Malyngamides O and P from the Sea Hare *Stylocheilus longicauda*

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Two new malyngamides, O (**1**) and P (**2**), were isolated from the sea hare *Stylocheilus longicauda*. The structures were elucidated by analysis of their 1D and 2D NMR spectra.

Fourteen malyngamides (A−N), constituents of blue-green and red algae cyanophytes, have been reported to date.[1−11] In 11 of the compounds, the acid moiety is 7-methoxytetradec-4-enoic acid. Two other acids are 7-methoxy-9-methylhexadec-4-enoic acid[2] and 7-methoxydodec-4-enoic acid.[7] The amine moiety is far more varied and interesting. Recurring features, which include rich oxygenation and chlorine substitution, lead one to surmise a biogenetic origin in some unconventional amino acids, generally a hallmark of blue-green algal constituents. Two acyclic serinol-derived compounds, found in an unidentified Australian blue-green alga, represent the most recent malyngamide-type isolates.[12] We now report the structures of two new malyngamides, O (**1**) and P (**2**), from the sea hare *Stylocheilus longicauda*, which is known to feed on *Lyngbya majuscula*. In these two compounds the standard C_{14} acid is linked to acyclic amines; one of these is the enol methyl ether (**1**) of the oxo function in the other (**2**).

Extraction of the freeze-dried mollusk with MeOH afforded a green gum, which was solvent-partitioned to yield hexane, CH_2Cl_2 and MeOH−H_2O fractions. Repeated Si gel chromatography and reversed-phase HPLC separation of the hexane fraction afforded malyngamides O (**1**) and P (**2**).

Malyngamide O (**1**) was obtained as a pale yellow oil. The molecular formula of $C_{25}H_{42}ClNO_5$ was deduced from the HRFABMS data ($[M + H]^+$, 472.2830 Da), implying five degrees of unsaturation. Analysis of the IR and ^1H and ^{13}C NMR spectral data provided evidence for two carbonyl groups, one attributable to an amide functionality (1654 cm^{-1}), and three double bonds. Complete 2D NMR analysis with HMBC, $^1H-^1H$ COSY, and NOESY data established the spin systems within the molecule. The ^1H NMR data also provided evidence for interconverting *tert*-amide conformers (see Table 1), as many of the resonances appeared as pairs with peaks in an approximate 2:1 ratio, a fairly common phenomenon among the malyngamides.[1,9,11] Diagnostic resonances for the 7-methoxytetradec-4(*E*)-enoyl moiety served to establish the acid portion of the compound.[4] The *N*-methyl residue (2.84/2.89, 3 H, s) was confirmed by HMBC correlations to the C-1′ amide carbonyl. HMBC couplings were observed between H-1 (4.17, 4.32 ppm) and the quaternary C-2 olefin at 133.62/134.55 ppm. HMBC cross-peaks of H-1 to the amide carbonyl (C-1′), the C-2/C-7 olefin and the two-proton methylene at 3.50/3.53 ppm (H-3) served to link three of the spin systems within the molecule. Further, cross-peaks from H-3 to C-4 and C-5 delineated the position of the third olefinic group within the molecule (Figure 1). The methoxy substituent at 3.64 ppm (4-OMe) exhibited a lone correlation to the downfield quaternary resonance at 171.45/171.64 ppm (C-4), while the methoxy substituent at 3.66/3.68 ppm was correlated to the ester carbonyl located at C-6.

Table 1. ^1H NMR, ^{13}C NMR, and HMBC Correlation Data for Malyngamide O (**1**)[a]

position	^{13}C	^1H	HMBC
1	45.24/48.87	4.17/4.32, 2H, s	C-1′, -2, -3, -7
2	133.62/134.55		
3	33.95[b]/34.36	3.53, 1H, d, J = 1.07 Hz 3.50, 1H, d, J = 1.29 Hz	C-1, -2, -4, -5, -7
4	171.45/171.64		
5	92.02/92.13	5.085/5.093, 1H, s	C-3, -4
6	167.52/167.56		
7	117.69/118.77	6.06/6.18, 1H, s	C-1, -2
1′	172.83/172.94		
2′	33.30/33.36	2.40, 2H, m	C-1′, -3′
3′	28.12/28.33	2.35, 2H, m	C-2′, -4′, -5′
4′	127.03/127.10	5.48, 1H, m	C-3′
5′	131.31/131.38	5.50, 1H, m	C-6′
6′	36.4	2.19, 2H, t, J = 5.64 Hz	C-4′, -5′, -7′, -8′
7′	80.8	3.14, 1H, t, J = 5.80 Hz	7′-OCH_3
8′	33.3	1.43, 2H, m	C-6′, -7′, -9′, -10′
9′	25.3	1.28, 2H, br s	
10′	29.7	1.28, 2H, br s	
11′	29.3	1.28, 2H, br s	
12′	31.8	1.28, 2H, br s	
13′	22.6	1.28, 2H, br s	
14′	14.1	0.87, 3H, t, J = 7.15 Hz	C-12′, -13′
N-CH_3	32.90/34.02[b]	2.84/2.89, 3H, s	C-1
7′-OCH_3	56.5	3.32, 3H, s	C-7′
4-OCH_3	55.71/55.75	3.64, 3H, s	C-4
6-OCH_3	50.86/51.04	3.66/3.68, 3H, s	C-6

