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Microsclerodermins F–I, Antitumor and Antifungal Cyclic Peptides from the Lithistid Sponge *Microscleroderma* sp.

Asfia Qureshi,[a] Patrick L. Colin[b] and D. John Faulkner[a,*]

[a]*Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0212, USA*
[b]*Coral Reef Research Foundation, P.O. Box 1765, Koror, PW 96940 Palau*

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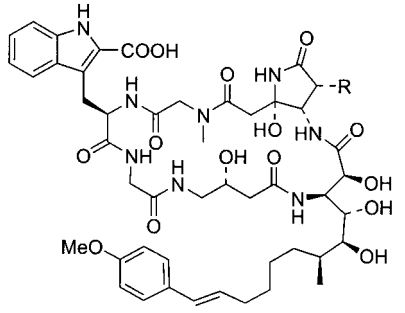
Abstract—Four new cyclic peptides, microsclerodermins F–I (**6**–**9**), were isolated from the deep water lithistid sponge *Microscleroderma* sp. from Palau. Their structures, which incorporate an unusual long chain β-amino acid, were elucidated using spectroscopic methods and chemical degradation.

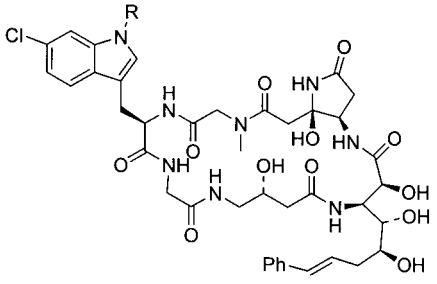
Introduction

Studies of sponges of the Order Lithistida have provided many bioactive metabolites, most of which are peptides that contain unusual amino acids.[1,2] It was recently shown that one such peptide, theopalauamide from *Theonella swinhoei*,[3] was located in and presumably produced by a symbiotic δ-proteobacterium, *Candidatus Entotheonella palauensis*, that is most closely related by DNA analysis to terrestrial myxobacteria.[4,5] While the extent of symbiont involvement in the production of peptides from lithistid sponges is uncertain, it appears likely that subtle structural variations in the peptides isolated from identical or closely related sponges may be due to variations in the symbiont populations. Detailed studies of the metabolites of different populations of lithistid sponges are therefore valuable in both the structure–activity and chemotaxonomic arenas.

The microsclerodermins are a growing family of cyclic peptides. We have previously reported the structures of microsclerodermins A (**1**) and B (**2**) from a Philippines *Microscleroderma* sp.[6] and microsclerodermins C–E (**3**–**5**) isolated from both a *Theonella* sp. and a *Microscleroderma* sp.,[7] also from the Philippines. The basic structural motif of the microsclerodermins consists of a 23-membered ring constructed from six amino acid residues three of which, glycine, *N*-methyl glycine and (3*R*)-4-amino-3-hydroxybutyric acid (GABOB), are common to all members of the family. The variable units are a modified tryptophan residue, an unusual 3-aminopyrrolidone-4-acetic acid moiety and various ω-aromatic 3-amino-2,4,5-trihydroxyacids. In this paper we report four additional members of the family, microsclerodermins F–I (**6**–**9**) from a deep-water specimen of *Microscleroderma* sp. from Palau.



1, R = OH
2, R = H



3, R = $CONH_2$
4, R = H

Keywords: sponge; marine natural products; cyclic peptides; cytotoxins.
* Corresponding author. Tel.: +1-858-534-4259; fax +1-858-534-2997; e-mail: jfaulkner@ucsd.edu

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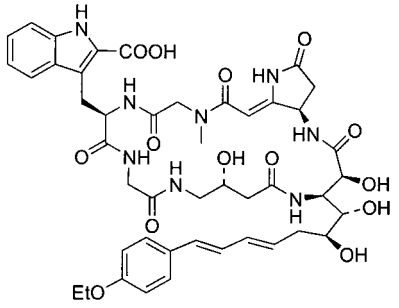

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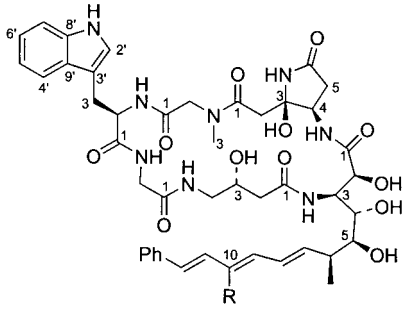
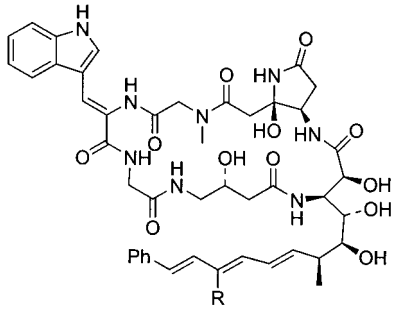


