Carriebowmide, a New Cyclodepsipeptide from the Marine Cyanobacterium *Lyngbya polychroa*

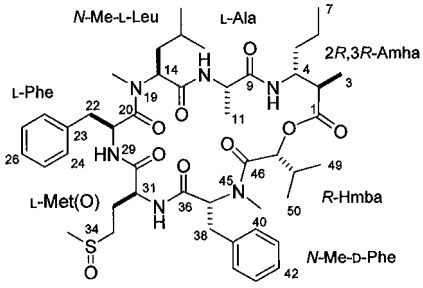
Sarath P. Gunasekera, Raphael Ritson-Williams, and Valerie J. Paul*

Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, Florida 34949

Received July 21, 2008

The new cyclodepsipeptide carriebowmide (**1**), which contains two rare amino acids, 3-amino-2-methylhexanoic acid and methionine sulfoxide, was isolated from the fish-deterrent lipophilic extract of the marine cyanobacterium *Lyngbya polychroa*, collected from the fore reef near the Smithsonian field station at Carrie Bow Cay, Belize. Its planar structure was determined by NMR spectroscopic techniques. The absolute stereochemistry of the hydroxy acid and all α-amino acid-derived units was ascertained by chiral HPLC analysis of the acid hydrolysate. The stereochemistry of the β-amino acid moiety, 3-amino-2-methylhexanoic acid, was established by Marfey analysis of the acid hydrolysate.

Marine cyanobacteria have emerged as a rich source of biologically and ecologically active secondary metabolites, which include lipopeptides, cyclic peptides, and depsipeptides.[1] While marine cyanobacteria are common inhabitants of tropical and subtropical coastal waters worldwide, under favorable environmental conditions, they can grow rapidly and form harmful algal blooms.[2] The chemical defenses of benthic marine cyanobacteria likely play a role in bloom formation and persistence because most generalist herbivores avoid consuming them.[2] In August 2005, large mats of *Lyngbya polychroa* were observed on the fore reef slope of the coral reef near the Smithsonian field station at Carrie Bow Cay, Belize. Smaller amounts were seen in August 2006, but very little in August 2007, suggesting that the large amounts found in 2005 were exceptional. Lipophilic extracts of the 2005 collections strongly deterred feeding by natural assemblages of reef fishes in Belize, indicating the presence of chemical defenses. Therefore, we investigated the natural products chemistry of this bloom-forming *Lyngbya* to better understand the chemical ecology of this species.



Carriebowmide (**1**)

Here, we report a novel cyclodepsipeptide, carriebowmide (**1**), as a component of the lipophilic extract of the 2005 collection of the marine cyanobacterium *L. polychroa*. This cyclodepsipeptide contains four common amino acid residues (alanine, *N*-methylleucine, phenylalanine, and *N*-methylphenylalanine), uncommon residue 2-hydroxy-3-methylbutyric acid (Hmba), and rare amino acids methionine sulfoxide [Met(O)] and the β-amino acid 3-amino-2-methylhexanoic acid (Amha). Methionine sulfoxides have previously been found in a few marine cyanobacterial metabolites: pompanopeptin A from *L. confervoides*,[3] somamide A from *L. majuscula*,[4] and symplostatin 2 from *Symploca hydnoides*.[5] The β-amino acid Amha has been reported from the cephalaspidean mollusk metabolite kulokekahilide-1,[6] *Symploca* metabolite malevamide B,[7] and *Lyngbya* metabolites lyngbyastatin 3[8] and ulongamides A−F.[9] Other β-amino acid units have previously been found in marine cyanobacterial metabolites[10,11] as well as in terrestrial cyanobacteria.[12]

The wet sample collected in August 2005 was stored frozen until freeze-drying. The freeze-dried material was extracted first with EtOAc−MeOH (1:1) and then with EtOH−H_2O (1:1). The fish feeding assays indicated the lipophilic extract strongly deterred feeding by natural assemblages of reef fishes in Belize. Therefore, the fish-deterrent lipophilic EtOAc−MeOH-soluble fraction was selected for chemical studies. Fractionation on repeated column chromatography followed by HPLC yielded purified carriebowmide (**1**).

