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Wewakamide A and Guineamide G, Cyclic Depsipeptides from the Marine Cyanobacteria *Lyngbya semiplena* and *Lyngbya majuscula*

Han, Bingnan[1]*, Harald Gross[2], Kerry L. McPhail[3], Doug Goeger[3], Claudia S. Maier[4], and William H. Gerwick[5]

[1]*Department of Ocean Science and Engineering, Zhejiang University, Hangzhou, China 310028*
[2]*Institute for Pharmaceutical Biology, University of Bonn, 53115 Bonn, Germany*
[3]*College of Pharmacy, and* [4]*Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, USA*
[5]*Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA 92093-0212, USA*

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Two new cyclic depsipeptides wewakamide A (1) and guineamide G (2) have been isolated from the marine cyanobacterium *Lyngbya semiplena* and *Lyngbya majuscula*, respectively, collected from Papua New Guinea. The amino and hydroxy acid partial structures of wewakamide A and guineamide G were elucidated through extensive spectroscopic techniques, including HR-FABMS, 1D ^{1}H and ^{13}C NMR, as well as 2D COSY, HSQC, HSQC-TOCSY, and HMBC spectra. The sequence of the residues of wewakamide A was determined through a combination of ESI-MS/MS, HMBC, and ROESY. Wewakamide A possesses a β-amino acid, 3-amino-2-methylbutanoic acid (Maba) residue, which has only been previously identified in two natural products, guineamide B (3) and dolastatin D (4). Although both new compounds (1,2) showed potent brine shrimp toxicity, only guineamide G displayed significant cytotoxicity to a mouse neuroblastoma cell line with LC_{50} values of 2.7 μM.

Keywords: *Lyngbya semiplena*, *Lyngbya majuscula*, cyclic depsipeptides, wewakamide A and guineamide G

Cyanobacteria, also known as "blue-green algae," are ancient aquatic and photosynthetic prokaryotes. Additionally, cyanobacteria, particularly of the genus *Lyngbya*, are phenomenal producers of structurally intriguing and biologically active secondary metabolites [13, 14, 16]. A major group of cyanobacterial secondary metabolites contain peptides as components of their structures, and these are incorporated both *via* nonribosomal peptide synthetases (NRPSs) as well as by ribosomal processes [7, 12]. In our ongoing program to explore the marine cyanobacteria of Papua New Guinea as sources for novel anticancer lead compounds, our investigations have led to the discovery of semiplenamides [3] and wewakpeptins [2] from *L. semiplena*, and the lyngbyabellins E-I [5], aurilides B and C [4], and a series of malyngamide analogs [6] from *L. majuscula*. However, a detailed re-investigation of the remaining fractions of the corresponding extracts led to the isolation of two new cyclic depsipeptides, wewakamide A (**1**) and guineamide G (**2**) (Fig. 1). Herein, we report the isolation, structure elucidation, and biological evaluation of these two new compounds.

*Corresponding author
Phone: +86-15257170118; Fax: +86-57187981748;
E-mail: hanbingnan@zju.edu.cn

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

Materials and Methods

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 Bruker Avance DPX 400 MHz and Bruker Avance 300 MHz spectrometers with the solvent $CDCl_3$ used as an internal standard (δ_H at 7.26, δ_C at 77.4). High-resolution mass spectra were recorded on a Kratos MS-50 TC mass spectrometer. Tandem mass spectrometric data were obtained on an electrospray ionization (ESI) quadrupole ion trap mass spectrometer (Finnigan LCQ, San Jose, CA). For ESI-MS/MS analysis, samples were injected onto a C18 trap column for desalting and introduced into the mass spectrometer by isocratic elution using 50% acetonitrile containing 0.1% formic acid. For MS/MS investigations, the protonated molecular ion clusters were isolated in the ion trap and collisionally activated with different collision energies to find optimal fragmentation conditions. For the most intense fragment ions, MS^3 and MS^4 experiments were performed. Chiral GC–MS analysis was accomplished on a Hewlett-Packard gas chromatograph 5890 Series II with a Hewlett-Packard 5971 mass selective detector using an Alltech capillary column

