JOC Article

Lobocyclamides A−C, Lipopeptides from a Cryptic Cyanobacterial Mat Containing *Lyngbya confervoides*

John B. MacMillan, Michael A. Ernst-Russell, Jeffrey S. de Ropp, and Tadeusz F. Molinski*

Department of Chemistry, One Shields Avenue, University of California, Davis, California 95616

tfmolinski@ucdavis.edu

Received July 12, 2002

The structures of lipopeptides lobocyclamides A (**1**), B (**2**), and C (**3**) were solved using a combination of mass spectrometry, 2D NMR spectroscopy, and degradative analysis. Lobocyclamides B and C are the first peptides reported with the unusual amino acid 4-hydroxythreonine and also incorporate the rare homologous long-chain β-amino acids 3-aminooctanoic acid and 3-aminodecanoic acid, respectively. The absolute configurations of the amino acid residues in each compound were assigned, after acid hydrolysis, by either direct chiral HPLC comparison with authentic standards or by prior derivatization by Marfey's method and reversed-phase HPLC. Both compounds exhibited moderate antifungal activity against a panel of *Candida* spp., including two fluconazole-resistant strains. When tested as a mixture, lobocyclamides A and B displayed synergistic in vitro antifungal activity, a phenomenon noted earlier for the related peptides laxaphycins A and B.

Introduction

Filamentous cyanobacteria of the genus *Lyngbya* express a prodigious array of secondary metabolites—mainly peptides and occasionally polyketides or other alkaloid metabolites.[1] *Lyngbya* spp. is commonly found growing on coral reefs in the form of thin diaphanous red or brown "veils". Cyanobacteria are also known to cohabit marine sponges in highly conserved symbiotic associations. For example, a sample of the tropical sponge *Dysidea herbacea* (comprising ~40% *Oscillatoria* sp. by tissue mass) contains the same compound, neodysidenin,[2] that was isolated from a free-living form of *L. majuscula* found in shallow waters in Panama.[3] Evidence suggests that a large abundance of cyanobacteria may be present in laminate marine sediments and shallow-ocean coral sand sediments.[4] Since some cyanobacteria (e.g., *Oscillatoria* spp.) carry out both photosynthesis and nitrogen fixation, such mats may be significant contributors to nutrient cycling in coral reef environments. Our interest in the symbiosis of cyanobacteria (*Oscillatoria* and *Lyngbya*) with sponges prompted us to examine a limited collection of cyanobacteria samples obtained during an expedition in the Southern Bahamas. We report the structures of new antifungal lipopeptides, lobocyclamides A−C (**1**−**3**, Figure 1), from a benthic sample of *Lyngbya confervoides* collected at Cay Lobos, Bahamas. Only one prior report describes a natural product from *L. confervoides*—the depsipeptide obyanamide.[5] To the best of our knowledge, **2** and **3** are the first peptides reported to contain the novel amino acid 4-hydroxythreonine.[6]

Methanol extracts obtained by mechanical agitation of solvent with lyophilized coral sand sediment and *Lyngbya confervoides* filaments showed significant in vitro antifungal activity against *Candida albicans*. The extract was separated by silica gel chromatography and reversed-phase HPLC to obtain pure samples of lobocyclamides A (**1**), B (**2**), and C (**3**). The molecular formula of lobocyclamide A (**1**) was established as $C_{59}H_{95}N_{11}O_{15}$ by HREIMS. The ^{1}H NMR spectrum (DMSO-d_6, Table 1) exhibited signals characteristic of a peptide including NH and OH resonances (δ 6−11), α-hydrogen signals (δ 4−6), and Me doublets and triplets (δ 0.7−1.0), characteristic of a peptide containing numerous aliphatic and hydroxylated residues. The downfield pair of doublets at δ 6.62 (d, 2H, J = 8.4 Hz) and 7.15 (d, 2H, J = 8.4 Hz) were characteristic of the para-substituted phenyl ring of tyrosine. Additional evidence for the peptide nature of **1** was seen in the presence of 11 amide carbonyl resonances (δ 166.8−172.6 ppm) suggesting an undecapeptide.

Automated amino acid analysis of **1** confirmed the presence of the proteinogenic amino acids Gly, Tyr, Leu

* To whom correspondence should be addressed. Tel: 530 752 6358. Fax: 530 752 8995.

(1) For recent examples, see: (a) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65* (1), 29−31 (b) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65* (1), 16−20. For a recent review, see: (c) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. In *The Alkaloids*; Academic Press: San Diego, 2001; Vol. 57, pp 75−184.

(2) MacMillan, J. B.; Trousedale, E. K.; Molinski, T. F. *Org. Lett.* **2000**, *21*, 2721−2723.