Carriebowmide (**1**) was obtained as a white solid. HRESI-TOFMS supported the molecular formula of $C_{46}H_{69}N_6O_9S$ [$(M + H)^+$ *m/z* at 881.4838]. The IR spectrum displayed strong absorption bands at 1738 and 1650 cm^{-1}, indicating the presence of ester and amide functionalities. The ^1H and ^{13}C NMR spectra were indicative of a depsipeptide (Table 1). Following the interpretation of DQF COSY, edited HSQC, and HMBC experiments, the ^1H and ^{13}C NMR signals were assignable to seven partial structures, which accounted for all of the atoms in **1**. Four standard amino acid residues were deduced as alanine (Ala), *N*-methylleucine (*N*-Me-Leu), phenylalanine (Phe), and *N*-methylphenylalanine (*N*-Me-Phe). The existence of the uncommon 2-hydroxy-3-methylbutyric acid (Hmba) moiety was revealed by the presence of a broad signal (δ_H 5.15, δ_C 76.0) corresponding to an α-hydroxymethine proton.[6] The expected cross-peak observed in the COSY spectrum for vicinal coupling between H-47 and H-48 was relatively weak and seen only at the noise level. The small coupling constant of H-47 and the high multiplicity of H-48 contribute to the weak cross-peak seen in the COSY spectrum. Therefore, the presence of an Hmba moiety was established by detailed HMBC analysis (Table 1). Another uncommon amino acid residue was elucidated by analyzing the 2D NMR data. Connecting COSY cross-peaks led to the two spin systems of CH_3-CH and $NH-CH-CH_2-CH_2-CH_3$. Although no COSY cross-peak was seen between the two methine protons, HMBC correlations between H-2/C-4 and C-5 and between H_3-3/C-4 confirmed the connectivity between C-2 and C-4. Further long-range CH coupling between H_3-3 and the carbonyl carbon at C-1 (δ_C 175.6) established the structure of 3-amino-2-methylhexanoic acid. The remaining partial structure unit consisted of a methine H-31 (δ_H 4.62) and two methylenes H_2-32 (δ_{Ha} 2.04/1.98 and δ_{Hb} 1.84/1.87) and H_2-33 (δ_{Ha} 2.57/2.55 and δ_{Hb} 2.48/2.44) based on COSY analysis, and the methylene carbon C-33 (δ 50.4/49.9) showed HMBC correlation to a methyl singlet H_3-S-34 (δ_H 2.62/ 2.61; δ_C 38.4/37.9). These data are in agreement with a methionine sulfoxide [Met(O)] residue as in somamide A,[4] symplostatin 2,[5] and pompanopeptin A.[3] The doubling of signals in the ratio of 1:1 suggested the occurrence of a mixture of *R* and *S* sulfoxide

* To whom correspondence should be addressed. Tel: (772) 462-0982. Fax: (772) 461-8154. E-mail: paul@si.edu.

10.1021/np800453t CCC: $40.75
Published on Web 11/14/2008

Table 1. NMR Spectroscopic Data (600 MHz, CD_3OD) for Carriebowmide (**1**)