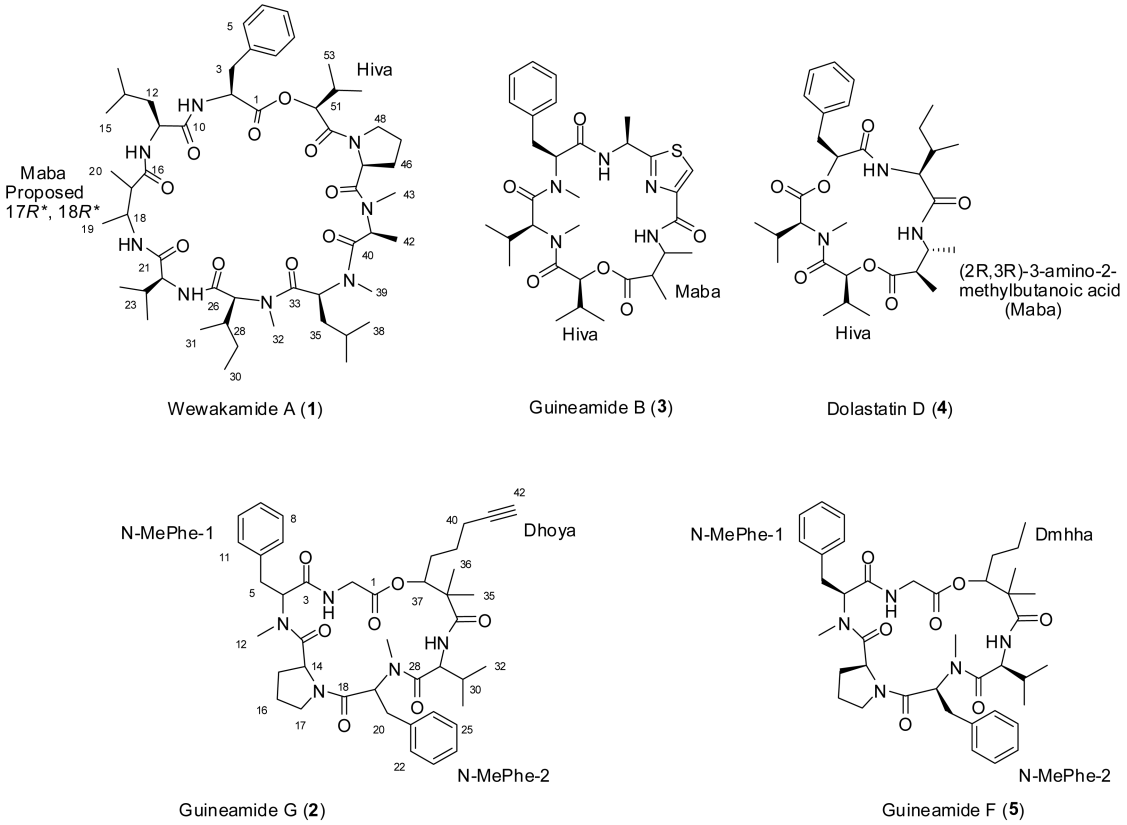

Fig. 1. Structures of wewakamide A (**1**), guineamide G (**2**), and related compounds isolated from marine cyanobacteria.

(CHIRASIL-VAL phase 25 m × 0.25 mm). HPLC was performed using Waters 515 HPLC pumps and a Waters 996 photodiode array detector.

Collection

The marine cyanobacterium *Lyngbya semiplena* was collected from shallow waters (1–3 m) in Wewak Bay, Papua New Guinea, in 1999. A shallow water (1–3 m) strain of *L. majuscula* was collected by hand from Alotau Bay, Papua New Guinea, in 2002. Taxonomy was assigned by microscopic comparison with the description given by Desikachary [1]. The material was stored in 2-propanol at –20°C until extraction.

