Three New Malyngamides from a Papua New Guinea Collection of the Marine Cyanobacterium *Lyngbya majuscula*

Kerry L. McPhail and William H. Gerwick*

College of Pharmacy, Oregon State University, Corvallis, Oregon 97331

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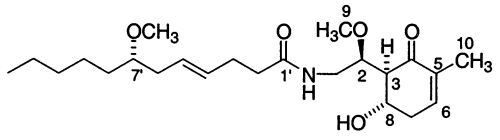
Three new malyngamides, U (**1**), V (**2**), and W (**3**), all possessing an uncommon oxygenation pattern, have been isolated from the marine cyanobacterium *Lyngbya majuscula* collected in Papua New Guinea. The planar structures of these compounds were deduced from NMR data. Aspects of their relative stereochemistry were investigated using selective ^{1}H decoupling, 1D and 2D NOESY, and E-COSY experiments in three different NMR solvents.

Marine cyanobacteria rich in secondary metabolites proliferate in shallow tropical waters. Since 1998, we have made several collections of *Lyngbya* species on diving expeditions to Papua New Guinea. Chemical investigations of *Lyngbya* species continue to yield a structurally diverse array of bioactive secondary metabolites typified by lipopeptides.[1] In August 2000, we made a shallow water collection of *Lyngbya majuscula* Harvey ex Gomont (Oscillatoriaceae), which subsequently has yielded three new malyngamides, U (**1**), V (**2**), and W (**3**), whose planar structure and relative stereochemistry were deduced from 1D and 2D NMR data. The malyngamides comprise an extensive series of lipopeptides that have been isolated primarily from *L. majuscula* collected globally.[1,2]



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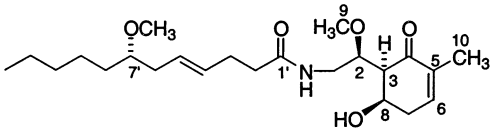

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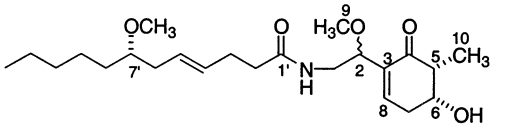

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Vacuum-liquid chromatography followed by solid-phase extraction and C_{12} reversed-phase HPLC of the organic algal extract gave malyngamides U (**1**) and V (**2**), which were subsequently further purified by C_{18} reversed-phase HPLC. Examination of the ^{1}H NMR spectrum for **1** readily suggested a lipopeptide structure. In support of this, HRFABMS data for **1** yielded a molecular formula of $C_{23}H_{40}NO_5$ with an inherent five degrees of unsaturation. In the ^{1}H NMR spectrum of **1**, a terminal methyl triplet (δ 0.89), a prominent aliphatic chain resonance ($\sim\delta$ 1.30), and a 2H olefinic proton multiplet (δ 5.49), in combination with a methine pentet (δ 3.16) and a methoxy methyl singlet (δ 3.33), delineated a methoxy-substituted unsaturated fatty acid moiety, which is characteristic of the malyngamides. The remaining three double-bond equivalents indicated by the molecular formula of **1**, in conjunction with a deshielded carbonyl carbon resonance (δ 198.0) and two olefinic carbon resonances (δ 136.5 and 142.6) in the ^{13}C NMR spectrum, suggested a monocyclic amine portion of the molecule containing an α,β-unsaturated carbonyl group. Such an arrangement is also a common motif in the malyngamides and is often found adjacent to either a vinyl chloride moiety (e.g., malyngamide S)[2] or an exomethylene (e.g., malyngamide H).[3] In the COSY spectrum of **1**, a continuous spin system was evident in which a broad amide proton triplet (δ 5.90) was coupled to two mutually coupled midfield methylene resonances (δ 3.48 and 3.57, H_2-1), which in turn showed couplings to an oxymethine ^{1}H resonance (δ 4.23, H-2). The latter resonance and a partially overlapping multiplet (δ 4.27, H-8) both appeared to be coupled to a double doublet at δ 2.82, which itself showed no other COSY correlations, thus suggesting that it was flanked by the two oxymethine protons. This spin system could be traced further through a coupling from the signal at δ 4.27 (H-8) to H_2-7, and from the latter protons to the olefinic proton H-6. Two- and three-bond HMBC correlations from H-3 to C-1, C-2, and C-8 confirmed the connectivity deduced from COSY data. H-3 also showed an HMBC correlation to C-4, thereby completing the cyclohexenone ring. Similarly, HMBC correlations from CH_3-10 to C-4, C-5, and C-6 positioned the olefinic methyl group (δ 1.77) at C-5.

