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New Malyngamides from the Hawaiian Cyanobacterium *Lyngbya majuscula*

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Isomalyngamides A and B (**1**, **2**) were isolated and characterized from a collection of the cyanobacterium *Lyngbya majuscula* from Hawaiian waters. These compounds represent a new type of malyngamide, similar to malyngamides Q and R, in which the conformation of the chloromethylene group is opposite from the majority of previously reported malyngamides. The geometry of the chloromethylene moiety was elucidated from the long-range coupling constants ($^3J_{C-H}$) obtained from editing-HETLOC and phase-sensitive HMBC experiments. Isomalyngamides A and B (**1**, **2**) showed lethal toxicity to crayfish.

Cyanobacteria of the genus *Lyngbya* are a rich source of bioactive secondary metabolites.[1] Malyngamides are common metabolites of *L. majuscula*.[2] The structures of malyngamides have been characterized as *N*-substituted amides of 7(*S*)-methoxytetradec-4(*E*)-enoic acid (**5**) or 7(*S*)-methoxy-9-methylhexadec-4(*E*)-enoic acid.[2−4] The majority of the known malyngamides, including malyngamides A (**3**)[2a] and B (**4**),[2b] possess a terminal chloromethylene group having an *E* conformation. Recently, Milligan et al. reported two new types of malyngamides, malyngamides Q and R,[2o] which possess a chloromethylene moiety with a *Z* conformation. This research describes the isolation and characterization of isomalyngamides A (**1**) and B (**2**) from the Hawaiian *L. majuscula*, which also possess a chloromethylene moiety in the *Z* configuration. Long-range coupling constants ($^3J_{C-H}$) obtained from NMR experiments were used in determining the trisubstituted olefin configuration.

Results and Discussion

The structure of isomalyngamide A (**1**) was elucidated by the analysis of COSY, TOCSY, editing-HSQC,[5] HMBC, and HRMS data. NMR analysis revealed the existence of two interconverting amide isomers in solution as observed for other malyngamides.[2l,o] Although the *E* configuration of the C4/C5 olefin of **1** was assigned from the H4/H5 coupling constant (15.4 Hz), the configuration of the C5′/C6′ trisubstituted olefin was identified as *E* from the NOESY spectra (cross-peak between H6′ and H12′). Previously isolated malyngamides possessing an *E* chloromethylene moiety exhibited NOE or ROE correlations between H3′ and H1′ and/or H3′ and H13′.[2a,d,j,l] No correlation between H3′ and H1′ and/or H3′ and H13′ was observed in **1**. However, the NOESY spectra of **1** showed a cross-peak between H3′ and H4′. These NOESY results suggested that the chloromethylene moiety was in the *Z* configuration. Long-range coupling constants ($^3J_{C-H}$) were measured to confirm the chloromethylene configuration. The long-range coupling constant between H3′ and C1′ was observed to be +7.0 Hz with the editing-HETLOC[6] experiment (Figure 1b, Figure 2), while the cross-peak between H3′ and C4′ was not observed. Because the editing-HETLOC experiment is a hetero half-filtered version of ^{1}H−^{1}H TOCSY, $^{2-3}J_{C-H}$ through a quaternary carbon cannot effectively be detected. Coupling between H3′and C1′ through C2′ is thought to be via a *W*-type H−H coupling of H3′ and H1′. The coupling constant between H3′ and C4′ was revealed to be 4.3 Hz by the phase-sensitive HMBC (PS-HMBC) experiment[7] (Figure 1b). The smaller coupling constants observed

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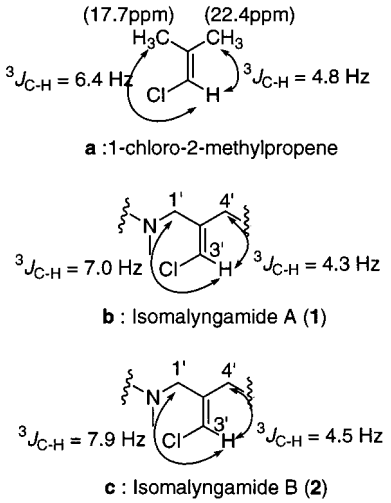


Figure 1. Comparison of the long-range coupling between an authentic sample (**a**; 1-chloro-2-methylpropene) and isomalyngamides (**b**, **c**).