Extraction and Isolation

Approximately 138 g and 150 g (dry wt) of *L. semiplena* and *L. majuscula*, respectively, were extracted repeatedly with CH_2Cl_2/MeOH (2:1) to produce 3.05 g (*L. semiplena*) and 3.30 g (*L. majuscula*) of crude organic extracts. The extracts were fractionated respectively by silica gel vacuum liquid chromatography using a stepwise gradient solvent system of increasing polarity starting from 10% EtOAc in hexanes to 100% MeOH. The fractions eluting with 100% MeOH were found to be active at 10 ppm in the brine shrimp toxicity assay. Further chromatography was achieved on Mega Bond RP_{18} solid-phase extraction (SPE) cartridges using a stepwise gradient solvent system of decreasing polarity starting from 80% MeOH in H_2O to 100% MeOH. The most active fractions after SPE (85% toxicity at 10 ppm to brine shrimp) were then purified by HPLC [Phenomenex Sphereclone 5 µm ODS (250 × 10 mm), 9:1 MeOH/H_2O, detection at 211 nm] to give compound **1** (2.5 mg) and compound **2** (1.0 mg) from *L. semiplena* and *L. majuscula*, respectively.

Wewakamide A (1): glassy oil; $[\alpha]^{22}_D$ -83 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} 215 nm (log ε 4.6); IR (neat) 3,317, 2,966, 2,922, 2,854, 1,736, 1,657, 1,546, 1,461, 1,238 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* $[M+H]^+$ 995.6544 (calculated for $C_{53}H_{87}N_8O_{10}$, 995.6545).

Guineamide G (2): white amorphous solid; $[\alpha]^{22}_D$ -50 (*c* 0.37, $CHCl_3$); UV (MeOH) λ_{max} 215 nm (log ε 3.0); IR (neat) 3,356, 2,962, 2,872, 1,712, 1,661, 1,640, 1,524, 1,196, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* $[M+H]^+$ 742.4176 (calculated for $C_{42}H_{56}N_5O_7$, 742.4179).

Absolute stereochemistry of 1

Wewakamide A (**1**, 500 µg) was hydrolyzed in 6 N HCl at 105°C for 16 h, then dried under a stream of N_2, and further dried under vacuum. The residue was reconstituted with 300 µl of H_2O prior to chiral HPLC analysis. For column Phenomenex Chirex 3126 (D), 4.6 × 250 mm; UV 254 nm detector, mobile phase I: 100% 2 mM $CuSO_4$ in H_2O, flow rate 0.7 ml/min; mobile phase II: 2 mM $CuSO_4$ in MeCN/H_2O (15:85), flow rate 0.8 ml/min; mobile phase III: 2 mM $CuSO_4$ in MeCN/H_2O (5:95), flow rate 1 ml/min. For column, Phenomenex Chirex 3126 (D), 4.6 × 50 mm; UV 254 nm detector, mobile phase IV: 2 mM $CuSO_4$ in MeCN/H_2O (15:85), flow rate 0.8 ml/min. Mobile phase I elution times (t_R, min) of authentic standards: L-Me-Ala (16.0), D-Me-Ala (16.5), L-Pro (28.4), D-Pro (63.0), L-Val (38.4), D-Val (68.5). Mobile phase II elution times (t_R, min) of authentic standards: L-Me-Leu (12.5), D-Me-Leu (14.1), L-

Table 1. NMR data for wewakamide A (**1**) at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$.

