Malyngamides M and N from the Hawaiian Red Alga *Gracilaria coronopifolia*

Yukiko Kan,[†] Tsuyoshi Fujita,[†] Hiroshi Nagai,*[,†,‡] Bryan Sakamoto,[‡] and Yoshitsugi Hokama[‡]

Suntory Institute for Bioorganic Research, Wakayamadai, Shimamoto, Mishima-gun, Osaka 618, Japan, and John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii 96822

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Two new malyngamides, M and N (**1**, **2**), were isolated along with malyngamide I acetate (**3**) from the Hawaiian red alga *Gracilaria coronopifolia*. Our results suggest that malyngamide N (**2**) is a revised structure of deacetoxystylocheilamide (**5**). The absolute configuration of malyngamide I acetate was deduced to be **3** using the reversed octant rule.

Aplysiatoxin and its derivatives were first identified as the causative agents of the red alga *Gracilaria coronopifolia* J. Agardh (Gracilariaceae) poisoning in Hawaii in 1994.[1,2] In our previous study, samples of *G. coronopifolia*, which were collected from the site of the original toxic specimen, were analyzed on a continuous basis. During the course of that study, we isolated some malyngamides from *G. coronopifolia*. Malyngamides are known as blue-green algal metabolites and generally are *N*-substituted amides of 7(*S*)-methoxytetradec-4(*E*)-enoic acid that frequently possess a terminal chloromethylene group.[3−11] In the present report, we describe the isolation and characterization of two new malyngamides M and N (**1**, **2**) from *G. coronopifolia* and the probable structural revision of deacetoxystylocheilamide (**5**)[12] to **2**. We also report that the absolute configuration of malyngamide I acetate,[10] which may represent a revised structure of stylocheilamide (**6**),[12] is deduced to be **3** using the reversed octant rule.

Malyngamide M (**1**) was obtained as a white solid. The molecular formula $C_{26}H_{40}ClNO_3$ of malyngamide M was determined from HRFABMS data. Structure elucidation of malyngamide M was determined from the ^{1}H-NMR, ^{13}C-NMR, COSY, ROESY, HSQC, and HMBC spectra. The ^{1}H-NMR spectrum indicated the presence of a phenol moiety [δ 8.97 (s, 5′-OH), 7.18 (d, H-7′), 6.85 (d, H-9′), 6.73 (t, H-8′)] and a linear alkyl group [δ 0.87 (t, 3H, H-14) and 1.24−1.50 (12H)]. The presence of an amide functionality was suggested by the IR absorption at 1652 cm^{-1} and a carbon signal at δ 174.3 ppm. The proton signals δ 3.27 (s, 3H) and 3.30 (s, 3H) were assigned to $-OCH_3$ (δ 56.6, C-15) and $-NCH_3$ (δ 38.2, C-11′), respectively, based on a HSQC experiment. In the HMBC spectrum, a correlation between $-OCH_3$ (δ 3.27, 3H, H-15) and C-7 (δ 81.5) was observed, suggesting that a methoxy group was attached to C-7. The geometry of the C-4−C-5 olefin was determined to be *E*, because the coupling constant between H-4 and H-5 was 15.4 Hz. ROE between H-3′ and H-11′ confirmed that the geometry of C-2′−C-3′ olefin is a *Z* configuration. C-4′ of the phenol moiety was connected to C-2′, because a cross peak between H-9′(δ 6.85) and C-2′ (δ 138.7) was observed in the HMBC spectrum. In the HMBC spectrum, correlations between H-10′ (δ 2.21) and C-5′ (δ 153.7), C-6′ (δ 126.2), and C-7′ (δ 131.6) were observed. Thus, the methyl group (C-10′) was concluded to be attached to C-6′ on the aromatic ring. Further analysis of the HMBC spectrum led to the elucidation of the planar structure of **1**. Malyngamide M (**1**) was isolated from the *G. coronopifolia* along with malyngamide I acetate (**3**, $[\alpha]_D = +9.3°$, MeOH, *c* 0.13 (lit.[10] $[\alpha]_D = +14.4°$, MeOH, *c* 1.54)),[10] which also includes a 7-methoxytetradec-4(*E*)-enoyl moiety. The configuration of **3** at C-7 has been proposed to be *S*.[10] Similarly, previous reports have suggested that malyngamides include 7(*S*)-methoxytetradec-4(*E*)-enoic acid.[5,7−11] Thus, **1** appears to possess the same *S* configuration at C-7 as **3**. Malyngamide M (**1**) is the first example of a natural aromatized malyngamide. Previous studies have reported only artifactual aromatized malyngamides.[12]

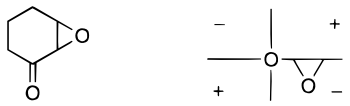

Figure 1. Reversed octant rule of an epoxy−ketone ring system.

