Theonegramide, an Antifungal Glycopeptide from the Philippine Lithistid Sponge *Theonella swinhoei*

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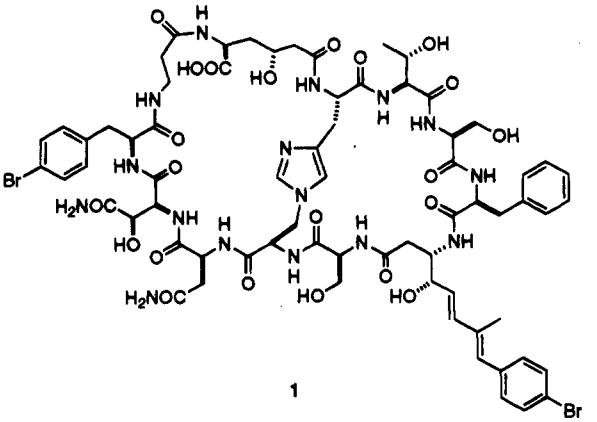
Theonegramide (**2**) is an antifungal glycopeptide consisting of arabinose joined to a bicyclic dodecapeptide that contains the unusual amino acids β-hydroxyasparagine, isoserine, α-aminoadipic acid, 4′-bromo-3-methylphenylalanine, 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid, and the same histidinoalanine moiety previously observed in theonellamide F (**1**). The structure of theonegramide (**2**) was elucidated by analysis of spectral data and its absolute configuration was determined by GC-MS analysis using a chiral column packing.

Sponges of the order Lithistida,[1] particularly those of the genus *Theonella*, provide some of the most interesting biologically-active marine natural products[2] such as swinholide,[3] the cyclotheonamides,[4] the onnamides,[5] and theonellamide F (**1**).[6] We are studying lithistid sponges because they contain symbiotic filamentous bacteria[7] that may play a role in the production of peptides such as the cyclotheonamides and theonellamide F (**1**).[8] In this paper, we report the structural elucidation of theonegramide (**2**),[9] which is the first glycopeptide from a lithistid sponge to be fully characterized.[10]

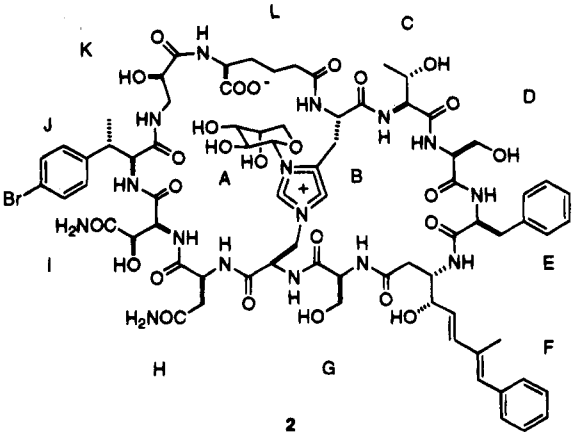
The lithistid sponge *Theonella swinhoei* was collected by hand using SCUBA (−20 m) at Antolang, Negros Island, The Philippines. The aqueous CH_3CN (1:1) soluble material from the lyophilized sponge was purified by chromatography on reversed-phase C-18 silica using a gradient from 10 to 60% CH_3CN in 0.05% aqueous TFA to obtain theonegramide (**2**, 0.2% dry wt).

Theonegramide (**2**) (Chart 1), $[\alpha]_D$ + 19° (c = 0.39), was isolated as a white powder of molecular formula $C_{75}H_{97}BrN_{16}O_{26}$ [for $C_{75}H_{98}{}^{79}BrN_{16}O_{26}$, m/z = 1717.6080 (M + H)$^+$]. The IR spectrum contained typical peptide bands at 3300, 1600, and 1540 cm^{-1} and the UV absorptions at 278 (ε 12 700), 288 (ε 12 900), and 305 (sh, ε 7100) nm indicated the presence of aromatic residues. Reaction of **2** with diazomethane produced a monomethyl ester [m/z = 1734; ^{1}H NMR δ 3.69 (s, 3 H)], suggesting the presence of one free carboxylic acid. Standard amino acid analysis showed the presence of 2 equiv of ammonia and serine, together with *allo*-threonine, asparagine (or aspartic acid), and phenylalanine. The uncommon amino acids β-hydroxyasparagine, isoserine, α-aminoadipic acid, 4′-bromo-3-methylphenylalanine, 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid, and the same histidinoalanine moiety previously observed in theonellamide F (**1**)[6] were identified by analysis of the ^{1}H and ^{13}C NMR data (Table 1) using DQFCOSY, TOCSY, HMQC, HMBC,