Unit	Position	δ_C	δ_H (*J* in Hz)	HMBC[a]
Phe	1	170.1		
	2	54.4	4.66, m	1, 3
	3	40.9	3.18, 2.89, dd (6.0, 3.2)	1, 2, 4, 5/9
	4	137.4		
	5	129.1	7.33	3, 6
	6	129.6	7.35	4, 5
	7	127.6	7.28	6, 8
	8	129.6	7.35	4, 9
	9	129.1	7.33	3, 7, 8
	NH		6.45, d (7.1)	1, 2, 10
Leu	10	169.30		
	11	50.5	4.73, dd (6.7, 6.1)	12, 13, 17
	12	38.7	1.76, 1.32	10, 11, 13, 14, 15
	13	25.8	1.78	11
	14	21.5	1.05	12, 13, 15
	15	21.3	1.01	12, 13, 14
	NH		7.12 d (7.8)	11, 16
Maba	16	174.3		
	17	44.7	2.32, dq (2.8, 7.0)	16, 18, 19, 20
	18	47.9	4.04, ddq (9.8, 2.8, 7.0)	17, 19, 20
	19	14.2	0.90, d (7.0)	17, 18
	20	13.8	1.07, d (7.0)	16, 17, 18
	NH		8.30, d (9.8)	18, 21, 22
Val	21	170.4		
	22	61.8	3.66, dd (8.5, 7.5)	21, 23, 24, 25, 26
	23	29.8	2.01, m	22, 24, 25
	24	20.2	1.20, d (6.8)	22, 23, 25
	25	20.0	1.10	22, 23, 24
	NH		5.85, d (7.0)	22, 23, 26
N-Me-Ile	26	172.9		
	27	61.3	5.51, d (6.0)	28, 29, 31, 32
	28	36.7	2.29	26, 29, 31
	29	28.1	1.56, 1.36	28, 30
	30	13.2	0.97	28
	31	16.4	1.04	27, 28
	32	33.2	3.08	27, 33
N-Me-Leu	33	176.4		
	34	55.3	5.53	33, 35, 39, 40
	35	38.3	1.76, 1.69	34, 36, 37, 38
	36	25.5	1.48, m	
	37	24.4	1.09, d (6.6)	35, 36, 38
	38	24.3	0.98, d (6.6)	35, 36, 37
	39	30.8	2.32	34, 40, 41
N-Me-Ala	40	171.3		
	41	50.4	5.66, q (7.0)	40, 42, 43
	42	15.0	1.32, d (6.6)	40, 41
	43	30.3	3.02	41, 44
Pro	44	169.1		
	45	59.8	2.86	44, 46, 47
	46	31.6	1.81, 1.61	45, 48
	47	23.1	1.79, 1.59	45
	48	47.1	3.36, 3.57, m	49
Hiva	49	172.6		
	50	75.8	5.07, d (9.2)	1, 51, 52, 53
	51	30.6	2.37	49, 50
	52	19.3	1.08	50, 53
	53	18.3	0.98	50, 52

[a]Proton showing HMBC correlation to indicated carbon.

Table 2. NMR data for guineamide G (**2**) at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$.

Unit	Position	δ_C	δ_H (*J* in Hz)	HMBC[a]
Gly	1	170.8		
	2a	41.3	3.24, m	1, 3
	2b	41.3	4.72, dd (16.9, 9.6)	
	NH		8.85, d (9.4)	3
N-Me-Phe-1	3	169.0		
	4	64.2	3.89, dd (10.2, 3.1)	3, 5, 6, 12
	5a	34.3	2.90, dd (13.7, 10.2)	3, 4, 6, 7/11
	5b	34.3	3.70, dd (14.5, 3.1)	
	6	138.5		
	7/11	129.7	7.10, m	5, 9
	8/10	127.2	7.23, m	
	9	127.4	7.23, m	
	12	31.5	3.03, s	3, 4
Pro	13	171.3		
	14	57.8	3.41, m	5, 6, 7
	15a	30.2	0.74, m	3, 6, 7
	15b	30.2	−0.03, m	
	16a	22.2	1.27, m	4, 7
	16b	22.2	1.27, m	
	17a	46.5	3.20, m	5, 6
	17b	46.5	3.39, m	
N-Me-Phe-2	18	168.9		
	19	54.2	5.14, dd (10.2, 4.9)	8, 20, 21, 27, 28
	20a	37.9	2.78, dd (12.6, 4.9)	18, 19, 21, 22
	20b	37.9	3.12, dd (12.6, 10.3)	
	21	137.2		19, 20, 22
	22/26	129.3	7.00, m	20, 23, 24
	23/25	129.2	7.23, m	21
	24	127.5	7.19, m	
	27	31.7	3.44, s	29, 28
Val	28	173.2		
	29	55.2	4.54, brt (7.4)	33, 28, 30, 31
	30	31.0	1.93, m	28, 29, 31
	31	18.5	0.95, m	29, 30, 32
	32	19.3	0.95, m	30, 31
	NH		5.85, d (7.6)	33
Dhoya	33	175.8		
	34	47.1		
	35	26.1	1.24, s	33, 34
	36	18.3	1.26, s	33, 34
	37	77.1	5.27, brt (9.0)	1, 33, 35, 36, 39
	38a	29.3	1.85, m	37, 39
	38b	29.3	1.69, m	
	39a	25.0	1.55, m	37, 40
	39b	25.0	1.55, m	
	40	18.4	2.27, m	39, 42
	41	83.7		
	42	69.5	1.99	

