New Congeners of Bistheonellides from Okinawan Marine Sponges of the Genus *Theonella*

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Four new bistheonellide-related compounds, bistheonellide C, isobistheonellide A and bistheonellic acids A and B, have been isolated from Okinawan marine sponges of the genus *Theonella*, and their structures elucidated by spectral and chemical means.

Macrolides from marine organisms are of current interest because of their significant biological activities.[1–9] During our studies on bioactive metabolites from Okinawan marine organisms,[10] we have isolated two new dimeric macrolides, bistheonellide C **1** and isobistheonellide A **2** and the related compounds bistheonellic acids A **3** and B **4** from Okinawan marine sponges of the genus *Theonella*. This paper deals with the isolation and structure elucidation of compounds **1**–**4**.

The methanol extract of the sponge *Theonella* sp. collected off Ishigaki Island, Okinawa, was partitioned between ethyl acetate and water. The ethyl acetate-soluble material was repeatedly subjected to column chromatography on silica gel [MeOH–$CHCl_3$ (0:100–50:50); acetone–hexane (10:90–60:40); and hexane–ethyl acetate–MeOH (8:2:1)] to give bistheonellide C **1** (0.000 85%, wet weight) and isobistheonellide A **2** (0.000 16%) as well as bistheonellides A **5** (0.027%) and B **6** (0.0012%) previously isolated from a *Theonella* sp. collected off Hachijo Island.[2] The ^1H NMR spectra, in $CDCl_3$ of compounds **1** and **2** were similar to those of compounds **5** and **6**, indicating that both new products **1** and **2** were the congeners of the bistheonellides **5** and **6**.

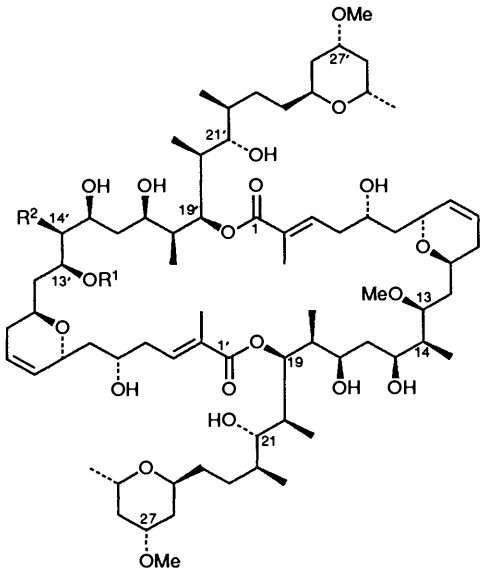
The FAB-MS [*m/z* 1428 (M + diethanolamine + H)$^+$] of bistheonellide C **1** showed that the molecular weight was 14 mass units less than that of bistheonellide A **5**. The ^1H (Table 1) and ^{13}C NMR spectra of compound **1** revealed the presence of three methoxy groups [δ_H 3.36(×1), δ_H 3.34 (×2); δ_C 57.2 (×1), δ_C 55.3 (×2)]. The combination of the ^1H–^1H COSY spectrum and the proton-decoupling experiments led to the assignment of all the proton signals, showing that the half-portion of product **1** was identical with that of compound **5**; however, in the other half-portion the signal of 13′-H was observed at δ 3.97, to lower field than that for 13-H (δ 3.82). Therefore, the structure of bistheonellide C was assigned as an unsymmetrical 40-membered dimeric macrolide **1** containing one hydroxy group instead of the methoxy group at C-13′ in bistheonellide A **5**.

The molecular formula of isobistheonellide A **2** was the same as that of bistheonellide A **5**, as indicated by the FAB-MS data [*m/z* 1442 (M + diethanolamine + H)$^+$]. The ^1H NMR spectrum showed an unsymmetrical structure for compound **2** because of the more complicated spectrum. An extensive NMR study including ^1H–^1H COSY and decoupling experiments resulted in the complete assignment of signals (Table 1), indicating that a half-portion of compound **2** was the same as that of bistheonellide A **5** and that the other portion had an ester linkage at a different position. The chemical shifts of 19′-H (δ 4.00) and 21′-H (δ 4.92) suggested that the ester linkage was formed at C-21′, and therefore the structure of isobistheonellide A was concluded to be the 42-membered macrodiolide **2**.

