Fonds Documentaire IRD
Cote : B*26199 Ex : 1

Reprinted from the Journal of the American Chemical Society, 1994, *116*.

Copyright © 1994 by the American Chemical Society and reprinted by permission of the copyright owner.

Microsclerodermins A and B. Antifungal Cyclic Peptides from the Lithistid Sponge *Microscleroderma* sp.

Carole A. Bewley,† Cécile Debitus,‡ and D. John Faulkner*,†

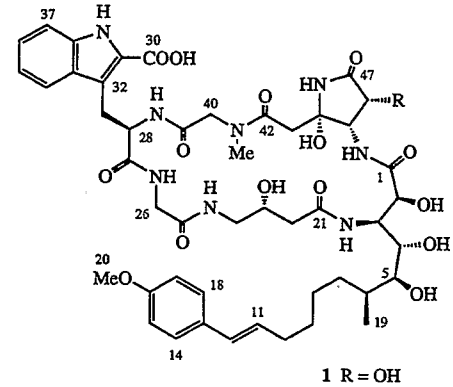
Contribution from the Scripps Institution of Oceanography, 0212, University of California at San Diego, La Jolla, California 92093-0212, and ORSTOM, Centre de Noumêa, B. P. A5 Noumêa Cédex, New Caledonia

Received April 11, 1994®

Abstract: The microsclerodermins are cyclic hexapeptides from a deep water sponge of the genus *Microscleroderma*. The structures of microsclerodermins A (**1**) and B (**2**) were determined by interpretation of spectral data. The absolute stereochemistry at each of the 10 asymmetric centers was determined by interpretation of NMR data, particularly the nOe data, and by chemical degradation followed by analysis of the resulting amino-acids by GC-MS using a chiral column. Microsclerodermins A and B inhibit the growth of *C. albicans* at 2.5 μg/disk. Examination of the symbiotic filamentous micro-organisms associated with the sponge revealed that they were eubacteria similar in appearance to members of the family Beggiatoaceae.

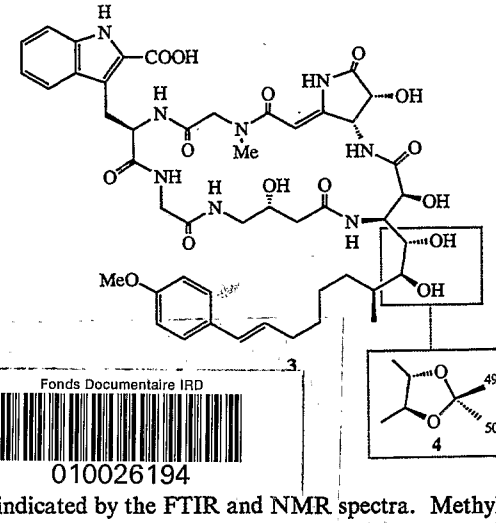
Sponges of the order Lithistida produce some of the most bioactive and complex marine natural products, among which are the cyclotheonamides,[1] the calyculins,[2] discodermolide,[3] the onnamides,[4] theonellamide F,[5] and swinholide.[6] We became interested in lithistid sponges because they often contain symbiotic filamentous bacteria in the interior of the sponge. Our aim is to investigate the role of symbionts, such as the filamentous bacteria, in the production of sponge metabolites by determining the cellular location of the metabolites. At the same time we recognized that the structural elucidation of the antifungal cyclic peptides from lithistid sponges would present an interesting challenge. In this paper we report the structural elucidation of two new cyclic peptides microsclerodermins A (**1**) and B (**2**) from a deep-water sponge and comment on the nature of the symbiotic filamentous bacterium associated with the sponge.

A specimen of the lithistid sponge *Microscleroderma* sp. was collected by dredging at a depth of 1000 ft off the Norfolk Rise near New Caledonia. The sponge was freeze-dried and then extracted sequentially with hexanes, ethyl acetate, and 50% aqueous acetonitrile. The crude aqueous extract showed antifungal activity against *Botrytis cinerea*, *Candida albicans*, *Fusarium oxysporum*, *Helminthosporium sativum*, and *Pyricularia oryzae*. Bioassay-guided fractionation of the aqueous extract using *C. albicans* as the test organism[7] resulted in the isolation of two polar antifungal metabolites, microsclerodermins A (**1**, 0.005% dry wt) and B (**2**, 0.001% dry wt).



1 R = OH
2 R = H

Microsclerodermin A (**1**) has the molecular formula $C_{47}H_{62}N_8O_{16}$, as determined by HRMS [m/z = 995.4395 $(M + H)^+$], but readily loses one molecule of water on treatment with mild acid or dehydrating agents to obtain dehydromicrosclerodermin A [**3**, m/z = 977 $(M+H)^+$]. The peptidal nature of the compound



was indicated by the FTIR and NMR spectra. Methylation of **1** with diazomethane in ethanol produced a monomethyl ester (m/z = 1008) while acetylation of either **1** or **3** with acetic anhydride in pyridine containing DMAP gave the same pen-

Fonds Documentaire IRD
010026194

† University of California at San Diego.
‡ ORSTOM.
® Abstract published in *Advance ACS Abstracts*, July 15, 1994.

(1) Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. *J. Am. Chem. Soc.* **1990**, *112*, 7053. Hagihara, H.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6570.
(2) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Koseki, K. *J. Org. Chem.* **1988**, *53*, 3930. Matsunaga, S.; Fujiki, H.; Sukata, D.; Fusetani, N. *Tetrahedron* **1991**, *47*, 2999.
(3) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912; **1991**, *56*, 1346.
(4) Matsunaga, S.; Fusetani, N.; Nakao, Y. *Tetrahedron* **1992**, *48*, 8369.
(5) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Walchli, M. *J. Am. Chem. Soc.* **1989**, *111*, 2582.
(6) Kobayashi, M.; Tanaka, J.; Katori, T.; Matsuura, M.; Yamashita, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2409. Kobayashi, M.; Tanaka, J.; Katori, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2960. Tsukamoto, S.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Chem. Soc., Perkin Trans. 1* **1991**, 3185.
(7) Acar, J. F.; Goldstein, F. W. In *Antibiotics in Laboratory Medicine*, 2nd ed.; Lorian, V., Ed.; Williams & Wilkins: Baltimore, MD, 1986; Chapter 2.