Chart 1



1



2

A = D-arabinose
B = histidino-alanine
C = *allo*-threonine
D = G = serine
E = phenylalanine
F = 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid
H = asparagine
I = β-hydroxy-asparagine
J = 4′-bromo-3-methyl-phenylalanine
K = isoserine
L = α-amino-adipic acid

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(1) The order Lithistida (class Demospongiae) represents a polyphyletic assemblage of sponges possessing a distinct spicule type called a desma. The desmas are fused to form a rigid skeleton. Lithistids include the chemically rich genera *Corallistes*, *Discodermia*, *Microscleroderma*, and *Theonella*.

(2) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793.

(3) 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. I* **1991**, 3185.

(4) 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.

(5) Matsunaga, S.; Fusetani, N.; Nakao, Y. *Tetrahedron* **1992**, *48*, 8369.

(6) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Walchli, M. *J. Am. Chem. Soc.* **1989**, *111*, 2582.

(7) Examination of the filamentous bacteria by transmission electron microscopy revealed that they are not cyanobacteria as had been previously presumed (for reviews, see refs 2 and 8).

(8) Faulkner, D. J.; He, H.; Unson, M. D.; Bewley, C. A.; Garson, M. J. *Gazz. Chim. Ital.* **1993**, *123*, 301.

(9) The name theonegramide incorporates the genus (*Theonella*), the location (Negros Island), and the type of molecule.

(10) Two glycopeptides have been isolated from an *Aciculites* sp. but the structures have not been fully defined.[8]

0022-3263/94/1959-4849$04.50/0

Table 1. Correlated ^1H and ^{13}C Spectral Data and Proton–Proton NOE Data for Theonegramide (2)[a]