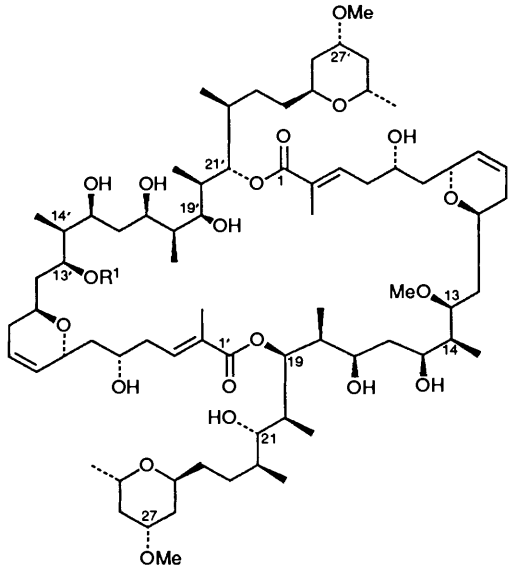
The methanol extract of the sponge *Theonella* sp. collected off Zamami Island, Okinawa, was partioned between ethyl acetate and water. The ethyl acetate-soluble fraction was subjected to silica gel column chromatography [MeOH–$CHCl_3$ (15:85)] followed by gel filtration on Sephadex LH-20 [MeOH–$CHCl_3$ (1:1)] and C_{18} reversed-phase column chromatography (80% MeOH) to give bistheonellic acids A **3** (0.005%, wet weight) and B **4** (0.002%) along with bistheonellide A **5** (0.01%).[2]

The molecular formula of compound **3** was determined to be $C_{74}H_{130}O_{21}$ on the basis of HR-FAB-MS data [*m/z* 1461.0000 (M + diethanolamine + H)$^+$, $C_{78}H_{142}NO_{23}$, Δ +2.7 mmu], implying that the molecular formula of compound **3** is larger by one H_2O unit than that of bistheonellide A **5**. Although compound **5** possesses a symmetrical dimeric structure, the ^1H and ^{13}C NMR spectra of bistheonellic acid A **3** suggested that it was also dimeric but that it possessed incomplete molecular symmetry. The ^1H NMR spectrum of compound **3** exhibited two split signals due to 3-H [δ 7.00 br t (3-H) and 6.85 br t (3′-H)]. The integral values for 19-H at δ 5.30 and 21-H at δ 3.05 both corresponded to one proton. The 19′-H and 21′-H signals in the other half-portion were shown to be at δ 4.00 and δ 3.30, respectively, by a ^1H–^1H COSY experiment. From these observations, along with the fact that compound **3** showed high polarity on TLC examination, bistheonellic acid A was determined to be a seco acid in which one of the two ester bonds of bistheonellide A **5** is cleaved. The presence of a carboxylic acid group in compound **3** was verified by the formation of a methyl ester [**7**, *m/z* 1474 (M + diethanolamine + H)$^+$] on treatment with diazomethane.