0002-7863/94/1516-7631$04.50/0 © 1994 American Chemical Society

taacetate [m/z = 1187 (M + H)$^+$], indicating the presence of one carboxylic acid and six hydroxyl groups in **1**. The ^{1}H NMR spectrum in DMSO-d_6 contained six amide NH signals between δ 7.15 and 8.05, an N-methyl signal at 2.85, and an indole NH signal at 10.5, accounting for all eight of the nitrogen atoms. The ^{13}C NMR spectrum revealed eight carbonyls, seven involved in amide linkages and one carboxylic acid, that together with an O-methyl group and the six hydroxyl groups account for all of the oxygen atoms.

Standard amino acid analysis revealed that glycine was the only common amino acid in **1**. *N*-Methylglycine and 4-amino-3-hydroxybutyric acid were easily identified by interpretation of the NMR data (see Table 1). Analysis of the aromatic region of the ^{1}H and ^{13}C NMR spectra provided evidence for an indole ring system that was substituted at both the 2- and 3-positions, C-31 and C-32, respectively. The HMBC[8] spectrum showed correlations between both H-29 signals and C-27, C-28, C-31, C-32, and C-33, which indicated alkyl substitution at the 3-position (C-32). The assignment of a carboxylic acid residue at the 2-position (C-32) was based on comparison of the ^{13}C chemical shift of C-31 at δ 117.5 with those of model compounds, such as 3,5-dimethylpyrrole-2-carboxylic acid (C-2:δ 117.4)[9a] and 3-methylindole-2-carboxylic acid (C-2:δ 117.8).[9b] These data fully support the presence of a tryptophan-2-carboxylic acid residue, which appears to be undescribed. The identity of the pyrrolidone ring system within the C-42 to C-47 portion of **1** was elucidated by analysis of the NMR spectra, particularly the HMBC data, and by comparing the NMR data of **1** with those of **3**. The isolated methylene group appeared in the ^{1}H NMR spectrum as two doublets at δ 2.52 and 2.78 which, in the HMBC spectrum, are correlated with C-42, C-44, and C-45. The chemical shift of C-44 at δ 82.0 (s) is appropriate for a carbon bearing both a hydroxyl and an N-acyl group. The HMBC correlations from H-45 to all of the ring carbons and the correlations between NH-44 and the ring carbons defined the pyrrolidone ring system. On dehydration, a new double bond is formed from C-43 (δ 88.8) to C-44 (δ 152.8); the NMR data of **3** (see Table 2) are consistent with the formation of a 3-hydroxy-4-amido-5-vinylpyrrolidone ring system.[10]

The aromatic region of the ^{1}H NMR spectrum contained signals for a *p*-substituted benzene ring at δ 6.84 (d, 2 H, J = 8.5 Hz) and 7.28 (d, 2 H, J = 8.5 Hz) and a disubstituted olefin at δ 6.28 (d, 1 H, J = 15.5 Hz) and 6.10 (dt, 1 H, J = 15.5, 7 Hz) that, on analysis of the ^{13}C NMR and HMBC data, were shown to constitute a *p*-methoxy-*trans*-styrene moiety. The C-1 to C-6 portion of the molecule was difficult to establish by NMR spectroscopy because the signals at δ 4.42 (br s, 1 H) and 4.15 (dd, 1 H, J = 10, 9 Hz) are not coupled, implying that the dihedral angle between H-2 and H-3 is about 90°. However, the ROESY[11] spectrum clearly indicated the proximity of these hydrogens. The placement of an amide functionality at C-3 and hydroxyls at C-2, C-4, and C-5 was based on the ^{1}H and ^{13}C NMR chemical shifts. The isolation of the intact C-1 to C-4 unit as β-hydroxyaspartic acid (see below) confirmed the C-1 to C-5 assignments. A methyl group was positioned at C-6 on the basis of HMBC data. This left only four methylene signals in the ^{13}C spectrum unassigned, and the COSY, TOCSY, and HMBC data were used to place them as a methylene chain from C-7 to C-10. At this stage we have identified six amino acid residues and, by analysis of the unsaturation equivalents, determined that **1** was a cyclic peptide. Linkages between the amino acid residues were established by interpretation of the HMBC and ROESY data to obtain the planar structure for microsclerodermin A (**1**).

The stereochemistry of **1** was determined by a series of degradation experiments coupled with analysis of NOE data. In each of the sub-milligram-scale degradation reactions, the amino acids were analyzed as their pentafluoropropionamide *N*-isopropyl ester derivatives by GC-MS using a chiral capillary column. Hydrolysis of **1** using 6 N hydrochloric acid at 100 °C in a sealed tube gave racemic 3-hydroxy-4-aminobutyric acid,[12] but treatment of **1** with neat methanesulphonic acid at 110 °C in a sealed tube gave (3*R*)-3-hydroxy-4-aminobutyric acid. The (*R*)-stereochemistry at the α-carbon in the tryptophan-2-carboxylic acid residue was established by ozonolysis of **1**, followed by an oxidative workup and hydrolysis of the product with 6 N hydrochloric acid to obtain (*R*)-aspartic acid. Ozonolysis of **3** followed by hydrolysis of the reaction product with 6 N hydrochloric acid gave (2*S*,3*R*)-3-hydroxyaspartic acid, indicating that the stereochemistry in the pyrrolidone ring must be (45*S*,46*R*). The (44*S*)-stereochemistry was determined by the observation of ROESY correlations between H-43, H-45, and H-46. Cleavage of the C-4, C-5 diol with sodium periodate, followed by oxidative workup and hydrolysis with 6 N hydrochloric acid, gave (2*S*,3*S*)-3-hydroxyaspartic acid, which requires the (2*S*,3*R*)-stereochemistry in **1**. The stereochemistry about the C-4, C-5 diol was established by treating **3** with dimethoxypropane in DMF to obtain the acetonide **4**.[13] The ROESY experiment on the acetonide indicated a *threo* relationship between the hydroxyl groups at C-4 and C-5. Furthermore, examination of molecular models showed that the observed NOE correlations were consistent with the (2*S*,3*R*,4*S*,5*S*)-stereochemistry but not the (2*S*,3*R*,4*R*,5*R*)-stereochemistry (see Figure 1). The (6*S*)-stereochemistry was also assigned from the ROESY data. Examination of the molecular model showed that the correlations between H-6 and both H-4 and H-5 and between the methyl group at C-6 and H-5 were only possible for the (6*S*)-stereochemistry (see Figure 1).