[a] Spectra determined in $CDCl_3$; data reported in ppm. [b] Signals may be interchanged.

Orientation of the C-4/C-5 olefin was determined to be *E* due to observed NOESY correlations between the 4-OMe substituent and the adjacent H-5 methine. The H-7 vinylic proton exhibited NOE interactions with the methylene protons on C-3, suggesting a *Z* orientation of the C-2/ C-7 olefin.

Malyngamide P (**2**) was isolated as a colorless oil. The molecular formula of $C_{24}H_{40}ClNO_5$ was identified by HREIMS analysis, which also determined the parent ion at *m*/*z* 457.2644 whose mass differed from **1** by 14 daltons ($-CH_2$). Similar ^1H and ^{13}C NMR resonances and HMBC cross-peaks were observed in **2** (Table 2) as had been identified in **1** (Figure 1), indicating a similarity in their gross structures. Fragmentation at *m*/*z* 143 ($C_9H_{19}O$), corresponding to the allylic cleavage at C-6′, is a typical pattern in the malyngamides.[4] Isomeric pairing of the signals due to interconversion of the amide bond was not observed in **2**. The presence of the 7-methoxytetradec-4(*E*)-enoyl group was demonstrated by characteristic resonances in the ^1H and ^{13}C NMR spectra. Downfield ^{13}C NMR shifts attributable to amide (173.4 ppm) and ester (167.6 ppm) carbonyl functionalities were evident in the spectra. Another car-

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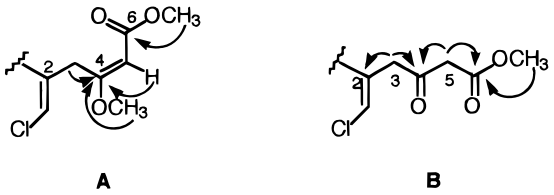


Figure 1. Partial structures A and B from key HMBC correlations in malyngamides O (**1**) and P (**2**), respectively.

Table 2. ^{1}H NMR, ^{13}C NMR, and HMBC Correlation Data for Malyngamide P (**2**)[a]

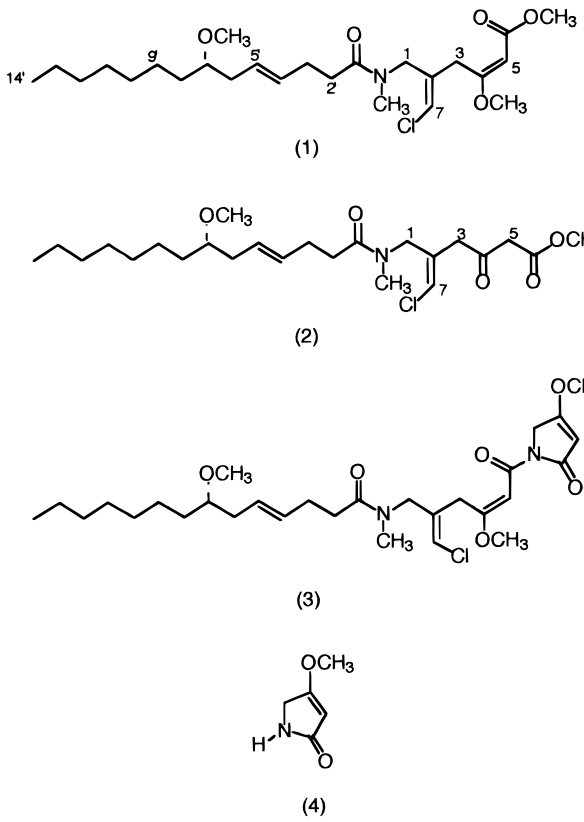
position	^{13}C	^{1}H	HMBC
1	46.38	4.21, 2H, s	C-1′, -2, -3, -7, *N*-CH_3
2	131.5		
3	46.29	3.28, 2H, s	C-1, -2, -4, -7
4	199.3		
5	48.9	3.50, 2H, s	C-4, -6
6	167.6		
7	120.4	6.10, 1H, s	C-1, -2, -3
1′	173.4		
2′	33.2	2.33, 2H, br s	C-1′, -3′
3′	28.0	2.33, 2H, br s	
4′	127.2	5.50, 1H, m	
5′	131.2	5.50, 1H, m	
6′	36.4	2.19, 3H, t, J = 5.59 Hz	C-4′, -5′, -7′, -8′
7′	80.8	3.15, 1H, t, J = 5.80 Hz	7′-OCH_3, C-9′
8′	33.3	1.43, 2H, br t, J = 5.37 Hz	
9′	25.3	1.26, 2H, br s	
10′	29.8[b]	1.26, 2H, br s	
11′	29.3[b]	1.26, 2H, br s	
12′	31.8	1.26, 2H, br s	
13′	22.7	1.26, 2H, br s	
14′	14.1	0.88, 3H, t, J = 7.1 Hz	C-12′, -13′
N-CH_3	35.2	2.91, 3H, s	C-1, -1′
7′-OCH_3	56.5	3.32, 3H, s	C-7′
6-OCH_3	52.3	3.73, 3H, s	C-6