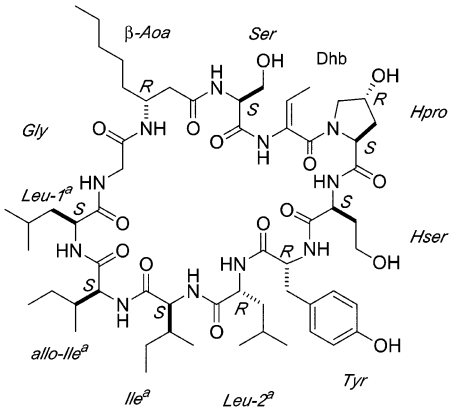
(3) Jiménez, J.; Scheuer, P. J. *J. Nat. Prod.* **2001**, *64*, 200−203.

(4) The highly ordered and metabolically dynamic populations of cyanobacteria that occur in stratified microbial communities of shallow coastal mud sediments are well-described (Stal, L. J. *New Phytol.* **1995**, *131*, 1−32), but less is known of open-ocean sand-sediment cyanobacterial communities.

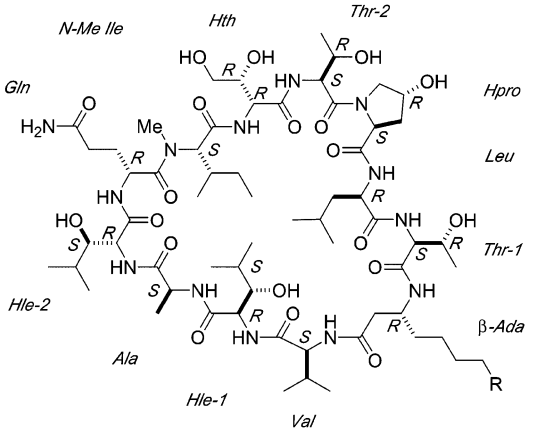
(5) (a) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 29−31. See also our preliminary communication: (b) MacMillan, J. B.; Molinski, T. F. *Org. Lett.* **2002**, *4*, 1883−1886.

(6) 4-Hydroxythreonine is an intermediary metabolite in the biosynthesis of vitamin B_6: (a) Hill, R. E.; Himmeldirk, K.; Kennedy, I. A.; Pauloski, R. M.; Sayer, B. G.; Wolf, E.; Spenser, I. D. *J. Biol. Chem.* **1996**, *271* (48), 30426−30435. (b) Pirrung, M. C.; Nunn, D. S.; McPhail, A. T.; Mitchell, R. E. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2095−2098.

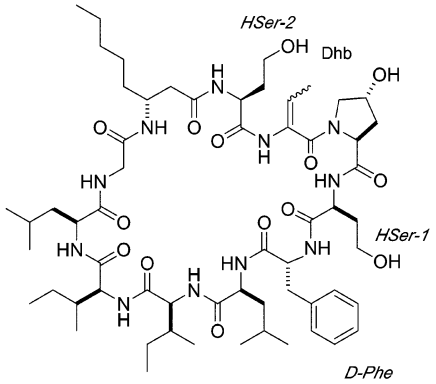
10.1021/jo0261909 CCC: $22.00
Published on Web 10/23/2002



1 *Lobocyclamide A*



2 R = Et *Lobocyclamide B*
3 R = H *Lobocyclamide C*



4 *Z*-Dhb *Hormothamnin A*
5 *E*-Dhb *Laxaphycin A*

FIGURE 1. Structures of lobocyclamides A (**1**), B (**2**), C (**3**), hormothamnin A (**4**), and laxaphycin A (**5**). The latter two structures are annotated at amino acid residues that differ from **1**. The sequence of the tetrad of four nonpolar acids in **1** was not rigorously assigned but assumed to be same as that of **4** and **5**. See Gerwick et al., ref 7.

(× 2), Ile (× 2), Ser, and *trans*-4-hydroxyproline (Hpr). The presence of the nonstandard α-amino acid residues 2,3-dehydro-2-aminobutanoic acid (Dab) and homoserine (Hse) was deduced from interpretation of 2D NMR spectra (600 MHz, DMSO-d_6), in particular TOCSY spectra, and in the latter case, confirmed by HPLC comparison of the acid hydrolysate with an authentic standard. Dab was identified by the ^{1}H NMR and gHMBC, which revealed ^{1}H signals for the characteristic propylidene group (δ 5.59, q, 1H, J = 7.2 Hz H_3-4; 1.70, d, 3H, J = 7.2 Hz) and polarized vinyl ^{13}C signals (119.8, s, C3; 131.0, s, C2). The presence of the β-amino acid residue 3-aminooctanoic acid, Aoa (**6**), was suggested from TOCSY and COSY data, which showed sequential spin correlations starting with the diastereotopic α-CH_2 proton signals (δ 1.86 bd, 1H, J = 13.8 Hz; 1.57, m, 1H), β-CH signal (δ 4.26, m, 1H), γ-CH_2 signals (δ 1.23, m, 1H; 1.32, m, 1H), and the β-NH signal (δ 6.80, d, 1H, J = 9.2 Hz). The identity and configuration of the Aoa residue was confirmed as (*R*)-(+)-**6** by hydrolysis of **1** and HPLC comparison with an authentic sample prepared by conventional methods (see below).

