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MALYNGAMIDE H, AN ICHTHYOTOXIC AMIDE POSSESSING A NEW CARBON SKELETON FROM THE CARIBBEAN CYANOBACTERIUM *LYNGBYA MAJUSCULA*

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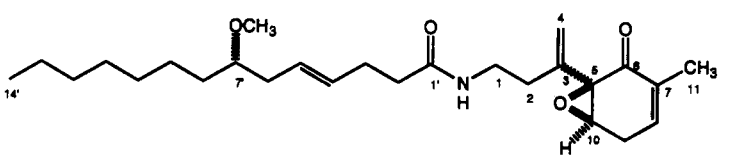
ABSTRACT.—Guided by ichthyotoxic activity against goldfish, a new lipopeptide, malyngamide H [**1**], and its corresponding free acid, 7-methoxytetradec-4(*E*)-enoic acid [**2**], have been isolated from the tropical marine cyanobacterium *Lyngbya majuscula*. The structure of the new carbon skeleton borne by malyngamide H was elucidated on the basis of spectroscopic analysis, mainly 2D nmr. The absolute stereochemistry of the cyclohexenone moiety of malyngamide H [**1**] was deduced by a combination of 2D NOESY and exciton chirality circular dichroism spectroscopy.

Prompted by our recent discovery of curacin A, a novel antimitotic metabolite from *Lyngbya majuscula* Gomont (Oscillatoriaceae) collected in Curaçao (1), we are currently undertaking a more in-depth investigation of Caribbean varieties of *L. majuscula*. Biological evaluation of a crude CH_2Cl_2/MeOH extract of a Curaçao collection of *L. majuscula* led to the detection of a potent ichthyotoxic effect to goldfish ($LC_{100}<25$ μg/ml). Investigation of the extract by 2D-tlc analysis disclosed the presence of curacin A (1). The ichthyotoxicity of the crude extract could not, however, be attributed to curacin A, since this metabolite showed no such effect at 50 μg/ml. Bioassay-guided fractionation on Si gel, Sephadex LH-20, and ODS Si gel led to the isolaton of a new metabolite, malyngamide H [**1**], as an active principle ($LC_{50}=5$ μg/ml, $EC_{50}=2$ μg/ml), as well as to the isolation of the previously described free 7-methoxytetradec-4(*E*)-enoic acid [**2**]. Following tradition with the previously reported amides of 7-methoxytetradec-4(*E*)-enoic acid from *L. majuscula*, we propose the trivial name malyngamide H (2–8).

Malyngamide H [**1**] was obtained as a pale yellow oil. The hreims of **1** gave a $[M]^+$ peak at *m/z* 431.3036, consistent with the molecular formula $C_{26}H_{41}NO_4$. Its ir spectrum contained absorptions due to amide proton (3310 cm^{-1}), conjugated carbonyl (1670 cm^{-1}), and amide carbonyl (1650 cm^{-1} and 1540 cm^{-1}) moieties. The uv spectrum exhibited a maximum at 243 nm, indicative of an α,β-unsaturated carbonyl moiety.

Of the seven degrees of unsaturation implied by the molecular formula, five could be accounted for by examination of the ^{13}C-nmr spectral data: an α,β-unsat-



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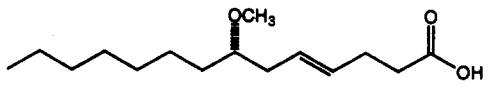

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urated carbonyl (δ 194.9), an amide carbonyl (δ 172.5), and six olefinic carbons forming three double bonds (Table 1); hence compound **1** was bicyclic.

