Dragonamides C and D, Linear Lipopeptides from the Marine Cyanobacterium Brown *Lyngbya polychroa*

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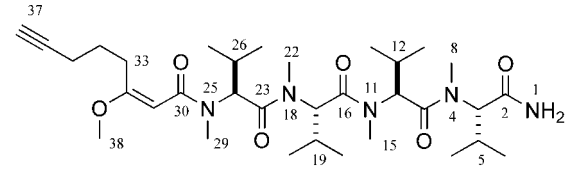
Two new linear lipopeptides, **1** and **2**, and a known compound, curacin D, have been isolated from a marine cyanobacterium, brown *Lyngbya polychroa*, collected from Hollywood Beach, Fort Lauderdale, Florida. Their planar structures were elucidated by 1D and 2D NMR techniques, and absolute configurations were assigned using chiral HPLC. The new compounds were assigned the trivial names dragonamide C (**1**) and dragonamide D (**2**), as their peptide moiety is related to previously reported dragonamides A and B.

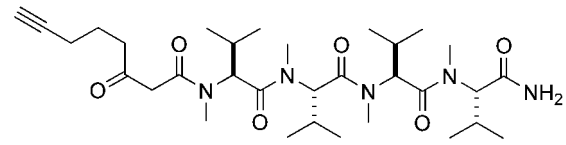
Marine cyanobacteria, or blue-green algae, have proven to be a rich source of novel biologically active secondary metabolites, in particular small modified peptides. Several recent review articles and related publications have shown that the genus *Lyngbya* has yielded an impressive array of structurally diverse secondary metabolites.[1] As part of our ongoing efforts to discover cytotoxic compounds produced by marine cyanobacteria around the south Florida coast, we have collected a sample of brown *Lyngbya polychroa* from Hollywood Beach, Fort Lauderdale, Florida. The lipophilic extract of this cyanobacterium led us to identify two new lipopeptides, dragonamides C (**1**) and D (**2**), and the antimitotic agent curacin D, which was previously reported from the marine cyanobacterium *L. majuscula*.[2] Several linear and cyclic depsipeptides of 7-octynoic acid and linear depsipeptides of 7-octenoic acid have been reported from *L. majuscula*.[3–6] Structurally similar C_8-alkynoate units have been reported from *Symploca laete-viridis*[7] and marine mollusks.[8] Dragonamides C (**1**) and D (**2**) represent two new linear tetrapeptide-octynoates.[3]

The wet sample collected in October 2006 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 EtOAc−MeOH-soluble fraction was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was further separated by repeated column chromatography to give 94 mg of curacin D as a pale yellow oil and a peptide-enriched fraction, which, after HPLC, yielded purified dragonamide C (**1**) and dragonamide D (**2**).

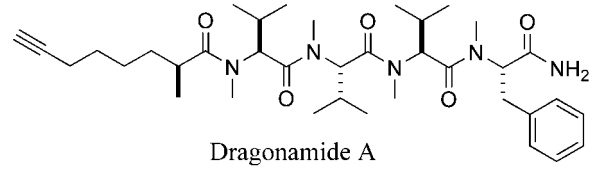
Dragonamide C (**1**) was obtained as a colorless, amorphous solid. HRESI/APCIMS supported the molecular formula of $C_{33}H_{57}N_5O_6$ [$(M + Na)^+$ *m/z* at 642.4223]. A strong IR absorption at 1636 cm^{-1} implied the presence of amide functionalities. The ^1H and ^{13}C NMR spectra indicated the presence of a minor conformer (17% in CD_3OD and $CDCl_3$). However, NMR analysis of the major conformer was not obstructed. The ^1H NMR spectrum (Table 1) for **1** revealed the presence of four singlets corresponding to *N*-methyl amide substituents (δ 3.11, 3.05, 3.04, 2.97) and another low-field singlet for an *O*-methyl substituent (δ 3.66), eight high-field methyl doublets (δ 0.96, 0.91, 0.90, 0.87, 0.85, 0.79, 0.77, 0.76), two high-field multiplets (δ 2.33 for 3H), and four low-field doublets for four α-protons of amino acid residues (δ 5.19, 5.18, 5.17, 4.65). In addition, the ^1H NMR spectrum indicated an olefinic singlet (δ 5.33), three methylene multiplets (δ 2.70−2.15), and a characteristic triplet for a terminal acetylenic proton [δ 2.20 (J = 2.7 Hz)]. Examination of the ^{13}C NMR (Table 1) and multiplicity-edited HSQC spectra revealed quaternary and methine ^{13}C signals at δ 84.5 and 69.8, respectively, consistent with the terminal acetylenic group,[9] and six putative carbonyl signals at δ 174.2, 173.6, 172.7, 172.6, 172.0, and 170.6. Examination of the HMBC data (Table 1) connected four of these carbonyl signals (δ 174.2, 172.7, 172.6, and 170.6) to the four *N*-methyl amide groups in the molecule. Corroborated by HMBC data (Table 1), the putative carbonyl at δ 173.6 belonged to a conjugated enol methyl ether group along with the methine ^{13}C NMR signals at δ 92.2.[10] These data accounted for the eight degrees of unsaturation inherent in the molecular formula. Analysis of the 2D DQF COSY and edited HSQC spectra revealed the presence of four isopropyl spin systems for four *N*-Me valine residues, and the order of these residues was assigned by analysis of the HMBC data (Table 1). The presence of a free amide group at