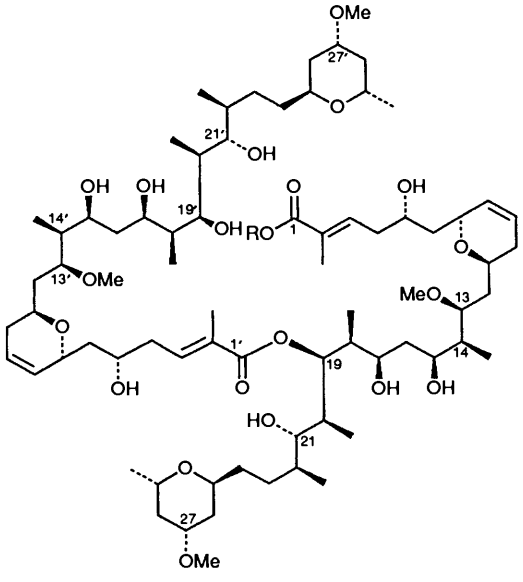
Compound **4** was shown to have the molecular formula $C_{37}H_{66}O_{11}$ by HR-FAB-MS data [negative mode: *m/z* 685.4523 (M − H)$^-$, $C_{37}H_{65}O_{11}$, Δ −0.4 mmu]. The ^1H and ^{13}C NMR spectra of compound **4** showed that it possessed a monomeric structure. The ^1H NMR spectrum of compound **4** was, however, quite similar to that of bistheonellide A **5**. Analysis of the ^1H NMR spectrum of compound **4** aided by the ^1H–^1H COSY spectrum revealed some differences from that of bistheonellide A **5** as follows. The signal for 3-H of compound **4** was shifted to δ 6.82 from δ 7.00 for compound **5**. The signal for a methyl group on C-2 of compound **4** appeared at δ 1.82, slightly shifted from δ 1.85 for bistheonellide A **5**. The signals observed at δ 5.30 and 3.05 for compound **5**, which were assignable to 19-H and 21-H, respectively, disappeared from the ^1H NMR spectrum of bistheonellic B **4**, and were found at δ 4.00 and 3.30, respectively. The molecular formula of bistheonellic acid **4** corresponded to half that of compound **5** together with one molecule of water. Compound **4** also showed high polarity on TLC. From these results bistheonellic acid **4** was assigned to be a monomeric seco acid of compound **5**. Compound **4** was also treated with diazomethane



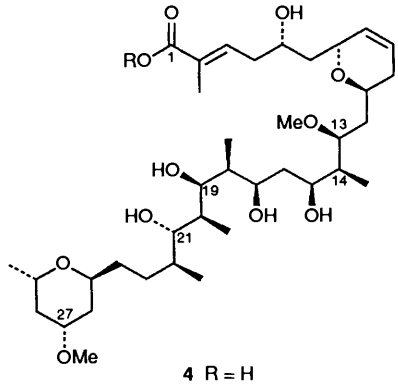
1 R^1 = H, R^2 = Me
5 R^1 = Me, R^2 = Me
6 R^1 = Me, R^2 = H



2



3 R = H
7 R = Me



4 R = H
8 R = Me

to afford a methyl ester [**8**, *m/z* 701 $(M + H)^+$], confirming the presence of a carboxylic acid group in compound **4**. This monomeric methyl ester **8** has previously been obtained by treatment of bistheonellide A **5** with MeONa–MeOH.[2] The spectral data of ester **8** derived from acid **4** were completely identical with those described in the literature.[2]

Bistheonellide A **5** was treated with 2 mol dm^{-3} KOH–MeOH at room temperature for 5 h to afford bistheonellic acid B **4**, which was also obtained from bistheonellic acid A **3** by treatment with 2 mol dm^{-3} KOH–MeOH at room temperature for 2.5 h. These observations further confirmed the structures of compounds **3** and **4** to be dimeric and monomeric seco acids of bistheonellide A **5**, respectively.