The ^{1}H NMR data for malyngamide V (**2**) were similar to those obtained for **1**. HRFABMS data for compound **2** suggested that the two compounds had the same molecular formula ($C_{23}H_{40}NO_5$), while connectivities established from the 2D NMR data for compound **2** indicated that both shared the same planar structure. However, significant chemical shift differences and variations in the splitting pattern of similarly assigned protons in the ^{1}H NMR spectra of **1** and **2** were evident for H-3, H-7, and H-8 (Table 1). As described below, the relative stereochemistry at C-3 and C-8 in compounds **1** and **2** was inferred from a combination of coupling constant data, selective ^{1}H decoupling experiments, an E-COSY experiment, and 1D and 2D NOESY experiments.

The relative stereochemistry of the amine portion of malyngamide U (**1**) was initially investigated in $CDCl_3$. In this solvent, equatorial H-7a and axial H-7b could be assigned by irradiation of H-6, which resulted in the

* To whom correspondence should be addressed. Tel: (541) 737-5801. Fax: (541) 737-3999. E-mail: Bill.Gerwick@oregonstate.edu.

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Table 1. ^{1}H NMR Data for Malyngamides U (**1**), V (**2**), and W (**3**) at 600 MHz in $CDCl_3$

atom number	**1** δ_H (mult., *J*/Hz)	**2** δ_H (mult., *J*/Hz)	**3** δ_H (mult., *J*/Hz)
1	3.48 (m)	3.33 (m)	3.40 (dd, 4.9, 6.0)
	3.57 (m)	3.70 (p, 7.0)	
2	4.23 (m)	4.25 (m)	4.31 (td, 1.1, 4.7)
3	2.82 (dd, 3.4, 10.9)	2.58 (br s)	
4			
5			2.70 (dq, 3.0, 7.0)
6	6.68 (dm, 1.3, 5.2)	6.60 (m)	4.27 (m)
7a	2.73 (dt, 5.6, 18.2)	2.59 (m)	2.66 (m)
b	2.38 (m)		
8	4.27 (m)	4.68 (br s)	6.67 (br t, 4)
9	3.47 (s)	3.52 (s)	3.28 (s)
10	1.77 (d, 1.2)	1.83 (d, 1.7)	1.22 (d, 7.0)
1′			
2′	2.25 (m)	2.27 (m)	2.20 (m)
3′	2.35 (m)	2.34 (m)	2.31 (m)
4′	5.49 (m)	5.48 (m)	5.47 (m)
5′	5.49 (m)	5.48 (m)	5.47 (m)
6′	2.20 (m)	2.19 (m)	2.20 (m)
7′	3.16 (p, 5.8)	3.16 (p, 5.7)	3.16 (m)
8′	1.44 (m)	1.43 (m)	1.44 (m)
9′	1.30 (m)	1.29 (m)	1.29 (m)
10′	1.28 (m)	1.28 (m)	1.27 (m)
11′	1.31 (m)	1.32 (m)	1.31 (m)
12′	0.89 (t, 6.8)	0.90 (t, 6.9)	0.91 (t, 7.3)
13′	3.33 (s)	3.33 (s)	3.33 (s)
OH		3.93 (br s)	1.81 (br s)
NH	5.90 (br s)	5.81 (br t, 5.9)	5.75 (br t, 5.5)