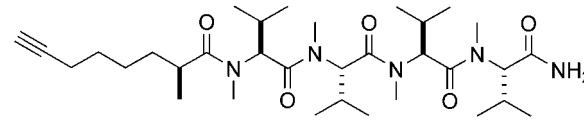
Dragonamide C (**1**)



Dragonamide D (**2**)



Dragonamide A



Dragonamide B

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Table 1. NMR Spectroscopic Data (600 MHz, CD_3OD) for Dragonamides C (**1**) and D (**2**)

		dragonamide C (**1**)			dragonamide D (**2**)		
unit	position	δ_C mult.	δ_H (J in Hz)	HMBC[a]	δ_C mult	δ_H (J in Hz)	HMBC[a]
NH_2[b]	1	N	5.02, s	2[b]	N	5.28, s	
			6.06, s			6.05, s	
N-Me Val-1	2	174.2, qC			174.2, qC		
	2[b]	171.5, qC					
	3	63.1, CH	4.65, d (10.2)	2, 5, 6, 7, 8	63.1, CH	4.65, d (10.9)	2, 5, 8
	4	N					
	5	27.4, CH	2.21, m	6, 7	27.4, CH	2.21, m	2
	6	18.8, CH_3	0.76, d (6.5)	3	18.8, CH_3	0.76, d (6.9)	3
	7	19.7, CH_3	0.96, d (6.5)	3	19.7, CH_3	0.96, d (6.9)	3
	8	31.4, CH_3	3.11, s	3, 9	31.4, CH_3	3.11, s	3, 9
N-Me Val-2	9	172.6 qC			172.7, qC		
	10	59.7, CH	5.19, d (10.8)	9, 13, 14, 15	59.7, CH	5.19, d (10.9)	9, 12, 15
	11	N			N		
	12	28.8, CH	2.33, m		28.8, CH	2.34, m	9
	13	18.4, CH_3	0.79, d (6.4)	10	18.5, CH_3	0.78, d (6.9)	10
	14	19.6, CH_3	0.91, d (6.4)	10	19.6, CH_3	0.91, d (6.5)	10
	15	32.1, CH_3	3.04, s	10, 16	31.2, CH_3	3.04, s	10, 16
N-Me Val-3	16	172.7, qC			172.1, qC		
	17	59.7, CH	5.17, d (10.2)	16, 19, 22	59.9, CH	5.18, d (10.9)	16, 19, 22
	18	N			N		
	19	28.5, CH	2.33, m		28.5, CH	2.35, m	16
	20	18.6, CH_3	0.85, d (6.7)	17	18.4, CH_3	0.87, d (6.7)	17
	21	18.6, CH_3	0.77, d (6.7)	17	18.4, CH_3	0.82, d (6.8)	17
	22	31.6, CH_3	2.97, s	17, 23	31.1, CH_3	3.04, s	17, 23
N-Me Val-4	23	170.6, qC			171.8, qC		
	24	59.9, CH	5.18, d (10.8)	23, 27, 28, 29	60.0, CH	5.13, d (10.9)	23, 26, 29
	25	N			N		
	26	28.5, CH	2.33, m		28.8, CH	2.31, m	
	27	19.9, CH_3	0.87, d (6.4)	24	19.9, CH_3	0.87, d (6.5)	24
	28	20.0, CH_3	0.90, d (6.5)	24	20.0, CH_3	0.88, d (6.5)	24
	29	31.3, CH_3	3.05, s	24, 30	31.5, CH_3	2.88, s	24, 30
MeO-Oya-2-ene	30	172.0, qC			170.5, qC		
	31	92.2, CH	5.33, s	30, 32, 33	49.6, CH_2	3.85, d (−16.5)	30, 32
						3.67, d (−16.5)	
	32	173.6, qC			205.5, qC		
	33	32.3, CH_2	2.70, m	31, 32, 34, 35	42.4, CH_2	2.69, m	32, 34, 35
			2.66, m	31, 32, 34, 35			
	34	27.9, CH_2	1.74, m	32, 33, 35, 36	23.4, CH_2	1.75, m	32, 33, 35, 36
	35	18.9, CH_2	2.15, dt (2.7, 7.5)	33, 34, 36, 37	18.3, CH_2	2.20, dt (2.7, 7.5)	33, 34, 36, 37
	36	84.5, qC			84.1, qC		
	37	69.8, CH	2.20, t (2.7)		70.3, CH	2.24, t (2.7)	
	38	55.9, CH_3	3.66, s	31, 32			