Malyngamide N (**2**) was obtained as a white solid. The molecular formula $C_{26}H_{40}ClNO_4$ of **2** was determined from the HRFABMS data. In the ^{1}H- and ^{13}C-NMR spectra of **2**, some of the peaks appeared as pairs or broadened due to the presence of two interconverting *tert*-amide isomers. On the other hand, interconversion was not observed in malyngamide M (**1**). Thus, hydrogen bonding appears to have limited the malyngamide M molecule to only one conformation. The NMR spectra of **2** showed a close resemblance to those of **3**. Comparison of the ^{1}H-NMR spectra of **2** and **3** revealed that **2** has an olefin methyl (δ 1.768 and 1.775, dt, $J = 2.2$, 1.2 Hz, H-10′; δ 16.6, q, C-10′) but no acetoxy group on the cyclic portion of the molecule. The cross peak between H-1′ and C-4′ in the HMBC spectrum enabled the cyclohexyl moiety to be connected at C-2′. The planar structure of **2** was elucidated based on the ^{1}H-NMR, ^{13}C-NMR, COSY, NOESY, editing-HSQC,[13] and HMBC spectra. Malyngamide N (**2**) has a 7-methoxytetradec-4(*E*)-enoyl moiety similar to **1** and **3**. Malyngamide N (**2**) is assumed to have an *S* configuration at C-7 similar to that of **1**. The empirical reversed octant rule can be applied to elucidate the absolute configu-

* To whom correspondence should be addressed. Suntory Institute for Bioorganic Research, Wakayamadai, Shimamoto, Mishima-gun, Osaka 618, Japan. Phone: +81 (75) 962-3743. FAX: +81 (75) 962-2115. E-mail: Hiroshi_Nagai@suntory.co.jp.

[†]Suntory Institute for Bioorganic Research.

[‡] John A. Burns School of Medicine, University of Hawaii at Manoa.

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Table 1. ^{1}H- and ^{13}C-NMR Data for Malyngamide M (**1**)[a]

position	^{1}H NMR	^{13}C NMR	HMBC	ROE
1		174.3		
2	2.52 (2H, t, 7.3 Hz)	33.7	C-4, C-3, C-1	H-11′
3	2.30 (2H, q, 7.3 Hz)	28.8	C-5, C-4	
4	5.50 (dt, 15.4, 6.6 Hz)	132.1	C-3, C-6	
5	5.54 (dt, 15.4, 7.0 Hz)	128.3	C-3, C-6	
6	2.15 (H-6a, dt, 13.5, 7.0 Hz) 2.19 (H-6b, m)	37.3	C-5, C-7	
7	3.15 (m)	81.5		
8	1.43 (2H, m)	34.2		
9		26.1		
10		30.0		
11	1.24−1.40 (H-9−H-13, 10H)	30.0		
12		32.7		
13		23.4		
14	0.87 (3H, t, 7.1 Hz)	14.4	C-12, C-13	
15 (−OCH_3)	3.27 (3H, s)	56.6	C-7	
1′	4.10 (2H, br s)	53.9		
2′		138.7		
3′	6.39 (t, 1.7 Hz)	116.8	C-1′, C-2′, C-4′	H-11′
4′		123.1		
5′		153.7		
6′		126.2		
7′	7.08 (d, 7.5 Hz)	131.6	C-5′, C-9′, C-10′	
8′	6.73 (t, 7.5 Hz)	119.7	C-4′, C-6′	
9′	6.85 (d, 7.5 Hz)	128.3	C-2′, C-5′, C-7′	
10′	2.21 (3H, s)	16.6	C-5′, C-6′, C-7′	
11′ (−NCH_3)	3.30 (3H, s)	38.2	C-1, C-1′	H-3′, H-2
5′-OH	8.97 (s)			

[a] Spectra determined in Me_2CO-d_6; data reported in ppm.

Table 2. ^{1}H- and ^{13}C-NMR Data for Malyngamide N(**2**)[a]