6, R = H
8, R = CH_3



7, R = H
9, R = CH_3

Results and Discussion

Specimens of the lithistid sponge *Microscleroderma* sp. were collected by hand using a closed circuit rebreathing apparatus (−125 m) at Short Dropoff, Koror, Palau, 1997, and were kept frozen until extraction. The ethyl acetate fraction, obtained by partitioning the initial methanol extract between ethyl acetate and water, was further partitioned between hexane and methanol. The methanol soluble material showed antifungal activity against *Candida albicans*, and was purified by chromatography on reversed-phase C_{18} silica, followed by Sephadex LH-20 and finally C_{18} reversed-phase HPLC using 40% aqueous acetonitrile to obtain four polar antifungal metabolites, microsclerodermin F (**6**, 0.002% wet wt.), G (7, 0.001% wet wt.), H (**8**, 0.004% wet wt.) and I (**9**, 0.002% wet wt.).

Microsclerodermin F (**6**) was isolated as a white powder having the molecular formula $C_{45}H_{56}N_8O_{12}$, which was established by HRFABMS [m/z=923.3915 $(M+Na)^+$]. The IR spectrum contained typical peptide bands at 3370, 1650 and 1540 cm^{-1} and UV absorptions at 317 (ϵ 30 400) and 332 nm (ϵ 23 000) that were assigned to aromatic systems. The ^1H NMR spectrum in DMSO-d_6 (Table 1), contained six amide NH signals between δ 7.45 and 8.67, an *N*-methyl signal at 2.94 and an indole NH signal at 10.93, accounting for all eight nitrogen atoms. The ^{13}C NMR spectrum (Table 1) indicated the presence of seven carbonyl carbons corresponding to the amide groups and five carbons attached to hydroxyl groups that together accounted for all twelve oxygen atoms in the molecule.

The ^1H, ^{13}C, HMQC and HMBC data allowed identification of the glycine, *N*-methyl glycine and tryptophan residues. The COSY and TOCSY data were used to identify glycine and *N*-methyl glycine while analysis of the aromatic region of the ^1H and ^{13}C NMR spectra provided evidence for an indole ring system. The indole unit was assigned as part of a tryptophan residue using HMBC data that showed correlations between H-3 and C-2′, C-3′ and C-9′, while additional correlations (Fig. 1) confirmed the indole ring system. The identification of the pyrrolidone ring system relied heavily on HMBC correlations. The methylene protons at δ 2.29 (H-5) were correlated to both C-3 and C-4 while the H-2 protons at 2.72 (d, 1H, J=17 Hz) and 2.86 (d, 1H, J=17 Hz), were assigned on the basis of HMQC correlations to δ_C 38.7 and HMBC correlations from OH-3 to C-2, C-3 and C-4. The chemical shift of C-3 at δ 85.7 is appropriate for a carbon bearing both a hydroxyl and an amide group.[6,7] Further HMBC correlations from NH-3 to C-3, C-4 and C-5 defined the pyrrolidone ring system. The relative stereochemistry of the pyrrolidone ring was established using ROESY data, specifically a correlation between the H-2 and H-4, which requires OH-3 and NH-4 to be on the same face of the pyrrolidone ring. 4-Amino-3-hydroxy butyric acid (GABOB) was also identified by interpretation of the NMR data, but since few HMBC correlations were observed, the most notable being that of OH-3 to C-2 and C-3, the COSY and TOCSY data, particularly the correlations from the –OH signal to the H-2, H-3 and H-4 signals, as well as the ROESY data (Fig. 2) were essential to assigning this moiety. However, HMBC experiments defined the partial amino acid sequence Gly-GABOB-AMPTD. The remaining residue in the molecule, 3-amino-6-methyl-12-phenyl-2,4,5-trihydroxydodeca-7,9,11-trienoic acid (AMPTD), differs from the ω-aromatic β-amino acid residues APTO and AETD in microsclerodermins C–E (**3**–**5**)[7] and the *p*-methoxy-*trans*-styrene moiety in microsclerodermins A (**1**) and B (**2**).[6] The aromatic region of the ^1H NMR spectrum contains signals at δ 7.45 (m, 2H), 7.34 (m, 2H) and 7.24 (m, 1H) assigned to a phenyl group which, from HMBC data, is conjugated to an olefin. Furthermore, using the COSY and TOCSY experiments, signals at δ 6.55 (H-12) and 6.94 (H-11), 6.34 (H-10) and 6.37 (H-9), and 6.12 (H-8) and 5.92 (H-7) were assigned to the hydrogens on three conjugated *trans*-disubstituted olefins, the geometry of which was determined from the ROESY data. The C-6 methyl group was assigned on the basis of TOCSY and HMBC data, and further TOCSY correlations between H-6 to Me-6 and H-5 to OH-5