[a]Proton showing HMBC correlation to indicated carbon.

Leu (16.0), D-Leu (17.1), L-Phe (39.8), D-Phe (41.3). Mobile phase III elution times (t_R, min) of authentic standards: L-*allo*-Me-Ile (17.7), L-Me-Ile (18.8), D-*allo*-Me-Ile (27.7), D-Me-Ile (28.2). Mobile phase IV elution times (t_R, min) of authentic standards: L-Hiv (9.2), D-Hiv (14.5). The hydrolysate was chromatographed alone and co-injected with standards to confirm assignments (L-Me-Ala, L-Pro, L-Val, L-Me-Leu, L-Leu, L-Phe, L-Me-Ile, L-Hiv).

Biological Activity

Brine shrimp (*Artemia salina*) toxicity was measured as previously described [4]. After a 24 h hatching period, aliquots of a 10 mg/ml stock solution of compound A was added to test wells containing 5 ml of artificial seawater and brine shrimp to achieve a range of final concentrations from 0.1 to 100 ppm. After 24 h, the live and dead shrimp were tallied. Modulation of the voltage-sensitive sodium channel in mouse neuro-2a neuroblastoma cells was also examined as previously described [17].

The cytotoxic effects of compounds 1 and 2 upon NCI-H460 human lung tumor cells and neuro-2a mouse neuroblastoma cells were measured by a cell viability assay using MTT as previously described [5]. Cells were seeded in 96-well plates at 6,000 cells/well in 180 µl of medium. Twenty-four hours later, the test chemical was dissolved in DMSO and diluted into medium without fetal bovine serum and then added at 20 µg/well. DMSO was less than 0.5% of the final concentration. After 48 h, the medium was removed and cell viability determined.

Results and Discussion

The extracts of collections of *L. semiplena* were found to be toxic to brine shrimp, and this biological property was used to guide the isolation of wewakamide A (**1**), which possessed an $LC_{50} = 5$ ppm. Whereas the IR spectrum displayed absorption bands at 1,736 and 1,657 cm^{-1} for both ester and amide functionalities, the HRFABMS of compound **1** analyzed for $C_{53}H_{86}N_8O_{10}$ indicated a molecule of mostly peptide origin. Of the 53 carbon resonances in its ^{13}C NMR spectrum (Table 1), nine amide/ester carbonyls in the δ 165–180 range as well as six characteristic low-field aromatic carbon resonances were observed, which accounted for 13 of the 15 degrees of unsaturation implied by the molecular formula. Its peptidic nature was further supported by the presence of four amide NH signals (δ 5.85, 6.45, 7.12, and 8.30) and three *N*-methylamide signals (δ 2.32, 3.02, and 3.08) in the ^1H NMR spectrum. Both the 1D and 2D NMR spectra of **1** were well dispersed in $CDCl_3$ (Table 1), allowing for construction of nine partial structures. Seven relatively standard amino acids were deduced as proline (Pro), valine (Val), leucine (Leu), phenylalanine (Phe), *N*-methyl-alanine (*N*-Me-Ala), *N*-methyl-isoleucine (*N*-Me-Ile), and *N*-methyl-leucine (*N*-Me-Leu). Further 2D NMR analysis also revealed the presence of a 2-hydroxyisovaleric acid (Hiva), and a β-amino acid, 3-amino-2-methylbutanoic acid (Maba), as components of wewakamide A. The Hiva subunit was