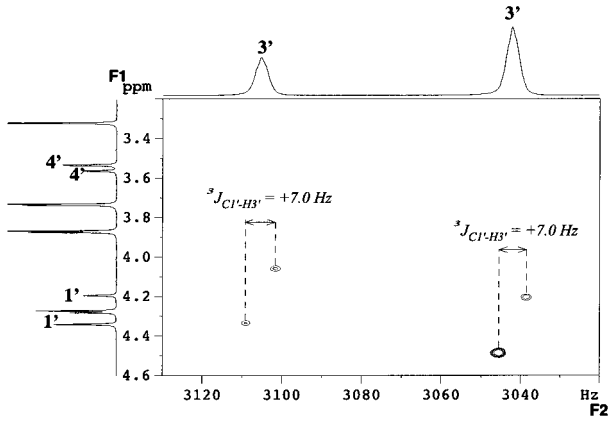


Figure 2. Expanded editing-HETLOC spectrum of isomalyngamide A (**1**) in $CDCl_3$. The measurement was carried out at 298 K, mixing time (MLEV17) 50 ms. with a Bruker DMX-500 spectrometer. F2 axis of the digital resolution was 0.77 Hz/point.

(compared to usual olefin *J* values) may be attributed to the effects of the electronegative substituents on the coupling pathway.[2o,8] The $^3J_{C-H}$ of a model compound, 1-chloro-2-methylpropene (Figure 1a, commercially available from Aldrich), was measured by ^{13}C NMR without ^1H decoupling. The *E* $^3J_{C-H}$ value was 6.4 Hz, and the *Z* $^3J_{C-H}$ value was 4.8 Hz (Figure 1a). These values were comparable to those of **1**, thus confirming the *Z* geometry of C4′/H3′ for **1**. Compound **1** was hydrolyzed under basic conditions to determine the configuration at C7. The optical rotation of the hydrolysis product (**5**) {$[\alpha]^{25}_D$ −4.8° (*c* 0.22, $CHCl_3$)} was comparable to the reported value of 7(*S*)-methoxytetradec-4(*E*)-enoic acid {$[\alpha]^{26}_D$ −11.1° (*c* 3.9, $CHCl_3$)},[2c] thus establishing a 7(*S*) configuration for **1**.

The geometries of the three double bonds in **2** were confirmed by using the same methods described for **1**. Editing-HETLOC experiments showed that the coupling constant between H3′ and C1′ was +7.9 Hz, and PS-HMBC experiments showed that the value between H3′ and C4′ was 4.5 Hz (Figure 1c). The absolute configuration at C10′ of **2** was determined by the modified Mosher's method.[9] Compound **2** was converted to (*S*)- and (*R*)-MTPA esters. The chemical shifts of H9′ and H11′ were assigned for each ester from the COSY data. The $\Delta\delta$ ($=\delta_S - \delta_R$) values (+0.102 for H9′a; +0.031 for H9′b; −0.104 for H11′a; −0.024 for H11′b) revealed a C10′ configuration as *R* for **2**. It was assumed that the absolute configuration of C7 for isomalyngamide B (**2**) was the same as that of isomalyngamide A (**1**) based on their co-occurrence in *L. majuscula*.

Table 1. ^1H, ^{13}C, and ^{15}N NMR Data for Isomalyngamide A (**1**)[a]

position	^1H NMR[b] (750.13 MHz)	^{13}C NMR[c] (188.62 MHz)	^{15}N NMR[d] (76.02 MHz)
1		172.76; 172.37	
2	2.42 (2H, t, 7.3 Hz); 2.39 (2H, t, 7.3 Hz)	33.93; 32.87	
3	2.34 (2H, q, 7.3 Hz)	28.26; 28.06	
4	5.52 (1H, dt, 15.3, 6.5 Hz)	131.31; 131.25	
5	5.46 (1H, dt, 15.3, 6.7 Hz)	126.96; 126.89	
6	2.18 (2H)	36.33; 36.30	
7	3.14 (1H, m)	80.71; 80.68	
8	1.43 (2H, m)	33.93	
9	1.35 (1H, m); 1.30 (1H, m)	25.16	
10		29.64	
11	1.22−1.29 (8H, m, H10−H13)	29.18	
12		31.72	
13		22.54	
14	0.88 (3H, t, 7.1 Hz)	13.98	
15	3.32 (3H, s)	56.37	
1′	4.34 (2H, s); 4.20 (2H, s)	48.81; 45.41	
2′		134.40; 133.72	
3′	6.21 (1H, s); 6.08 (1H, s)	118.32; 117.45	
4′	3.56 (2H, s); 3.53 (2H, s)	35.59; 34.97	
5′		172.83; 172.69	
6′	6.88 (1H, s); 6.86 (1H, s)	94.39	
7′		164.40; 164.37	
8′		170.08	
9′	5.12 (1H, s); 5.11 (1H, s)	94.84; 94.77	
10′		175.71; 175.55	
11′	4.28 (2H, s); 4.27 (2H, s)	48.55	
12′	3.74 (3H, s); 3.73 (3H, s)	55.99; 55.93	
13′	2.91 (3H, s); 2.85 (3H, s)	33.93; 32.87	
14′	3.88 (3H, s); 3.87 (3H, s)	58.50; 58.46	
N-a			107.4; 106.3
N-b			159.5