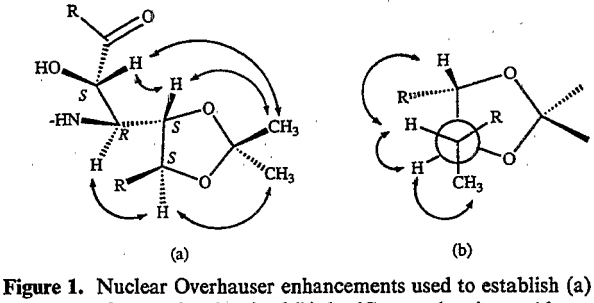


Figure 1. Nuclear Overhauser enhancements used to establish (a) the 2*S*,3*R*,4*S*,5*S* stereochemistry and (b) the 6*S* stereochemistry. Alternative stereochemical arrangements cannot account for all of the observed NOE correlations.

Microsclerodermin B (**2**) has the molecular formula $C_{47}H_{62}N_8O_{15}$, as revealed by the low-resolution mass spectrum (m/z = 978.4) and the high-resolution mass spectrum of the corresponding dehydro derivative [m/z = 961.4256 (M + H)$^+$]. Analysis of the NMR data revealed that the only change was the replacement of the hydroxyl group at C-46 by hydrogen. The key differences in the NMR data are that the signals at δ_C 70.9 and δ_H 4.12 (br d, 1 H, J = 7.5 Hz) in **1** are replaced by signals at δ_C 32.6 and δ_H 2.43 (dd, 1 H, J = 17, 5.5 Hz) and 2.75 (dd, 1 H, J = 17, 10 Hz) in **2**. Otherwise, the similarity between the spectral data of **1** and **2** strongly suggests that the stereochemistry of microsclerodermin B (**2**) is the same as that of microsclerodermin A (**1**).

(8) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.

(9) Sadtler standard carbon-13 NMR spectra, Sadtler Research Laboratories, Philadelphia, PA. (a) # 23,174C. (b) # 19,719C; the spectrum should be assigned as follows: δ 163.8 (COOH), 136.3 (C-7′), 128.0 (C-3), 124.4 (C-3′ and C-6), 120.0 (C-4), 119.0 (C-5), 117.8 (C-2), 112.3 (C-7), 9.7 (CH_3).

(10) For ^{1}H and ^{13}C chemical shifts of model compounds possessing similar pyrrolidinone moieties, see: Orr, D. E. *Synthesis* **1984**, *7*, 618. Hart, D. J. *J. Org. Chem.* **1981**, *46*, 367.

(11) (a) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811. (b) Kessler, H.; Griesinger, C.; Kerssebaum, R.; Wagner, K.; Ernst, R. *J. Am. Chem. Soc.* **1987**, *109*, 607.

(12) Rossiter, B. E.; Sharpless, K. B. *J. Org. Chem.* **1984**, *49*, 3707.

(13) Alternative acetonides were ruled out by analysis of the ^{1}H NMR spectrum.

Table 1. Correlated ^{13}C and ^{1}H NMR Spectral Data and Proton–Proton NOE Data for Microsclerodermin A (**1**)