position	^{1}H NMR	^{13}C NMR	HMBC	NOE
1		175.5, 175.8[b]		
2	2.55 (H-2a, dt, 15.4, 7.5 Hz)[b] 2.50 (H-2b, dt, 15.4, 7.3 Hz)[b] 2.41 (H-2a and H-2b, t, 7.3 Hz)[b]	33.8, 34.5[b]	C-1, C-3, C-4	
3	2.30 (H-3a and H-3b, m)[b] 2.27 (H-3a and H-3b, m)[b]	29.4, 29.6[b]	C-1, C-4, C-5	
4	5.52 (m)	132.5, 132.6[b]	C-6	
5	5.48 (m)	128.2, 128.6[b]	C-3	
6	2.18 (2H, m)	37.5, 37.6[b]	C-8, C-7, C-5, C-4	
7	3.19 (br quintet, 5.5 Hz)	82.5, 82.5[b]	C-5, C-9	
8	1.44 (2H, m)	34.6	C-7, C-9	
9		26.5, 26.5[b]		
10		30.6[c]		
11	1.23−1.40 (H-9−H-13, 10H)	31.0[c]		
12		33.2		
13		23.9		
14	0.891 (H-14, t, 7.0 Hz)[b] 0.893 (H-14, t, 7.0 Hz)[b]	14.6	C-12, C-13	
15 (−OCH_3)	3.32 (3H, s)	57.0	C-7	
1′	4.25 (H-1′a, dd, 17.0, 1.5 Hz)[b] 4.23 (H-1′a, br s)[b] 4.02 (H-1′b, dd, 17.0, 1.5 Hz)[b] 4.00 (H-1′b, br s)[b]	49.3[d], 52.4[b]	C-1, C-2′, C-3′, C-4′, C-11′	H-3′
2′		133.8		
3′	6.39 (t, 1.5 Hz)	122.2, 122.6[b]	C-1′, C-2′, C-4′	H-1′, H-11′
4′		60.0		
5′		194.3		
6′		132.7		
7′	6.47 (br s)[b] 6.43 (br s)[b]	140.4, 140.7[b]		
8′	2.90 (m)	28.1		H-9′, H-7′
9′	3.74 (quintet, 1.0 Hz)[b] 3.79 (quintet, 1.0 Hz)[b]	62.5	C-7′, C-8′	H-8′
10′	1.768 (H-10′, dt, 2.2, 1.2 Hz)[b] 1.775 (H-10′, dt, 2.2, 1.2 Hz)[b]	16.6	C-5′, C-6′, C-7′	H-7′
11′ (−NCH_3)	3.04 (H-11′, s)[b] 3.23 (H-11′, s)[b]	34.5[e], 35.6[b,e]	C-1, C-1′	H-3′, H-9′

[a] Spectra determined in CD_3OD; data reported in ppm. [b] Signals appeared as pairs. [c] Assignments may be interchanged. [d] Chemical shifts determined from HMBC experiment. [e] Chemical shifts determined from HSQC experiment.

ration of an epoxy−ketone ring system (Figure 1).[14] Therefore, the negative Cotton effect of malyngamide N, λ_{ext} 332 nm ($\Delta\epsilon$ −1.76) in MeOH indicates that the absolute configuration of the epoxide is 4′*S* and 9′*S*, as

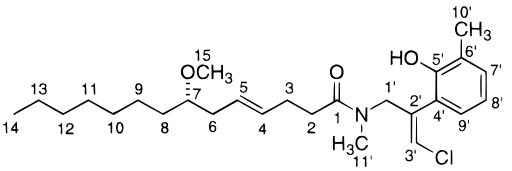
seen in **2**. Furthermore, malyngamide I acetate also showed a negative Cotton effect, λ_{ext} 298 nm ($\Delta\epsilon$ −1.38) in MeOH. Thus, the absolute configuration of malyngamide I acetate, which may represent a revised structure of stylocheilamide (**6**),[10,12] was deduced to be **3**.

Malyngamide N (**2**) and malyngamide I acetate (**3**) showed moderate cytotoxicity to mouse neuroblastoma (NB) cells in the tissue culture. The IC_{50} values of **2** and **3** were 12 μM (4.9 μg/mL) and 12 μM (7.1 μg/mL), respectively. In contrast, malyngamide M (**1**) showed rather weak cytotoxicity to NB cells (IC_{50} > 20 μM).

Stylocheilamide (**6**) and deacetoxystylocheilamide (**5**) were previously isolated together from the sea hare *Stylocheilus longicauda*.[12] Todd and Gerwick reported that malyngamide I acetate (**3**) likely represents a revised structure of stylocheilamide (**6**).[10] Furthermore, the physicochemical properties of malyngamide N (**2**) (see Experimental Section) bear a close resemblance to those of deacetoxystylocheilamide (**5**).[12] These results suggest that malyngamide N (**2**) represents a revised structure of deacetoxystylocheilamide (**5**) similar to that of stylocheilamide.

The aromatized stylocheilamide (**4**) was formerly reported as an artifact derivatized from stylocheilamide (**6**) during column chromatography;[12] however, **1** exists as a natural product in the algal sample, because **1** was observed in the HPLC profile of the crude algal extract.

Aplysiatoxin and its derivatives were obtained along with malyngamides **1**−**3** from the same *G. coronopifolia* sample (see Experimental Section). Malyngamides are known as metabolites of blue-green algae, in particular *Lyngbya majuscula*.[3−11] Aplysiatoxin and related compounds are also known as products of blue-green algae including *L. majuscula*.[15,16] Furthermore, it has been reported that epiphytes such as blue-green algae grow on *Gracilarlia*.[1,17] Therefore, the true origin of **1**, **2**, and **3** is likely a blue-green alga that grows on *G. coronopifolia*.



