 mg, 72%). The pentaacetate **7**: Colorless glassy solid, $[\alpha]_D^{27}$ −22° (c=0.62, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2980, 2940, 1735, 1620, 1435, 1370, 1020. UV λ_{max}^{MeOH} nm (ε): 264 (18100). ^{1}H-NMR ($CDCl_3$) δ: Table I, 7.33 (1H, d, J=15.7 Hz), 5.93 (1H, dd, J=7.0, 7.0 Hz), 5.82 (1H, d, J=15.7 Hz), 5.63 (1H, br d, J=10.4 Hz), 4.99 (1H, d, J=10.1 Hz), 4.73 (1H, br dd, J=7.0, 7.0 Hz), 4.67 (1H, dd, J=8.2, 4.6 Hz), 4.22 (1H, br d, J=8.5 Hz), 3.67 (1H, ddq, J=9.5, 3.0, 6.5 Hz), 3.52 (1H, dddd, J=10.0, 10.0, 4.5, 4.5 Hz), 2.61 (1H, ddd, J=14.0, 7.0, 7.0 Hz), 2.55 (1H, ddd, J=14.0, 7.0, 7.0 Hz), 1.20 (3H, d, J=6.5 Hz), 0.96 (3H, d, J=7.0 Hz), 0.92 (3H, d, J=6.7 Hz), 0.89 (6H, d, J=6.0 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: Table II. FABMS (G): m/z 937 $(M+H)^+$.

Methanolysis of Swinholide C (3) A 28% solution of sodium methoxide in methanol (100 μl) was added dropwise to a stirred solution of swinholide C (**3**) (73 mg) in methanol (6 ml). The mixture was stirred for 1 d at room temperature under a nitrogen atmosphere, then partitioned into a mixture of ethyl acetate and water. The ethyl acetate layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude product (76 mg) was subjected to HPLC [Cosmosil $5C_{18}$, MeOH–H_2O (10 : 1)] to furnish the methyl ester **6** (23 mg, 60%) and **4** (25 mg, 62%). The methyl ester **6**: A white amorphous powder, $[\alpha]_D^{20}$ −33° (c=0.70, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 3000, 1940, 1700, 1620, 1455, 1380, 980. UV λ_{max}^{MeOH} nm (ε): 266 (20000). ^{1}H-NMR (C_6D_6) δ: Table I, 7.64 (1H, d, J=15.8 Hz), 6.08 (1H, dd, J=7.0, 7.0 Hz), 5.90 (1H, d, J=15.8 Hz), 5.56 (1H, d, J=10.4 Hz), 4.67 (1H, d, J=10.1 Hz), 4.34 (1H, d, J=8.5 Hz), 4.29 (1H, d, J=10.1 Hz), 4.15 (1H, br dd, J=7.0, 7.0 Hz), 3.90 (1H, br dd, J=7.0, 7.0 Hz), 2.38 (1H, ddd, J=14.7, 7.0, 7.0 Hz), 2.27 (1H, ddd, J=14.7, 7.0, 7.0 Hz), 1.21 (3H, d, J=6.1 Hz), 1.15 (3H, d, J=7.0 Hz), 0.87 (3H, d, J=6.7 Hz), 0.86 (3H, d, J=6.7 Hz), 0.78 (3H, d, J=7.0 Hz). ^{13}C-NMR (C_6D_6) δ_C: Table II. FABMS (G): m/z 713 $(M+H)^+$. The methyl ester **4**: The spectral data (IR, UV, ^{1}H- and ^{13}C-NMR, FABMS, and optical rotation) were identical with those reported earlier.[3]

Acetylation of Methyl Ester 6 to Give the Hexaacetate 9 **6** (4 mg) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml), and the mixture was stirred at room temperature overnight. The reaction mixture was then partitioned into a mixture of ethyl acetate and water. The ethyl acetate layer was dried over $MgSO_4$, and evaporated under reduced pressure. The crude product (6 mg) was purified by HPLC [Hibar RP-18, MeCN–H_2O (10 : 1)] to give the hexaacetate **9** (4 mg, 81%). The hexaacetate **9**: Colorless glassy, $[\alpha]_D^{27}$ −60° (c=0.14, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2995, 2915, 1720, 1360, 1010. UV λ_{max}^{MeOH} nm (ε): 264 (24800). ^{1}H-NMR ($CDCl_3$) δ: Table I, 7.33 (1H, d, J=15.6 Hz), 5.93 (1H, dd, J=7.0, 7.0 Hz), 5.82 (1H, d, J=15.6 Hz), 5.62 (1H, br d, J=10.4 Hz), 5.06 (1H, dddd, J=10.0, 10.0, 5.0, 5.0 Hz), 4.99 (1H, d, J=10.1 Hz), 4.74 (1H, br dd, J=7.0, 7.0 Hz), 4.67 (1H, dd, J=8.2, 4.9 Hz), 4.22 (1H, br d, J=7.3 Hz), 2.61 (1H, ddd, J=14.0, 7.0, 7.0 Hz), 2.55 (1H, ddd, J=14.0, 7.0, 7.0 Hz), 1.19 (3H, d, J=6.4 Hz), 0.97 (3H, d, J=6.7 Hz), 0.92 (3H, d, J=7.0 Hz), 0.89 (6H, d, J=6.7 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: Table II. FABMS (G): m/z 965 $(M+H)^+$.