amino acid		δ^b ^{13}C	δ^b ^1H	*m*	*J* (Hz)	HMBC	ROESY[c]
allo-threonine	1	173.7					
	2	58.7	4.51	dd	9.5, 9.5	69.2, 169.8, 173.7	1.15
	3	69.2	3.76	m		58.7	4.74
	4	21.9	1.15	d	6.0	58.7, 69.2	3.81, 7.54
	NH		7.54	d	9.5	169.8	1.15, 3.81, 5.01
serine 1	1	170.0					
	2	57.8	4.74	m		62.3	3.71, 3.84
	3	62.3	3.71	m		57.8, 170.0	4.74, 3.97
			3.84	m			
	NH		8.94	d	7.5	57.8, 173.7	3.71, 3.84, 4.51, 4.74, 8.42
phenylalanine	1	172.2					
	2	54.8	4.87	m		39.9, 137.6, 170.0, 172.2	2.75, 2.96
	3	39.9	2.75	m		128.7, 137.6, 172.2	4.87
			2.96	m			
	4	137.6					
	5,9	128.7	7.29	d	7.5	39.9, 127.2, 128.7, 137.6	8.42
	6,8	130.0	7.25	*d*		127.2, 128.7, 137.6	
	7	127.2	7.21	*d*		130.0	
	NH		8.42	d	9.5	170.0	3.84, 4.74, 6.10, 7.29, 8.94
AHMP[e]	1	172.6					
	2	37.9	2.34	dd	12.5, 3.0	53.6, 68.9, 172.6	4.43, 4.49, 5.44
			2.66	br d	12.5		
	3	53.6	4.43	m			2.34
	4	68.9	4.49	m		37.9, 134.0, 135.8	1.78, 2.34
	5	134.0	5.44	d	8.5	134.1	1.78, 2.34
	6	135.8					
	7	134.1	6.82	d	16.5	134.0, 135.8, 138.2	4.49, 5.44, 7.53
	8	128.2	6.59	d	16.5	135.8, 138.2, 127.0	1.78, 7.53
	9	138.2					
	10,14	127.0	7.53	dd	7.5, 1.0	127.0, 127.9	6.59, 6.82
	11,13	129.2	7.37	*d*		127.0, 129.2, 138.2	
	12	127.9	7.24	*d*		127.0, 129.2	
	15	13.0	1.78	s		134.0, 135.8	
	NH		8.44	d	11.0	172.2	4.87, 8.08
serine 2	1	171.9					
	2	57.5	3.97	m		61.4, 171.9	2.66, 3.67, 3.95, 8.44
	3	61.4	3.67	m		57.5, 171.9	
			3.95	m			
	NH		8.08	br s		57.5, 61.4, 172.6	2.66, 3.67, 3.95, 8.44
alanine	1	169.5					
	2	51.3	5.24	br d	10.0	169.5, 171.9	8.26
	3	50.6	4.38	dd	13.0, 3.5	51.3, 137.8, 169.5	9.28
			5.08	br d	13.0		
	NH		8.50	d	10.0	51.3, 171.9	3.67, 3.95, 8.26, 9.28
asparagine	1	172.1					
	2	52.5	4.67	m		37.1, 171.3, 172.1	2.46, 7.46
	3	37.1	2.46	dd	16.0, 3.0	52.5, 171.3	4.67, 7.66
			2.84	dd	16.0, 9.5		
	4	171.3					
	NH		8.26	d	3.5	169.5	2.84, 4.67, 5.24, 8.50
	NH_2		6.97	br s			2.46, 2.84
			7.66	br s			
β-hydroxyasparagine	1	171.3					
	2	54.5	5.72	br t	9.5	72.1, 171.3, 172.1, 174.1	5.01, 7.43, 7.46
	3	72.1	4.19	br t	9.0	54.5, 174.1	7.46
	4	174.1					
	NH		8.59	d	9.5	172.1	2.46, 4.19, 4.67, 5.72, 7.46
	NH_2		7.46	*d*		174.1	4.19, 5.72, 8.59
4′-bromo-3-methylphenylalanine	1	171.1					
	2	59.0	4.76	m		18.0, 38.6, 141.4, 171.1, 171.3	1.16
	3	38.6	3.73	m		18.0, 59.0, 131.4, 141.4	1.16, 4.76
	4	18.0	1.16	d	7.5	38.6, 59.0, 141.4	3.73, 4.76, 7.09, 8.02
	5	141.4					
	6,10	131.4	7.09	d	8.5	38.6, 120.6, 131.6	1.16, 3.76, 4.76, 8.02
	7,9	131.6	7.38	d	8.5	120.6, 131.4, 141.4	8.02
	8	120.6					
	NH		8.02	d	7.5	171.3	1.16, 4.19, 4.76, 5.72
isoserine	1	171.7					
	2	70.3	4.27	br s		171.7	3.12
	3	44.4	3.12	m		70.3, 171.7	4.27
			3.96	m			
	NH		7.38	m			1.16, 3.12, 4.76

Table 1 (Continued)

amino acid		δ^b ^{13}C	^1H	*m*	*J* (Hz)	HMBC	ROESY[c]
α-aminoadipic acid	1	173.9					
	2	51.9	4.36	m		22.2, 31.9, 173.9	1.00, 1.77
	3	31.9	1.63	m			4.36
			1.77	m			
	4	22.2	1.00	m		173.4	
			1.38	m			
	5	34.8	1.76	m		173.4	
			2.26	m			
	6	173.4					
	NH		7.62	d	14.0	14.0, 51.9, 171.7, 173.9	4.27, 4.36
histidine	1	169.8					
	2	54.7	5.01	m		27.4, 169.8	3.01, 7.54
	3	27.4	3.01	m		54.7, 124.6, 131.8, 169.8	3.81, 5.01
			3.52	m			
	4	131.8					
	5	124.6	7.44	s		131.8, 137.8	3.01, 4.38, 5.01, 5.08
	7	137.8	9.28	s		124.6, 131.8	3.81, 4.38, 5.08, 5.27, 8.50
	NH		8.79	d	7.5	27.4, 173.4	2.26, 3.01, 3.52
arabinose	1	88.0	5.27	d	8.0	70.6, 131.8, 137.8	3.52, 3.58, 4.01, 9.28
	2	70.6	3.81	dd	8.0, 9.0	73.8, 88.0	1.15, 3.52, 7.54, 8.42, 9.28
	3	73.8	3.58	dd	9.0, 2.5	70.6	4.01, 5.27
	4	69.3	3.88	br s		70.6, 73.8	
	5	69.4	4.01	br s		69.3, 73.8, 88.0	3.58, 5.27