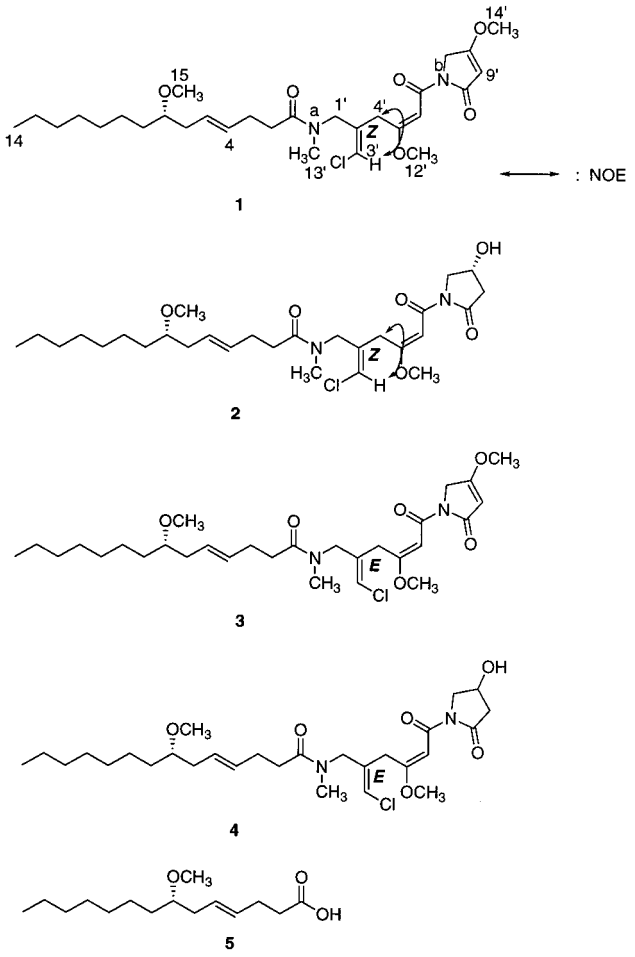
[a] Spectra determined in $CDCl_3$ at 25 °C; data reported in ppm. [b] TMS (0.00 ppm) was used as a reference. [c] $CDCl_3$ (77.0 ppm) was used as a reference. [d] CH_3NO_2 (379.6 ppm) was used as a reference. The assignments were achieved by $^1H-^{15}N$ HMBC.

The long-range coupling constants ($^3J_{C-H}$) obtained by editing-HETLOC and PS-HMBC experiments were effective in determining the geometry of the chloromethylene moiety in compounds **1** and **2**. These NMR techniques have been used to obtain $^{2-3}J_{C-H}$ values to determine the stereochemistry of other acyclic structures.[10] The work presented in this paper demonstrated that $^3J_{C-H}$ values were effective, too, in elucidating the configuration of trisubstituted olefins. Milligan et al. also recently employed $^3J_{C-H}$ couplings to elucidate the configuration of a trisubstitued olefin in malyngamides.[2o]

Isomalyngamides A (**1**) and B (**2**) were lethal to the crayfish *Procambarus clarkii* by intraperitoneal injection at 250 and 500 μg/kg, respectively. A dietary preference study using the sea hare *Stylocheilus longicauda* shows that the closely related malyngamides A (**3**) and B (**4**) increase feeding at low concentrations and inhibit feeding at higher concentrations.[11] The toxicity of malyngamides[2i−k] may be associated with the feeding inhibition at the higher malyngamide concentrations.