In the ^{1}H- and ^{13}C-nmr spectra of **1**, signals characteristic of a 7-methoxytetradec-4(*E*)-enoic acid moiety, as seen for compound **2**, were observed (Table 1, fragment **A**, Figure 1)(3). From the DQF-COSY and ^{1}H-^{13}C-COSY spectra of malyngamide H, it was possible to establish two partial structures in the amide portion of the molecule. Fragment **B** (Figure 1), was delineated by the correlation cross-peaks between the exchangeable amide proton at δ 6.14 and H_2-1 (δ 3.43), which in turn showed cross-peaks to the methylene protons at H_a-2 (δ 2.47) and H_b-2 (δ 2.34). Additionally, these latter signals showed allylic coupling to one of the exomethylene protons (H_a-4, δ 5.18). Further, resonances characteristic of an allylic methyl group (H_3-11, δ 1.82) showed allylic coupling to H-8 (δ 6.34), confirming its location on the $\Delta^{7,8}$

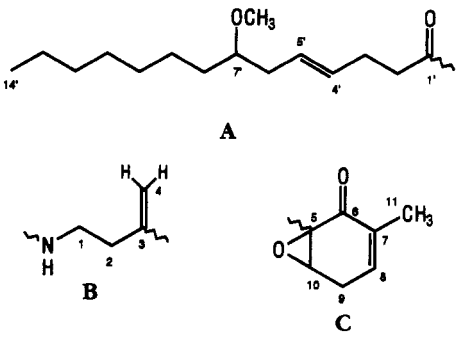

FIGURE 1. Substructures of malyngamide H [**1**].

TABLE 1. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-Nmr Data (100 MHz) for Malyngamide H [**1**].

Position	δ (int., mult., *J* in Hz)	δ (mult.)	HMBC
1a	3.43 (1H, m)	37.2 (t)	3, 1′, 2
1b	3.36 (1H, m)		
2a	2.47 (1H, dt, 14.5, 6.0)	33.0 (t)	5, 4, 3
2b	2.34 (m)		
3	—	140.5 (s)	
4a	5.27 (1H, s)	116.5 (t)	5, 2
4b	5.18 (1H, br s)		5, 2, 3
5	—	62.7 (s)	
6	—	194.9 (s)	
7	—	133.4 (s)	
8	6.34 (1H, br s)	138.0 (d)	
9a	2.95 (1H, dm, 21.0)	27.2 (t)	5
9b	2.82 (1H, dm, 21.0)		5
10	3.56 (1H, br s)	60.8 (d)	8, 9
11	1.82 (1H, br s)	16.4 (q)	6, 7, 8
1′	—	172.5 (s)	
2′	2.26 (m)	36.6 (t)	1′, 3′, 4′
3′	2.34 (m)	28.7 (t)	4′, 5′
4′	5.48 (m)	130.9 (d)	
5′	5.48 (m)	127.4 (d)	
6′	2.18 (1H, m)	36.4 (t)	7′, 5′, 4′, 8′
7′	3.15 (1H, quint., 6.0)	80.8 (d)	9′, 5′
8′	1.41 (2H, m)	33.4 (t)	9′, 10′
9′	1.27 (m)	25.3 (t)	
10	1.27 (m)	29.8[a] (t)	
11′	1.27 (m)	29.3[a] (t)	
12′	1.27 (m)	31.8 (t)	
13′	1.27 (m)	22.6 (t)	
14′	0.88 (3H, br t, 6.8)	14.1 (q)	12′, 13′
OCH_3	3.31 (3H, s)	56.5 (q)	7′
NH	6.14 (1H, m)	—	

[a]Interchangeable.

double bond. The proton at C-8, in turn, showed further couplings to H_a-9 (δ 2.94) and H_b-9 (δ 2.82), both of which were further coupled to H-10 (δ 3.56). These data, together with the previously delineated α,β-unsaturated carbonyl moiety (from uv) and the ^{13}C-nmr spectral data, accounted for all of the atoms in malyngamide H [**1**] except for one oxygen atom. Consideration of the ^{13}C-nmr chemical shifts of C-5 (δ 62.7) and C-10 (δ 60.8) indicated that this unassigned oxygen atom was part of an epoxide functionality, completing partial structure **C** (Figure 1). The chemical shift of the β proton (H-10, δ 3.56) on the resulting α,β-epoxycyclohexenone agreed well with the values reported in the literature for the α,β-epoxycyclohexanone in malyngamide C (5).

