Diverse Secondary Metabolites from a Puerto Rican Collection of *Lyngbya majuscula*

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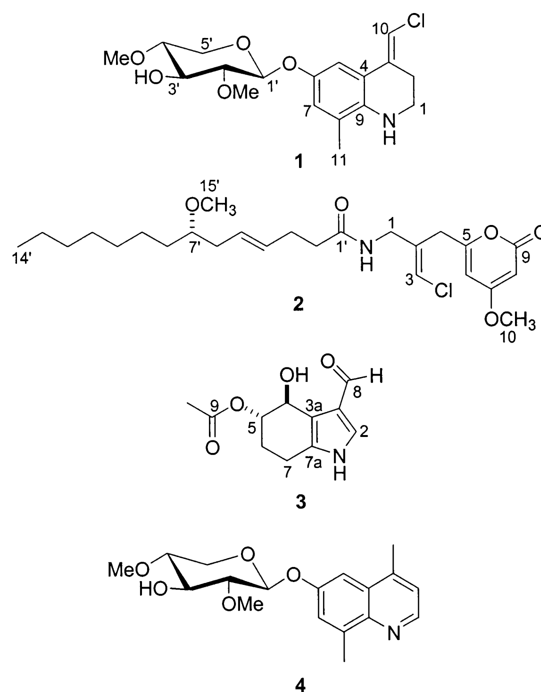
Received July 25, 2002

Extensive fractionation of the crude organic extract from a Puerto Rican collection of *Lyngbya majuscula* led to the discovery of three new secondary metabolites: a quinoline alkaloid (**1**), malyngamide T (**2**), and a tryptophan derivative (**3**). In addition, several previously reported compounds, including the potent neurotoxins antillatoxin, antillatoxin B, and kalkitoxin, were identified. The structures of **1**, **2**, and **3** were deduced by NMR and mass spectral data interpretation and suggest the existence of a convergent biosynthetic pathway for these new and unusual metabolites.

Lyngbya majuscula Gomont (Oscillatoriaceae) is a prolific producer of secondary metabolites displaying significant structural diversity and biological activity.[1] While the malyngamides are an abundant and interesting class of *L. majuscula* compounds which combine a lipid portion (typically, 7(*S*)-methoxytetradec-4(*E*)-enoic acid = lyngbic acid) with a variety of amine-derived moieties,[1,2] quinoline alkaloids have rarely been observed from this marine cyanobacterium.[3] In the present work, a chemically rich collection of *L. majuscula* was acquired from shallow waters off the coast of Puerto Rico in September 1997. The organic extract exhibited activity in several biological assays (brine shrimp toxicity, sodium channel modulation, cytotoxicity) and led to the isolation of the potent neurotoxins antillatoxin, antillatoxin B, and kalkitoxin.[4–6] During this extensive fractionation process, several additional compounds previously reported, including malyngamides C, C acetate, F, F acetate, J (**9**), and K, the quinoline alkaloid **4**, and hermitamides A and B (**7**), were also detected.[2b,c,e,3,7] Moreover, three new secondary metabolites, a quinoline alkaloid derivative (**1**), malyngamide T (**2**), and a new tryptophan derivative (**3**), were discovered. This paper describes the chromatographic isolation and structure elucidation of these three new compounds and proposes that a common biogenic connection exists among most of the enormously diverse collection of metabolites isolated from this single *L. majuscula* species.



Results and Discussion

In our continuing efforts to characterize the extraordinary chemistry of marine cyanobacteria, a detailed examination of the crude organic extract from a Puerto Rican collection of *L. majuscula* led to the isolation of three new secondary metabolites (**1**–**3**) using vacuum liquid chromatography and reversed-phase HPLC. Compound **1** showed a HRCIMS $[M]^+$ ion at *m/z* 369.1339, for a molecular formula of $C_{18}H_{24}NO_5{}^{35}Cl$, and therefore possessed seven degrees of unsaturation. Both ^1H and ^{13}C NMR spectra were well dispersed in C_6D_6 and indicated the presence of four carbon–carbon double bonds. Thus, three rings were required to account for the remaining degrees of unsaturation.