Bistheonellide C **1** and isobistheonellide A **2** exhibited almost the same cytotoxicity as bistheonellides A **5** and B **6** against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro*. Bistheonellic acids A **3** and B **4** were, however, less cytotoxic than bistheonellide A **5**.*

Experimental

Optical rotations were determined on a JASCO DIP-370 or DIP-4 polarimeter. UV and IR spectra were measured on a

* Inhibition against L1210 and KB cells at 10 μg cm^{-3}: bistheonellide C **1**, 71.1 (IC_{50} 5.6 μg/cm^{-3}) and 37.2 (IC_{50} > 10 μg cm^{-3})%, respectively; isobistheonellide A **2**, 95.9 (IC_{50} 0.76 μg cm^{-3}) and 97.0 (IC_{50} 1.3 μg cm^{-3})%, respectively; bistheonellic acid A **3**, 41.0 and 29.9%, respectively; bistheonellic acid B **4**, 2.7 and 1.1%, respectively; bistheonellide A **5**, 89.9 (IC_{50} 2.4 μg cm^{-3}) and 64.2 (IC_{50} 6.4 μg cm^{-3}) %, respectively; bistheonellide B **6**, 86.6 (IC_{50} 3.4 μg cm^{-3} and 43.6 (IC_{50} > 10 μg cm^{-3})%, respectively.

Table 1 ^{1}H NMR data for bistheonellide A **5**, bistheonellide C **1** and isobistheonellide A **2** in $CDCl_3$ [*J*-values in Hz] *

Atom	Bistheonellide A **5**	Bistheonellide C **1**		Isobistheonellide A **2**	
		n-H	*n'*-H	*n*-H	*n'*-H
2-Me	18.6s	1.86s (6 H)		(1.87s[a]	1.86s[b])
3	6.97br t [7.0]	(6.97br t [7.0]	6.98br t [7.0])	(6.96br t [7.0][a]	6.90br t [7.0][b])
4	2.26br d [15]	2.27m (2 H)		(2.25m[a]	2.28m[b])
	2.35dt [7.0, 8.4]	2.33m (2 H)		(2.41m[a]	2.35m[b])
5	4.11br t [10]	4.11br t [9] (2 H)		(4.09m[a]	4.10m[b])
6	1.52m	1.57m (2 H)		(1.52m[a]	1.56m[b])
	1.68m	1.68m (2 H)		(1.73m[a]	1.66m[b])
7	4.51br d [11]	4.51br d [11] (2 H)		(4.55br d [11][a]	4.51br d [11][b])
8	5.67dd [10, 2.1]	5.68br d [10] (2 H)		(5.65br d [11][a]	5.68br d [11][b])
9	5.78br d [10]	5.78br d [10] (2 H)		(5.80br d [11][a]	5.78br d [11][b])
10	1.86m	1.87 m (2 H)		(1.84m[a]	1.88m[b])
	2.22m	2.21 m (2 H)		(2.25m[a]	2.16m[b])
11	3.83m	3.84m (2 H)		(3.84m[a]	3.77m[b])
12	1.49m	1.49m (2 H)		(1.48m[a]	1.50m[b])
	2.07ddd [14, 10, 4.2]	2.09m (2 H)		(2.12m[a]	2.08m[b])
13	3.79m	3.82m	3.97m	3.88m (2 H)	
13-OMe	3.36s	3.36s (3 H)		(3.34s	3.35s)
14	1.70m	1.72m (2 H)		(1.69m[c]	1.60m[d])
14-Me	0.84d [6.3]	(0.84d [6.3]	0.85d [6.3])	(0.81d [6.3][c]	0.80d [6.3][d])
15	3.76m	3.77m (2 H)		3.81m (2 H)	
16	1.59m	1.58m (2 H)		1.60m (2 H)	
	1.59m	1.58m (2 H)		1.60m (2 H)	
17	3.86m	3.88m (2 H)		3.70m (2 H)	
18	1.79m	(1.79m	1.81m)	1.76m	1.86m
18-Me	0.92d [6.7]	0.92d [6.3] (6 H)		0.89d [6.3]	0.87d [6.3]
19	5.28br d [11]	(5.26br d [11]	5.32br d [11])	5.32br d [11]	4.00m
20	1.90m	1.93m (2 H)		1.89m	1.98m
20-Me	0.85d [6.3]	(0.88d [6.3]	0.89d [6.3])	0.82d [6.3]	0.83d [6.3]
21	3.05dd [10, 2.1]	3.04m (2 H)		2.95m	4.92br t [6.3]
22	1.64m	1.67m (2 H)		1.66m	2.08m
22-Me	0.98d [6.3]	(0.97d [6.3]	0.98d [6.3])	0.98d [6.3]	0.94d [6.3]
23	1.29m	1.28m (2 H)		1.33m (2 H)	
	1.39m	1.39m (2 H)		(1.37m[e]	1.39m[f])
24	1.22m	1.24m (2 H)		(1.22m[e]	1.23m[f])
	1.86m	1.90m (2 H)		1.89m (2 H)	
25	4.00m	4.00m (2 H)		4.00m (2 H)	
26	1.59m	1.60m (2 H)		1.59m (2 H)	
	1.80m	1.81m (2 H)		1.80m (2 H)	
27	3.54tt [10, 4.5]	3.54tt [10, 4.5] (2 H)		3.53m (2 H)	
27-OMe	3.34s	3.34s (6 H)		3.34s (6 H)	
28	1.16m	1.16m (2 H)		1.14m (2 H)	
	1.98m	1.98m (2 H)		1.98m (2 H)	
29	3.69ddq [11, 2.8, 6.2]	3.69m (2 H)		(3.68m[g]	3.61m[h])
29-Me	1.20d [6.2]	(1.18d [6.3]	1.20d [6.3])	(1.20d [6.3][g]	1.14d [6.3][h])