At this point, it was clear that the composition of **1** was very similar to the known peptides hormothamnin (**4**) isolated from *Hormothamnion enteromorphoides*[7] and laxaphycin A, isolated from the terrestrial cyanobacterium *Anabaena laxa*.[8] The differences in amino acid composition are substitution of Hpr, Phe, and Hse-1 in **4** for Pro, Tyr, and Ser in **1**, respectively. The bond geometry for Dab was *Z* in **4** but *E* in **1** (ROESY, see below). Chiral HPLC amino acid analysis (Chirex D-penicillamine) revealed that one of the two Ile residues (designated here as Ile-1 and Ile-2) was the *allo*-L-diastereomer and that the two Leu residues were of opposite absolute configurations (L and D). Assignment of NMR signals and elucidation of the amino acid sequence of **1** was carried out by a combination of gHMBC, gHSQC−TOCSY, and ROESY (mixing time = 500 mS). Strong ROESY cross-peaks between the α-H signal for residue *i* and the amide NH signal for the *i* + 1 residue were particularly useful for sequence assignment. Dipolar correlations (Figure 1) were observed

(7) Gerwick, W. H.; Jiang, Z. D.; Agarwal, S. K.; Farmer, B. T. *Tetrahedron* **1992**, *48*, 2313−2324.

(8) (a) Frankmölle, W. P.; Knübel, G.; Moore, R. E.; Patterson, G. M. L. *J. Antibiot.* **1992**, *45*, 1458−1466. (b) Frankmölle, W. P.; Larsen, L. K.; Caplan, F. R.; Patterson, G. M. L.; Knübel, G.; Levine, I. A.; Moore, R. E. *J. Antibiot.* **1992**, *45*, 1451−1457. (b) Bonnard, I.; Rolland, M.; Francisco, C.; Banaigs, B. *Lett. Pept. Sci.* **1997**, *4*, 289−292.

TABLE 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) of 1 and 2 in DMSO-d_6