[a] All spectra recorded at 125 MHz for ^{13}C and 500 MHz for ^1H. [b] Referenced to residual solvent DMF-d_7 at 25 °C. [c] Mixing time = 100 ms; field strength = 1900 Hz. [d] Multiplicities not assigned due to extensive overlap of aromatic and amide signals between δ 7.2 and 7.4. [e] AHMP = 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid.

and ROESY experiments.[11] The (5*E*,7*E*) geometry of the 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid moiety was elucidated from the ^{13}C NMR chemical shift of the 6-methyl signal at δ 13.0 and the proton coupling constant ($J_{7,8}$ = 16.5 Hz) associated with the *trans*-disubstituted olefin. A single HMBC experiment (*J* = 6 Hz) showed all of the correlations required to sequence the molecule and, in particular, showed that the α-aminoadipic acid was linked through the 6-carboxylic acid. The HMBC experiment also indicated the presence of asparagine and β-hydroxyasparagine rather than aspartic acid and β-hydroxyaspartic acid. The sequence of peptide bonds identified in the HMBC experiment was confirmed by a ROESY experiment. The HMBC correlation from a proton signal at δ 5.27 to carbon signals at δ 131.8 and 137.8 indicated that a pentose sugar, identified from coupling constants as arabinose, was attached at C-1 to the remaining nitrogen on the imidazole ring of the histidinoalanine residue.

The absolute configurations of L-*allo*-threonine, L-α-aminoadipic acid, L-asparagine, (2*S*,3*R*)-3-hydroxyasparagine, L-phenylalanine, and two L-serine residues were determined by hydrolysis of **2** using 6 N hydrochloric acid at 110 °C, followed by derivatization to form the pentafluoropropionylamide isopropyl esters and analysis by GC-MS using a chiral column. The retention times are shown in Table 2. (2*R*)-Isoserine was detected by coinjection of the derivatized hydrolysate with the derivatives of both racemic and (2*S*) isoserine, prepared by the method of Miyazawa *et al.*[12] Hydrogenation of **2** in 50% aqueous acetonitrile at atmospheric pressure over a 5% palladium on charcoal catalyst removed the bromine substituent from the 4′-bromo-3-methylphenylalanine residue and reduced the olefinic bonds in the 3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid residue to obtain a single diastereoisomer of unknown absolute configuration at C-6. The product was hydrolyzed, derivatized, and analyzed by GC-MS on a chiral column to identify (2*S*,3*S*)-3-methylphenylalanine and one C-6 diastereoisomer of (3*S*,4*S*)-3-amino-4-hydroxy-6-methyl-8-phenyloctanoic acid. The same sequence of reactions was performed on an authentic sample of theonellamide F (**1**) to obtain both C-6 diastereoisomers of (3*S*,4*S*)-3-amino-4-hydroxy-6-methyl-8-phenyloctanoic acid; the GC peak of the major diastereoisomer (85%) corresponded exactly to the peak obtained from theonegramide (**2**). Hydrolysis of **2** for 16 h in 5 N hydrochloric acid at 104 °C followed by ozonolysis for 30 min at −70 °C in methanol solution, oxidative workup using hydrogen peroxide at 25 °C for 45 min, derivatization, and chiral GC-MS analysis produced (*R*)-diaminopropionic acid and additional L-aspartic acid (1.5 equiv total). The alanine portion of histidinoalanine must therefore have the (*R*)-configuration while the histidine portion has the (*S*)-

Table 2. Retention Times (in min) of Derivatized Amino Acid Residues

D-threonine	13.04
L-threonine	13.60
D-serine	16.70
D-*allo*-threonine	16.89
L-serine	17.13
L-*allo*-threonine	17.20
(2*S*,3*R*)-3-hydroxyaspartic acid	21.60
(2*R*,3*S*)-3-hydroxyaspartic acid	21.93
(*S*)-isoserine	22.56
(*R*)-isoserine	22.75
D-aspartic acid	23.20
L-aspartic acid	23.36
(2*S*,3*S*)-3-hydroxyaspartic acid	23.90
(2*R*,3*R*)-3-hydroxyaspartic acid	24.11
(2*R*,3*R*)-3-methylphenylalanine	30.87
(2*S*,3*S*)-3-methylphenylalanine	31.09
(2*R*,3*S*)-3-methylphenylalanine	32.22
(2*S*,3*R*)-3-methylphenylalanine	32.71
D-2-aminoadipic acid	35.25
D-2,3-diaminopropionic acid	35.33
L-2-aminoadipic acid	35.51
L-2,3-diaminopropionic acid	35.77
(3*S*,4*S*)-3-amino-4-hydroxy-6-methyl-8-phenyloctanoic acid	40.79