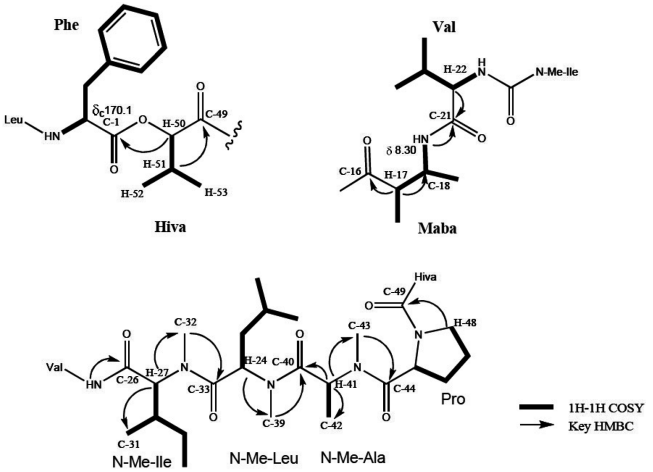

Fig. 2. Key ^1H-^1H COSY and HMBC correlations of compound **1**.

assembled from sequential ^1H-^1H-COSY correlations from H-50 (δ 5.07; δC 75.8, indicating an α-hydroxy acid) to a higher field methine (H-51, δ 2.37) and then to two high field methyl groups (H_3-52/53, δ 1.08 and δ 0.98). For the Maba fragment, COSY correlations were observed from a downfield NH proton (δ 8.30) to a deshielded methine (H-18, δ 4.04), and from the latter, correlations were observed to both a higher field methine proton (H-17, δ 2.32) and a methyl group (H_3-19, δ 0.90). Finally, a COSY correlation was observed from H-17 to a second methyl resonance (H_3-20, δ 1.07), thereby identifying the Maba fragment. HMBC correlations from H-17 to the carbonyl resonance at C-16 (δ 174.3) and the methine carbon at C-18 (δ 47.9) supported this fragment structure (Fig. 2).

Determination of the sequence and connection of amino acid residues and other units (Hiva, Maba) in **1** was achieved primarily by long-range ^{13}C-^1H correlation experiments (HMBC) with different mixing times and a ROESY experiment. The α-proton (H-50, δ 5.07) of Hiva showed a cross-peak to the C-1 (δ 170.1) carbonyl carbon of phenylalanine (Phe), and the NH proton (δ 8.30) of Maba showed a correlation with the C-21 (δ 170.4) carbonyl carbon of valine (Val) (Fig. 2). Together, these connections completed a 28-membered ring, which along with a proline (Pro) moiety accounted for the 15 degrees of unsaturation present in wewakamide A (**1**). Further evidence supporting this sequence of amino acid residues was developed from $(MS)^n$ experiments (Fig. 3).

The absolute configuration of **1** was established by analysis of degradation products. A small sample of **1** was hydrolyzed with 6N HCl to its constituent amino and hydroxy acid units. These were analyzed by chiral HPLC as well as chiral GC–MS and compared with the retention times of authentic standards. All of the proteinogenic amino acids and *N*-methylated amino acids as well as the Hiva unit were shown to possess L-configuration. The absolute