[a] HMBC correlations, optimized for $^{2/3}J_{CH} = 8$ Hz, are from proton(s) stated to the indicated carbon. [b] Data recorded in $CDCl_3$.

the C-terminal end was evident from the presence of two exchangeable singlets (δ 5.02 and 6.02 in $CDCl_3$), which are mutually coupled and showed long-range correlations to the C-terminal amide carbonyl group (δ 171.5 in $CDCl_3$, Table 1). Further analysis of the 2D DQF COSY and edited HSQC spectra indicated that the terminal acetylenic proton (H-37) and the three sequentially coupled methylene groups (H_2-33 to H_2-35) constitute a H_{33} to H_{37} spin system, as evidenced by a long-range coupling between H_2-35 and H-37 ($J = 2.7$ Hz). The HMBC spectrum exhibited three-bond coupling between H_2-35 (δ 2.15)/terminal C-37 (δ 69.8) and methylene C-33 (δ 32.3), H_2-34 (δ 1.74)/C-36 acetylenic qC (δ 84.5) and C-32 olefinic qC (δ 173.6), H_2-33 (δ 2.70, 2.66)/C-35 (δ 18.9) and olefinic C-31 (δ 92.2), H_3-38 (*O*-Me, δ 3.66)/C-32 olefinic qC (δ 173.6), H-31 (δ 5.33)/C-33 (δ 32.3), and two-bond coupling between H-31 (δ 5.33)/C-32 (δ 173.6) and C-30 amide carbonyl group (δ 172.0). Combination of the above data established the connectivity between C-30 and C-38 and thus confirmed the structure of the terminal fatty acid moiety as 3-methoxy-2-en-7-octynoic acid. Further, the HMBC spectrum exhibited three-bond coupling between H_3-29 (*N*-Me Val-4, δ 3.05)/C-30 amide carbonyl signal (δ 172.0) and thus established the connectivity between the *N*-Me Val-4 moiety and the terminal acid group in the molecule. The stereochemistry of the C-31 double bond was assigned on the basis of correlations seen in the NOESY spectrum. Strong correlation between H-31 and *O*-Me-38, but not between H-31 and H_2-33, assigned an *E* geometry to the C-31 double bond.