(11) The choice of DMF-d_7 as NMR solvent was the key to obtaining sharp NMR signals. We tried most common NMR solvents and combinations of solvents without success. For example, a ^{13}C NMR spectrum in DMSO-d_6 showed only aromatic and methyl signals.

(12) Miyazawa, T.; Akita, E.; Ito, T. *Agric. Biol. Chem.* **1976**, *40*, 1651.

configuration. Mild acid hydrolysis in 4 N hydrochloric acid at 70 °C for 12 h followed by derivatization with pentafluoropropionic anhydride and analysis by chiral GC-MS produced peaks that were identical to those obtained by identical treatment of an authentic specimen of D-arabinose.[13]

The structure of theonegramide (**2**) is similar in many respects to that of theonellamide F (**1**) except that four of the twelve amino acid residues are different and that **2** contains an arabinose moiety. In addition, 4′-bromo-3-methylphenylalanine has not been reported previously as a natural product. Theonegramide (**2**) inhibits the growth of *Candida albicans* (ATCC 32354) in the standard disk assay at a loading of 10 μg/disk.

Experimental Section

Isolation of Theonegramide (2). The sponge *Theonella swinhoei* (voucher specimens are deposited at Silliman University and Scripps Institution of Oceanography, acquisition number NCI-036) was collected by hand using SCUBA at a depth of 20 m at Antolang, Negros Island, The Philippines. The sponge was frozen and stored at −20 °C until being freeze-dried prior to extraction. The lyophilized sponge (70 g) was extracted with 1:1 hexanes:CH_2Cl_2 (3 × 700 mL) to yield a brown oil (1.45 g), EtOAc (2 × 500 mL) to yield an amber oil (140 mg), and 1:1 EtOAc:acetone (2 × 500 mL) to yield a pale yellow oil (240 mg), followed by exhaustive extraction with 50% aqueous CH_3CN until antifungal activity was no longer detected in the extract. CH_3CN was removed from the aqueous extract under reduced pressure until the solution started to turn cloudy; it was then centrifuged and loaded onto a reversed-phase preparative cartridge (Waters Bondapak C18, 47 × 300 mm) equilibrated in 0.05% TFA. The column was washed with four column volumes (ca. 2 L) of 0.05% TFA before running a linear gradient from 20 to 60% CH_3CN in 0.05% TFA over 60 min (70 mL/min, UV detection at 230 nm). Fractions showing antifungal activity against *C. albicans* that were >97% pure (Vydac C18 analytical column, 38% CH_3CN in 0.05% TFA at 1.5 mL/min, UV detection at 215 nm) were lyophilized. Fractions that were antifungal but <97% pure were combined, repurified using the same conditions, and lyophilized to obtain theonegramide (**2**, 195 mg total, 0.2% dry wt).

Theonegramide (2): white powder; $[\alpha]_D = +19°$ ($c = 0.39$, 1:1 CH_3CN:H_2O; UV (MeOH) 200 (ε 18 600), 278 (ε 12 700), 288 (ε 12 900), 305 nm (sh, ε 7100); IR (KBr) 3300, 2920, 1660, 1540 cm^{-1}; ^1H NMR (DMF-d_7) see Table 1; ^{13}C NMR (DMF-d_7) see Table 1; HRFABMS, m/z = 1717.6080 $(M + H)^+$, $C_{75}H_{98}{}^{79}BrN_{16}O_{26}$ requires m/z = 1717.6020.

Methylation of Theonegramide (2). A solution of theonegramide (**2**, 2 mg) in DMF (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 continued for 6 h followed by lyophilization. The reaction product was submitted for FABMS without further purification: m/z 1734 $(M + H)^+$. The ^1H NMR spectrum contained a new methyl signal at δ 3.69 (s, 3 H).