amino acid	assignment	δ[a] ^{13}C (mult)[b]	δ[a] ^{1}H (mult, J (Hz))[c]	HMBC[c]	NOE[c,d]
AMMTD[e]	1	173.5 (s)			
	2	69.3 (d)	4.42 (brs)	C1	3, 4, NH3, NH45, OH2
	3	53.5 (d)	4.15 (dd, 10.0, 9.0)	C:4, 21	2, NH3,[g] OH4
	4	68.4 (d)	3.42 (brd, 9.0)	C3	2, 5, 19, NH3, OH2, OH4, OH5
	5	72.9 (d)	3.05 (m)		4, 6,[g] 7′,[g] 19, NH3,[g] OH5
	6	34.6 (d)	1.56 (m)	C:5, 8	4, 5,[g] 19, OH4, OH5[g]
	7	32.7 (t)	1.00 (m)	C8	5,[g] 6,[g] 7′, 8′
			1.70 (m)	C8	5, 7′, OH5[g]
	8	26.0 t	1.19 (m)	C7	6, 7′, 9, 10
			1.37 (m)		6, 7′, 9, 10
	9	29.6 (t)	1.38 (m)	C:8, 10	10, 11
	10	32.5 (t)	2.12 (m)	C:8, 9, 11	5, 9, 11, 12
	11	128.4 (d)	6.10 (ddd, 15.5, 7.0, 7.0)	C13	9, 10
	12	128.9 (d)	6.28 (d, 15.5)	C:13, 14, 18	10
	13	130.3 (s)			
	14	126.9 (d)	7.28 (d, 8.5)	C:12, 15, 16, 18	
	15	113.9 (d)	6.84 (d, 8.5)	C:13, 16, 17	20
	16	158.0 (s)			
	17	114.0 (d)	6.84 (d, 8.5)	C:13, 15, 16	20
	18	126.9 (d)	7.28 (d, 8.5)	C:12, 14, 16, 17	
	19	15.5 (q)	0.75 (d, 6.0)	C:5, 6, 7	4, 5, 6
	20	55.1 (q)	3.72 (s)	C16	22′, NH3,[g] NH24, OH5
	NH3		7.25 (d, 10.0)	C21	4, 22, 23[g]
	OH2		6.28		2, 4, NH3, NH24
	OH4		4.28		2, 3,[g] 6
	OH5		4.08		3, 5, 6,[g] 7′[g]
GABOB[f]	21	172.5 (s)			
	22	40.9 (t)	2.11 (dd, 10.5, 14.0)	C:21, 23	22′, 23
			2.39 (dd, 2.5, 14.0)	C:21, 23	22, 23, 24, 41,[g] NH3, NH24
	23	66.9 (d)	3.72 (m)		22′, NH3,[g] NH24, OH23
	24	45.2 (t)	2.60 (m)	C:22, 23	24′, NH24
			3.36 (m)	C:22, 23	24, NH24
	NH24		7.40 (dd, 5.5, 5.5)		22′, 23, 24, 24′, 26′,[g] OH23
	OH23		4.80		22, 23, NH3, NH24
Gly	25	169.0 (s)			
	26	42.8 (t)	3.13 (dd, 17.5, 5.5)	C:25, 27	26′, NH26
			3.55 (dd, 17.5, 5.5)	C:25, 27	26, NH24, NH26[g]
	NH26		8.30 (dd, 5.5, 5.5)	C:26, 27	26, 28
Trp-2COOH	27	171.7 (s)			
	28	56.0 (d)	4.09 (ddd, 5.0, 5.5, 5.5)	C:27, 29	29, 29′, 34
	29	25.4 (t)	3.38 (dd, 13.0, 5.5)	C:27, 28, 31, 32, 33	28, 29′, 34
			3.46 (dd, 13.0, 5.5)	C:27, 28, 31, 32, 33	29, 34
	30	163.5 (s)			
	31	117.5 (s)			
	32	125.2 (s)			
	33	127.9 (s)			
	34	120.2 (d)	7.52 (d, 8.0)	C:36, 38	28, 29, 29′, 40′
	35	119.4 (d)	7.05 (dd, 8.0, 8.0)	C:33, 37	
	36	124.3 (d)	7.21 (dd, 8.0, 8.0)	C:34, 38	
	37	112.3 (d)	7.38 (d, 8.0)	C:33, 35	
	38	135.9 (s)			
	NH28		8.72 (d, 5.0)	C39	28, 40′
	indoleNH		11.50 (s)	C:31, 32, 33, 38	37
	COOH30		13.00 (br s)		
NMeGly	39	170.6 (s)			
	40	49.3 (t)	3.53 (d, 16.0)	C:39, 41, 42	40′, 41, NH24
			4.42 (d, 16.0)	C:39, 41, 42	40
	41	36.2 (q)	2.89 (s)	C:40, 42	40, 43
pyrrolidone	42	170.7 (s)			
	43	39.5 (t)	2.52 (d)	C:42, 44, 45	41, 43′, 45, 46, NH44
			2.78 (d)	C:42, 44, 45	41, 43
	44	82.0 (s)			
	45	59.2 (d)	4.22 (dd, 8.0, 7.5)	C:43, 44, 46, 47	43, NH44,[g] NH45, OH46
	46	70.9 (d)	4.12 (brd, 7.5)	C:45, 47	43, 45, NH44,[g] NH45, OH46
	47	173.5 (s)			
	NH44		8.05 (s)	C:44, 45, 46	43, 45,[g] 46[g]
	OH44		*h*		
	NH45		7.45 (d, 8.0)	C:1, 45, 46	2, 45,[g] 46,[g] OH2
	OH46		5.56	C45	45, 46

[a] Referenced to residual solvent DMSO-d_6. [b] ^{13}C spectra recorded on a Bruker WP200SY at 50 MHz. [c] ^{1}H spectra recorded on a Varian Unity 500 at 500 MHz. [d] Spin lock used. [e] AMMTD = (2*S*,3*R*,4*S*,5*S*,6*S*,11*E*)-3-amino-6-methyl-12-(*p*-methoxyphenyl)-2,4,5-trihydroxydodec-11-enoic acid. [f] GABOB = (3*R*)-4-amino-3-hydroxybutyric acid. [g] Weak NOE. [h] Not observed.

Both microsclerodermins A (**1**) and B (**2**) inhibit the growth of *C. albicans* at a loading of 2.5 μg/disk in the standard disk assay.[7] We have examined a number of lithistid sponges from both shallow-water and deep-water environments and found that

Table 2. Correlated ^{13}C and ^{1}H NMR Spectra Data and Proton–Proton NOE data for Dehydromicrosclerodermin A (3)