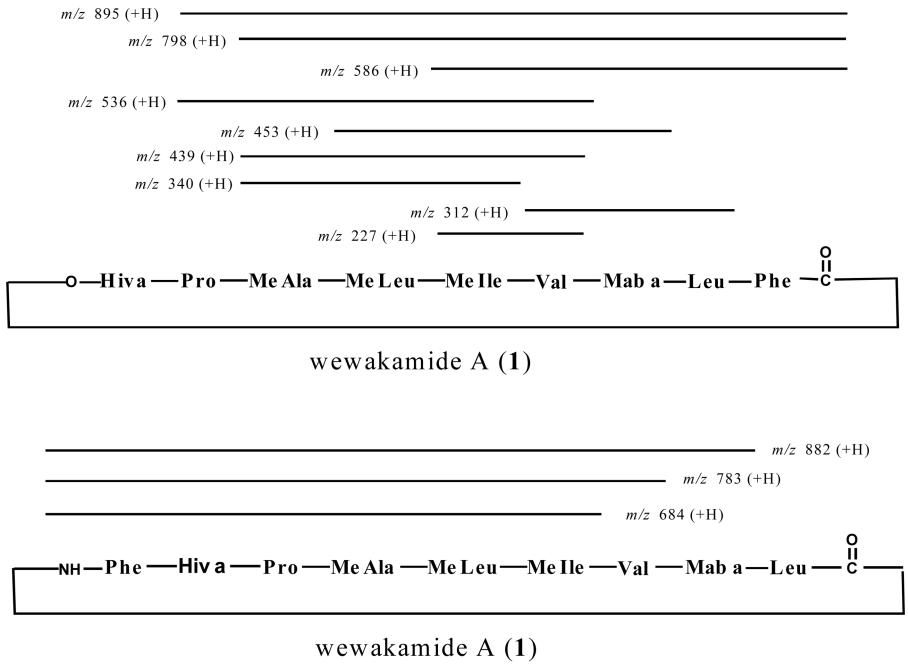

Fig. 3. Key fragments from collisionally induced ESI-MS/MS experiments with wewakamide A (**1**).

configuration of the Maba unit was not determined owing to the unavailability of standards. However, a literature search indicated that the Maba unit has been previously identified in two cyanobacterial natural products, guineamide B (**3**) [18] and dolastatin D (**4**) [15]. Diagnostic NOEs from H-17 to H-18 in wewakamide A were indicative of the relative stereochemistry 17*R**, 18*R**, the same as in dolastatin D (**4**) (Fig. 1). Because these two metabolites both derive from cyanobacteria and have comparable spectroscopic properties, we propose that the Maba unit in compound **1** is of the same absolute configuration as dolastatin D, namely 17*R*,18*R*.

Wewakamide A (**1**) was evaluated for biological activity using brine shrimp toxicity [10], sodium channel modulation [9], and both the NCI-H460 human lung cancer and the neuro-2a mouse neuroblastoma cell lines. Curiously, wewakamide A was inactive in all of these assays, except for brine shrimp toxicity evaluation, with an LC_{50} = 5 ppm. The four co-metabolites from this extract of *L. semiplena*, wewakpeptins A–D, are cyclic depsipeptides of similar overall size to wewakamide A, but have quite different residues; interestingly, these latter metabolites all displayed potent cytotoxicity against both the NCI-H460 human lung cancer and the neuro-2a mouse neuroblastoma cell lines [5].

A strain of the Papua New Guinea marine cyanobacterium *L. majuscula* was also investigated as part of the current study. Previously, this strain had yielded the lyngbyabellins E-I [5], aurilides B and C [4], and malyngamide analogs [6]. Continued investigation has now provided a new guineamide congener, guineamide G (**2**), which is structurally related to guineamide F. An $[M + H]^+$ peak observed by HR FABMS of guineamide G (**2**) suggested a molecular formula of $C_{42}H_{55}N_5O_7$, indicating 18 degrees of unsaturation. The peptidic nature of this molecule was again established from diagnostic exchangeable NH protons resonating at δ 8.85 and δ 5.85 in the ^{1}H NMR data. Similarly to guineamide F (**5**) [18], four singlet methyl proton signals (δ 3.03, δ 3.44, δ 1.24, and δ 1.26) were present in the ^{1}H NMR spectrum (Table 2), and the two lower field of these suggested *N*-methyl groups. Six distinct low-field carbon signals, due to ester of amide carbonyls, were observed in the ^{13}C NMR data for guineamide G (**2**). A number of olefinic carbon signals were present in the 128–132 ppm range, and several of these appeared to represent two or more carbon signals that overlapped owing to an apparent symmetry. Taken together with the low-field aromatic proton signals (6.8–7.4 ppm), two monosubstituted phenyl groups were implicated in guineamide G. Additionally, the ^{13}C NMR spectrum of **2** showed two distinctive carbon signals at δ 83.7 and δ 69.5, consistent with a terminal acetylene functionality. As previously observed for the guineamides, the carbon signal at δ 69.5 exhibited weak HSQC correlations but showed a $^1J_{CH}$ coupling of 249 Hz to a methine proton at δ 1.99 in the HMBC spectrum [15, 18]. This proton also exhibited a $^2J_{CH}$ HMBC correlation to the quaternary carbon at δ 83.7, confirming the acetylene functionality.