		lobocyclamide A (**1**)					lobocyclamide B (**2**)[d]				
		δ ^{13}C NMR (mult)	^{1}H NMR	mult (*J*, Hz)	ROESY[c]			δ ^{13}C NMR (mult)	^{1}H NMR	mult (*J*, Hz)
Aoa	1	168.8 (s)				Ada	1	171.4 (s)[b]		
	2	40.2 (t)	1.86, 1.57	d (13.8)			2	39.7 (t)	2.32, 2.44	d (13.8)
	3	44.7 (d)	4.26	m			3	45.9 (d)	4.08	m
	4	35.0 (t)	1.32, 1.23	m, m			4	33.5 (t)	1.26	m, m
	5	26.2 (t)	0.90	m			5	25.2 (t)	1.21	m
	6	30.8 (t)	0.84	m			6	28.7 (t)	1.18	m
	7	25.2 (t)	0.79	m			7	28.5 (t)	1.18	m
	8	13.9 (q)	0.70	t (6.6)			8	31.8 (t)	1.18	m
	NH		6.80	d (9.2)	4.26, 1.23, 8.64		9	22.0 (t)	1.21	m
							10	13.8 (q)	0.83	t
Gly	1	167.3 (s)					NH		7.67	d (9.2)
	2	42.2 (t)	3.80, 3.14	dd (8.4, 17.4)						
	NH		8.64	bt	6.80 (w), 3.95, 3.14 (w)	Thr1	1	168.7 (s)		
							2	58.2 (d)	4.08	m
							3	66.3 (d)	3.96	m
Leu 1	1	172.4[a] (s)					4	19.6 (t)	1.01	m
	2	53.0 (d)	3.95	m			NH		7.83	d
	3	38.8 (t)	1.55, 1.34	m, m			OH		4.85	m
	4	24.1 (d)	1.62	m						
	5	22.7 (q)	0.90	d (6.6)		Leu	1	171.7 (s)		
	6	21.4 (q)	0.84	d			2	51.2 (d)	4.37	dd (8.4, 17.4)
	NH		8.49	d (4.0)	4.62, 3.95 (w), 2.01, 1.55		3	41.2 (t)	1.45	bt
							4	24.2 (d)	1.32	m
							5	22.8 (q)	0.87	m
Ile 1	1	172.6 (s)[a]					6	21.7 (q)	0.81	m
	2	53.5 (d)	4.62	dd (4.2, 9.6)			NH		8.03	m
	3	37.0 (d)	2.01	m						
	4	21.3 (t)	1.20, 1.12	m, m		Hpr	1	171.5 (s)[b]		
	5	11.1 (q)	0.83	t			2	58.7 (d)	4.38	m
	6	14.6 (q)	0.80	d			3	37.7 (t)	1.81, 1.98	m, m
	NH		8.35	d (9.2)	4.69, 4.62, 1.81		4	68.6 (d)	4.30	m
							5	55.4 (t)	3.56, 3.68	d (6.6)
Ile 2	1	172.6 (s)[a]					OH		5.11	m
	2	55.7 (d)	4.69	bd (8.4)						
	3	38.9 (d)	1.81	m		Thr2	1	168.9 (s)		
	4	21.6 (t)	1.09, 0.86	m, m			2	55.9 (d)	4.45	dd (4.2, 9.6)
	5	11.6 (q)	0.74	t (7.2)			3	66.5 (d)	3.89	m
	6	15.5 (q)	0.71	d (6.0)			4	18.9 (t)	1.04	m, m
	NH		6.34	bs			NH		7.38	d (9.2)
							OH		4.91	m
Leu 2	1	171.5 (s)								
	2	51.6 (d)	4.23	m		Hth	1	170.2 (s)		
	3	39.2 (t)	1.23, 1.06	m, m			2	55.5 (d)	4.38	bd (8.4)
	4	24.1 (d)	1.53	m			3	62.3 (d)	3.90	m
	5	22.9 (q)	0.78	d (6.6)			4	70.5 (t)	3.27	m, m
	6	20.3 (q)	0.71	d (6.0)			NH		7.74	bs
	NH		7.20	bd	4.23		3-OH		4.61	t (7.2)
							4-OH		4.90	d (6.0)
Tyr	1	172.5 (s)[a]								
	2	57.0 (d)	4.15	ddd (3.0, 7.8, 13.8)		*N*-Me Ile	1	170.1 (s)		
	3	36.3 (t)	2.99, 2.80	dd (3.0, 12.8)			2	59.6 (d)	4.78	m
	4	128.3 (s)					3	31.8 (d)	1.81	m, m
	5,9	130.1 (d)	7.15	d (8.4)			4	23.9 (t)	0.89, 1.27	m
	6,8	114.9 (d)	6.62	d (8.4)			5	15.2 (q)	0.81	d (6.6)
	7	155.7 (s)					6	10.4 (q)	0.78	d (6.0)
	OH		9.15	s			N-Me	30.2 (q)	3.01	bd
	NH		7.90	d (8.0)	7.15, 4.29 (w), 4.15, 2.99					
						Gln	1	172.6 (s)		
							2	49.1	4.60	ddd (3.0, 7.8, 13.8)
Hse	1	172.3 (s)[a]					3	26.3 (t)	1.59, 1.96	dd (3.0, 12.8)
	2	48.7 (d)	4.29	m			4	30.4 (t)	2.12, 2.23	
	3	33.8 (t)	2.03, 1.93	m, m			5	174.6 (s)		d (8.4)
	4	57.0 (t)	3.50	10.8, 6.4, 4.8			NH		7.64	d (8.0)
			3.33	7.2, 6.4, 4.8			NH_2		6.96, 7.38	d (8.4)
	OH		4.35	t (4.8)						
	NH		7.15	d (overlap)	4.49, 4.29	Hle2	1	170.7 (s)		
							2	54.4 (d)	4.29	m
Hpr	1	170.1 (s)					3	75.8 (d)	3.46	m, m
	2	59.4 (d)	4.49	dd (9.6)			4	29.9 (d)	1.56	m, m
	3	38.1 (t)	2.26, 1.75	dd (7.8, 12.6)			5	18.9 (q)	0.74	m
	4	68.1 (d)	4.21	m			6	18.6 (q)	0.88	m
	5	57.0 (t)	4.05, 3.19	bd (9.6)			NH		7.75	d (7.2)
	OH		5.14	d (2.8)			OH		5.12	t (4.8)

Table 1. (Continued)

		lobocyclamide A (**1**)					lobocyclamide B (**2**)[d]				
		δ ^{13}C NMR (mult)	^{1}H NMR	mult (*J*, Hz)	ROESY[c]			δ ^{13}C NMR (mult)	^{1}H NMR	mult (*J*, Hz)
Dab	1	166.8 (s)				Ala	1	172.6 (s)		
	2	131.0 (s)					2	49.4 (d)	4.22	dd (9.6)
	3	119.8 (d)	5.59	q (7.2)			3	17.7 (q)	1.32	dd (7.8, 12.6)
	4	12.2 (q)	1.70	d (7.2)			NH		7.99	d (7.6)
	NH		10.90	s	5.59, 4.37					
						Hle1	1	171.4 (s)[b]		
Ser	1	172.7 (s)[a]					2	55.2 (d)	4.39	
	2	53.7 (d)	4.37	q (8.4)			3	76.6 (d)	3.49	q (7.2)
	3	60.6 (t)	3.62	m			4	30.6 (d)	1.60	m
	OH		5.15	t (5.2)			5	19.3 (q)	0.91	m
	NH		6.79	d (9.2)	4.37, 1.57		6	18.7 (q)	0.77	m
							NH		8.08	s
							OH		4.91	d (7.2)
						Val	1	171.4 (s)[b]		
							2	58.9 (d)	4.14	q (8.4)
							3	29.4 (d)	1.96	m
							4	18.9 (q)	0.85	m

[a,b] Interchangeable. [c] w; weak dipolar coupling. [d] Two conformers are present in a 1:3 ratio. Values listed are for the major isomer. In CD_3OH the ratio is 1:1.6.