amino acid	assignment	δ[a] ^{13}C (mult)[b]	δ[a] ^{1}H (mult, J (Hz))[c]	HMBC[c]	NOE[c,d]
AMMTD[e]	1	174.5 (s)			
	2	69.5 (d)	4.45 (brs)	C:1, 3	3, NH45, OH2
	3	54.0 (d)	4.08 (dd, 10.0, 9.0)	C:4, 21	2, 5, NH3
	4	68.7 (d)	3.41 (brd, 9.0)		5, 6, NH3, OH2, 4
	5	73.0 (d)	3.05 (m)	C6	4, 6, 7, NH3, OH5
	6	34.1 (d)	1.56 (m)		5, 8, 7, 19
	7	32.8 (t)	1.00 (m)		5, 8
			1.70 (m)		5, 8
	8	26.3 (t)	1.18 (m)		6, 7, 9
			1.38 (m)		6, 7, 9
	9	29.7 (t)	1.38 (m)	C:8, 10	10, 11
	10	32.6 (t)	2.14 (m)	C:8, 9	9, 11
	11	128.5 (d)	6.10 (ddd, 15.5, 7.0, 7.0)	C:9, 10, 13	9, 10
	12	129.0 (d)	6.30 (d, 15.5)	C:10, 13, 14, 18	9, 10
	13	130.3 (s)			
	14	127.2 (d)	7.28 (d, 8.5)	C:12, 15, 16, 18	11, 12, 15
	15	114.0 (d)	6.84 (d, 8.5)	C:13, 16, 17	14, 20
	16	158.0 (s)			
	17	114.0 (d)	6.84 (d, 8.5)	C:13, 15, 16	18, 20
	18	127.2 (d)	7.28 (d, 8.5)	C:12, 14, 16, 17	11, 12, 17
	19	15.5 (q)	0.75 (d, 6.0)	C:5, 6	4, 5, 6, 7
	20	55.3 (q)	3.70 (s)	C16	15, 17
	NH3		7.15 (d, 10.0)	C21	4, 5, 22, 22′, NH45, OH2, OH5
	OH2		6.25	C:1, 2, 3	4, 5, 6, 7
	OH4		4.30		3, 4
	OH5		4.10		
GABOB[f]	21	170.3 (s)			
	22	41.0 (t)	2.08 (dd, 10.5, 14.0)	C:21, 23	22′, 24, 43,[g] NH3
			2.30 (dd, 2.5, 14.0)	C:21, 23	22, 23, 41,[g] 43,[g] NH24
	23	67.1 (d)	3.65 (m)		22′, 43,[g] NH24
	24	45.8 (t)	2.60 (m)		24′, NH24
			3.40 (m)		24, 43, NH24
	NH24		7.44 (dd, 5.5, 5.5)	C25	22′, 23, 24, 24′, NH45[g]
	OH23		5.05		23, 24′
Gly	25	169.3 (s)			
	26	43.0 (t)	3.28 (dd, 17.5, 5.5)	C:25, 27	26′, NH26
			3.60 (dd, 17.5, 5.5)	C:25, 27	26, NH26
	NH26		8.28 (dd, 5.5, 5.5)	C:26, 27	26, 26′, 28, NH26
Trp-2COOH	27	171.3 (s)			
	28	56.0 (d)	4.00 (ddd, 5.0, 5.5, 5.5)		29, NH26, 28
	29	25.1 (t)	3.37 (dd, 13.0, 5.5)	C:27, 28, 31, 33	28, 29′, 34, NH26
			3.53 (dd, 13.0, 5.5)	C:27, 28, 31, 33	28, 29
	30	163.5 (s)			
	31	117.5 (s)			
	32	125.2 (s)			
	33	127.9 (s)			
	34	120.2 (d)	7.52 (d, 8.0)	C:35, 36, 38	
	35	119.4 (d)	7.05 (dd, 8.0, 8.0)	C:33, 37	
	36	124.3 (d)	7.21 (dd, 8.0, 8.0)	C:34, 38	
	37	112.3 (d)	7.38 (d, 8.0)	C:33	
	38	135.9 (s)			
	NH28		8.72 (d, 5.0)	C39	28, 29, 40′
	indoleNH		11.50 (s)	C:31, 32, 33, 38	
	COOH30		13.00 (br s)		
NMeGly	39	170.6 (s)			
	40	49.5 (t)	3.36 (d, 16.0)	C:39, 41, 42	40′
			4.48 (d, 16.0)	C:39, 41, 42	NH28
	41	36.5 (q)	2.85 (s)	C:40, 42	40, 43, 22′[g]
pyrrolidinone	42	168.0 (s)			
	43	88.8 (d)	5.10 (d, 1.5)	C:42, 45	22,[g] 24,[g] 41, NH45
	44	152.8 (s)			
	45	55.1 (d)	4.82 (ddd, 8.0, 7.5, 1.5)	C:44, 46	43, NH44,[g] NH45, OH46
	46	71.6 (d)	4.40 (brd, 7.5)	C:45, 47	45, NH44,[g] NH45, OH46
	47	174.5 (s)			
	NH44		10.50 (s)	C:44, 45, 46, 47	45,[g] 46[g]
	OH45		*h*		
	NH45		8.37 (d, 8.0)	C:1, 45	2, 43, 45, 46, NH3, OH2
	OH46		6.05		45, 46

[a] Referenced to residual solvent DMSO-d_6. [b] ^{13}C spectra recorded on a Bruker WP200SY at 50 MHz. [c] ^{1}H spectra recorded on a Varian Unity 500 at 500 MHz. [d] Spin lock used. [e] AMMTD = (2*S*,3*R*,4*S*,5*S*,6*S*,11*E*)-3-amino-6-methyl-12-(*p*-methoxyphenyl)-2,4,5-trihydroxydodec-11-enoic acid. [f] GABOB = (3*R*)-4-amino-3-hydroxybutyric acid. [g] Weak NOE. [h] Not observed.

not all lithistid sponges contain symbiotic filamentous microorganisms. However, it may be significant that, in the relatively small sample of lithistid sponges that we have examined (17 in total), there is a correlation between antifungal activity, which is attributed to the cyclic peptides possessing the ω-phenyl-β-amino acids of various chain lengths, and the presence of the

filamentous symbionts. Because of the similarities between compounds reported from lithistid sponges and those found in certain cyanobacteria, it had been presumed that the symbionts were filamentous cyanobacteria. We became suspicious of this assignment because the filaments in the specimens we have studied do not autofluoresce and are found only in the interior of the sponge. In addition, lithistid sponges are often found in environments where there is little light and their crude extracts do not appear to contain chlorophyll. Examination of the filaments by transmission electron microscopy clearly reveals that they are not cyanobacteria because they lack the characteristic thylakoid structures. Analysis of the TEM pictures suggests that the filamentous bacteria may be most closely related to those belonging to the family Beggiatoaceae,[14] such as *Beggiatoa* sp., a filamentous bacterium that occurs as mats in deep-water environments such as the deep-sea vents.

Experimental Section

Instrumentation. UV and IR spectra were recorded on Perkin-Elmer Lambda 3B and 1600 series spectrometers, respectively. Optical rotations were measured on a Rudolph Autopol III polarimeter (*c* g/100 mL) at 589 nm. NMR spectra were recorded on Varian Unity 500 and Bruker WP200SY spectrometers. Effective field strengths and mixing times used in the TOCSY[15] and ROESY[11] experiments were 70 ms at 8.5 kHz and 100 ms at 2.0 kHz (Kessler spinlock used, $\beta = 30°$), respectively. Chiral GC-MS experiments were performed on a Hewlett Packard 5890A gas chromatogram fitted with an Alltech Chirasil-Val capillary column (0.32 mm × 25 m) and interfaced to a Hewlett Packard 5988A mass spectrometer. The oven temperature was ramped from 50 to 210 °C at 4 °C/min and a mass range of 50–600 Da was recorded every 1.96 s. FAB mass spectra were measured on a VG ZAB mass spectrometer at the Regional Mass Spectrometry Facility, UC Riverside.