Table 2. ^{13}C NMR Data for Malyngamides U (**1**), V (**2**), and W (**3**) at 600 MHz in $CDCl_3$

atom number	**1** δ_C mult.	**2** δ_C mult.	**3** δ_C mult.
1	40.0 t	40.5 t	43.0 t
2	80.3 d	78.3 d	76.9 d
3	54.7 d	53.5 d	136.3 s
4	198.0 s	198.0 s	200.1 s
5	136.5 s	135.7 s	48.0 d
6	142.6 d	141.0 d	71.5 d
7	34.7 t	34.2 t	33.74 t
8	68.9 d	68.6 d	140.4 d
9	58.6 q	59.5 q	57.4 q
10	16.1 q	16.4 q	11.0 q
1′	172.8 s	173.2 s	172.8 s
2′	36.9 t	36.9 t	36.9 t
3′	28.9 t	28.9 t	29.1 t
4′	131.2 d	131.1 d	131.2 d
5′	127.9 d	128.2 d	127.9 d
6′	36.6 t	36.7 t	36.8 t
7′	81.1 d	81.0 d	81.1 d
8′	33.7 t	33.7 t	33.69 t
9′	25.4 t	25.4 t	25.4 t
10′	32.4 t	32.4 t	32.4 t
11′	23.0 t	23.0 t	23.0 t
12′	14.4 q	14.4 q	14.4 q
13′	56.9 q	56.8 q	56.9 q

collapse of the more deshielded H-7a (δ 2.73, J = 18.2, 5.6, 4.9 Hz) from a double doublet of doublets to a double doublet (J = 18.2, 4.9 Hz). The small vicinal coupling indicated a *cis* relationship between equatorial H-7a and oxymethine H-8. A coupling constant of approximately 10 Hz between the upfield H-7b resonance (δ 2.38) and H-8, obtained by analysis of an E-COSY experiment ($CDCl_3$), was in agreement with an *anti* relationship between these two protons. Unfortunately, in the ^{1}H NMR spectra for **1**, overlap of the signals for H-2 and H-8 in $CDCl_3$ and C_6D_6 hampered the assignment of the coupling constants between these two resonances and H-3, despite their being readily observed in the H-3 signal ($CDCl_3$, δ 2.82, J = 3.4, 10.9 Hz). However, H-2, H-3, and H-8 were all well resolved

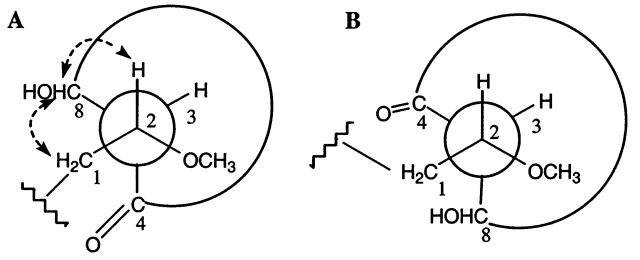

Figure 1. Two possible configurations for the C-2/C-3 relative stereochemistry in malyngamides U (**1**) and V (**2**). Part A shows key 1D NOESY enhancements (dashed arrows) which defined this configuration to be correct for both **1** and **2**.

in pyridine-d_5 (see Supporting Information), and irradiation of H-2 in a ^{1}H decoupling experiment produced an H-3 doublet with $J_{H-3/H-8}$ = 7.8 Hz, while irradiation of H-8 resulted in a broad H-3 singlet. Thus, these data defined an *anti* relationship between H-3 and H-8 in **1**. In considering the relative stereochemistry at C-2, enhancements of both H-2 and methoxy CH_3-9 upon selective irradiation of H-3 permitted two possible configurations (Figure 1). In pyridine-d_5, selective excitation of H-8 in a 1D DPFGSE NOESY experiment produced enhancements in the signals for H-2, H_2-1, and equatorial H-7, as well as a small enhancement for H-3. These data were consistent with 2D NOESY data and led us to assign the relative stereochemistry of the amine portion in **1** as 2*S**, 3*S**, 8*S** (Figure 1A).