Two-dimensional NMR experiments, including HSQC, COSY, and HSQMBC,[8] were mainly used for the structure elucidation of **1** (Table 1). Proton NMR coupling constants and COSY spectral data were used to construct the 2,4-dimethoxyxylose moiety (Figure 1), a residue previously observed in the *L. majuscula* metabolites malyngamide J[2e] and the quinoline alkaloid **4**,[3] both of which were also isolated during the fractionation of this extract. The remaining signals in **1** were assembled to form a quinoline-like structure similar to compound **4**. However, signals forming the methyl pyridine ring in **4** were replaced by two coupled methylene signals (CH_2-1, δ_C 41.6, δ_H 2.75 and CH_2-2, δ_C 26.4, δ_H 2.61) and an exocyclic olefin possessing a vinyl chloride (CH-10, δ_C 111.3, δ_H 6.34), reminiscent of the malyngamide class of secondary metabolites.[1,2]

The sugar and quinoline units were connected through an HSQMBC correlation observed from H-1′ to C-6 (Figure 1). A 4.2 Hz heteronuclear coupling constant between H-10 and C-4 and a 6.8 Hz coupling constant between H-10 and C-2 were also measured from the HSQMBC experiment,[8] establishing an *E* geometry for the vinyl chloride functionality of **1** and completing the planar structure and relative stereochemistry of this unusual tetrahydroquinoline.

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10.1021/np020332c CCC: $25.00 © 2003 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/16/2003

Table 1. NMR Data for Quinoline Alkaloid **1** in C_6D_6

position	^{1}H (*J* in Hz)	^{13}C	HSQMBC[a]
1	2.75, brt (5.9)	41.6	2, 3, 9
2	2.61, brt (5.9)	26.4	1, 3, 10
3		135.1	
4		119.3	
5	7.14	111.8	3, 6, 7, 9
6		149.6	
7	6.90, d (1.5)	121.5	5, 6, 9, 11
8		124.0	
9		139.4	
10	6.34, s	111.3	2, 3, 4
11	1.66, brs	17.4	7, 8, 9
1′	4.78, d (7.4)	103.9	6, 2′, 5′
2′	3.32, dd (8.8, 7.4)	83.8	1′, 3′, 2′-OMe
3′	3.66, dd (8.8, 8.8)	76.4	2′, 4′, 5′
4′	3.21, ddd (9.6, 8.8, 5.1)	79.6	3′, 5′, 4′-OMe
5a′	2.98, dd (11.8, 9.6)	63.6	1′, 3′, 4′
5b′	3.81, dd (11.8, 5.1)		1′, 3′, 4′
2′-OMe	3.60, s	60.7	2′
4′-OMe	3.16, s	58.6	4′

[a] Proton showing long-range correlation to indicated carbon.

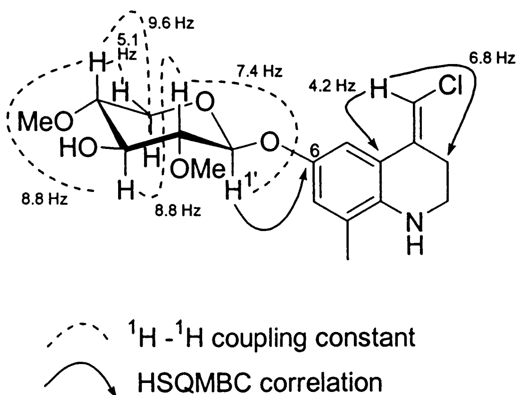

Figure 1. Key coupling constants and HSQMBC correlations for quinoline alkaloid **1**.

Compound **2** produced an $[M + H]^+$ peak at *m*/*z* 468.2518 by HRFABMS for a molecular formula of $C_{25}H_{38}NO_5{}^{35}Cl$ (7° unsaturation). The 1D and 2D NMR data suggested a strong resemblance between **2** and the malyngamide family of metabolites (Table 2), including the presence of the 7(*S*)-methoxytetradec-4(*E*)-enoate moiety (i.e., lyngbic acid (**5**)), a common feature of this structural class.[1,2,9]