* Data in parentheses: chemical shifts could not be assigned to *n*-H or *n'*-H exclusively or unambiguously. [a–h] The connectivities were shown by COSY or decoupling experiments.

Shimadzu UV-220 and a JASCO IR Report-100 spectrometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GX-270 or a JEOL EX-400 spectrometer. Mass spectra were obtained on a JEOL HX-110 spectrometer by using glycerol or diethanolamine as matrix.

Isolation.—The sponge *Theonella* sp. (0.8 kg, wet weight) collected by SCUBA off Ishigaki Island, Okinawa, was kept frozen until used. The sponge was extracted with methanol (1 dm^3 × 2). After evaporation, a dark yellow extract (39.1 g) was suspended in aq. 1 mol dm^{-3} NaCl (500 cm^3), which was then extracted with ethyl acetate (500 cm^3 × 3). The combined extracts were evaporated to give a yellow residue (5.58 g), which was subjected to column chromatography on silica gel (Wako gel C-300, Wako Pure Chemical) eluted with 0–50% MeOH–$CHCl_3$ to give two fractions. The first eluted one was rich in bistheonellide A **5**, and further purification by silica gel column chromatography with acetone–hexane (10, 20 and 40%, successively) afforded pure compound **5** (217.4 mg). The later eluted one, containing minor congeners, *viz.* bistheonellides B **6** and C **1** and isobistheonellide A **2**, was chromatographed on silica gel [20, 40 and 60% acetone–hexane and hexane–ethyl acetate–MeOH (8:2:1), separately] to give pure compounds **6** (9.3 mg), **1** (6.8 mg) and **2** (1.3 mg).

The sponge *Theonella* sp. (1.1 kg) collected by SCUBA off Zamami Island, Okinawa, was kept frozen until used. The methanol (1.1 dm^3 × 2) extract of the sponge was evaporated under reduced pressure to afford a residue (54.6 g), which was dissolved in a mixed solvent of ethyl acetate (400 cm^3) and aq. mol dm^{-3} NaCl (400 cm^3) and the mixture was then extracted with ethyl acetate (400 cm^3 × 3). The ethyl acetate-soluble material (6.35 g) was partly (0.6 g) subjected to silica gel flash column chromatography (22 × 380 mm; Wako gel C-300) and eluted with 5–100% MeOH–$CHCl_3$. The fraction eluted after 150–170 cm^3 [MeOH–$CHCl_3$ (5:95)] was further purified on a silica gel column (22 × 340 mm) with acetone–hexane (40:60) to give bistheonellide A **5** (0.01% wet weight) in the 200–250 cm^3 fraction. The fraction (22 mg) of the first silica column eluting after 350–440 cm^3 [MeOH–$CHCl_3$ (15:85)] was separated on a Sephadex LH-20 column (15 × 850 mm) with