unit	position	δ mult	δ_H (*J* in Hz)	COSY[a]	HMBC[b]	NOESY[c]
Amha	1	175.6, qC			2, 3, 4, 47	
	2	43.2, CH	2.62, m	3	3, 4	3, 4, 5a
	3	9.3, CH_3	0.89, d (6.9)	2	2, 4	2, 5b
	4	51.9, CH	4.27, m	5	2, 3, 5a, 5b, 6a, 6b	2, 5a
	5a	34.9, CH_2	1.50, m	4, 6	2, 3, 6a, 6b, 7	2, 4, 5a
	5b		1.40, m	4, 6		2, 5b
	6a	20.6, CH_2	1.39, m	5, 7	4, 7	7
	6b		1.32, m	5, 7		7
	7	13.8, CH_3	0.93, t (6.9)	6a, 6b	5	6a, 6b
	8-NH[d]		7.38, d (8.2)	4		
Ala	9	174.1 qC			4, 10, 11	
	10	49.0, CH	4.48, q (6.9)	11, 12[d]	11	11
	11	16.5, CH_3	1.16, d (6.9)	10		10
	12-NH[d]		8.76, d(8.8)	10		
N-Me-Leu	13	170.7, qC			10, 14, 15ab	
	14	59.9, CH	4.67, m	15a, 15b, 17, 18	15a, 15b, 19-*N*-Me	18, 21
	15a	37.9, CH_2	1.74, m	14, 15b, 16	14, 17, 18	15b, 19-*N*-Me
	15b		−0.13, m	14, 15a, 16		15a, 19-*N*-Me
	16	25.8, CH	1.40, m	15, 17, 18	14, 15a, 15b, 17, 18	
	17	21.9, CH_3	0.75, d (6.8)	16	15, 18	15b, 16
	18	23.8, CH_3	0.70, d (6.8)	16	15, 17	15a, 15b, 16
	19-*N*-Me	29.7, CH_3	2.60, s			15a, 15b
Phe	20	173.9, qC			14, 19- *N*-Me, 22a, 22b	
	21	52.7, CH	4.78, dd (9.6, 6.1)	22a, 22b, 29[d]	22a, 22b	14, 24/28
	22a	38.7, CH_2	3.10, m	21, 22b	21, 24/28	21, 24/28
	22b		3.05, m	21, 22a		21, 24/28
	23	137.3, qC			21, 22, 25/27	
	24/28	130.5, CH	7.19, d (7.5)	25/27	22, 26	25/27
	25/27	129.9, CH	7.29, t, (7.5)	24/26/28	27/25	24/28, 26
	26	128.3, CH	7.24, t (7.5)	25, 27	24/28	25/27
	29-NH[d]		8.80, d (8.8)	21		
Met(O)	30	172.3, qC			21, 31, 32	
	31	51.7, CH	4.62, m	32a, 32b, 35[d]	32a, 32b, 33a, 33b	
	32a	26.3, CH_2	2.04, m	31, 32b, 33	31, 33	32b
	32b		1.84, m	31, 32a, 33		32a
	32a	27.2[e]	1.98, m	31, 32b, 33	31, 33	32b
	32b		1.87, m	31, 32a, 33		32a
	33a	50.4, CH_2	2.57, m	32, 33b	31, 32, *S*-Me	32a, 32b, *S*-Me
	33b		2.48, m	32, 33a		32b, 33a
	33a	49.9[e]	2.55, m	32, 33b	31, 32, *S*-Me	
	33b		2.44, m	32, 33a		
	34-*S*-Me	38.4	2.62, s			33a
		37.9[e]	2.61, s			
	35-NH[d]		8.62, d (7.7)	31		
N-Me-Phe	36	170.1, qC			31, 37, 38a, 38b	
	37	62.2, CH	4.63, m	38a, 38b	38a, 38b, 45-*N*-Me	40/44, 45-*N*-Me
	38a	37.3, CH_2	3.38, dd (−13.7, 8.2)	37, 38b	37, 40/44	45-*N*-Me
	38b		2.94, dd (−13.7, 6.0)	37, 38a		45-*N*-Me
	39	138.3, qC			37, 38, 41/43	
	40/44	130.6, CH	7.25, d (7.5)	41/43	38, 42	37, 41/43
	41/43	130.0, CH	7.35, t (7.5)	40/44, 42	43/41	40/44, 42
	42	128.3, CH	7.27, d (7.5)	41, 43	40/44	41, 43
	45-*N*-Me	30.0, CH_3	3.03, s			38a, 38b
Hmba	46	172.1, qC			37, 45-*N*-Me, 47	
	47	76.0, CH	5.15, br s		49, 50	37, 48, 49, 50
	48	30.7, CH	1.52, m	49, 50	47, 49, 50	47, 49, 50
	49	19.8, CH_3	1.10, d (6.9)	48	47, 50	47, 50
	50	16.8, CH3	0.80, d (6.9)	48	47, 49	47, 49

[a] $^1H-^1H$ COSY correlations are from proton(s) stated to the indicated proton(s). [b] HMBC correlations, optimized for $^{2/3}J_{CH} = 8$ Hz, are from proton(s) stated to the indicated carbon. [c] NOESY correlations are from proton(s) stated to the indicated proton(s). [d] Observed only in CD_3OH. [e] Indicates diastereomers at chiral *S**.