Correlations observed in the HMBC were essential in constructing the remaining portion of **2**, as homonuclear couplings were virtually absent (Table 2). In the COSY spectrum, one cross-peak was observed between an amide proton (δ_H 5.89) and a low-field methylene signal (CH_2-1, δ_C 42.9, δ_H 3.94). The HMBC data showed this methylene was separated from a second low-field methylene (CH_2-4, δ_C 33.2, δ_H 3.38) by a vinyl chloride functionality, also common in the malyngamides. Both UV spectral data and HMBC connectivities were used to assemble the α-pyrone ring of **2**, notably H-8 to C-9, C-7, and C-6 and H-6 to C-8, C-7, and C-5. This substructure has been observed in several metabolites from red algae and marine bacterial species, but only the γ-pyrone metabolite kalkipyrone has been isolated previously from a marine cyanobacterium.[10,11] The placement of the *O*-methyl substituent on the α-pyrone ring was determined by an HMBC correlation observed between the *O*-methyl protons (H_3-10, δ 3.80) and C-7 as well as the homonuclear *meta* coupling (1.9 Hz) measured between H-8 and H-6.

The above subunits were linked by HMBC correlations seen from both the amide proton (δ_H 5.89) and the H_2-1 methylene protons to C-1′. The *E* geometry of the vinyl chloride in **2** was deduced by measurement of a 3.9 Hz coupling between H-3 and C-1 and a 7.1 Hz coupling between H-3 and C-4 by HSQMBC, completing the structure of malyngamide T (**2**).

Table 2. NMR Data for Malyngamide T (**2**) in $CDCl_3$

position	^{1}H (*J* in Hz)	^{13}C	HMBC[a]
1	3.94, d (6.1)	42.9	2, 3, 1′
2		133.4	
3	6.27, s	119.9	1, 2, 4, 5
4	3.38, s	33.2	1, 2, 3, 5, 6
5		160.8	
6	5.92, d (1.9)	100.8	4, 5, 7, 8
7		171.3	
8	5.42, d (1.9)	87.8	6, 7, 9
9		164.4	
10	3.80, s	55.8	7
NH	5.89, brt (6.0)		1, 1′
1′		172.8	
2′	2.27, m	36.2	1′, 3′, 4′
3′	2.34, m	28.4	1′, 2′, 4′, 5′
4′	5.48, m	130.6	2′, 3′, 6′
5′	5.48, m	127.8	3′, 6′
6′	2.18, m	36.1	4′, 5′, 7′, 8′
7′	3.16, m	80.7	5′, 8′, 9′, 15′
8′	1.43	33.2	6′, 7′, 9′
9′	1.33	25.4	10′
10′	1.28	29.4	
11′	1.28	29.9	
12′	1.29	22.7	13′, 14′
13′	1.27	31.8	12′
14′	0.89, t (7.0)	14.3	12′, 13′
15′	3.31, s	56.3	7′

[a] Proton showing long-range correlation to indicated carbon.

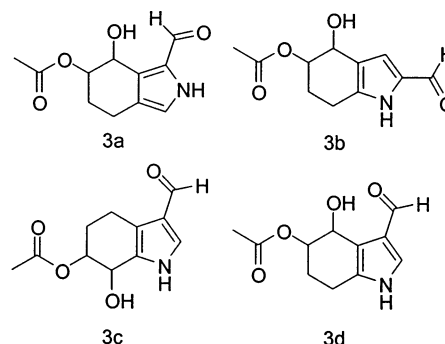

Figure 2. Candidate structures for tryptophan derivative **3**.

Compound **3** was assigned a molecular composition of $C_{11}H_{13}NO_4$ by HRFABMS (*m*/*z* $[M + H]^+$ 224.0920). The ^{1}H and ^{13}C NMR spectra revealed the presence of aldehyde and ester functionalities as well as two olefins. This accounted for four of the six degrees of unsaturation in **3** and indicated the presence of two rings. Both HSQC-TOCSY and HMBC experiments were used to sequence a hydroxy-substituted methine signal (CH-4, δ_C 67.8, δ_H 4.92) adjacent to a second, acetoxy-substituted methine (CH-5, δ_C 75.9, δ_H 5.06), which in turn was adjacent to two consecutive methylene signals (CH_2-6, δ_C 26.4, δ_H 2.20,1.94 and CH_2-7, δ_C 20.5, δ_H 2.77, 2.64).