Isolation. The sponge *Microscleroderma* sp. (reference number R1466, ORSTOM Centre in Nouméa) was collected by dredging at ca. 1000 ft on the Norfolk Rise off the coast of New Caledonia. Following sorting, the sponge was immediately frozen and stored at –20 °C until being freeze-dried prior to extraction. The lyophilized sponge (600 g) was extracted with hexanes (2 × 800 mL to yield 600 mg of brown oil) and ethyl acetate (3 × 800 mL to yield 850 mg of amber oil), followed by exhaustive extraction with 50% aqueous CH_3CN (until antifungal activity was no longer detected in the extract) to yield a salty powder (ca. 12 g). The aqueous extract was applied in 12 portions to C-18 Sep-paks (Millipore-Waters, 10-g size) that were preconditioned by washing with 75 mL of 80% and 40% aqueous CH_3CN, followed by equilibration with 150 mL of water. Following loading, the columns were washed with water (50 mL) and eluted sequentially with 10%, 20%, 30%, 50%, 60%, and 80% aqueous CH_3CN (25 mL of each except for 50 mL of 50%). Fractions eluting with 50% aqueous CH_3CN showed reproducible activity against *C. albicans* and were combined for further purification on reversed-phase HPLC (YMC ODS-AM (300 mm × 10 mm); 40% CH_3CN in 0.05% TFA at 3.8 mL/min; UV detection, 215 nm) to obtain microsclerodermins A (**1**, 27 mg, 0.005% dry wt) and B (**2**, 6 mg, 0.001% dry wt).

Microsclerodermin A (1): white powder; $[\alpha]_D = -113°$ (*c* 0.53, 0.1 M NH_4HCO_3, pH 7.0),[16] UV (MeOH) (nm) 208 (ϵ 7600), 260 (ϵ 2500), 290 (ϵ 1900); IR (KBr) 3310, 3070, 2930, 1660, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 1; HRFABMS obsd $m/z = 995.4395$ $(M+H)^+$, $C_{47}H_{63}N_8O_{16}$ requires $m/z = 995.4362$.

Microsclerodermin B (2): white powder; $[\alpha]_D = -64°$ (*c* 0.23, 0.1 M NH_4HCO_3, pH 7.0); UV (MeOH) (nm) 204 (ϵ 6100), 260 (ϵ 2100), 290 (ϵ 1700); IR (KBr) 3310, 3070, 2930, 1660, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.75 (d, 3 H, $J = 6$ Hz, H-19), 1.00 (m, 1 H, H-7), 1.18 (m, 1 H, H-8), 1.37 (m, 1 H, H-8′), 1.38 (m, 1 H, H-9), 1.56 (m, 1 H, H-6), 1.70, (m, 1 H, H-7′), 2.03 (dd, 1 H, $J = 14.5$, 12 Hz, H-22), 2.14 (m, 1 H, H-10), 2.32 (dd, 1 H, $J = 12$, 1.5 Hz, H-22′), 2.43 (dd, 1 H, $J = 17$, 5.5 Hz, H-46), 2.60 (m, 1 H, H-24), 2.75 (dd, 1 H, $J = 17$, 10 Hz, H-46′), 2.85 (s, 3 H, H-41), 3.05 (m, 1 H, H-5), 3.28 (dd, 1 H, $J = 17.5$, 5.5 Hz, H-26), 3.32 (d, 1 H, $J = 16$ Hz, H-40), 3.37 (dd, 1 H, $J = 13$, 5.5 Hz, H-29), 3.39 (br d, 1 H, $J = 13.5$ Hz, H-4), 3.40 (m, 1 H, H-24′), 3.51 (dd, 1 H, $J = 13.0$, 5.5 Hz, H-29′), 3.60 (dd, 1 H, $J = 17.5$, 5.5 Hz, H-26′), 3.65 (m, 1 H, H-23), 3.70 (s, 1 H, H-20), 3.98 (ddd, 1 H, $J =$ 5.5, 5.5, 5 Hz, H-28), 4.08 (dd, 1 H, $J = 13.5$, 6 Hz, H-3), 4.10 (br s, 1 H, OH-5), 4.30 (br s, 1 H, OH-4), 4.42 (br s, 1 H, H-2), 4.55 (d, 1 H, $J = 16$ Hz, H-40′), 4.82 (ddd, 1 H, $J = 10$, 9, 5.5 Hz, H-45), 5.05 (br s, 1 H, OH-23), 5.10 (s, 1 H, H-43), 6.10 (ddd, 1 H, $J = 15.5$, 7, 7 Hz, H-11), 6.25 (br s, 1 H, OH-2), 6.30 (d, 1 H, $J = 15.5$ Hz, H-12), 6.84 (d, 2 H, $J = 8.5$ Hz, H-15, -17), 7.05 (dd, 1 H, $J = 8$, 8 Hz, H-35), 7.08 (d, 1 H, $J = 6$ Hz, NH-3), 7.22 (dd, 1 H, $J = 8$, 8 Hz, H-36), 7.28 (d, 2 H, $J = 8.5$ Hz, H-14, –18), 7.38 (d, 1 H, $J = 8.0$ Hz, H-37), 7.42 (dd, 1 H, $J = 5.5$, 5.5 Hz, NH-24), 7.52 (d, 1 H, $J = 8$ Hz, H-34), 8.30 (dd, 1 H, $J = 5.5$, 5.5 Hz, NH-26), 8.37 (d, 1 H, $J = 9$ Hz, NH-45), 8.62 (d, 1 H, $J = 5$ Hz, NH-28), 10.5 (s, 1 H, NH-44); ^{13}C NMR (DMSO-d_6) δ 15.5 (q), 25.1 (t), 26.3 (t), 29.7 (t), 32.6 (t), 32.8 (t), 34.1 (d), 36.5 (q), 41.0 (t), 43.0 (t), 45.4 (t), 45.8 (t), 49.5 (t), 54.0 (d), 55.1 (d), 55.3 (d), 56.0 (d), 67.1 (d), 68.7 (d), 69.5 (d), 74.2 (d), 87.6 (d), 112.3 (d), 114.0 (d, 2 C), 117.5 (s), 119.4 (d), 120.2 (d), 124.3 (d), 125.2 (s), 127.2 (d, 2 C), 127.9 (s), 128.5 (d), 129.0 (d), 130.0 (s), 135.9 (s), 152.8 (s), 158.0 (s), 163.5 (s), 168.0 (s), 169.3 (s), 170.3 (s), 170.6 (s), 171.3 (s), 174.5 (s, 2 C); MS $m/z = 978.4$ (M^+); HRFABMS obsd $m/z = 961.4256$ $(M + H - H_2O)^+$, $C_{47}H_{61}N_8O_{14}$ requires $m/z = 961.4307$.