MeOH–$CHCl_3$ (1:1) to give bistheonellic acid A **3** (0.005%) in the 110–140 cm^3 fraction. The 140–150 cm^3 fraction of the LH-20 column was further purified on a C_{18} reversed-phase column (15 × 100 mm; YMC-GEL I-40/60 ODS, Yamamura Chemical) eluted with 80% MeOH to give bistheonellic acid B **4** (0.002%) in the 4–16 cm^3 fraction.

Bistheonellide C **1**.—An amorphous solid; $[\alpha]_D^{16}$ −22° (*c* 0.68, EtOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3400 and 1680; λ_{max}(EtOH)/nm 222 (log ε 4.2); δ_H (Table 1); $\delta_C(CDCl_3)$ 170.2, 170.1, 142.2 (2 C), 130.0, 129.9, 128.5 (2 C), 123.4 (2 C), 75.2 (2 C), 74.1 (2 C), 73.3 (2 C), 72.3 (C-13′), 71.4 (2 C), 70.5 (2 C), 66.5 (2 C), 66.4, 66.3, 65.2 (2 C), 64.7, 64.4, 57.2 (13-OMe), 55.3 (27-, 27′-OMe), 43.0 (C-14′), 41.3, 41.0, 40.9 (C-14), 40.6, 40.4, 38.6 (2 C), 38.2 (2 C), 37.4 (2 C), 37.2, 37.1, 37.0 (C-12′), 35.0 (2 C), 34.0 (C-12), 33.5, 33.3, 30.4 (2 C), 29.4, 29.3, 24.3, 24.2, 21.9, 21.8, 17.8 (2 C), 12.8 (2 C), 9.4, 9.3 and 9.2 (4 C); the signals for C-13, -21 and -21′ were not observed because of overlap with that of chloroform; HR-FAB-MS *m/z* 1428.9790 (M + diethanolamine + H)$^+$. $C_{77}H_{138}NO_{22}$ requires *m/z* 1428.9710.

Isobistheonellide A **2**.—An amorphous solid; $[\alpha]_D^{16}$ +43° (*c* 0.13, EtOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3400 and 1680; λ_{max}(EtOH)/nm 220 (log ε 4.3); δ_H (Table 1); HR-FAB-MS *m/z* 1442.9790 (M + diethanolamine + H)$^+$. $C_{78}H_{140}NO_{22}$ requires *m/z* 1442.9867.