Ten years ago, Moore et al. reported that malyngamides A (**3**) and B (**4**) were the major constituents of *L. majuscula* collected at Kahala Beach, Oahu, Hawaii.[12] From the differential NOE experiment, which showed a 9% NOE to the ^1H olefinic signal by irradiation of the *N*-methyl group, Moore et al. unambiguously determined the configuration of the chloromethylene moiety of **3** as *E*.[2a] The collection of *L. majuscula* from Kahala Beach, Oahu, used in this study revealed isomalyngamides A (**1**) and B (**2**) as major constituents.[13] In addition, malyngamides A (**3**) and B (**4**)

were undetectable. It is unclear why *L. majuscula* has altered its major secondary metabolite constituents during these 10 years.



Experimental Section

Instruments. NMR data were measured on a Bruker DMX-750 and DMX-500 spectrometer. FABMS were obtained on a JEOL JMS-HX/HX110A spectrometer. Optical rotations were determined on a JASCO DIP-1000 instrument.

Biological Material. *Lyngbya majuscula* (reference #99/6/18-2) was collected at Kahala Beach, Oahu, Hawaii, during June 1999.[14] A voucher specimen has been retained by the author (H. N.) and is available on request.

Extraction and Isolation. Freeze-dried *Lyngbya majuscula* (160 g) was extracted with MeOH (3 × 1 L), and the extract was evaporated to dryness. The residue was dissolved in 50% aqueous MeOH (50 mL) and applied to Sep-Pak ODS (10 g, Waters). The column was eluted with 50, 70, 90, and 100% MeOH (200 mL per MeOH concentration). Isomalyngamides A and B, majusculamides A and B, and lyngbyatoxin A eluted in the 70% and 90% aqueous MeOH fractions. These fractions were further purified with reversed-phase HPLC (CAPCELL PAK C_{18}, UG-50, 20 × 250 mm, solvent system; 60, 70, 80, or 90% aqueous CH_3CN isocratic elution) to yield lyngbyatoxin A (23 mg),[15a] majusculamides A and B (mixture, 73 mg),[16] and isomalyngamides A (**1**, 57.8 mg) and B (**2**, 10.1 mg).

Isomalyngamide A (1): $[\alpha]^{29}_D$ −4.8° (*c* 2.89, CH_2Cl_2); ^1H and ^{13}C NMR data, see Table 1; HRFABMS $[M + 1]^+$ *m/z* 553.3011 ($C_{29}H_{46}{}^{35}ClN_2O_6$, Δ −3.3 mmu of calcd).

Isomalyngamide B (2): $[\alpha]^{27}_D$ +46.0° (*c* 0.40, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 2; HRFABMS $[M + 1]^+$ *m/z* 541.3035 ($C_{28}H_{46}{}^{35}ClN_2O_6$, Δ −1.0 mmu of calcd).

Basic Hydrolysis of Isomalyngamide A. Compound **1** (16.2 mg) was dissolved in a 10-mL solution of 10% KOH in EtOH−H_2O (4:1) and refluxed for 15 h with constant stirring. The hydrolysate was concentrated in vacuo and partitioned between H_2O and CH_2Cl_2. The H_2O layer was isolated, acidified, and extracted with CH_2Cl_2 to yield 4.4 mg of **5**. The properties of compound **5** were as follows: $[\alpha]^{25}_D$ −4.8° (*c* 0.22, $CHCl_3$); HRFABMS $[M + 1]^+$ *m/z* 257.2107 ($C_{15}H_{28}O_3$, Δ −0.9 mmu of calcd).

Table 2. ^1H, ^{13}C, and ^{15}N NMR Data for Isomalyngamide B (**2**)[a]