Dehydration of Microsclerodermin A (1). A solution of microsclerodermin A (**1**, 11 mg) in DMSO (200 μL) was diluted with 1.0% aqueous TFA (to 2 mL). The resulting solution was warmed to 35 °C for 5 min, diluted with water to 5 mL, and lyophilized immediately. The reaction mixture was purified by HPLC (YMC ODS-AM; 45% CH_3CN in 0.05% TFA at 3.4 mL/min; UV detection, 215 nm) to obtain starting material (**1**, 1 mg) and dehydromicrosclerodermin A (**3**, 8 mg, 80% yield): white powder, ^1H NMR (DMSO-d_6) see Table 2; ^{13}C NMR (DMSO-d_6) see Table 2; HRFABMS obsd $m/z = 977.4285$ $(M + H)^+$, $C_{47}H_{61}N_8O_{15}$ requires $m/z = 977.4256$.

Acetylation of Microsclerodermin A (1). A solution of microsclerodermin A (**1**, 500 μg), acetic anhydride (95 μL), DMAP (100 μg), and pyridine (200 μL) was stirred at 4 °C overnight. The reagents were removed under high vacuum, and the residue was dissolved in aqueous CH_3CN (100 μL), filtered, and purified by reversed-phase HPLC eluting with a linear gradient from 30 to 70% CH_3CN in 0.05% aqueous TFA over 40 min at a flow rate of 3.4 mL/min. The major product eluted at 37.9 min and showed a mass of $m/z = 1187$, indicating the formation of a pentaacetate of dehydromicrosclerodermin A (**3**), which was not characterized further.

Acetylation of Dehydromicrosclerodermin A (3). A solution of dehydromicrosclerodermin A (**3**, 500 μg), acetic anhydride (95 μL), DMAP (100 μg), and pyridine (200 μL) was stirred at 4 °C overnight. The experiment was worked up as shown above to obtain a pentaacetate that showed identical HPLC behavior to that obtained from **1**.

Methylation of Microsclerodermin A (1). A solution of microsclerodermin A (**1**, 2 mg) in EtOH (1 mL) was placed in the collection chamber of an MNNG-diazomethane generator (Aldrich) and cooled to 0 °C before distilling CH_2N_2 directly into the solution. The solution was warmed to room temperature and the reaction left overnight. The EtOH was removed under high vacuum, and the residue was dissolved in aqueous CH_3CN (200 μL) and purified by reversed-phase HPLC eluting with a linear gradient from 30 to 70% CH_3CN in 0.05% aqueous TFA over 40 min at a flow rate of 3.4 mL/min. The major product eluted at 23.8 min and showed a mass of $m/z = 1008$, indicating the formation of a monomethyl ester. The ^1H NMR spectrum contained a new methyl signal at δ 3.67 (s, 3 H).

Acetonide of Dehydromicrosclerodermin A (3). Under an atmosphere of dry ntirogen, 2,2-dimethoxypropane (400 μL) was added to a stirred solution of dehydromicrosclerodermin A (**3**, 8 mg) and *p*-toluenesulfonic acid (2 mg) in dry DMF (2 mL). The reaction was stopped after 6 h, when no further reaction could be observed by TLC (silica gel, 2:1 EtOAc–MeOH), by addition of pyridine (20 μL) and removal of excess reagent in a stream of nitrogen. The solvent was removed under high vacuum. The residue was dissolved in 1:1 CH_3CN–H_2O (1 mL) and purified by HPLC (YMC ODS-AM; six injections; linear gradient, 30–50% CH_3CN in 0.01 M NH_4Cl, pH 5.0 over 30 min at 3.4 mL/min; UV detection, 220 nm), which gave the starting material **3** (10 min) and the acetonide **4** (22 min): IR (KBr) 3340, 2930, 1735, 1660, 1540 cm^{-1}; UV (MeOH) (nm) 205 (ϵ 11 100), 262 (ϵ 7000), 268 (sh, ϵ 6800), 285 (ϵ 4900); ^1H NMR (5:1 acetone-d_6–DMSO-d_6) δ 0.86 (d, 3 H, $J = 6.5$ Hz, H-19), 1.10 (m, 1 H, H-7), 1.12 (m, 1 H, H-8), 1.34 (s, 3 H, H-50), 1.38 (m, 2 H, H-9), 1.39 (m, 1 H, H-8′), 1.40 (s, 3 H, H-49), 1.42 (m, 1 H, H-7′), 2.15 (m, 2 H, H-10), 2.20 (m, 1 H, H-22), 2.30 (m, 2 H, H-22′, –24), 3.20 (s, 3 H, H-41), 3.26 (m, 1 H, H-24′), 3.30 (dd, 1 H, $J = 17.5$, 5.5

(14) Lawry, N. H.; Jani, V.; Jensen, T. E. *Curr. Microbiol.* **1981**, *6*, 71.
(15) (a) Braunschweiler, L.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229. (b) Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 2820.
(16) The 0.1 M NH_4NCO_3 buffer is used to increase the solubility of the peptide.