Bistheonellic Acid A **3**.—An amorphous solid; $[\alpha]_D^{20}$ −20° (*c* 0.45, $CHCl_3$); λ_{max}(MeOH)/nm 232 (log ε 3.8); ν_{max}(KBr)/cm^{-1} 3425, 2930, 1680, 1640, 1450, 1380 and 1080; $\delta_H(CDCl_3)$ 0.76 (3 H, d, 20′-Me), 0.84 (3 H, d, 20-Me), 0.84 (3 H, d, 18′-Me), 0.87 (6 H, d, 14- and 14′-Me), 0.89 (3 H, d, 18-Me), 0.98 (6 H, d, 22- and 22′-Me), 1.20 (6 H, d, 29- and 29′-Me), 1.64 (1 H, m, 22-H), 1.70 (3 H, m, 14-, 14′- and 18′-H), 1.75 (1 H, m, 22′-H), 1.78 (1 H, m, 18-H), 1.80 (1 H, m, 20′-H), 1.83 (3 H, s, 2-Me), 1.85 (3 H, s, 2′-Me), 1.90 (1 H, m, 20-H), 2.35 (2 H, m, 4- and 4′-H), 3.05 (1 H, br d, 21-H), 3.30 (1 H, m, 21′-H), 3.34 (6 H, s, 27- and 27′-OMe), 3.36 (6 H, s, 13- and 13′-OMe), 3.56 (2 H, m, 27- and 27′-H), 3.70 (2 H, m, 29- and 29′-H), 4.00 (1 H, m, 19′-H), 4.10 (2 H, m, 25- and 25′-H), 4.16 (2 H, m, 5- and 5′-H), 4.53 (2 H, m, 7- and 7′-H), 5.30 (1 H, br d, 19-H), 5.68 (2 H, br d, 9- and 9′-H), 5.82 (2 H, br d, 8- and 8′-H), 6.85 (1 H, br t, 3-H) and 7.00 (1-H, br t, 3′-H); δ_C* (C_6D_6) 9.6 (q, 18-Me), 9.7 (q, 20-Me), 9.8 (q, 20′-Me), 10.3 (q, 18′-Me), 10.8 (2 C, q, 14- and 14′-Me), 13.0 (2 C, q, 2- and 2′-Me), 17.0 (q, 22′-Me), 18.3 (q, 22-Me), 21.9 (2 C, q, 29- and 29′-Me), 24.6 (t, C-23), 28.2 (t, C-23′), 29.3 (t, C-24′), 29.5 (t, C-24), 31.2 (2 C, t, C-10 and -10′), 33.5 (2 C, d, C-22 and -22′), 35.1 (2 C, t, C-12 and -12′), 35.6 (2 C, t, C-26 and -26′), 37.5 (d, C-20′), 37.8 (d, C-20), 38.0 (t, C-4), 38.2 (t, C-4′), 39.0 (2 C, t, C-16 and -16′), 39.2 (2 C, t, C-28 and -28′), 41.0 (d, C-18), 41.4 (d, C-18′), 41.6 (2 C, d, C-14 and -14′), 41.8 (2 C, t, C-6 and -6′), 55.0 (2 C, q, 13- and 13′-OMe), 56.5 (q, 27′-OMe), 56.8 (q, 27-OMe), 64.5 (d, C-11′), 64.7 (d, C-11), 64.8 (d, C-29), 65.1 (d, C-29′), 66.6 (d, C-5′), 67.0 (d, C-5′), 68.2 (d, C-7′), 68.5 (d, C-7), 70.8 (2 C, d, C-25 and -25′), 71.3 (2 C, d, C-17 and -17′), 72.1 (d, C-19′), 73.5 (2 C, d, C-27 and -27′), 75.0 (2 C, d, C-15 and -15′), 76.4 (d, C-19), 76.9 (d, C-21), 77.8 (2 C, d, C-13 and -13′), 80.4 (d, C-21′), 123.6 (2 C, d, C-8 and -8′), 129.0 (s, C-2′), 129.7 (s, C-2), 130.6 (2 C, d, C-9 and -9′), 140.4 (d, C-3), 142.0 (d, C-3′), 170.3 (s, C-1′) and 170.5 (s, C-1); FAB-MS (negative) *m/z* 1353 (M − H)$^-$; HR-FAB-MS (positive) *m/z* 1461.0000 (M + diethanolamine + H)$^+$. $C_{78}H_{124}NO_{23}$ requires *m/z* 1460.9973.

* Assignments of the ^{13}C NMR signals of **3** and **4** are based on comparison with those of bistheonellide.