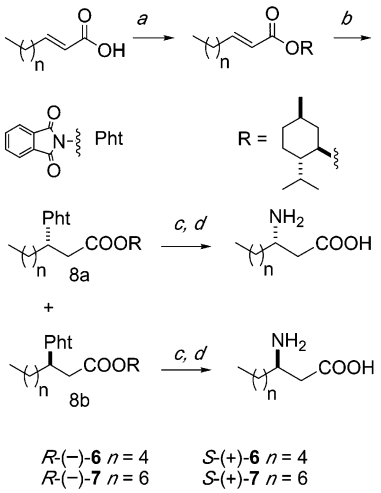
between the Gly NH (δ 8.64, bt) and Leu-1 H2 (δ 3.95); Leu-1 NH (δ 8.49, d, 1H, J = 4.0 Hz), Ile-1 H2 (δ 4.62, dd, 1H, J = 9.6, 4.2 Hz), and H3 (δ 2.01, m), and also between Ile-1 NH (δ 8.35, d, 1H, J = 9.2 Hz), Ile-2 H2 (δ 4.69, bd, 1H, J = 8.4 Hz), and H3 (δ 1.81). Correlations were also observed between signals for Dab NH (δ 10.90, bs) and Ser H2 (δ 4.37, d, 1H, J = 8.4 Hz); the Ser NH (δ 6.79, d, 1H, J = 9.2 Hz) and Aoa H2 at δ 1.57; Hse NH δ 7.15 and Hpr Hα (δ 4.49, d, 1H, J = 9.6 Hz); and between the Tyr NH (δ 7.90, d, 1H, J = 8.0 Hz) and Hse H2 (δ 4.29, m). An amide bond between the Dab and Hpr residues was identified by the observation of a gHMBC correlation between the ^{1}H NMR signal Hpr H2 and ^{13}C NMR signal for the Dab carbonyl signal (δ 166.8, s).

A strong cross-peak observed in the ROESY spectrum of **1** between the Dab NH (δ 10.90) and the sole vinyl proton signal (δ 5.59, q, 1H, J = 7.2 Hz) established the configuration of this moiety as *E*. Thus, the structure of lobocyclamide A was established as cyclo[Aoa-Hse-(*E*)-Dab-Hpr-Tyr-Leu2-Ile2-Ile1-Leu1-Gly]. Total hydrolysis of **1** (6 M HCl, 100 °C, 17 h) followed by chiral HPLC analysis (Chirex D-penicillamine column, 1−14% CH_3-CN/2 mM aq $CuSO_4$, 1 mL/min) and comparison of peak retention times with those of amino acid standards provided the assignment of absolute configuration of the α-amino acid residues. The presence of two pairs of stereoisomers in **1**—L-Ile and L-*allo*-Ile, and the L- and D-enantiomers of Leu (see configuration analysis below)—generates ambiguity in assignment of sequence of these four contiguous amino acids. This was not addressed explicitly, and we assume the sequence of the nonpolar residue tetrad is the same as that found in **4** and **5**. Aside from this assumption, the sequence of the remaining amino acid residues in **1** was determined explicitly from ROESY data and found to be the same as that of **4**.

HREIMS data established the molecular formula of lobocyclamide B (**2**) as $C_{65}H_{115}N_{13}O_{20}$. NMR spectra of **2** measured in CD_3OH solution revealed two major conformers of lobocyclamide B in a ratio of 1.6:1, which complicated analysis. Measurements of the ^{1}H NMR spectra of **2** or **3** in DMSO-d_6 gave a more manageable ratio of ∼3:1; therefore, this solvent was used for ROESY and heteronuclear 2D NMR experiments. Automated amino acid analysis of **2** identified seven proteinogenic amino acid residues: Ala, Leu, Val, *trans*-4-hydroxyproline (HPr), two threonine residues (Thr-1 and Thr-2), and Glu, which was derived from hydrolysis of Gln(see below). Analysis of the TOCSY spectrum of **2** established the identity of another three residues as *N*-methylisoleucine (*N*-MeIle) and β-hydroxyleucine (2 equiv, Hle-1 and Hle-2). Evidence for the presence of the rare amino acid, 4-hydroxythreonine moiety (Hth)—the first reported occurrence in a peptide—was substantiated by HSQC−TOCSY (600 MHz) which resolved sequential ^{1}H−^{1}H correlations associated with the α,β,γ, and OH signals from the heavily congested α-CH region between δ 3.2−4.4 ppm. The β-amino acid, (*R*)-3-(+)-aminodecanoic acid, Ada [(+)-**7**] was identified in the acid hydrolysate of **2** (110 °C HCl, 18 h) by HPLC comparison with authentic standards.[5b] TOCSY and ROESY also allowed assignment of the Gln spin network from the CdO signal (δ 172.6 ppm) through to the primary amide NH2 1H signals (δ 6.96, d, 1H, J = 8.4 Hz; 7.38, d, 1H, J = 8.4 Hz). The remaining ^{1}H and ^{13}C NMR signals, were assigned by gHMBC. Satisfactory resolution of some of the carbonyl ^{13}C NMR signals in **2** was not possible under our conditions which limited our ability to obtain sequence data from gHMBC alone. We elected, instead, to sequence the amino acid residues of **2** using ROESY data from sequential pairwise NH and α-CH signal correlations of i and i + 1 amino acid residues as described for **1**. The key ROESY correlations are summarized in Table 1.