These partial structures were connected by the interpretation of the long-range correlations observed in the HMBC spectrum (J=7 Hz) of **1** (Table 1). The correlation cross-peak observed from H_2-1 to C-1′ confirmed the amide linkage between the 7-methoxytetradec-4(*E*)-enoate moiety and fragment **B**. The correlation seen between H_a-4, as well as H_b-4, and C-5 connected fragment **B** to the cyclohexenone fragment **C**, and led to the assignment of the structure of malyngamide H [**1**]. Further, the correlation between H_3-11 to C-6, C-7, and C-8 confirmed the position of the α,β-unsaturated carbonyl moiety adjacent to the C-11 allylic methyl group.

The absolute stereochemistry of the α,β-epoxycyclohexenone moiety in **1** was deduced from a combination of exciton chirality and observation of an nOe between H_a-4 and H-10 (10). The two possible conformations that could reasonably display this nOe are shown in Figure 2. The positive first Cotton effect in the cd spectrum ($\Delta\epsilon_{253}$ max +1.7, MeOH) can only be explained by the right-hand figure, thus establishing the absolute stereochemistry at C-5 as *R*, and consequently, C-10 as *R*.

The stereochemistry at C-7 within the molecule of methyl 7-methoxytetradec-4(*E*)-enoate [**2**] was assigned as *S* based on optical rotation ($[\alpha]^{26}D$ −12.1° (c=1.1, $CHCl_3$), lit. (3) $[\alpha]^{26}D$ −11.1°). Biosynthetic considerations suggest that this moiety in malyngamide H [**1**] has the same configuration at position C-7′.

Malyngamide H [**1**] possesses a novel carbon skeleton in its amide portion relative to other malyngamides, and is the first non-chlorine-containing amide of 7(*S*)-methoxytetradec-4(*E*)-enoate from *L. majuscula*. The α,β-epoxycyclohexenone moiety of malyngamide H is structurally related to deacetoxystylocheila-

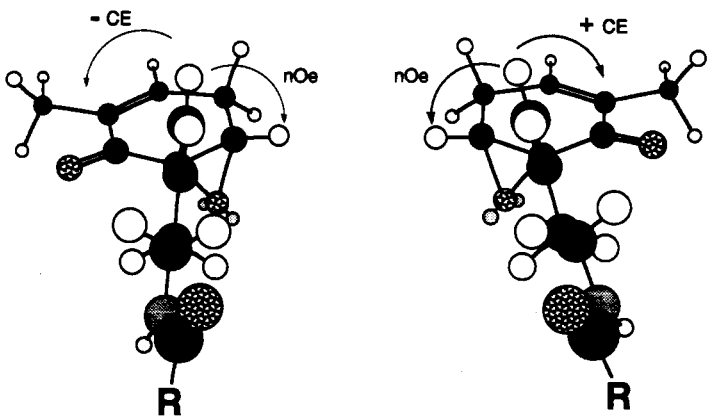

FIGURE 2. The two possible conformations for malyngamide H [**1**], displaying a nOe correlation between H_a-4 and H-10, and the predicted sign of the Cotton effect (CE).

mide, a chlorine-containing lipid from the sea hare *Stylocheilus longicauda* which is believed to have a dietary origin from *L. majuscula* (9). Malyngamide H was also found as a minor metabolite in a Caribbean collection of *L. majuscula* from St. Thomas (J. Todd, Oregon State University, personal communication).

The ichthyotoxic effect of malyngamide H [**1**] (LC_{50}=5 μg/ml, EC_{50}=2 μg/ml) and previous reports of the antifeedant effect of malyngamide A (11), a chlorine-containing amide from a Hawaiian *L. majuscula*, suggest these compounds may be part of the natural defense of this cyanobacterium. An interesting observation is the apparent selective activity of malyngamide H [**1**]; it displays a pronounced ichthyotoxic effect, but is not active in brine shrimp lethality or molluscicidal assays.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker AM-400 spectrometer operating at a basic frequency of 400 MHz. The solvent was used as an internal standard ($CDCl_3$ at δ 7.26 and δ 77.0). Mass spectra were recorded on a Varian-MAT 311 mass spectrometer. Uv and ir spectra were recorded on Hewlett-Packard 8452A uv-vis and Nicolet 510 spectrophotometers, respectively. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. Hplc separations were performed with a Waters M-6000A pump, a Rheodyne 7010 injector, and a R401 Waters differential refractometer. Merck aluminum-backed thin-layer chromatography sheets were used for tlc, and all solvents were distilled from glass prior to use.