Hz, H-26), 3.44 (d, 1 H, J = 16 Hz, H-40), 3.48 (dd, 1 H, J = 13, 5.5 Hz, H-29), 3.59 (dd, 1 H, J = 13, 5.5 Hz, H-29′), 3.74 (s, 3 H, H-20), 3.82 (dd, 1 H, J = 5.5, 5.5 Hz, H-5), 3.96 (dd, 1 H, J = 10, 5.5 Hz, H-4), 3.89 (dd, 1 H, J = 17.5, 5.5 Hz, H-26′), 4.02 (m, 1 H, H-23), 4.22 (ddd, 1 H, J = 5.5, 5.5, 5 Hz, H-28), 4.34 (d, 1 H, J = 16 Hz, H-40′), 4.44 (br s, 1 H, H-2), 4.45 (dd, 1 H, J = 10, 8.5 Hz, H-3), 4.48 (d, 1 H, J = 5.5 Hz, H-46), 4.92 (br d, 1 H, J = 5.5 Hz, H-45), 5.31 (s, 1 H, H-43), 6.12 (ddd, 1 H, J = 15.5, 7, 7 Hz, H-11), 6.32 (d, 1 H, J = 15.5 Hz, H-12), 6.84 (d, 2 H, J = 8.5 Hz, H-15, –17), 7.05 (dd, 1 H, J = 8, 8 Hz, H-35), 7.08 (dd, 1 H, J = 5.5, 5.5 Hz, NH-26), 7.17 (br t, 1 H, J = 5.5, 5.5 Hz, NH-24), 7.20 (dd, 1 H, J = 8, 8 Hz, H-36), 7.28 (d, 2 H, J = 8.5 Hz, H-14, –18), 7.45 (d, 1 H, J = 8 Hz, H-37), 7.50 (d, 1 H, J = 6 Hz, NH-3), 7.65 (d, 1 H, J = 8 Hz, H-34), 7.65 (d, 1 H, J = 5 Hz, NH-28), 8.10 (br s, 1 H, NH-45), 10.45 (s, 1 H, NH-44); FABMS m/z = 1017 $(M + H)^+$.

Determination of Absolute Configuration. (A) Hydrolyses with 6 N HCl. A solution of the peptide or degradation products in degassed 6 N hydrochloric acid (500–800 μL) was heated in a sealed tube for 12–24 h and then cooled. The solvent was removed in a stream of dry nitrogen, with heating, and under high vacuum.

(b) Derivatization. A premixed solution of acetyl chloride (1 part) in isopropanol (4 parts) (0.5–0.8 mL) was added to each of the hydrolysates in a 1-mL thick-walled reaction vial, and the vial was securely capped. The solution was heated to 100 °C for 45 min and cooled, and the solvent was removed in a stream of dry nitrogen. Pentafluoropropionic anhydride (400 μL) in CH_2CL_2 (400 μL) was added to the residue, the vial was capped, the solution was heated at 100 °C for 15 min and cooled, and the solvent was removed in a stream of dry nitrogen followed by high vacuum. The residue was dissolved in CH_2Cl_2 (100 μL) and immediately analyzed by GC-MS using a chiral column. The identity of each peak was confirmed by coinjection with a solution of a standard that had been derivatized in the same manner. Retention times (in minutes) for the standards, using the experimental protocol above, are as follows: glycine, 10.1; 3-hydroxy-4-aminobutyric acid, (3*R*) 29.15, (3S) 29.37; aspartic acid, (*R*) 26.63, (*S*) 26.83; 3-hydroxyaspartic acid, (2*R*,3*R*) 21.64, (2*S*,3*S*) 22.02, (2*R*,3*S*) 24.04, (2*S*,3*R*) 24.31.

(c) 3-Hydroxy-4-aminobutyric Acid. A degassed solution of microsclerodermin A (**1**, 200 μg) in neat methanesulfonic acid (100 μL) was heated at 110 °C in a sealed tube for 24 h. Because the solvent is essentially nonvolatile, approximately half of the reaction product was derivatized as described in b above. A 19:1 mixture of (*R*)- and (*S*)-3-hydroxy-4-aminobutyric acids was obtained.

(d) Ozonolysis of Microsclerodermin A (1). A stream of ozone in argon was bubbled into a cooled solution of microsclerodermin A (**1**, 400 μg) in dry MeOH (1.5 mL) at –70 °C for 20 min. The excess reagent was removed in a stream of dry nitrogen as the solution was warmed to 20 °C. The solvent was removed under reduced pressure, and the ozonide was redissolved in a 2:1 mixture of formic acid and hydrogen peroxide. The solution was heated over a period of 30 min to 80 °C and cooled, and excess reagents were removed under reduced pressure. The reaction product was hydrolyzed and derivatized as described in a and b above. A peak in the GC trace corresponding to (*R*)-aspartic acid was derived from (*R*)-tryptophan-2-carboxylic acid.

(e) Ozonolysis of Dehydromicrosclerodermin A (3). Dehydromicrosclerodermin A (**3**, 400 μL) was ozonized using the procedure described above (d). The reaction product was hydrolyzed and derivatized as described in a and b above. A peak in the GC trace corresponding to (2*S*,3*R*)-β-hydroxyaspartic acid was derived from carbons 44–47 in **3** (and **1**).

(f) Periodate Oxidation of Microsclerodermin A (1). A solution of microsclerodermin A (**1**, 2 mg) and sodium periodate (200 μg) in water (0.5 mL), adjusted to pH 4.0 with acetic acid, was stirred overnight at 25 °C. The solvent was lyophilized, and the residue was dissolved in hydrogen peroxide (2 mL) and formic acid (1 mL). The solution was heated at 70 °C for 20 min and cooled, and the solvent was evaporated under reduced pressure. The reaction product was hydrolyzed and derivatized as described in a and b above. A peak in the GC trace corresponding to (2*S*,3*S*)-β-hydroxyaspartic acid was derived from carbons 1–4 in **1**.

Acknowledgment. We thank Professor Klaus Bock[17] and Richard Reid for supplying optically pure samples of the enantiomers of 4-amino-3-hydroxybutyric acid, and the diastereomers of *erythro*-β-hydroxyaspartic acid, respectively, Dr. Claude Lévi for identifying the sponge, Professor Jean Rivier for amino acid analyses, and Mary Kay Harper for technical assistance. This research was generously supported by the National Science Foundation (Grant CHE 92-04647), the National Institutes of Health (Grant CA 49084), and the ORSTOM-CNRS SMIB program.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of compounds **1** and **3**, HMBC and ROESY spectra of dehydromicrosclerodermin A (**3**), and the ^1H NMR spectrum of acetonide **4** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(17) Bock, K.; Lundt, I.; Pedersen, D. *Acta Chem. Scand.* **1983**, *37*, 341.

22°25, 1'S 171°40, 7'