Due to its small molecular size and the presence of several quaternary carbons, four possible structure types were initially conceived for **3** (Figure 2). An intense HMBC cross-peak observed between H-2 (δ_H 7.39) and C-8 (δ_C 188.5) excluded isoindole structures of type 3a. Additionally, because the proton spectrum of **3** showed H-2 as a finely split doublet coupled to an NH proton (H-1, δ 9.31) in the HSQC-TOCSY experiment, structure 3b was also rejected. While structure types 3c and 3d differ only in the location of substituents about the six-membered ring, HMBC correlations observed from the C-6 methylene

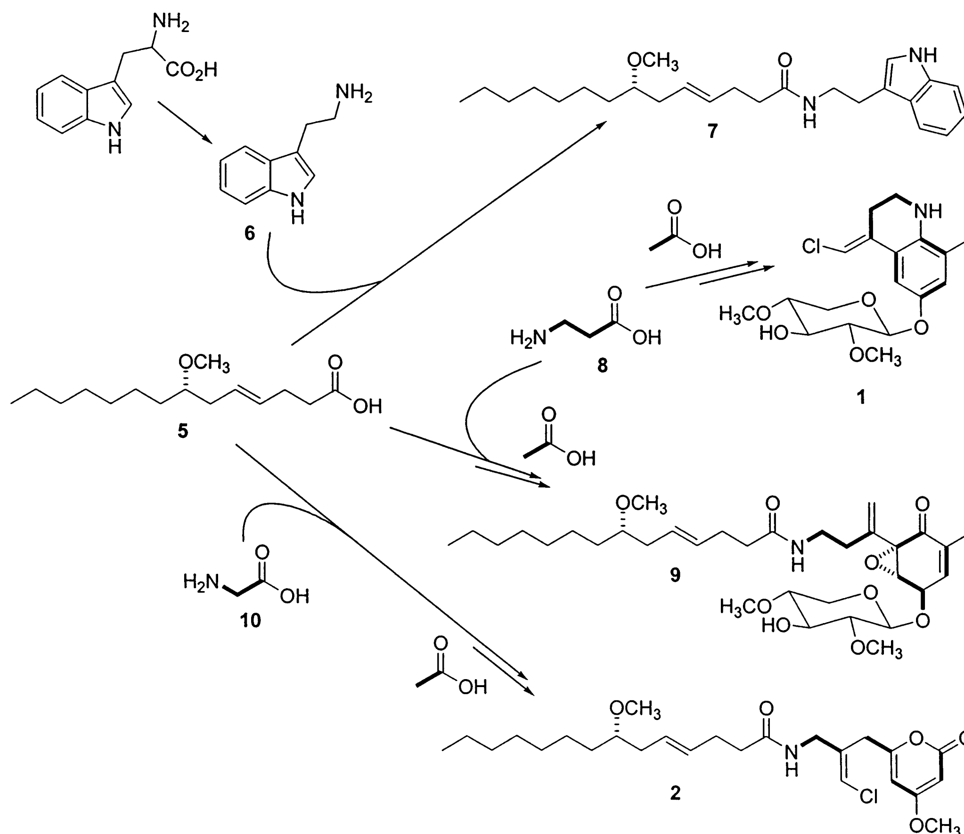

Figure 3. Proposed biosynthetic relationships between *L. majuscula* secondary metabolites isolated in this report.

protons to C-7a and from H-5 to C-3a identified 3d as the correct planar structure for **3**. The relative stereochemistry of H-4 and H-5 was deduced through proton coupling constant analysis. The H-5 proton appears as a ddd with ^3J coupling constants of 10.9 and 3.3 Hz to the C-6 methylene protons and 6.7 Hz to H-4, establishing an axial orientation for H-5 and indicating a *trans* relationship with H-4. In addition, no dipolar coupling was observed between H-4 and H-5 from 1D NOE experiments as anticipated for the *trans* geometry.

Isolation of these three new *L. majuscula* metabolites, in addition to 12 known compounds from this single collection, demonstrates the capability of this organism to produce a remarkable variety of structures. While this species was not viable in culture, it is interesting to speculate on the biogenesis of such an intriguing spectrum of metabolites. Lyngbic acid (**5**) appears to serve as substrate for extension by a variety of amino acids, producing a suite of related natural products (Figure 3). For example, hermitamide B (**7**) can be formed directly from condensation of **5** with tryptamine (**6**). Similarly, we envisage that **5** is condensed with either β-alanine (**8**) or glycine (**10**),[12] extended with additional malonyl-CoA-derived acetate units, and further elaborated with chlorination, methylation, and glycosylation events, to produce malyngamides J (**9**) and T (**2**), respectively.