Table 1. NMR data for microsclerodermins F (**6**) and G (**7**) in DMSO-d_6

Amino acid	C#	6		7	
		δ_C	δ_H	δ_C	δ_H
AMPTD	1	172.7		173.0	
	2	69.5	4.39 d, 6	69.3	4.41 d, 5.5
	3	53.3	4.12 m	53.4	4.17 m
	4	68.3	3.48 dd, 9.5, 9.5	68.5	3.38 m
	5	72.9	3.19 m	72.7	3.11 m
	6	40.8	2.44 m	40.5	2.54 m
	7	141.0	5.92 d, 7.5	141.0	5.92 d, 7.5
	8	129.0	6.12 dd, 7.5, 7.5	129.1	6.14 m
	9	134.6	6.37 m	134.6	6.35 m
	10	130.6	6.34 m	130.6	6.34 m
	11	129.6	6.94 dd, 15.5, 10.5	129.6	6.94 dd, 15.5, 9.5
	12	131.0	6.55 d, 15.5	131.0	6.55 d, 15.5
	13	137.3		137.4	
	14,18	126.1	7.45 m	126.2	7.45 m
	15,17	128.8	7.34 m	128.8	7.32 t, 7.5
	16	127.3	7.24 n	127.4	7.21 d, 7.5
	NH-3		7.45 d, 8.5		7.24 m
	OH-2		4.34 d, 4.5		4.21 d, 5.5
	OH-4		4.64 d, 8.5		4.56 d, 8.5
	OH-5		6.10 m		6.08 m
	Me-6	16.4	0.92 d, 6.5	16.4	0.93 d, 6.5
GABOB	1	172.9		173.0	
	2	41.0	2.18 m	41.3	2.16 m
			2.44 m		2.44 m
	3	67.2	3.79 d, 7	67.4	3.73 m
	4	45.0	2.67 m	45.0	2.59 m
			3.44 m		3.44 m
	NH-4		7.53 d, 8		7.68 m
	OH-3		4.88 d, 4.5		4.96 d, 5.5
Gly	1	169.1		169.7	
	2	42.6	3.38 m	43.2	3.69 m
			3.76 m		
	NH-2		8.59 t, 6, 6		8.30 t, 6.6
Trp	1	172.2		164.6	
	2	55.5	4.21 m	123.5	
	3	26.2	2.99 m	121.2	7.45 m
			3.12		
	2′	123.9	7.22 m	127.7	7.90 m
	3′	109.6		108.7	
	4′	118.3	7.53 m	118.2	7.65 m
	5′	118.4	6.99 dd, 7.5, 7.5	122.2	7.17 m
	6′	121.0	7.07 dd, 7.5, 7.5	120.3	7.12 m
	7′	111.5	7.34 m	112.0	7.44 m
	8′	136.2		135.7	
	9′	127.2		127.2	
	NH-2		8.67 d, 4		10.02 s
	NH-2′		10.93 s		11.82 s
N-Me-Gly	1	170.3		170.2	
	2	49.7	3.85 m	51.1	3.93 m
			4.13 m		4.14 m
	3	36.4	2.94 s	37.2	3.05 s
Pyrrolidone	1	170.6		171.3	
	2	38.7	2.72 d, 17	38.7	2.88 m
			2.86 d, 17		
	3	85.7		8.57	
	4	50.6	4.48 dd, 18, 9	51.2	4.52 m
	5	35.1	2.29 m	34.1	2.32 m
	6	173.4		173.2	
	NH-3		8.00 s		7.76 s
	OH-3		5.89		5.83 s
	NH-4		7.57 d, 8.5		7.54 m

established the C-5 to C-18 portion of the β-amino acid. The second half of the amino acid from C-1 to C-4 could also be assigned from HMBC correlations between C-1 to H-2 to C-3 to H-4. Unfortunately, the absence of coupling between H-4 and H-5 and a lack of HMBC correlations across the C-4/C-5 bond, other than a weak four bond HMBC correlation between H-2 and C-5, precluded a simple assignment of the AMPTD structure. The structure was deduced on the