diastereomers (Table 1) as reported earlier.[3] The sulfoxide has been suggested to be an artifact formed during isolation from a naturally occurring methione residue.[4] Recently, Baumann et al. reported that very little oxidation was observed for the methionine residue in the peptide planktocyclin when vented with argon for storage, thus confirming the process of oxidation of methionine under normal isolation conditions.[13] The residue sequence for **1** was determined by HMBC and supported by NOESY data (Table 1). HMBC correlation between H-47 (δ 5.15) of Hmba and C-1 (δ 175.6) of Amha connected these two residues by an ester linkage. Similarly, HMBC correlations between H-4 (δ 4.27)/C-9 (δ 174.1); H-10 (δ 4.48)/C-13 (δ 170.7); H-14 (δ 4.67)/C-20 (δ 173.9); and H-21 (δ 4.78)/C-30 (δ 172.3) or the very close carbon signal C-46 (δ 172.1) established a residue sequence of Amha-Ala-*N*-Me-Leu-Phe-Met(O) or Hmba. This latter ambiguity was clarified by a strong NOESY cross-peak observed between H-47 (δ 5.15) of Hmba and H-37 (δ 4.63) of *N*-Me-Phe, and an HMBC correlation observed between H-47 and C-46 confirmed the connection of *N*-Me-Phe to Hmba. Therefore, Phe is connected to Met(O) and not to Hmba in the above residue sequence. Further, HMBC correlations between H-31 (δ 4.62)/C-36 (δ 170.1) and H-37 (δ 4.63)/C-46 completed the sequence of all residues in the cyclodepsipeptide.

Next, the absolute configuration of the amino acid units was determined. The configuration of all α-amino acid residues in **1** was determined by chiral HPLC analysis, comparing the amino acid content in two acid hydrolysates with standards. Compound **1** was separately subjected to desulfurization[14] with Raney-Ni in order to convert Met(O) to 2-aminobutyric acid (Aba) prior to a second acid hydrolysis. Retention times established an L-configuration for Ala, *N*-Me-Leu, Phe, and Met(O) and the D-configuration for *N*-Me-Phe. The configuration of the hydroxy acid was determined using a different chiral column, and its retention time indicated the presence of *R*-Hmba. The configuration of the Amha residue was determined as 2*R*,3*R* by Marfey analysis[6] by using two standards (2*R*,3*R* and 2*S*,3*R*) of the four possible stereoisomers. Amha residues with 3*S* configuration have not been reported to date to occur in cyanobacterial metabolites. These data established the residue sequence of cyclo[2*R*,3*R*-Amha-L-Ala-*N*-Me-L-Leu-L-Phe-L-Met(O)-*N*-Me-D-Phe-*R*-Hmba] for carriebowmide (**1**).

When tested against a natural assemblage of reef fish, only the lipophilic extract reduced feeding on agar food pellets ($p < 0.001$). The fish consumed 3.35 ± 0.11 (mean ± SE) agar cubes of the control food, 2.25 ± 0.31 agar cubes of the food containing the hydrophilic extract, and 0.85 ± 0.22 of the agar cubes that contained the lipophilic extract. Unfortunately, not enough carriebowmide remained after structure elucidation to determine its feeding-deterrent properties.

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a Perkin-Elmer model 343 polarimeter. UV spectral data were acquired on a Hitachi U-3010 spectrophotometer. IR spectral data were obtained on a Bruker Vector 22 FT-IR spectrometer. NMR data were collected on a JEOL ECA-600 spectrometer operating at 600.17 MHz for ^{1}H and 150.9 MHz for ^{13}C. The edited-gHSQC experiment was optimized for J_{CH} = 140 Hz, and the gHMBC spectrum was optimized for $^{2/3}J_{CH}$ = 8 Hz. ^{1}H NMR chemical shifts (referenced to residual CH_3OH observed at δ 3.30) were assigned using a combination of data from 2D DQF COSY and gHMQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to CD_3OD observed at δ 49.0) were assigned on the basis of multiplicity-edited HSQC experiments. The HRMS data were obtained using an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector at the Mass Spectrometer Facility at the University of California, Riverside, CA. Silica gel 60 (EMD Chemicals, Inc. 230−400 mesh) and Varian BondElut octadecyl (C18) were used for column chromatography. All solvents used were of HPLC grade (Fisher Scientific).