Bistheonellic Acid B **4**.—An amorphous solid; $[\alpha]_D^{20}$ −7.5° (*c* 0.2, $CHCl_3$); λ_{max}(MeOH)/nm 218 (log ε 3.7); ν_{max}(KBr)/cm^{-1} 3400, 2920, 1700, 1640, 1450, 1380 and 1080; $\delta_H(CDCl_3)$ 0.76 (3 H, d, 20-Me), 0.84 (3 H, d, 18-Me), 0.96 (3 H, d, 22-Me), 1.19 (3 H, d, 29-Me), 1.70 (1 H, m, 18-H), 1.75 (1 H, m, 22-H), 1.82 (1 H, m, 20-H), 1.83 (3 H, s, 2-Me), 2.32 (1 H, m, 4-H), 3.30 (1 H, m, 21-H), 3.34 (3 H, s, 27-OMe), 3.36 (3 H, s, 13-OMe), 3.55 (1 H, m, 27-H), 3.73 (1 H, m, 29-H), 4.00 (2 H, m, 19- and 25-H), 4.12 (1 H, m, 5-H), 4.51 (1 H, m, 7-H), 5.67 (1 H, br d, 9-H), 5.80 (1 H, br d, 8-H) and 6.82 (1 H, br t, 3-H); δ_C* (67.5 MHz; C_6D_6) 9.9 (q, 20-Me), 10.7 (q, 18-Me), 11.3 (q, 14-Me), 13.2 (q, 2-Me), 17.1 (q, 22-Me), 21.8 (q, 29-Me), 27.8 (t, C-23), 29.3 (t, C-24), 31.5 (2 C, t, C-10 and d, C-22), 35.2 (t, C-12), 35.5 (d, C-26), 37.3 (d, C-10), 38.2 (t, C-4), 38.6 (t, C-16), 38.8 (t, C-28), 41.0 (d, C-18), 41.3 (d, C-14), 41.6 (t, C-6), 55.0 (q, 13-OMe), 56.7 (q, 27-OMe), 64.3 (d, C-11), 65.1 (d, C-29), 66.9 (d, C-5), 68.9 (d, C-7), 71.2 (2 C, d, C-17 and -25), 72.2 (d, C-19), 73.5 (d, C-27), 75.4 (d, C-15), 76.7 (d, C-13), 80.1 (d, C-21), 123.6 (d, C-8), 130.1 (s, C-2), 130.8 (d, C-9) and 140.0 (d, C-3); FAB-MS (negative) *m/z* 685 (M − H)$^-$; HR-FAB-MS (negative) *m/z* 685.4523 (M − H)$^-$. $C_{37}H_{65}O_{11}$ requires *m/z* 685.4527.

Methylation of Bistheonellic Acids A **3** *and B* **4**.—To a solution of bistheonellic acid A **3** (1.0 mg) in methanol (0.5 cm^3) was added an excess of diazomethane in diethyl ether (~1 cm^3). After 10 min storage at room temperature, the solution was evaporated under reduced pressure to give the methyl ester **7** (1 mg): $\delta_H(C_6D_6)$ 3.15 (3 H, s, CO_2Me), 3.20 and 3.30 (each 3 H, s, 13- and 13′-OMe), 3.45 and 3.50 (each 3 H, s, 27- and 27′-OMe), 4.65 (2 H, m, 7- and 7′-H), 5.45–5.65 (4 H, m, 8-, 8′-, 9- and 9′-H), 5.75 (1 H, d, 19-H) and 7.34 (1 H, br t, 3′-H); FAB-MS (positive) *m/z* 1474 (M + diethanolamine + H)$^+$.

By the same procedure, bistheonellic acid B **4** was converted into its methyl ester **8**, whose spectral data were identical with those described in the literature.[2]

Alkaline Hydrolysis of Bistheonellic Acid A **3** *and Bistheonellide A* **5**.—To a solution of bistheonellic acid A **3** (1.5 mg) in MeOH (1 cm^3) was added aq. 2 mol dm^{-3} KOH (0.5 cm^3). After being stirred at room temperature for 2.5 h, the reaction mixture was acidified with 2 mol dm^{-3} HCl, extracted with chloroform (2.5 cm^3 × 5), and dried over $MgSO_4$ to give bistheonellic acid B **4** (1.5 mg). A solution of bistheonellide A **5** (2.0 mg) in MeOH (1 cm^3) was also treated with 2 mol dm^{-3} KOH (0.5 cm^3) at room temperature for 5 h to afford bistheonellic acid B **4** (1.9 mg).

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