HREIMS data established the formula for lobocyclamide C (**3**), $C_{63}H_{111}N_{13}O_{20}$, as a lower homologue of **2**. The 600 MHz ^{1}H NMR spectra (CD_3OH) of **2** and **3** were almost indistinguishable. Amino analysis of the two homologues revealed the same composition of α-amino acids, which suggested the difference lay in the carbon chain length of the long-chain β-amino acid residue. The presence of (*R*)-(+)-2-aminooctanoic acid (Aoa, (−)-**6**) in cyclopeptide **3** was confirmed by hydrolysis (110 °C, 6 M

SCHEME 1



HCl, 18 h) followed Marfey's analysis[9] and comparison with authentic (+)-**6** and (−)-**6**.[5b,10] The latter amino acids, along with (*R*)-(−)-**7** and (*S*)-(+)-**7**, were prepared as follows (Scheme 1). Michael addition of phthalimide to chiral alkenoate esters of (−)-menthol (DBU, DMF, 130 °C) gave a mixture (~1:1) of phthalimides (+)-**8a** and (−)-**8b**. Separation of the diastereomers followed by separate treatments to remove N- and O-protecting groups (NH_2-NH_2, then NaOH, aq) gave pure (+)-**6a** and (−)-**6b** (Scheme 1).

The absolute configuration of the remaining amino acid residues in **2** were identified by Marfey's analysis or by direct analysis of the hydrolysates on a chiral HPLC column (Phenomenex D-penicillamine). Because the ^{1}H NMR spectra of compounds **2** and **3** did not reveal the relative stereochemistry within each β-hydroxy amino acid, we synthesized both enantiomers of each of the *threo* and *erythro* diastereomers of β-hydroxyleucine and β-hydroxythreonine for use as standards in Marfey's analysis. The synthesis, developed for the present purpose, employed a novel (−)-sparteine-mediated asymmetric aldol addition, the details of which were recently published.[5b]

A summary of the HPLC analysis of the Marfey's derivatives of peptide hydrolysates obtained from **2** and **3**, including retention times of standard amino acids, is found in Table 2 (Supporting Information). Quantitative assay of amino acids derived from **2** allowed a definitive configurational assignment of the β-substituted-α-amino acids and revealed 2 equiv each of *threo*-(2*R*,3*S*)-(+)-2-hydroxyleucine and (2*S*,2*R*)-threonine and 1 equiv each of *threo*-(2*R*,3*R*)-(+)-4-hydroxythreonine and *trans*-2*S*,3*R*-3-hydroxyproline. The remaining α-amino acids have the 2*S* configuration, and the configurations of the Aoa (**6**) or Ada (**7**) from their respective source peptides **1**−**3** were found to be *R*.[11] Thus, the complete configurations of **1**−**3** are as shown.

(9) Marfey, P. *Carlsberg. Res. Commun.* **1984**, *49*, 591−596.

(10) Jefford, C. W.; McNulty, J.; Lu, Z.-H.; Wang, J. B. *Helv. Chim Acta* **1996**, *79*, 1203−1216

(11) Cyclopeptides **2** and **3** are homologues that only differ in the long-chain β-amino acid residue. The specific rotations of **2** and **3** are very similar ([α]$_D$ −13 and −16, respectively), and the CD spectra (MeOH) are identical (Cotton effects: **2**, λ 230 ($\Delta\epsilon$ −20.7), **3** 230 nm ($\Delta\epsilon$ −19.3), see the Supporting Information). Thus, we infer that the corresponding configurations of the remaining amino acids in **1** and **2** are the same.

Biological Activity

Lobocyclamides A−C exhibited modest antifungal activity when tested in disk diffusion assays on Saboraub agar plates against fluconazole-resistant fungi *C. albicans* 96−489 (150 μg/disk, **1**; 7 mm zone; **2**, 8 mm; **3**, 10 mm) and *C. glabrata* (**2**, 6 mm; **3**, 8 mm, each at 150 μg/disk) or microbroth dilution assay (*C. albicans* 96−489 MIC **1**, 100 μg/disk; **2**, 30−100 μg/mL). Mixtures of **1** and **2** exhibited significant synergism with higher activity (e.g., 1:1 mixture of **1** + **2**, MIC 10−30 μg/mL) than either of the pure compounds alone, a phenomenon also noted by Moore and co-workers for the laxaphycins A and B.[8]