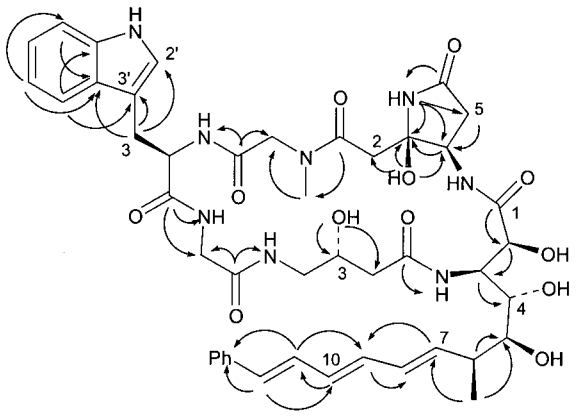


Figure 1. Key HMBC correlations used to determine the structure of microsclerodermin F (**6**).

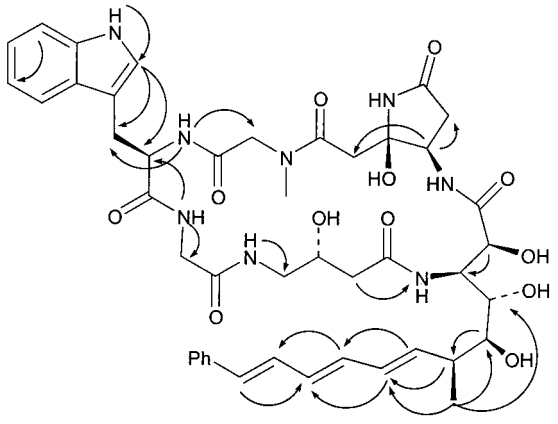


Figure 2. Key ROESY correlations used to determine the structure of microsclerodermin F (**6**).

basis of ROESY correlations (Fig. 2) but was not confirmed until the synthesis of the acetonide **11** from microsclerodermin H. To the best of our knowledge, the β-amino acid AMPTD has not been described

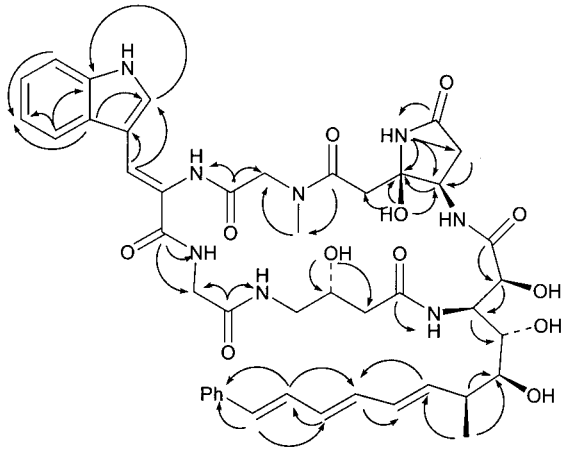


Figure 3. Key HMBC correlations used to determine the structure of microsclerodermin G (**7**).