In compound **2**, placement of H-8 *cis* to H-3 concurs with the broad singlet resonance observed for H-3 in C_6D_6 (δ 2.58, width at half peak height = 6.8 Hz). Once again, complete resolution of H-2 and H-8, and H-3 and H_2-7, was attained in pyridine-d_5. In the latter solvent, H-3 resonates as a doublet (J = 6.1 Hz) which collapsed to a singlet upon ^{1}H decoupling of H-2. Selective excitation of H-3 in a 1D NOE experiment produced a strong enhancement in the H-8 resonance, as well as enhancement of H-2 and the methoxy CH_3-9. In pyridine-d_5, a 2D NOESY experiment showed correlations from H-8 to H_2-1 and H-2, but not methoxy CH_3-9, implying that the relative stereochemistry between C-2 and C-3 is also as shown in Figure 1A. Therefore, we concluded that the relative stereochemistry of the amine portion in **2** is 2*S**, 3*S**, 8*R**, and thus, malyngamides U and V are C-8 epimers. Unfortunately, insufficient material for derivatization precluded determination of the absolute stereochemistry in these two new malyngamides.

Malyngamide W (**3**) was obtained as a pale yellow oil after C_{12} reversed-phase HPLC of a vacuum liquid chromatography fraction. From HRFABMS data, it was established that compound **3** had the same molecular formula ($C_{23}H_{39}NO_5$) as **1** and **2**. In the ^{1}H NMR spectrum of compound **3**, chemical shifts of the 7-methoxydodecenoic acid residue protons were apparent, as well as a broad amide NH resonance (δ 5.75). A second methoxy methyl singlet (δ 3.28) and a deshielded olefinic ^{1}H signal (δ 6.67) were also present, as in the spectra of **1** and **2**. Notably, the slightly split methyl doublet in the ^{1}H NMR spectra for **1** and **2** (δ 1.77, J = 1.7 Hz and 1.83, J = 1.2 Hz, respectively) was replaced by a shielded methyl doublet (δ 1.22, J = 7.0 Hz) in the spectrum of **3**, signifying the presence of a methine methyl in the latter compound. Examination of the HMBC data for **3** led to the placement of the methine methyl at C-5 (δ 48.0) and the hydroxyl group at C-6 (δ 71.5). Two- and three-bond HMBC correlations from the methine methyl to C-4, C-5, and C-6 were observed, while the hydroxy methine resonance showed COSY couplings to H-5 and H_2-7 (Table 1) as well as to the hydroxyl proton.

A 5*R**, 6*R** stereochemistry for **3** was assigned from the following data. Methylene protons H_2-7, which resonated together in $CDCl_3$ and C_6D_6, were resolved in pyridine-d_5 as a pair of broad doublets (J_{gem} = 18.4 Hz). The small H-6/H_2-7 couplings present in this signal are consistent with a pseudoequatorial H-6 and thus an axial hydroxyl group. A *cis* relationship between H-5 and H-6 was suggested by $J_{H5/H6}$ = 3.0 Hz observed in the H-5 ^{1}H signal (δ 2.40, dq) and is consistent with the reciprocal NOE enhancements observed between H-6 (δ 3.63) and H-5 (δ 2.40) and CH_3-10 (δ 1.40) in a series of 1D NOESY experiments. As predicted from this hypothesis, which places CH_3-10 pseudoequatorial, a small 1,3-diaxial NOE to H-7b was observed upon irradiation of H-5. While a reciprocal NOE was observed between the C-2 methoxy methyl and H-8 olefinic proton, the lack of any other NOE correlations to H-2 prevented assignment of its relative stereochemistry. Unfortunately, as with malyngamides U and V, the absolute stereochemistry of the amine portion of malyngamide W was not determined due to a lack of material.