Collection, Extraction, and Isolation. The sample of *L. polychroa* was collected on August 19, 2005, from Carrie Bow Cay fore-reef, Belize, at a depth of 10 m. The sample was identified by one of us (V.J.P.), and a voucher specimen is maintained at the Smithsonian Marine Station, Fort Pierce, FL. Filament width: 47.5 ± 5.53 μm (mean ± SD); cell width: 37.8 ± 5.33 μm; cell length: 7.00 ± 1.97 μm.

The freeze-dried material (57.3 g) was first extracted with EtOAc−MeOH (1:1) and then with EtOH−H_2O (1:1). Concentration of the extracts by rotary evaporation at 45 °C under reduced pressure furnished 7.1 g (12.3% yield) of the organic extract and 6.2 g (10.8% yield) of a polar extract. The fish feeding assays conducted at Golden Reef, Belize, in June 2006 indicated the less polar fraction significantly deterred feeding by a natural assemblage of reef fish. Therefore, the less polar EtOAc−MeOH (1:1)-soluble organic fraction was selected for chemical analysis. The EtOAc−MeOH (1:1)-soluble fraction (4.0 g) was chromatographed on a column of Si gel (30.0 g). The column was prepared in hexanes and eluted with a hexanes−EtOAc−MeOH step gradient system to give seven subfractions. Fraction 6 (0.90 g), which eluted with EtOAc−25% MeOH, showed a ^{1}H NMR pattern corresponding to a peptide. This fraction was rechromatographed on a column of C_{18} using a MeOH−H_2O step gradient system to give six subfractions. The subfraction 4 (18 mg), which eluted with MeOH−25% H_2O, was further separated by reversed-phase HPLC (semiprep, 5 μm, RP-18) using MeOH to give three subfractions. Subfraction 2 (4.0 mg) was further purified by reversed-phase HPLC using a MeOH−10% H_2O mixture to give 2.2 mg of carriebowmide (**1**, yield, 0.004% dry wt).

Carriebowmide (1): colorless, amorphous powder; $[\alpha]^{25}_D$ −40.0 (*c* 0.12, MeOH); UV (MeOH) λ_{max}(log ε) 218 (4.04), 258 (2.81) nm; IR (KBr film) ν_{max} 2957, 2870, 1738, 1650, 1646, 1538, 1453, 1410, 1385, 1367, 1315, 1279, 1240, 1215, 1186, 1083 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1, assignments were made by interpretation of 2D DQF COSY, edited-HSQC, HMBC, and NOESY data; HRESI/TOFMS *m*/*z* 881.4838 $[M + H]^+$ (calcd for $C_{46}H_{69}N_6O_9S$, 881.4847).

Acid Hydrolysis and Chiral HPLC Analysis. Compound **1** (0.1 mg) was suspended in 6 N HCl (0.3 mL) and heated at 115 °C for 18 h in a sealed tube. The hydrolysate was concentrated to dryness. The residue was reconstituted in 0.2 mL of H_2O and analyzed by chiral HPLC, comparing the retention times with those of authentic standards [Phenomenex Chirex (D) Penicillamine, 4.6 × 250 mm, 5 μm]; solvent 2.0 mM $CuSO_4$ and mixtures of 2.0 mM $CuSO_4$−MeCN (95:5, 90:10, or 85:15); detection at 254 nm. Using 2.0 mM $CuSO_4$ for D,L-alanine and 2.0 mM $CuSO_4$−MeCN (95:5) for D,L-*N*-Me-leucine, with a flow rate of 0.8 mL/min, the retention times (t_R, min) for authentic standards were L-Ala (11.0) and D-Ala (15.2), and *N*-Me-L-Leu (50.2) and *N*-Me-D-Leu (62.1), respectively. Similarly, using 2.0 mM $CuSO_4$−MeCN (90:10) for D,L-*N*-Me-phenylalanine and 2.0 mM $CuSO_4$−MeCN (85:15) for D,L-phenylalanine, with a flow rate of 1.0 mL/min, the t_R min for authentic standards were *N*-Me-L-Phe (59.5) and *N*-Me-D-Phe (69.8), and L-Phe (39.7) and D-Phe (41.8), respectively. The t_R (min) of the amino acids in the hydrolysate under the respective conditions were 11.0 (100:0), 50.2 (95:5), 69.8 (90:10), and 39.7 (85:15), indicating the presence of L-Ala, *N*-Me-L-Leu, *N*-Me-D-Phe, and L-Phe in the hydrolysate. The stereochemistry of the α-hydroxy acid was determined using a different chiral column for the HPLC analysis [Chiralpak MA (+) (4.6 × 50 mm), Diacel Chemical Industries, Ltd.; solvent, 2.0 mM $CuSO_4$−MeCN (90:10); flow rate, 1.0 mL/min; detection at 254 nm]. The t_R (min) for authentic standards were *R*-Hmba (10.3) and *S*-Hmba (13.8). The retention time of the α-hydroxyamino acid in the hydrolysate under these conditions was 10.3, indicating the presence of *R*-Hmba in the hydrolysate.