Table 2. NMR data for microsclerodermins H (**8**) and I (**9**) in DMSO-d_8

Amino acid	C#	**8**		**9**	
		δ_C	δ_H	δ_C	δ_H
AMPTD	1	172.8		173.0	
	2	69.6	4.40 m	69.3	4.43 m
	3	53.4	4.12 m	53.4	4.18 m
	4	68.4	3.48 m	68.5	3.48 m
	5	73.0	3.20 m	72.8	3.11 m
	6	40.4	2.42 m	40.5	2.54 m
	7	141.7	5.93 d, 7.5	141.6	5.95 dm 7
	8	125.5	6.39 dd, 10.5, 7.5	125.5	6.40 dd, 11.7
	9	133.3	6.25 d, 10.5	133.2	6.28 d, 11
	10	132.5		132.4	
	11	133.8	6.92 d, 16	133.7	6.98 d, 16
	12	126.6	6.55 d, 16	126.4	6.57 d, 16
	13	137.7		137.6	
	14, 18	126.3	7.47 m	126.2	7.46 m
	15, 17	128.8	7.32 m	128.8	7.34 t, 7.5
	16	127.2	7.18 m	127.1	7.23 m
	NH-3		7.47 d, 8		7.24 m
	OH-2		4.35 d, 5.5		4.23 d, 5
	OH-4		4.66 d, 8.5		4.62 m
	OH-5		6.14 br s		5.90 d, 8
	Me-6	16.6	0.94 d, 6.5	16.7	0.96 d, 6.
	Me-10	12.4	1.91 s	12.4	1.94 s
GABOB	1	173.0		173.0	
	2	41.0	2.18 m	41.3	2.19 m
			2.31 m		2.41 m
	3	67.3	3.73 m	67.5	3.74 m
	4	45.1	2.67 m	45.0	2.62 m
	NH-4		7.51 d, 8		7.69 t, 8
	OH-3		4.89 m		4.97 d, 5.5
Gly	1	169.2		169.7	
	2	42.7	3.35 m	43.2	3.69 m
			3.79 m		
	NH-2		8.59 t, 6		8.31 t, 6
Trp	1	172.2		164.6	
	2	55.5	4.18 m	123.5	
	3	26.3	2.97 m	121.2	7.46 m
			3.12 m		
	2′	123.9	7.23 m	127.7	7.92 m
	3′	109.7		108.8	
	4′	118.3	7.58 m	118.2	7.65 m
	5′	118.4	6.97 t, 7.5	122.2	7.17 m
	6′	121.1	7.05 t, 7.5	120.3	7.14 m
	7′	111.5	7.33 m	112.0	7.45 m
	8′	136.3		135.7	
	9′	127.3		127.2	
	NH-2		8.67 d, 4		10.05 s
	NH-2′		10.91 s		11.87 s
N-Me-Gly	1	170.4		170.2	
	2	49.8	3.86 m	51.1	3.96 m
			4.12 m		
	3	36.4	2.93 s	37.2	3.06 s
Pyrrolidone	1	170.7		171.3	
	2	38.7	2.72 m	38.7	2.86 m
			2.86 d, 17		
	3	85.8		85.7	
	4	50.6	4.46 dd, 17.5, 9	51.2	4.53 m
	5	35.4	2.31 m	34.1	2.33 m
	6	173.5		173.3	
	NH-3		8.00 s		7.79 s
	OH-3		5.88		5.86 s
	NH-4		7.59 m		7.55 t, 11.5

previously. The sequence of the amino acid residues in microsclerodermin F (**6**) was determined from the HMBC correlations from the NH or N–Me proton signal of one residue to the C-1 carbonyl signal of the adjacent

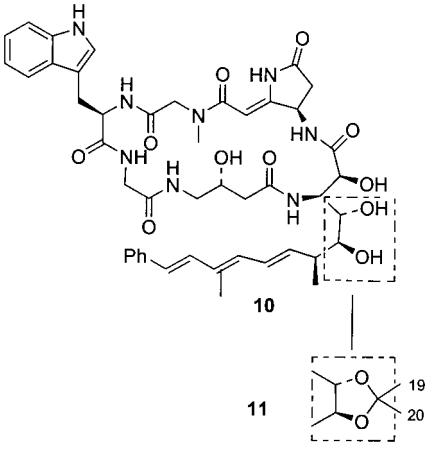
amino acid (Fig. 1) and was confirmed in most cases by ROESY data (Fig. 2).

Microsclerodermin G (**7**) was isolated as a white powder having the molecular formula $C_{45}H_{54}N_8O_{12}$, as revealed by the high resolution mass spectrum [m/z=921.3760 $(M+Na)^+$], which is 2 amu less than that of **6**. Extensive analysis of the NMR spectral data including COSY, HMQC, HMBC and ROESY experiments indicated that the only difference was the replacement of the tryptophan unit in **6** by a 2,3-dehydrotryptophan unit in **7**. In the ^{13}C NMR spectrum of **7**, the C-2 and C-3 signals are at δ 123.5 and 121.2, respectively, and replace signals at 55.5 and 26.2 in **6**. Other differences in the NMR data (Table 1) are that the signals at δ_C 172.7 (C-1), 123.9 (C-2′) and δ_H 7.22 (H-2′) are replaced by 164.6, 127.7 and 7.90, respectively. The signal at δ 121.2 (C-3) showed an HMBC correlation to 7.90 (H-2′), which confirmed the location of the double bond as shown (Fig. 3). The geometry of the 2,3 double bond of Δ-Trp was assigned as *Z* by comparison of the chemical shift of the β-proton (δ 7.45) with that of keramamide F (δ 7.83)[8] and the geometrical isomers of methyl-α-acetamido-6-methylindole-3-acrylate [δ 7.69 (*Z*) and 7.00 (*E*)].[9] This assignment was supported by the presence of a ROESY correlation between NH-2 (δ 10.02) and H-2′ (7.90) in **7**.