Moreover, while the *Lyngbya*-derived malyngamide and quinoline alkaloids may appear as disparate structural families (quinolines are known to derive from anthranilate biosynthetic origins in plants),[13] the discovery of **1** with its distinctive vinyl chloride functionality suggests that a potential biogenic relationship could exist between these classes. Our hypothesis concerning quinoline metabolite biosynthesis in *L. majuscula* is depicted in Figure 3. Should compounds of this nature be produced in cultured *L. majuscula*, exploration of their molecular assembly (through stable-isotope labeling experiments, for example) will determine the veracity of this proposed biosynthetic convergence.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. IR and UV spectra were recorded on Nicolet 510 and Beckman DU640B spectrophotometers, respectively. NMR spectra were recorded on a Bruker DRX600 spectrometer operating at a proton frequency of 600.01 MHz and a carbon frequency of 150.90 MHz, with the solvent ($CDCl_3$ at δ_C 77.2, δ_H 7.27 or C_6D_6 at δ_C 128.4, δ_H 7.16) used as an internal standard. Mass spectra were recorded on a Kratos MS50TC mass spectrometer, and HPLC isolations were performed using Waters Millipore model 515 pumps and a Waters Millipore Lambda-Max model 480 spectrophotometer.

Collection. The marine cyanobacterium *Lyngbya majuscula* (voucher specimen available from WHG as collection number PRLP-16 Sept 97-3) was obtained from shallow waters (0.5 m) near Collado Reef, Puerto Rico, on September 16, 1997. The material was stored in 2-propanol at −20 °C until extraction.

Extraction and Isolation. Approximately 170 g (dry wt) of the cyanobacterium was extracted repeatedly with 2:1 CH_2Cl_2/MeOH to produce 6.8 g of crude organic extract. A portion of the extract (5.9 g) was subjected to Si vacuum liquid chromatography (VLC, hexanes/EtOAc/MeOH) to produce 14 chemically distinct fractions. The fraction eluting with 25% EtOAc in hexanes was purified by a C_{18} solid phase extraction (SPE) cartridge (7:3 MeOH/H_2O) to yield 280.2 mg of lyngbic acid (**5**). The VLC fraction eluting with 35% EtOAc was subjected to C_{18} SPE (7:3 MeOH/H_2O) and repetitive reversed-phase HPLC (9:1 MeOH/H_2O, Phenomenex Sphereclone 5 μ ODS, followed by 17:3 MeOH/H_2O, Phenomenex Spherisorb 10 μ ODS) to obtain 2.8 mg of hermitamide A and 1.0 mg of kalkitoxin. Purification of the fraction eluting with 40% EtOAc by C_{18} SPE (4:1 MeOH/H_2O) and RP HPLC (9:1 MeOH/H_2O,

Table 3. NMR Data for Compound **3** in $CDCl_3$

position	^{1}H (J in Hz)	^{13}C	HMBC[a]
1	NH, 9.31, brs		
2	7.39, d (2.3)	132.5	3, 3a, 7a, 8
3		125.7	
3a		118.8	
4	4.92, d (6.7)	67.8	3a, 5, 7a
5	5.06, ddd (10.9, 6.7, 3.3)	75.9	4, 9
6	2.20, 1.94, m	26.4	4, 5, 7, 7a
7	2.77, ddd (15.9, 10.2, 5.5) 2.64, dt (15.9, 4.6)	20.5	3a, 5, 6, 7a
7a		132.1	
8	9.65, s	188.5	3, 3a
9		172.6	
10	2.11, s	21.2	9
OH	5.36, brs		4, 5

[a] Proton showing long-range correlation to indicated carbon.