Dragonamide D (**2**) was obtained as a colorless solid. HRESI/APCIMS supported the molecular formula of $C_{32}H_{55}N_5O_6$ [$(M + Na)^+$ m/z at 628.4067]. A strong IR absorption at 1631 cm^{-1} indicated the presence of amide functionalities. The ^1H and ^{13}C NMR spectra indicated the presence of a minor conformer (8% in CD_3OD and 12% $CDCl_3$). The ^1H NMR spectrum of the major conformer (Table 1) was similar to the ^1H NMR spectrum for **1**, including the presence of four tertiary *N*-methyl amides, methyl doublets suggestive of valine isopropyl groups, and the characteristic terminal acetylenic signal; however, an *O*-methyl singlet (δ 3.66) was absent (Table 1). In addition, the ^1H NMR spectrum for **2** showed a low-field methylene AB quartet (δ 3.85, 3.67, $J = -16.5$ Hz) instead of the olefinic proton and methoxy singlet observed in dragonamide C (**1**). The presence of a carbonyl signal at δ 205.5 in addition to the five amide carbonyl peaks in the ^{13}C NMR spectrum for **2** and the above ^1H NMR data suggested a possible keto–enol relationship between compounds **1** and **2**. HMBC analysis (Table 1) established the connectivity between C-30 and C-37 and thus established the structure of the terminal fatty acid moiety as 3-keto-7-octynoic acid. Further 2D DQF COSY, and HMBC analyses confirmed the connectivity of all amino acid residues in the molecule. Another batch of the same brown *Lyngbya polychroa* was

extracted with EtOAc. ^{1}H NMR analysis of this extract in $CDCl_3$ indicated the presence of the methoxy and other *N*-Me signals corresponding to dragonamide C (**1**). Further, we have noticed that dragonamide C (**1**) in $CDCl_3$ was stable for a long period of time, suggesting that dragonamide D (**2**) is probably not an acid-catalyzed artifact formed during MeOH extractions.

The configuration of the *N*-Me Val residues in **1** and **2** was assigned by chiral HPLC analysis, comparing the amino acid content in the acid hydrolysates with *N*-Me D- and L-valine standards. Their retention times established an L-configuration for all *N*-Me Val residues in **1** and **2**.[3]

^{1}H and ^{13}C NMR spectroscopic data of our isolated curacin D were identical to those data reported[2] for curacin D, previously isolated from the cyanobacterium *Lyngbya majuscula* collected from St. Croix in the U.S. Virgin Islands. The observed specific rotation value for our isolated curacin D ($[\alpha]_D$ +34) closely matched that reported[2] in the literature for curacin D ($[\alpha]_D$ +33), indicating that both compounds have the same absolute configuration, although not all stereocenters are determined yet. These data confirmed the structure of the isolate as curacin D. No other reported curacins were found in this collection.

Dragonamides C (**1**) and D (**2**) were tested for biological activity in cancer cell viability assays. Compounds **1** and **2** showed weak activity, with GI_{50} values of 56 and 59 μM against U2OS osteosarcoma cells, 22 and 32 μM against HT29 colon adenocarcinoma cells, and 49 and 51 μM against IMR-32 neuroblastoma cells, respectively. These data are similar to cytotoxicity data reported for dragonamides A and B against other cell lines.[3]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco DIP-370 digital polarimeter. IR spectra 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 experiments were optimized for J_{CH} = 140 Hz, and the gHMBC spectra were optimized for $^{2/3}J_{CH}$ = 8 Hz. ^{1}H NMR chemical shifts (referenced to residual $CHCl_3$ observed at δ 7.24 and 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 $CDCl_3$ observed at δ 77.0 and CD_3OD observed at δ 49.0) were assigned on the basis of multiplicity-edited HSQC experiments. The LRESIMS was obtained on a Finnigan LTQ LC-MS with an electrospray ionization detector. 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.