Microsclerodermin H (**8**) was isolated as a white powder. Its molecular formula, $C_{46}H_{58}N_8O_{12}$, was 14 amu heavier than that of microsclerodermin F (**6**). Analysis of the NMR data (Table 2) revealed that the only change was replacement of H-10 by a methyl group in the β-amino acid AMPTD. The signals at δ_C 130.6 and δ_H 6.34 in the NMR spectra of **6** were replaced by a fully substituted carbon signal at 132.5 and additional methyl signals at δ_C 12.4 and δ_H 1.91 (s, 3H). The new CH_3 signal showed HMBC correlations to C-9 and C-10 and the H-9 and H-11 signals showed long-range correlations to CH_3-10, thereby confirming the position of the methyl group at C-10. The remaining spectral data suggested that the rest of the molecule was identical to **6**.

Microsclerodermin I (**9**) was isolated as a white powder. The molecular formula $C_{46}H_{56}N_8O_{12}$ indicated that **9** was 2 amu less than that of **8**. Full spectral data (Table 2), including COSY, HMQC, HMBC and ROESY, indicated that **9** is identical to **8** except for the replacement of the tryptophan unit by a 2,3-dehydrotryptophan as with **6** and **7**.

The microsclerodermins are readily dehydrated under acidic conditions.[6,7] To form an acetonide, we found it best to first dehydrate microsclerodermin H (**8**) by the addition of TFA vapors to a solution of **8** in dry DMF to obtain anhydromicrosclerodermin H (**10**), which was immediately treated with 2,2 dimethoxypropane and a catalytic amount of pyridinium *p*-toluenesulfonate in dry DMF to obtain acetonide **11**. A ROESY experiment performed on **11** gave correlations that were very similar to those observed for the acetonides of dehydromicrosclerodermin A[6] and anhydromicrosclerodermin D,[7] indicating that the stereochemistry of the AMPTD group was (2*S*,3*R*,4*S*,5*S*,6*S*). Additionally, formation of the acetonide provided unequivocal confirmation of the proposed bond linking C-4 and C-5 in the AMPTD residue.



The stereochemistry of all four microsclerodermins was determined by a series of chemical degradation experiments together with the analysis of the ROESY data. Treatment of peptide **6** with vapors of TFA gave **10**, which was treated in situ with periodate, followed by ozonolysis using an oxidative work-up and acid-catalyzed hydrolysis to obtain a mixture of amino acids that was derivatized and analyzed by GC-MS. Comparison with derivatives of amino acid standards allowed the identification of (3*R*)-3-hydroxy-4-amino butyric acid, (2*S*,3*S*)-3-hydroxyaspartic acid and *R*-aspartic acid. The absolute configuration of each amino acid in **7**, **8** and **9** was shown to be identical to those in **6** by employing the same series of chemical degradations, followed by chiral GC-MS analysis of the resulting fragments.

Microsclerodermins F (**6**), G (**7**), H (**8**) and I (**9**) inhibited the growth of *Candida albicans* using a paper disk diffusion assay. Peptide **6** was the most active at 1.5 μg per disk followed by **7** at 3 μg, **8** at 12 μg and **9** at 25 μg, where the reported concentrations are the minimum at which inhibition was observed. All four microsclerodermins showed very similar cytotoxicity against the HCT-116 cell line with IC_{50}'s of 1.0 μg mL^{-1} (**8**), 1.1 μg mL^{-1} (**9**), 1.8 μg mL^{-1} (**6**) and 2.4 μg mL^{-1} (**7**).

Experimental

General experimental procedures

^1H, DQCOSY, GHMQC, GHMBC, TOCSY and ROESY NMR spectra were recorded on a Varian Inova 300 MHz spectrometer. ^{13}C and DEPT spectra were recorded on a Varian Unity 500 MHz spectrometer. All NMR data are reported in DMSO-d_6. TOCSY spectra were obtained with 80 ms mixing times, and ROESY spin locks were established by continuous pulsing for 200 ms. High resolution FABMS data were obtained on a VG ZAB mass spectrometer from the Mass Spectrometry Facility, University of California at Riverside. Optical rotations were measured on a Rudolph Autopol III polarimeter (*c* g/100 mL) at 589 nm. UV and IR spectra were recorded on Perkin–Elmer Lambda 3B and 1600 FT-IR series spectrometers, respectively.