Phenomenex Sphereclone 5 μ ODS) provided 6.3 mg of malyngamide C-acetate. The 45−50% EtOAc VLC fraction was also purified by C_{18} SPE (3:2 $MeOH/H_2O$) and RP HPLC (3:1 $MeOH/H_2O$, Phenomenex Spherisorb 10 μ ODS) to yield 1.3 mg of quinoline alkaloid **1**. In addition, the SPE fraction eluting with 7:3 $MeOH/H_2O$ was subjected to RP HPLC (4:1 $MeOH/H_2O$, Phenomenex Spherisorb 10 μ ODS) to produce 3.6 mg of antillatoxin and 15.1 mg of malyngamide F-acetate. Further RP HPLC purification of this subfraction (7:3 $MeOH/H_2O$, Phenomenex Spherisorb 10 μ ODS) led to the isolation of 0.3 mg of hermitamide B and 1.2 mg of malyngamide K. The 55−65% VLC fraction was crudely chromatographed over LH-20 using 100% MeOH (3 × 40 cm). The first fraction was purified by C_{18} SPE (7:3 $MeOH/H_2O$) and RP HPLC (4:1 $MeOH/H_2O$, Phenomenex Spherisorb 10 μ ODS), yielding 0.4 mg of antillatoxin B and an additional 7.0 mg of malyngamide F-acetate (RP HPLC, 9:1 $MeOH/H_2O$, Phenomenex Sphereclone 5 μ ODS). The second LH-20 fraction was also purified by C_{18} SPE (7:3 $MeOH/H_2O$) and RP HPLC (4:1 $MeOH/H_2O$, Waters SymmetryShield 5 μ ODS), affording 1.3 mg of quinoline alkaloid **4** and 2.1 mg of malyngamide C. Purification of the fraction eluting with 70−75% EtOAc by C_{18} SPE (3:2 $MeOH/H_2O$) and RP HPLC (3:1 $MeOH/H_2O$, Phenomenex Sphereclone 5 μ ODS) yielded 13.2 mg of tryptophan derivative **3**. Likewise, the fraction eluting with 80−90% EtOAc was also purified by C_{18} SPE (7:3 $MeOH/H_2O$) and RP HPLC (4:1 $MeOH/H_2O$, Phenomenex Spherisorb 10 μ ODS) to yield 1.1 mg of malyngamide F and 2.3 mg of malyngamide T (**2**). Finally, the VLC fraction eluting between 100% EtOAc and 5% MeOH in EtOAc was purified by LH-20 (100% MeOH), C_{18} SPE (4:1 $MeOH/H_2O$), and RP HPLC (7:3 $MeOH/H_2O$, Phenomenex Spherisorb 10 μ ODS), to give 11.9 mg of malyngamide J (**9**).

Quinoline alkaloid (1): white amorphous solid; $[\alpha]^{22}_D$ −20.8° (c 0.12, $CHCl_3$); UV (MeOH) λ_{max} 212 nm (log ϵ 4.58), 241 nm (log ϵ 4.29), 269 nm (log ϵ 3.90); IR (neat) 3407, 2922, 2853, 1632, 1091, 1074 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; LRCIMS m/z 371 (32), 369 (92), 263 (69), 209 (100); HRCIMS m/z $[M]^+$ 369.1339 (calcd for $C_{18}H_{24}NO_5{}^{35}Cl$, 369.1343).

Malyngamide T (2): pale yellow oil; $[\alpha]^{22}_D$ −13.5° (c 0.20, $CHCl_3$); UV (MeOH) λ_{max} 218 nm (log ϵ 4.45), 284 nm (log ϵ 4.14); IR (neat) 3309, 2927, 2855, 1725, 1650, 1567, 1457, 1415, 1247, 1095, 1037 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; LRFABMS m/z 470 (36), 468 (100), 438 (32), 436 (91), 367 (11), 325 (21), 230 (24), 213 (26), 143 (23); HRFABMS m/z $[M + H]^+$ 468.2518 (calcd for $C_{25}H_{39}NO_5{}^{35}Cl$, 468.2517).

Tryptophan derivative (3): white amorphous solid; $[\alpha]^{22}_D$ +104.6° (c 0.50, $CHCl_3$); UV (MeOH) λ_{max} 214 nm (log ϵ 4.39), 254 nm (log ϵ 4.04), 287 nm (log ϵ 3.67); IR (neat) 3274, 2935, 2890, 1731, 1637, 1245, 1042 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; LRFABMS m/z 224 (83), 206 (100), 163 (64), 146 (32); HRFABMS m/z $[M + H]^+$ 224.0920 (calcd for $C_{11}H_{14}NO_4$, 224.0923).

Acknowledgment. We thank S. Ketchum, M. A. Roberts, and J. Menino for the collection, identification, and extraction of this microalga, respectively. We also thank B. Marquez and the Biochemistry NMR Facility at OSU for 2D NMR spectral data assistance, B. Arbogast and the EIHS Center at OSU for mass spectral data, and the National Institute of Health (CA 52955and GM63554) for financial support of this work.

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NP020332C