Collection, Extraction, and Isolation. The sample of brown *Lyngbya polychroa* cyanobacterium was collected in October 2006 from Hollywood Beach, Fort Lauderdale, FL. 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. This specimen displayed an average cell width of 36.9 μm, cell length of 12.6 μm, and sheath width of 2.8 μm. The freeze-dried material (65 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 6.8 g (10.5% yield) of the organic extract and 7.5 g of a polar extract (11.6% yield). The EtOAc−MeOH-soluble fraction was partitioned between EtOAc and H_2O. The H_2O-soluble fraction was further partitioned between *n*-BuOH and H_2O. Concentration of these extracts furnished 1.7 g of an EtOAc-soluble fraction, 0.8 g of an *n*-BuOH-soluble fraction, and 3.9 g of a H_2O-soluble material. The EtOAc-soluble fraction (1.7 g) was chromatographed on a column of Si gel. The column was prepared in a mixture of hexanes−EtOAc (1:1) and eluted with a hexanes−EtOAc−MeOH step gradient system to give 10 fractions. Fraction 2, which eluted with hexanes−EtOAc (1:1), was rechromatographed on a column of Si gel using hexanes followed by 10% EtOAc−hexanes to give three subfractions. Subfractions 2 and 3 were combined and rechromatographed on a column of Si gel with hexanes followed by 5% EtOAc−hexanes to give 94 mg of curacin D (yield, 0.14% dry wt) as a pale yellow oil. Fraction 6, which eluted with 50% EtOAc−hexanes, was further separated by reversed-phase HPLC (semipreparative, 5 μm, RP-18) using a 20% H_2O−MeOH mixture to give 25 mg of dragonamide C (**1**, yield, 0.038% dry wt) and 4 mg of dragonamide D (**2**, yield, 0.006% dry wt).

Dragonamide C (1): colorless, amorphous solid; $[\alpha]^{25}_D$ −226 (*c* 1.66, MeOH); IR (KBr film) ν_{max} 2924, 2940, 1685, 1636, 1458, 1399, 1257, 1238, 1000 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1, assignments were made by interpretation of 2D DQF COSY, HSQC, and HMBC data; HRESI/APCIMS *m/z* 642.4223 $[M + Na]^+$ (calcd for $C_{33}H_{57}N_5O_6Na$, 642.4207).

Dragonamide D (2): colorless solid; $[\alpha]^{25}_D$ −250 (*c* 0.26, MeOH); IR (KBr film) ν_{max} 2925, 2940, 2930, 1690, 1631, 1467, 1420, 1388, 301, 1259, 1098 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1, assignments were made by interpretation of 2D-DQF-COSY, HSQC, and HMBC data; HRESI/APCIMS *m/z* 628.4067 $[M + Na]^+$ (calcd for $C_{32}H_{55}N_5O_6Na$, 628.4050).

Curacin D: pale yellow oil; $[\alpha]^{25}_D$ +34 (*c* 0.17, $CHCl_3$) [lit.[2] $[\alpha]_D$ +33 (*c* 0.14, $CHCl_3$)]; ^{1}H and ^{13}C NMR data are identical with those reported[2] in the literature; LRESIMS (positive ion) *m/z* 260.4 $[M + H]^+$.

Absolute Configurations of the Peptide Portions of Compounds 1 and 2. Each of compounds **1** and **2** (0.1 mg) was dissolved in 0.3 mL of 6 N HCl and heated in a sealed tube at 115 °C for 18 h. The product mixtures were dried, and each hydrolysate 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 $CuSO_4$−MeCN (95:5); detection 254 nm. The retention times (t_R min) for authentic standards were *N*-Me-L-Val (12.8) and *N*-Me-D-Val (17.5). The hydrolysates of **1** and **2** showed peaks at 12.8 min, but not at 17.5 min, indicating the presence of only *N*-Me-L-Val in both compounds.[3]

Cell Viability Assays. Cells were plated in 96-well plates (U2OS, 5000 cells; HT29, 10 000 cells; IMR-32, 30 000 cells) and 24 h later treated with various concentrations of dragonamides C and D or solvent control (1% EtOH). After 48 h of incubation, cell viability was measured using MTT according to manufacturer's instructions (Promega).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra in CD_3OD for dragonamides C (**1**) and D (**2**) and 2D-NOESY spectrum in CD_3OD for dragonamide C (**1**). ^{1}H and ^{13}C NMR spectra of curacin D in C_6D_6. This material is available free of charge via the Internet at http://pubs.acs.org.

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