The olefin stereochemistry of the fatty acid portion of malyngamides U (**1**), V (**2**), and W (**3**) was readily inferred from a simple ^{1}H decoupling experiment (olefinic C-4′ and C-5′). The olefinic proton signals of C-4′ and C-5′ for all three compounds were overlapped in $CDCl_3$ (Table 1) and pyridine-d_5, but resolved to a distinct pair of multiplets in C_6D_6 (see Supporting Information). In C_6D_6 the flanking methylene protons were decoupled (e.g., H_2-3′, δ 2.30 and H_2-6′, δ 2.20 in **1**) and a $J_{H4'/H5'}$ = 15.5 Hz was measured, confirming a 4′*E* geometry in all three compounds. The optical rotation of the co-occurring free 7-methoxydodecenoic acid was comparable to the literature value for 7-(*S*)-methoxydodec-*E*-enoic acid,[4] which is also the fatty acid component of malyngamides G[5] and S.[2] We assume that malyngamides U (**1**), V (**2**), and W (**3**) derive from this acid and therefore possess the same 7′(*S*) stereochemistry.

Compounds **1**−**3** were inactive in a brine shrimp toxicity assay.[6] Indeed, few malyngamides have been reported to exhibit more than moderate biological activity.[1] However, the free fatty acid was reported to show potent immunosuppressive properties[4] and, more recently, cytotoxicity toward the BSC-1 (monkey kidney) cell line and antimicrobial activity.[2] Besides malyngamides U, V, and W, only malyngamides G and S possess a 12-carbon fatty acid residue rather than the more common 14-carbon residue (lyngbic acid).[1] The occurrence of malyngamides with either the 12-carbon or 14-carbon aliphatic chain does not appear to be correlated to geography. The only other reported malyngamides that are oxygenated at C-2 are the 2-hydroxylated malyngamides D and E.[7] The isolation of malyngamides that are oxygenated at the 2 position lends credence to the proposal that glycine (or in some cases β-alanine) is the extension unit of an initial polyketide chain, which then undergoes further polyketide extension to form the cyclohexyl ring.[8] The probable mixed polyketide and peptide biosynthetic origin of the malyngamides is characteristic of *Lyngbya* species and warrants further investigation.

Experimental Section

General Experimental Procedures. Optical rotation measurements were recorded on a Perkin-Elmer model 243 polarimeter. FTIR and UV spectra were recorded on a Nicolet 510 and a Beckman DU640B spectrophotometer, respectively. NMR spectra were recorded on a Bruker Avance 400 MHz and a Bruker DRX 600 MHz spectrometer operating at proton frequencies of 400.13 and 600.01 MHz, respectively, and carbon frequencies of 100.61 and 150.90 MHz, respectively, with the solvent used as an internal standard ($CDCl_3$ at δ_C 77.4, δ_H 7.27; C_6D_6 at δ_C 128.4, δ_H 7.16; pyridine-d_5 at δ_H 7.22, 7.58, and 8.74). Mass spectra were recorded on a Kratos MS50TC mass spectrometer. HPLC separations were performed using a Waters 515 pump, a Rheodyne 7725i injector, and a Waters 996 photodiode array detector.

Collection, Extraction, and Isolation Procedures. The marine cyanobacterium *L. majuscula* was collected by hand from shallow water (1−5 m) on August 28, 2000, at Crown Island, Papua New Guinea (5°08.585 S, 146°57.955 E) and stored at −20 °C in 70% ethanol until workup. A voucher specimen is available from W.H.G. (collection number PNG-CI-8/28/00-9). Filaments from this collection were very long and without calyptra. The cells were encased in a sheath (ca. 4 μm wide) and showed measurements of 35 μm diameter by 5 μm length, well within the range for *L. majuscula*.[9]

The EtOH-preserved alga (372 g dry wt) was extracted with CH_2Cl_2−MeOH (2:1) six times to give a crude organic extract of 2.84 g. The extract (2.69 g) was fractionated on silica gel by normal-phase vacuum-liquid chromatography (NPVLC) using a stepwise gradient of hexanes−EtOAc and EtOAc−MeOH to give seven fractions. Fraction 6 (eluted 100% EtOAc) was further chromatographed on a Waters C_{18} solid-phase extraction cartridge (RP-18 SPE; 2 g) using a stepwise gradient from 3:2 MeOH−H_2O to 100% MeOH, followed by CH_2Cl_2. The fraction eluting in 7:3 MeOH−H_2O was subjected to reversed-phase HPLC (Phenomenex Synergi, 4 μm, 250 × 4.6 mm) in 80% MeOH−H_2O to yield pure **1** (1.2 mg, 0.04% of extract) and a mixture of **2** and **3** (0.7 mg, 0.02% of extract and 1.2 mg, 0.04% of extract, respectively). The latter two compounds were subsequently resolved using 50% MeCN−H_2O as the HPLC (Varian microsorb-mv, 5 μm, 250 × 4.6 mm) mobile phase. Crude fraction 3 (eluted 4:1 hexanes−EtOAc) was also chromatographed on a Waters RP-18 SPE, and the fraction eluting in 9:1 MeOH−H_2O yielded 7-methoxydodec-*E*-enoic acid (ca. 80 mg) after RPHPLC (Varian microsorb-mv, 5 μm, 250 × 4.6 mm, 8:2 MeOH−H_2O).