PLANT MATERIAL.—*L. majuscula* Harvey (Oscillatoriaceae) was collected from shallow water (0.1–1.0 m) on December 15, 1991, at Santa Barbara Beach, Curaçao, Netherlands Antilles, and stored in *i*-PrOH at low temperature until work-up. A voucher specimen is available from W.H.G. (collection NSB-15 Dec 91-2).

EXTRACTION AND ISOLATION.—A total of 295 g (dry wt) of the alga was extracted with CH_2Cl_2-MeOH (2:1) two times to give the crude extract (3.3 g). The crude extract possessed ichthyotoxic activity at 25 μg/ml. A portion of the crude extract (3.0 g) was fractionated using vlc on Si gel with a stepwise gradient of hexane/EtOAc and EtOAc/MeOH. Eluted material was collected in fifteen 200-ml fractions and monitored by tlc. Similar fractions were combined to give 8 fractions. Fractions 6 (127 mg, eluted with 75% EtOAc/hexane) and 7 (100 mg, eluted with 100% EtOAc) both showed an ichthyotoxic effect at 10 μg/ml.

Fraction 7 was further fractionated by cc on Sephadex LH-20 using EtOAc/MeOH as eluent to give 18×10 ml fractions. Fractions similar by tlc were combined to yield five fractions. Fraction 3 (41 mg) was ichthyotoxic at 10 μg/ml. This fraction was further purified by hplc on ODS using MeOH-H_2O (4:1) as eluent to yield malyngamide H [**1**], 9.7 mg. Fraction 4 (35 mg) from the Sephadex column was also further purified by hplc on ODS using MeOH-H_2O (4:1) as eluent to yield trans-7-methoxytetradec-4-(E)-enoic acid [**2**], which showed no ichthyotoxic effect at 10 μg/ml.

Malyngamide H [**1**].—Isolated as a yellowish oil (9.7 mg, 0.30%); $[\alpha]^{26}D$ +26.1° (*c*=0.5, $CHCl_3$); uv λ max (MeOH) 243 nm (ε 6,460); ir ν max (film) 3310, 2930, 2860, 1670, 1650, 1540, 1190 cm^{-1}; eims *m/z* $[M]^+$ 431 (16), 289 (34), 235 (13), 176 (36), 143 (47), 113 (19), 111 (13), 98 (14), 69 (100); hreims measurement for $C_{26}H_{41}NO_4$: *m/z* 431.3036 (Δ=0.1 mmu); cd $\Delta\epsilon_{253}$ max +1.7, MeOH; ^1H- and ^{13}C-nmr data, see Table 1.

(−)-trans-7-Methoxytetradec-4-(E)-enoic acid [**2**].—Colorless oil (12.4 mg, 0.41%); $[\alpha]^{26}D$ −12.1° (*c*=1.1, $CHCl_3$); spectroscopic data (^1H-nmr, ^{13}C-nmr, eims) were identical to previously reported data (3).

BIOASSAY FOR ICHTHYOTOXICITY.—Ichthyotoxic potential of the crude extract, chromatography fractions, and pure compounds was determined as detailed in Ref. (12). The test organism was goldfish (*Carassius auratus*). The samples were dissolved in 20 μl of EtOH and then diluted to 20 ml with distilled H_2O. The assay was carried out for 1 h after which the fish were examined. End points in this assay were death (lack of breathing) and inability to swim against a manually induced current for LD_{50} and EC_{50} measurements, respectively.

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