Advanced Marfey Analysis.[6] A portion of the acid hydrolysate (0.05 mg) and the standards (2*R*,3*R*)-Amha and (2*S*,3*R*)-Amha were separately derivatized by treatment with 1 M $NaHCO_3$ (50 μL) and a 1% solution of 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide in acetone (50 μL). After heating at 45 °C for 1 h, the reaction mixtures were cooled, acidified with 2 N HCL (25 μL), and diluted with MeCN (100 μL). These solutions were subjected to reversed-phase HPLC analysis [C18 (3.0 × 250 mm), 5 μ, Atlantis; flow rate 1.0 mL/min, detection at 340 nm] using 50 mM aqueous NH_4COOCH_3−MeCN (70:30). The t_R (min) of L-FDAA derivatives of (2*R*,3*R*)-Amha and (2*S*,3*R*)-Amha were 53.5 and 59.5, respectively. The t_R (min) of the Amha derivative in the hydrolysate under these conditions was 53.5, indicating the presence of (2*R*,3*R*)-Amha in the hydrolysate.

Desulfurization, Acid Hydrolysis, and Chiral HPLC Analysis. Compound **1** (0.2 mg) was dissolved in EtOH (0.5 mL) and treated with an excess of fresh Raney-Ni (2400) as a slurry in H_2O (0.1 mL) and refluxed for 0.5 h. The product was filtered, concentrated, and subjected to acid hydrolysis as above at 115 °C for 12 h in a sealed tube. The hydrolysate was concentrated to dryness. The residue was reconstituted in 0.2 mL of H_2O and analyzed by chiral HPLC, comparing the retention times with those of authentic standards [Phenomenex Chirex (D) penicillamine, 4.6 × 250 mm, 5 μm]; solvent 2.0 mM $CuSO_4$; detection at 254 nm. The t_R (min) for authentic standards were L-Aba (16.8) and D-Aba (27.7). The t_R (min) of the amino acid in the hydrolysate under this condition was 16.8, indicating the presence of L-Aba in the hydrolysate.

Preparation and Identification of the Standard Samples of (2*S*,3*R*)-Amha and (2*R*,3*R*)-Amha. Samples (0.2 mg) of lyngbyastatin 3 and ulongamide A from our pure sample repository were separately subjected to acid hydrolysis as above. The hydrolysates were dried, and each was reconstituted in H_2O (100 μL) for separation. Each hydrolysate was separated by reversed-phase HPLC (Phenomenex analytical, 250 × 4.6 mm, 5 μm, phenyl-hexyl) using MeCN−90% H_2O. Lyngbyastatin 3 and ulongamide A hydrolysates furnished six fractions and three fractions, respectively. The retention times of the standards identified the common amino acids glycine, *N*-Me-valine, *N*-Me-leucine, and *N*-Me-alanine in the lyngbyastatin 3 hydrolysate and *N*-Me-valine and phenylalanine in the ulongamide A hydrolysate. A portion from the hydrolysates of lyngbyastatin 3 and ulongamide A

was further analyzed by negative ion LRESIMS to identify the fraction that contained (2*S*,3*R*)-Amha (*m*/*z* 144) and (2*R*,3*R*)-Amha (*m*/*z* 144), respectively.