Methanolysis of Isoswinholide A (10) A 28% solution of sodium methoxide in methanol (100 μl) was added dropwise to a stirred solution of isoswinholide A (**10**) (47 mg) in methanol (1 ml). The reaction mixture was stirred for 5 h at room temperature under a nitrogen atmosphere, then partitioned into a mixture of ethyl acetate and water. The organic layer was washed with brine, and dried over $MgSO_4$, then the solvent was removed under reduced pressure. The crude product (51 mg) was purified by HPLC [Cosmosil $5C_{18}$, MeOH–H_2O (10 : 1)] to give **4** (32 mg, 65%) as a sole product. The methyl ester **4**: The spectral data were identical (IR, UV, ^{1}H- and ^{13}C-NMR, FABMS, and optical rotation) with those reported earlier.[3]

Acid Treatment of Swinholide A (1) A solution of **1** (563 mg) in $CHCl_3$ (10 ml) was treated with *p*-toluenesulfonic acid monohydrate (30 mg). The reaction mixture was stirred for 7 h at room temperature and partitioned into a mixture of ethyl acetate and saturated aqueous $NaHCO_3$. The ethyl

acetate layer was washed with brine, and dried over $MgSO_4$, then the solvent was removed under reduced pressure. The crude product (544 mg) was subjected to HPLC [Hiber RP-18, MeOH–H_2O (10 : 1)] to furnish **11** (40 mg, 7%), swinholide A (**1**, 337 mg, 60%), isoswinholide A (**10**, 65 mg, 12%), and **12** (33 mg, 6%) in order of elution. **11**: A white amorphous powder, $[\alpha]_D^{23}$ −39° (c=2.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2940, 1680, 1615, 1380, 980. UV λ_{max}^{MeOH} nm (ε): 268 (31000). ^{1}H-NMR ($CDCl_3$) δ: 7.39 (1H, d, J=15.6 Hz), 7.38 (1H, d, J=15.6 Hz), 6.09 (1H, dd, J=7.0, 7.0 Hz), 6.04 (1H, dd, J=7.0, 7.0 Hz), 5.86 (1H, d, J=15.6 Hz), 5.84 (1H, d, J=15.6 Hz), 5.81 (2H, m), 5.67 (2H, m), 5.39 (1H, d, J=10.4 Hz), 5.08 (1H, m), 4.50 (2H, m), 3.36 (3H, s), 3.35 (3H, s), 3.34 (6H, s), 3.08 (1H, br s), 1.84 (3H, s), 1.80 (3H, s), 1.21 (3H, d, J=6.2 Hz), 1.20 (3H, d, J=6.2 Hz), 0.99 (3H, d, J=7.0 Hz), 0.98 (3H, d, J=7.0 Hz), 0.94 (3H, d, J=6.8 Hz), 0.91 (3H, d, J=6.8 Hz), 0.87 (3H, d, J=6.6 Hz), 0.86 (3H, d, J=6.6 Hz), 0.82 (3H, d, J=6.6 Hz), 0.74 (3H, d, J=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: 169.4 (s), 167.6 (s), 151.1 (d), 150.2 (d), 139.3 (d), 138.7 (d), 134.9 (s), 134.8 (s), 129.9 (d), 129.7 (d), 123.9 (d), 123.9 (d), 116.0 (d), 115.2 (d). **12**: A white amorphous powder, $[\alpha]_D^{25}$ +16° (c=2.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2940, 1680, 1620, 1380, 980. UV λ_{max}^{MeOH} nm (ε): 268 (37000). ^{1}H-NMR ($CDCl_3$) δ: 7.52 (1H, d, J=15.6 Hz), 7.34 (1H, d, J=15.6 Hz), 6.23 (1H, br dd, J=7.0, 7.0 Hz), 6.02 (1H, br dd, J=7.0, 7.0 Hz), 5.83 (1H, d, J=15.6 Hz), 5.78 (1H, d, J=15.6 Hz), 5.78 (2H, m), 5.67 (2H, m), 5.66 (1H, m), 4.96 (1H, br d, J=8.6 Hz), 4.51 (2H, m), 4.24 (1H, d, J=10.3 Hz), 3.36 (3H, s), 3.33 (6H, s), 3.32 (3H, s), 1.80 (3H, s), 1.79 (3H, s), 1.21 (3H, d, J=6.0 Hz), 1.20 (3H, d, J=6.0 Hz), 0.93 (3H, d, J=6.0 Hz), 0.90 (6H, d, J=7.0 Hz), 0.89 (3H, d, J=7.0 Hz), 0.83 (3H, d, J=6.0 Hz), 0.82 (3H, d, J=6.0 Hz), 0.78 (3H, d, J=7.2 Hz), 0.77 (3H, d, J=7.2 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: 169.6 (s), 168.8 (s), 151.8 (d), 150.9 (d), 140.2 (d), 140.0 (d), 134.5 (s), 134.4 (s), 130.0 (d), 129.8 (d), 123.9 (d), 123.4 (d), 115.2 (d), 114.7 (d). Both **1** and **10** were identical with authentic samples as determined by HPLC and ^{1}H-NMR comparisons.

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References and Notes

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