Malyngamide U (1): colorless oil; $[\alpha]^{18}_D$ −15.8° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 203 (3.48), 233 (3.54); IR ν_{max} (film) 3315, 2928, 1671, 1547, 1450, 1376, 1087 cm^{-1}; ^{1}H and ^{13}C NMR data in $CDCl_3$, see Table 1; ^{1}H NMR in C_6D_6 and pyridine-d_5, see Table S1; HRFABMS (3-NBA) obsd $[M + H]^+$ *m/z* 410.2905 (calcd for $C_{23}H_{40}NO_5$, 410.2907).

Malyngamide V (2): colorless oil; $[\alpha]^{18}_D$ +4.3° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 203 (3.48), 231 (3.48); IR ν_{max} (film) 3454, 2930, 1664, 1547, 1453, 1383, 1097, 972 cm^{-1}; ^{1}H and ^{13}C NMR data in $CDCl_3$, see Table 1; ^{1}H NMR in C_6D_6 and pyridine-d_5, see Table S1; HRFABMS (3-NBA) obsd $[M + H]^+$ *m/z* 410.2904 (calcd for $C_{23}H_{40}NO_5$, 410.2907).

Malyngamide W (3): colorless oil; $[\alpha]^{18}_D$ −15.0° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 203 (4.08), 227 (3.88); IR ν_{max} (film) 3478, 2928, 1655, 1546, 1449, 1368, 1091, 971 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 8.80 (1H, br s, N*H*), 6.86 (1H, br s, H-8), 5.66 (1H, m, H-4′), 5.63 (1H, m, H-5′), 4.73 (1H, br s, H-2), 4.45 (1H, br s, H-6), 3.94 (1H, m, H-1), 3.70 (1H, dt, J = 4.0, 13.6 Hz, H-1), 3.28 (3H, s, H_3-9), 3.15 (3H, s, H_3-13′), 3.14 (1H, p, J = 5.5 Hz, H-7′), 2.93 (1H, br d, J = 4.0 Hz, H-5), 2.81 (br d, J = 18.4 Hz, H-7a), 2.75 (br d, J = 18.4 Hz, H-7b), 2.59 (2H, m, H_2-3′), 2.54 (2H, m, H_2-2′), 2.24 (2H, m, H_2-6′), 1.45 (1H, m, H-8′), 1.39 (1H, m, H-8′), 1.32−1.11 (6H, m, H_2-9′, H_2-10′, H_2-11′), 1.48 (3H, d, J = 6.4 Hz, H_3-10), 0.82 (3H, t, J = 7.2 Hz, H_3-12′); HRFABMS (3-NBA) obsd $[M + H]^+$ *m/z* 410.2901 (calcd for $C_{23}H_{40}NO_5$, 410.2907).

7-(*S*)-Methoxydodec-*E*-enoic acid: $[\alpha]^{18}_D$ −6.2° (*c* 0.1, $CHCl_3$) (lit. $[\alpha]^{20}_D$ −8° (*c* 1.8, $CHCl_3$));[4] ^{1}H NMR ($CDCl_3$) data are in agreement with literature values.[4]

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Supporting Information Available: ^{1}H NMR data in C_6D_6 for malyngamides U (**1**), V (**2**), and W (**3**) and in pyridine-d_5 for malyngamides U (**1**) and V (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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