Fish Feeding Assay. Fish feeding assays were conducted with a natural assemblage of reef fish in the field at Golden Reef (7 m depth) in Belize (GPS: N 16°48.575′, W 088°05.138′). Feeding assays consisted of three treatments of agar-based food, which was composed of 5 g of fish food (Kent Platinum Reef Herbivore), 1.25 g of agar, and 1.25 g of carrageenan, mixed with 100 mL of boiling H_2O. This mixture was poured warm into 1 cm^3 molds and allowed to cool. The two treatment foods were made by incorporating a natural concentration of the extract (lipophilic or hydrophilic) dissolved in 5 mL of EtOH into the agar mixture before it was poured. The control food was made with 5 mL of EtOH added to control for any deterrent effect of the solvent itself. Four agar food pellets were attached with safety pins to each polypropylene rope and offered to fish in groups of three (two treatments, one control), which was replicated 20 times. Fish including *Thalassoma bifasciatum*, *Scarus iserti*, and *Acanthurus chirurgus* were observed feeding on the pellets. The number of agar cubes eaten was recorded in the field and was subsequently analyzed with Friedman's random block test.[15]

Acknowledgment. This research was supported in part by the National Oceanic and Atmospheric Administration's ECOHAB program (the Ecology and Oceanography of Harmful Algae Blooms) Project NA05NOS4781194 and the Smithsonian Marine Science Network. We thank the Harbor Branch Oceanographic Institute at Florida Atlantic University spectroscopy facility for 600 MHz NMR spectrometer time, low-resolution MS, and UV measurements. We also thank the Department of Chemistry, University of Florida, for IR measurements and Florida Atlantic University, Jupiter Campus, for optical rotation measurements. K. Arthur assisted with measurements and identification of the *Lyngbya*. The high-resolution mass spectrometric analysis was performed by the UCR mass spectrometer facility, Department of Chemistry, University of California at Riverside. This is contribution number 749 from the Smithsonian Marine Station at Fort Pierce and 836 from the Caribbean Coral Reef Ecosystems Program.

Supporting Information Available: ^1H, ^{13}C, COSY, and NOESY NMR spectra in CD_3OD for carriebowmide (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Blunt, J. W.; Copp, B. R.; Hu, W-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2008**, *25*, 35–94, and references therein. (b) Gunasekera, S. P.; Ross, C.; Paul, V. J.; Matthew, S.; Luesch, H. *J. Nat. Prod.* **2008**, *71*, 887–890. (c) Tan, L. T. *Phytochemistry* **2007**, *68*, 954–979.
(2) Paul, V. J.; Arthur, K. E.; Ritson-William, R.; Ross, C.; Sharp, K. *Biol. Bull.* **2007**, *213*, 226–251.
(3) Matthew, S.; Ross, C.; Paul, V. J.; Luesch, H. *Tetrahedron* **2008**, *64*, 4081–4089.
(4) Nogle, L. M.; Williamson, R. T.; Gerwick, W. H. *J. Nat. Prod.* **2001**, *64*, 716–719.
(5) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 655–658.
(6) Kimura, J.; Takada, Y.; Inayoshi, T.; Nakao, Y.; Goetz, G.; Yoshida, W. Y.; Scheuer, P. J. *J. Org. Chem.* **2002**, *67*, 1760–1767.
(7) Horgen, F. D.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **2000**, *63*, 461–467.
(8) Williams, P. G.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 1356–1363.
(9) Luesch, H.; Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 996–1000.
(10) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 29–31.
(11) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236–241.
(12) Golakoti, T.; Ogino, J.; Heltzel, C. E.; Husebo, T. L.; Jenson, C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1995**, *117*, 12030–12049.
(13) Baumann, H. I.; Keller, S.; Wolter, F. E.; Nicholson, G. J.; Jung, G.; Süssmuth, R. D.; Jüttner, F. *J. Nat. Prod.* **2007**, *70*, 1611–1615.
(14) Crossley, N. S.; Djerassi, C.; Kielczewski, M. A. *J. Chem. Soc.* **1965**, 6253–6264.
(15) Sokal, R. R.; Rohlf, F. J. *Biometry: The Principles and Practice of Statistics in Biological Research*, 2nd ed.; Freeman W. H. and Company: New York, 1981; Chapter *13*, pp 446−447.

NP800453T