Chiral GC-MS experiments were performed on a Hewlett Packard 5890A gas chromatograph with an Alltech Chirasil-Val capillary column (0.32 mm×25 m) interfaced to a Hewlett–Packard 5988A mass spectrometer. The oven temperature was ramped from 50 to 200°C at 4°C min^{-1}.

Isolation of microsclerodermins F–I (6–9)

The sponge *Microscleroderma* sp. was collected on 29 October 1997, at a depth of 370 feet (125 m) at Short Drop-off, Koror, Palau. The sponge (640 g wet wt.) was frozen and stored at −20°C until it was diced and soaked in MeOH (3×500 mL). The combined methanol extracts were concentrated and partitioned between 1:1 ethyl acetate–water (3×200 mL). The ethyl acetate fraction was concentrated and further partitioned between hexane and methanol. The methanol extract was chromatographed on a C_{18} SepPak using a stepwise gradient elution of 20% aqueous CH_3CN to 100% CH_3CN. Fractions obtained with 20–50% CH_3CN were further chromatographed on Sephadex LH-20 with 80% aqueous CH_3CN followed by RP HPLC with 40% aqueous acetonitrile as solvent to yield microsclerodermin F (**6**, 12 mg, 0.002% wet wt.), microsclerodermin G (**7**, 6 mg, 0.001% wet wt.), microsclerodermin H (**8**, 27 mg, 0.004% wet wt.) and microsclerodermin I (**9**, 15 mg, 0.002% wet wt.).

Microsclerodermin F (6). White powder; $[\alpha]_D$ −19° (*c* 0.62, 1:1 MeOH/H_2O); UV (1:1 MeOH/H_2O) ν_{max} 317 nm (ϵ 30 400), 332 (23 000); IR (film) λ_{max} 3370, 2350, 1650, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 1; HRFABMS obsd *m/z* 923.3915 $(M+Na)^+$, $C_{45}H_{56}N_8O_{12}Na$ requires *m/z* 923.3874.

Microsclerodermin G (7). White powder; $[\alpha]_D$ −20° (*c* 0.31, 1:1 MeOH/H_2O); UV (1:1 MeOH/H_2O) ν_{max} 317 nm (ϵ 43 100), 332 (36 600); IR (film) λ_{max} 3310, 2350, 1650, 1530, 1430, 1280 cm^{-1}; ^1H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 1; HRFABMS obsd *m/z* 921.3760 $(M+Na)^+$, $C_{45}H_{54}N_8O_{12}Na$ requires *m/z* 921.3759.

Microsclerodermin H (8). White powder; $[\alpha]_D$ −13° (*c* 0.95, 1:1 MeOH/H_2O); UV (1:1 MeOH/H_2O) ν_{max} 319 nm (ϵ 51 800), 334 (38 000); IR (film) λ_{max} 3330, 1680, 1630, 1550, 1500, 1430, 1190 cm^{-1}; ^1H NMR (DMSO-d_6) see Table 2; ^{13}C NMR (DMSO-d_6) see Table 2; HRFABMS obsd *m/z* 937.4072 $(M+Na)^+$, $C_{46}H_{58}N_8O_{12}Na$ requires *m/z* 937.4098.

Microsclerodermin I (9). White powder; $[\alpha]_D$ −35° (*c* 0.08, 1:1 MeOH/H_2O); UV (1:1 MeOH/H_2O) ν_{max} 319 nm (ϵ 30 200), 334 (25 000); IR (film) λ_{max} 3310, 2350, 1660, 1530, 1430, 1280, 1080, 970, 750 cm^{-1}; ^1H NMR (DMSO-d_6) see Table 2; ^{13}C NMR (DMSO-d_6) see Table 2; HRFABMS obsd *m/z* 935.3949 $(M+Na)^+$, $C_{46}H_{56}N_8O_{12}Na$ requires *m/z* 935.3915.