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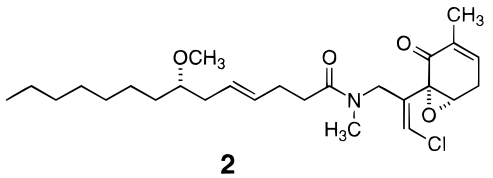

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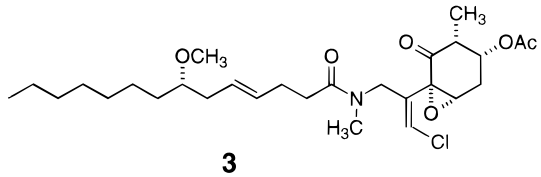

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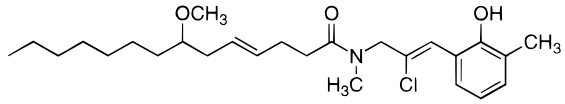

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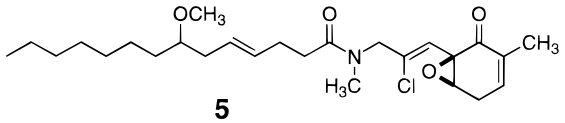

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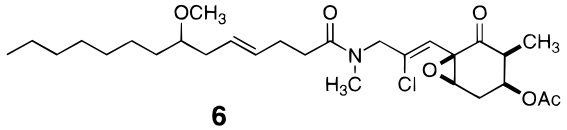

6

Experimental Section

Instruments. UV spectra were recorded on a Shimadzu UV-250 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker DMX-750 spectrometer. FABMS were obtained on a JEOL JMS-HX/HX110A spectrometer. Optical rotations were determined on a JASCO DIP-1000 instrument. CD spectra were recorded on a JASCO J-600 spectropolarimeter.

Algal Material. *G. coronopifolia* (4.8 kg, wet wt) was collected one week after the *G. coronopifolia* food poisonings [18] (September 1994) at the same site in Waiehu, Maui, where the toxic specimen was collected. The sample was transferred to the University of Hawaii, cooled with ice, and then kept at −15 °C until extraction could be carried out.

Extraction and Isolation. The sample (4.8 kg) was thawed and lightly washed in distilled H_2O. The algae were soaked in 6 L of Me_2CO overnight at room temperature, and the Me_2CO extract was filtered. The sample was further extracted twice with Me_2CO and twice with MeOH. The extracts were combined and evaporated. The dried residue was partitioned between H_2O (600 mL) and $CHCl_3$ (400 mL). The H_2O phase was evaporated to eliminate $CHCl_3$ and extracted with EtOAc (500 mL) three times. The EtOAc fraction was evaporated to dryness and dissolved in small amounts of MeOH. The solution was subjected to HPLC purification on a TSK-GEL ODS 120-T column (7.8 × 300 mm; TOSOH Inc., Japan) with 80 or 90% CH_3CN in H_2O. Malyngamide M (**1**, 0.6 mg), malyngamide N (**2**, 0.7 mg), and malyngamide I acetate (**3**, 2.7 mg) were isolated. Aplysiatoxin (6.7 mg), debromoaplysiatoxin (3.2 mg), and manauealide C (1.25 mg) were also isolated from the same sample.[2]

Malyngamide M (1): UV (MeOH) λ_{max} nm (ϵ) 279 (3940), 302 (3410), 317 (3050); FTIR (film) 3590, 1699, 1652, 1515, 1185, 1108, 1053, 971, 842 cm^{-1}; $[\alpha]_D$ −35.0° (*c* 0.06, MeOH); ^{1}H and ^{13}C NMR (Me_2CO-d_6), see Table 1; HRFABMS *m/z* 450.2792 (calcd for $C_{26}H_{41}{}^{35}ClNO_3$, 450.2775).

Malyngamide N (2): UV (MeOH) λ_{max} nm (ϵ) 237 (4600); FTIR (film) 2924, 2856, 1650, 1459, 1401, 1100, 1088, 969, 845 cm^{-1}; $[\alpha]_D$ −11.4° (*c* 0.07, MeOH); CD (MeOH) λ_{ext} 332 nm ($\Delta\epsilon$ − 1.76); ^{1}H and ^{13}C NMR (CD_3OD), see Table 2; FABMS (positive ion) $[M + Na]^+$ 488, $[M + H]^+$ 466; HRFABMS *m/z* 466.2722 (calcd for $C_{26}H_{41}{}^{35}ClNO_4$, 466.2724).

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