Experimental Section

General Methods. General procedures can be found elsewhere.[12] Amino acid analyses were performed by the UC Davis Protein Laboratory. (2*S*,3*R*)-*trans*-Hydroxy-L-proline, *allo*-isoL-leucine, *N*-methyl-L-leucine, and Marfey's reagent (2,4-dinitro-5-fluorophenylamino)-L-alaninamide were used as received from a chemical supplier. The corresponding D-reagent was synthesized from D-alaninamide according to Marfey.[9] Asymmetric syntheses of the *threo* and *erythro* diastereomers of 4-hydroxythreonine and 3-hydroxyleucine were carried out as previously described.[5b]

Animal Material. A mixture of *L. confervoides* and coral sand was skimmed from sand substrate by hand (scuba, sample 99-08-041) at Cay Lobos, Southern Bahamas (lat. 22° 22.770′ N; long. 77° 35.847′ W) at a depth of −19.2 m (August 1999) and kept at −20 °C until required. A sample was analyzed by microscopy and the cyanobacterial determined to be largely *L. confervoides* with minor amounts (up to 10%) of *Anabaena* sp. and *Oscillatoria* spp. (Valerie Paul, University of Guam). A type sample is preserved and archived at the University of California, Davis.

Extraction and Isolation. The lyophilized cyanobacterium (794.1 g dry weight) was extracted with CH_2Cl_2/MeOH followed by MeOH (× 2). The CH_2Cl_2/MeOH extract was then reduced in volume to remove CH_2Cl_2, and the water content (v/v) of the resultant MeOH solution was adjusted, followed by sequential partioning against increasingly polar solvents. Concentration of each sequential organic extract afforded *n*-hexane (10% v/v, 212 mg) and $CHCl_3$ (40% v/v, 1.19 g) soluble fractions. MeOH was removed from the aqueous phase under reduced pressure, and the remaining solvent partitioned against *n*-butanol (846 mg). The aqueous phase was removed under vacuum (7.44 g). The extracts were screened for antifungal activity in the disk diffusion assay (150 μg/ disk) against *C. albicans* (ATCC 14503), *C. albicans* (96-489), *C. glabrata*, and *C. krusei*, and the $CHCl_3$ fraction was found to be most active. A portion of the $CHCl_3$ extract (843 mg) was then separated using Sephadex LH-20 with MeOH as eluant. The fractions with antifungal activity were combined (264 mg) and further purified by vacuum chromatography on a C_{18} reversed-phase support using a stepwise gradient from H_2O to MeOH as eluant. The active fraction (55 mg) was separated by on a C_{18} reversed-phase column (10 × 250 mm, 1:1 CH_3CN/H_2O) yielded cyclic peptides lobocyclamides A (**1**, 20 mg, 0.0025% dry weight), B (**2**, 16 mg, 0.0021%) and C (**3**, 4 mg, 0.0005%) with retention times t_R = 10.2, 9.6, and 6.2 min, respectively.

Lobocyclamide A (1): amorphous white powder; [α]$_D$ +33 (*c* 0.17, MeOH); IR (ZnSe) ν_{max} 3308, 2958, 1651, 1645 cm^{-1}; ^{1}H and ^{13}C NMR (400 MHz, DMSO-d_6) see Table 1; ESIMS *m*/*z* [M$^+$] 1197.5; HRMS *m*/*z* [M + Na]$^+$ 1220.6867 (calcd for $C_{59}H_{95}N_{11}O_{15}Na$, 1220.6901).

Lobocyclamide B (2): amorphous white powder; [α]$_D$ −13 (*c* 0.22, MeOH); CD (MeOH) λ 230 ($\Delta\epsilon$ −20.7); IR (ZnSe) ν_{max} 3340,1680, 1660 cm^{-1}; ^{1}H and ^{13}C NMR (400 MHz, DMSO-d_6,

(12) Moon, S. S.; MacMillan, J. B.; Olmstead, M. M.; Anh Ta, T.; Pessah, I. N.; Molinski, T. F. *J. Nat. Prod.* **2002**, *65* (3), 249−254.

see Table 1); ^{1}H and ^{13}C NMR (400 MHz, DMSO-d_6); ESIMS m/z [M$^+$] 1397.5; HRMS m/z [M + Na]$^+$ 1420.8220 (calcd for $C_{65}H_{115}N_{13}O_{20}Na$, 1420.8274).

Lobocyclamide C (3): amorphous white powder; $[\alpha]_D$ −16 (c 0.20, MeOH); CD (MeOH) λ 230 ($\Delta\epsilon$ −19.3); ^{1}H and ^{13}C NMR (400 MHz, CD_3OH) almost identical with that of **2** (see Table 1); ESIMS m/z [M$^+$] 1369.5; HRMS m/z [M + Na]$^+$ 1392.7935 (calcd for $C_{63}H_{111}N_{13}O_{20}Na$, 1420.7961).

Total Acid Hydrolysis of Peptides: Determination of Configuration of 1−3. Lobocyclamide A (**1**), B (**2**), or C (**3**) (∼0.2−1 mg) was separately suspended in 6 N HCl (1 mL) and hydrolyzed by heating in a sealed tube at 100−108 °C for 17 h. Each solution was evaporated under a N_2 stream to obtain the hydrolysate, which was used either for direct analysis by chiral HPLC or Marfey's analysis of amino acid configuration.