Determination of Absolute Configuration. (a) Hydrolyses with 6 N HCl. A solution of peptide or reaction products in degassed 6 N HCl (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 N_2, with heating, and then under high vacuum.

(b) Derivatization and Analysis. A premixed solution of acetyl chloride (1 part) in 2-propanol (4 parts) (500−800 μL) 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 N_2. Pentafluoropropionic anhydride (PFPA, 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 N_2. The residue was dissolved in CH_2Cl_2 (100 μL) and immediately analyzed by GC-MS using an Alltech Chirasil-Val capillary column (0.32 mm × 25 m). The oven temperature was ramped from 50 to 120 °C at 3°/min continuing to 210 °C at 5°/min, and a mass range of 50 to 600 Da was recorded every 1.96 s. The identity of each peak was confirmed by coinjection with a solution of a standard that had been derivatized in the same manner (see Table 2).

(c) Ozonolysis and Oxidation of Hydrolysate of Theonegramide (2). A 500-μL sample of the hydrolysate prepared from theonegramide (**2**, 1.5 mg) in 5 N HCl (1.0 mL) was evaporated to dryness and twice relyophilized from water. The material was redissolved in dry MeOH (1.5 mL) and cooled to −70 °C, and a stream of ozone was bubbled into the cooled solution for 20 min. After the solution was warmed to room temperature, 50% hydrogen peroxide solution (1.5 mL) was added to the solution, and the resulting solution was stirred for an additional 40 min, after which excess reagents were removed under high vacuum. The mixture was derivatized as described in (b) above. Two new peaks were observed in the GC trace corresponding to (*R*)-diaminopropionic acid, derived from the alanine portion of histidinoalanine, and (*S*)-aspartic acid (ca. 1.5 equiv vs 1 equiv in the original hydrolysis experiment), derived from the histidino portion. No (*S*)-diaminopropionic acid was detected.[6]

(d) Hydrogenation of Theonegramide (2). A solution of theonegramide (**2**, 3 mg) in 50% aqueous CH_3CN (1 mL) containing 5% Pd/C catalyst was stirred under an atmosphere of hydrogen for 16 h. The solution was centrifuged and the supernatant was filtered and lyophilized. The ^1H NMR spectrum showed the absence of the diene signals at δ 5.44, 6.59, and 6.82. A sample (600 μg) of the hydrogenated product was hydrolyzed and the products were derivatized as described in (a) and (b) above. A peak in the GC trace corresponding to (2*S*,3*S*)-3-methylphenylalanine was derived from (2*S*,3*S*)-4′-bromo-3-methylphenylalanine. A single peak eluting at 40.79 min, which corresponds to one of the C-6 diastereoisomers of (3*S*,4*S*)-3-amino-4-hydroxy-6-methyl-8-phenyloctanoic acid [*m/z* 392, 274, 230, 216, 190, 169, 145, 131, 119, 105, 104, 91 (100%)], was also observed in the GC trace (see below).

(e) Hydrogenation of Theonellamide F (1). Theonellamide F (**1**, 800 μg) was hydrogenated, hydrolyzed, and derivatized as described in (d) above. The GC trace of the hydrolyzed hydrogenation product showed two peaks with identical mass spectra that corresponded to the two possible C-6 diastereoisomers of (3*S*,4*S*)-3-amino-4-hydroxy-6-methyl-8-phenyloctanoic acid. The major peak (85%), which corresponds to the C-6 diastereoisomer obtained above, eluted after 40.79 min and the minor peak (15%) eluted after 40.45 min. Neither peak was observed among the hydrolysis products of theonellamide F (**1**) or theonegramide (**2**).

(f) Analysis for Arabinose. A solution of theonegramide (500 μg) in degassed 4 N HCl (1 mL) was heated to 70 °C for 12 h. Excess HCl was removed under high vacuum and the hydrolysate derivatized with PFPA (400 μL) in CH_2Cl_2 (400 μL) at 100 °C for 15 min. Excess reagents were removed under a stream of dry nitrogen and the derivative analyzed by chiral GC-MS. The peaks observed by GC-MS were identical to those of D-arabinose.[13]

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of theonegramide (**2**), expansions of the TOCSY spectrum in F2, and regions of the HMBC and ROESY spectra used for sequencing theonegramide (**2**) (7 pages). This material is contained in 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.

(13) König, W. A.; Benecke, I.; Bretting, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 693.