position	^1H NMR[b] (750.13 MHz)	^{13}C NMR[c] (188.62 MHz)	^{15}N NMR[d] (76.02 MHz)
1		173.31; 173.22	
2	2.37 (2H, t, 6.9 Hz)	33.38; 33.32	
3	2.32 (2H, m)	28.36; 28.30	
4	5.51 (1H, dt, 15.1, 6.2 Hz)	131.22; 131.12	
5	5.46 (1H, dt, 15.1, 6.4 Hz)	127.17; 127.04	
6	2.18 (2H, m)	36.34; 36.17	
7	3.17 (1H, m)	80.86; 80.80	
8	1.43 (2H, m)	33.28; 33.11	
9	1.34 (1H, m); 1.30 (1H, m)	25.31	
10		29.72	
11	1.22−1.30 (8H, m, H10−H13)	29.29	
12		31.82	
13		22.64	
14	0.88 (3H, t, 7.1 Hz)	14.08	
15	3.32 (3H, s)	56.43; 56.35	
1′	{4.37 (1H, d, 14.1 Hz); 4.18 (1H, d, 14.1 Hz)};	48.94; 44.55	
2′	4.17 (2H, s)	134.11; 133.34	
3′	6.19 (1H,s); 6.13 (1H,s)	118.79; 118.57	
4′	3.92 (1H, d, 14.8 Hz); 3.77 (1H, d, 14.8 Hz), 3.26(1H, d, 14.8 Hz); 3.25 (1H, d, 14.8 Hz)	35.54; 34.93	
5′		171.91; 171.87	
6′	6.76 (1H, s); 6.71 (1H, s)	94.93; 94.65	
7′		166.08; 165.90	
8′		173.67; 173.53	
9′	a: 2.87 (1H, dd, 17.5, 6.2 Hz); 2.86 (1H, dd, 17.5, 6.2 Hz)	44.13	
	b: 2.64 (1H, br d, 17.5 Hz); 2.62 (1H, br d, 17.5 Hz)		
10′	4.43 (1H, br q)	63.05; 62.86	
11′	a: 3.87 (1H, dd, 12.8, 3.8 Hz); 3.85 (1H, dd, 12.8, 3.8 Hz)	54.70; 54.62	
	b: 4.02 (1H, d, 12.8 Hz); 4.00 (1H, d, 12.8 Hz)		
12′	3.72 (3H, s); 3.71 (3H, s)	56.10; 56.02	
13′	2.88 (3H, s); 2.79 (3H, s)	33.95; 32.99	
N-a			108.3; 107.5
N-b			165.8

[a] Spectra determined in $CDCl_3$ at 25 °C; data reported in ppm. [b] TMS (0.00 ppm) was used as a reference. [c] $CDCl_3$ (77.0 ppm) was used as a reference. [d] CH_3NO_2 (379.6 ppm) was used as a reference. The assignments were achieved by $^1H-{}^{15}N$ HMBC.

Preparation of (*R*)-MTPA Ester of 2. Compound **2** (1.2 mg) was dissolved in CH_2Cl_2 (50 μL), trimethylamine (0.46 μL), and a catalytic amount of 4-DMAP. The addition of (+)-MTPA chloride (0.62 μL) followed. The solution was incubated at room temperature for 2 h. The reaction was terminated with the addition of MeOH (100 μL). After 10 min., the solvent was evaporated. The residue was subjected to preparative TLC (hexanes−EtOAc 1:1) to yield the (*R*)-MTPA ester of **2** (1.0 mg, 60.1%): HRFABMS $[M + 1]^+$ *m/z* 757.3469 ($C_{38}H_{53}{}^{35}ClF_3N_2O_8$, Δ 2.6 mmu of calcd).

Preparation of (*S*)-MTPA Ester of 2. CH_2Cl_2 (80 μL) containing DCC (1.5 mg), 4-DMAP (0.65 mg), and (−)-MTPA acid (1.4 mg) was added to **2** (1.2 mg). The solution was incubated for 7.5 h at room temerature. The reaction mixture was subjected to preparative TLC (hexane−EtOAc 1:1) to yield the (*S*)-MTPA ester of **2** (0.7 mg, 42.1%): HRFABMS $[M + 1]^+$ *m/z* 757.3459 ($C_{38}H_{53}{}^{35}ClF_3N_2O_8$, Δ 1.7 mmu of calcd).

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(13) We asked Prof. Moore (University of Hawaii) for authentic samples or spectra of malyngamides A and B (**3**, **4**) to compare with isomalyngamides A (**1**) and B (**2**). Unfortunately, he does not have good ^{1}H and ^{13}C NMR spectra for malyngamides A and B nor any authentic samples left, because the work was done some time ago. Therefore, we could not compare isomalyngamides A and B (**1**, **2**) with authentic malyngamides A and B (**3**, **4**).
(14) During the collection, the authors (H.N.; B.S.) experienced "swimmer's itch"[15] suggesting lyngbyatoxin A was present in the sample. After showering, symptoms dissipated within a few hours.
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