Careful analyses of COSY, HSQC, and HMBC data for guineamide G (**2**) revealed that it consisted of five common amino acid residues, as for guineamide F (**5**). Two of these

were *N*-Me-Phe and then one residue each of Pro, Val, and Gly. However, the 2,2-dimethyl-3-hydroxyhexanoic acid (Dmhha) residue in guineamide F (**5**) was replaced by 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) in guineamide G (**2**), and accounted for all of the atoms in the molecular formula. A highly shielded proton signal at δ −0.03 was assigned as one of the methylene protons on β-carbon of the Pro unit (H-15b). Such a shielding effect could arise from the influence of nearby aromatic amino acids, such as *N*-Me-Phe-1 and *N*-Me-Phe-2 in the molecule. The sequence of most of the residues in guineamide G (**2**) was determined from correlations observed by HMBC (Table 2), namely Pro-*N*-Me-Phe1-Gly-Dhoya-Val-*N*-Me-Phe2. Although no HMBC correlations were detected between *N*-Me-Phe2 and the Pro residue, this final linkage is required from a consideration of the molecular formula and resulting degrees of unsaturation. Stereoanalysis of guineamide G (**2**) was not undertaken owing to the small quantity isolated and the desire to evaluate its biological properties.

Several of the initially reported guineamides possess moderate cytotoxicity to a mouse neuroblastoma cell line with IC_{50} values of 15 and 16 μM, respectively [18]. Guineamide G was more potent in its cytotoxicity to this mouse neuroblastoma cell line, with an LC_{50} value of 2.7 μM.

Marine cyanobacteria continue to be extraordinarily prolific as a source of new and highly elaborate secondary metabolites, many of which are exquisitely bioactive. The investigation described herein further explores the chemical diversity of organic molecules produced by marine cyanobacteria of the genus *Lyngbya*. Indeed, a total of 21 new compounds representing seven separate chemical classes were identified from the organic extracts of two species of *Lyngbya* from Papua New Guinea, including semiplenamides A to G, wewakpeptins A to D, lyngbyabellins E-I, aurilides B and C, isomalyngamide K, wewakamide A, and guineamide G. All of the secondary metabolites found in this work are nitrogen-containing compounds with molecular sizes ranging from 325 Da (semiplenamide C) to 1,008 Da (wewakpeptin D). Among them, aurilides were the most potent compounds to the mouse neuroblastoma cell line with LC_{50} values between 0.01 and 0.04 μM, followed by lyngbyabellins and wewakpeptins with LC_{50} values between 0.2 and 10.7 μM. Guineamide G was more potent compared with the most structurally related compound guineamide F, with an LC_{50} value of 2.7 μM, perhaps as a result of its acetylene functionality.

Many of the highly cytotoxic metabolites from cyanobacteria function through antitubulin or antiactin mechanisms, and thus, there has been much interest in these metabolites for their potential utility to the chemotherapy of neoplastic diseases [11]. However, a growing number of cyanobacterial natural products operate through unknown mechanisms, such as the apratoxins [8, 16], and these will certainly provide a rich resource of novel pharmacological tools for the future. Moreover, additional insights into the mode of action of marine cyanobacterial toxins will come from chemical–ecological and genomic studies of these organisms.

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