Preparation of acetonide 11. Trifluoroacetic acid vapor was added to a solution of peptide **8** (7 mg) in dry DMF (100 μL) in a conical vial. The vial was left for 5 min, then dried in vacuo to yield the anhydromicrosclerodermin H (**10**). To the dry product was added a solution of dry DMF (100 μL) containing dimethoxypropane (400 μL) and a catalytic amount of pyridinium *p*-toluensulfonate. The solution was stirred overnight, then dried under a stream of nitrogen and in vacuo to obtain a quantitative yield of pure acetonide **11** (7 mg): white powder; ^1H NMR (DMSO-d_6) AMPTD: 1.01 (d, 3H, *J*=6.3 Hz, Me-6), 1.33 (s, 3H, Me-20), 1.37 (s, 3H, Me-19), 1.94 (s, 3H, Me-10), 2.44 (m, 1H, H-6), 3.74 (m, 1H, H-4), 3.93 (m, 1H, H-5), 4.18 (m, 1H, H-2), 4.31 (dd, 1H, *J*=10.3, 10.3 Hz, H-3), 5.78 (m, 1H, H-7), 6.30 (d, 1H, *J*=11.2 Hz, H-9), 6.53 (m, 1H, H-8), 6.60 (d, 1H, *J*=16.1 Hz, H-12), 6.96 (br s, 1H, H-11), 7.08 (m, 1H, NH-3), 7.11 (m, 1H, H-16), 7.34 (dd, 2H, *J*=14.1, 7.3 Hz, H-15, H-17), 7.48 (d, 2H, *J*=7.8 Hz, H-14, H-18); GABOB: 2.03 (m, 1H, H-2), 2.28 (m, 1H, H-2′), 3.01 (m, 1H, H-4), 3.22 (m, 1H, H-4′), 3.82 (m, 1H, H-3), 7.39 (m, 1H, NH-4); Gly: 2.73 (m, 1H, H-2), 2.89 (m, 1H, H-2′), 7.96 (m, 1H, NH-2); Trp: 3.17 (m, 2H, H-3), 4.13 (m, 1H, H-2), 7.00 (m, 1H, H-6′), 7.08 (dd, 1H, *J*=6.8, 6.8 Hz, H-5′), 7.22 (d, 1H, *J*=7.8 Hz, H-2′), 7.25 (m, 1H, H-4′), 7.54 (d, 1H, *J*=7.8 Hz, H-7′), 8.77 (d, 1H, *J*=4.4 Hz, NH-2), 10.90 (s, 1H, NH-2′); NMeGly: 3.04 (s, 3H, N-Me), 3.42 (m, 1H, H-2′), 4.56 (m, 1H, H-2); pyrrolidone: 2.44 (m, 1H, H-5), 2.73 (m, 1H, H-5′), 5.23 (s, 1H, H-2), 5.24 (m, 1H, H-4), 8.37 (m, 1H, NH-4), 10.42 (s, 1H, NH-3); HRFABMS obsd *m/z* 959.4265 $(M+Na)^+$, $C_{46}H_{60}N_8O_{11}Na$ requires *m/z* 959.4279.

Absolute stereochemistry of microsclerodermins F–I (6–9)

TFA vapors were added to a solution of peptide **6** (300 μg) in dry DMF (100 μL) in a conical vial. The vial was left for 5 min, then dried in vacuo to yield anhydromicrosclerodermin F which was immediately dissolved in water (100 μL, acidified to pH=4 with AcOH), and $NaIO_4$ (300 μg) was added. The reaction was stirred overnight, then lyophilized. The periodate cleavage product was dissolved in MeOH (200 μL) and ozonized at −78°C for 60 min. The reaction was quenched with 50% H_2O_2 (5 drops), brought to room temperature, and allowed to stand for 1 h. The reaction mixture was dried under a stream of nitrogen followed by lyophilization, then hydrolyzed using 6N hydrochloric acid as described previously.[6] Using standard procedures,[6] the resulting amino acids were converted into their *N*-pentafluoropropionyl isopropyl ester derivatives. After drying, the residue was dissolved in dry CH_2Cl_2 (GC-MS grade) and subjected to GC-MS analysis using a chiral column under standard conditions.[6] Comparison of retention times and MS fragmentation patterns with those of derivatized standards [retention times: *N*-MeGly, 9.00 min; Gly, 10.95 min; (*R*)-Asp, 19.30 min; (*S*)-Asp, 19.50 min; (*R*)-GABOB, 20.40 min; (*S*)-GABOB, 20.55 min; (2*R*,3*R*)-β-OHAsp, 15.85 min, (2*S*,3*S*)-β-OHAsp, 16.10 min, (2*R*,3*S*)-β-OHAsp, 17.50 min, (2*S*,3*R*)-β-OHAsp, 17.65 min] allowed the identification of (3*R*)-3-hydroxy-4-aminobutyric acid, (2*S*,3*S*)-3-hydroxy-aspartic acid, and (*R*)-aspartic acid. Microsclerodermins G–I (7– 9) were analyzed in an identical manner.

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