Amino Acid Configurations of Amino Acids in Lobocyclamides A (1) and B (2). Chiral HPLC. An aliquot (∼0.1 mg) of the cyclopeptide hydrolysate of **2** was dissolved in 0.1 M HCl and analyzed by HPLC using a chiral column (D-penicillamine). UV detection at 254 nm, flow rate 1.15−1.20 mL min^{-1}. L-Gln in **2** was analyzed as Glu. To resolve overlapped peaks, samples of peptide hydrolysate of **1** or amino acid standards were run under one or more of two sets of conditions optimized for different amino acid polarities: Condition A: 2 mM aq $CuSO_4$/CH_3CN (1:99) flow rate 1.20 mL min^{-1}, L-Ala (t_R 6.2), D-Ala (7.2), L-HSer (9.3), D-Hser (9.8), L-Ser (7.4), d-Ser (8.8), L-Thr (6.4), D-Thr (7.3)), L-*allo*-Ile (27.1), D-*allo*-Ile (35.6), L-Val (18.6 min), D-Val (29.2). Condition B: 2 mM aq $CuSO_4$/CH_3CN (95:5) flow rate 1.15 mL min^{-1}, L-Glu (t_R 33.2 min), D-Glu (37.0), L-Ile (31.1), D-Ile (42.2), L-Leu (35.9), D-Leu (47.0), L-*allo*-Ile (27.1), D-*allo*-Ile (35.6), L-Tyr (46.0), D-Tyr (56.1), hydrolysate of **2** (36.5 min). Condition C: 2 mM aq $CuSO_4$/CH_3CN (86:14), flow rate 1.18 mL min^{-1}, D-Leu (t_R 15 min). The following configurational assignments for **2** were made by co-injection of hydrolysate with authentic standards (L-Ileu, L-*allo*-Ile, D- and L-Leu, D-Tyr, L-Hse, L-Hpro, and L-Ser).

Amino Acid Configurations of Lobocyclamide B (2) and C (3). Marfey's Method. The hydrolysate (∼0.1 mg) was redissolved in water and an aliquot (100 μL) heated for 10 min at 80 °C with a 1% acetone solution of 2,4-dinitrophenyl-5-fluoro-L-alanamide, 2,4-dinitrophenyl-5-fluoro-D-alanamide, or 2,4-dinitrophenyl-5-fluoro-L-leucinamide (200 μL) in the presence of 1.0 M $NaHCO_3$ (20 μL). The mixture was cooled and quenched with 1 N HCl (20 μL) prior to analysis by reversed-phase HPLC (reversed-phase C_{18} column, 4.8 × 250 mm, 1 mL/min, 340 nM, gradient of 9:1 50 mM triethylammonium phosphate (TEA, pH 3.0)/acetonitrile to 1:1% TEA phosphate (50 mM)/acetonitrile over 40 min). See Table 2 (Supporting Information) for summary of HPLC retention times of hydrolysate and standard amino acid Marfey's derivatives.

Biological Activity. Antifungal susceptibility assays were performed as previously described with minor modifications of the published procedure.[13] *C. krusei* and *C. glabrata* were strains obtained the University of Texas Medical Center. *C. albicans* UCD FR1 was a strain raised in-house by repetitive passage through Saboraub media containing inhibitory concentrations of fluconazole.

Acknowledgment. We are grateful to Karl Bailey for assistance with collections, Valerie Paul, University of Guam, for identification of *Lyngbya confervoides*, Scott Stanley, Equine Chemical Analysis Laboratory, UC Davis, for some ion-trap MS data, and the UC Riverside Mass Spectrometry laboratory for HRMS data. Funding for instruments used in this project is acknowledged from NSF CHE 9808183 (400 MHz NMR spectrometer, Department of Chemistry), NIH RR11973 (600 MHz NMR spectrometer, UC Davis NMR Facility), and NIH (LCMS, S10 RR14701-01). This work was supported by the National Institutes of Health (AI 39987).

Supporting Information Available: Experimental details for the syntheses of *S*-(+)- and *R*-(−)-**6**, ^{1}H (DMSO-d_6 and CD_3OH) and ^{13}C NMR, COSY, TOCSY, gHSQC, and gHMBC spectra for **1** and **2**, HSQC-TOCSY for **2**, ^{1}H and ^{13}C NMR, DEPT, and TOCSY (CD_3OH) for **3**, CD spectra for **2** and **3**, and HPLC retention times for Marfey's analyses (Table 2). This material is available free of charge via the Internet at http://pubs.acs.org.

JO0261909

(13) Jones, R. N.; Barry, A. L.; Gavan, T. L.; Washington, J. A., III. In *Manual of Clinical Microbiology*, 4th ed.; Lennette, A., Balows, A., Hausler, W. J., Shadomy, H. J., Eds.; American Society of Microbiology: Washington, DC, 1985.