[a] Spectra determined in $CDCl_3$; data reported in ppm. [b] Signals may be interchanged.

bonyl group resonated at 199.3 ppm, indicative of a ketone. Evidence for the presence of two double bonds was provided by the identification of four olefinic signals in the carbon spectrum between 120.4 and 131.5 ppm.

Methylene singlet protons at 3.28 ppm (H-3) exhibited HMBC correlations to the olefinic carbons, C-2 and C-7, and the carbonyl at 199.3 ppm (C-4). Based on the correlation of a second methylene at 3.50 ppm (H-5) to this C-4 carbonyl, it was deduced that this ketone was not α,β-unsaturated, but β,γ-substituted by the C-2/C-7 olefin. Irradiation of the vinylic proton of H-7 resulted in enhancement of the H-3 protons, thus establishing the *Z* orientation of the olefin. Further, the H-5 two-proton singlet exhibited cross-peak connectivity to C-6 (167.6 ppm), thus establishing the location of the ester carbonyl with respect to this methylene signal. An HMBC correlation of the methoxy signal at 3.73 ppm to the ester carbonyl confirmed the methyl ester and hence the structure of malyngamide P (**2**). The stereochemistry at C-7′ for **1** and **2** is presumed to be *S* and the orientation of the C-4′−C-5′ double bond to be *E* based on literature precedence[9] with the malyngamides.

Malyngamides O and P represent rare examples of malyngamides without alicyclic or aromatic rings. The orientation of the C-2/C-7 olefin precludes the hypothesis that malyngamide O could be a product of amide hydrolysis of malyngamide A (**3**) in which the 4-methoxy-Δ^3-pyrrolin-2-one portion is being replaced by a methoxyl group. The free 4-methoxy-Δ^3-pyrrolin-2-one (**4**) has, however, been isolated as a natural product from *L. majuscula*.[1] The discovery of these compounds could serve to identify another natural synthon for the construction of this interesting class of compounds.



The bioassay assessment with mouse lymphoma (P-388), human lung carcinoma (A-549), and human colon carcinoma (HT-29) indicated that there was moderate activity, IC_{50} 2 μg/mL with malyngamide O (**1**). Malyngamide P (**2**) was not tested due to the inadequate quantity of the sample. Information on biological activity of malyngamides A−N is spotty.[1−11]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C spectra were recorded on either a General Electric GN Omega 500 spectrometer or a Varian Unity INOVA 400 MHz instrument. Ultraviolet spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer. Mass spectral data were measured on a VG 70ZAB2SE mass spectrometer. Optical rotations were determined on a JASCO DIP-370 polarimeter. Infrared spectra were obtained on a Perkin-Elmer 1600 FTIR instrument.

Animal Material. The sea hare *S. longicauda* was collected by snorkeling at Black Point, Oahu, in February 1999, where the animals were seen mating in huge numbers. The animals were freeze-dried prior to extraction. The sea hare was identified by Professor Alison E. Kay, Department of Zoology, University of Hawaii.

Biological Assays. Assays for IC_{50} values (recorded in μg/mL) of selected cancer cell lines were determined against mouse lymphoma (P-388, ATCC: CCL 46), human lung carcinoma (A-549, ATCC: CCL 8), and human colon carcinoma (HT-29, ATCC: HTB 38).

Extraction and Isolation. The freeze-dried animals (327.5 g lyophilized wt) were exhaustively extracted with MeOH to yield a green gum (127.4 g) on evaporation of the solvent in vacuo. A portion (30.8 g) of the dried residue was dissolved in 90% MeOH−H_2O followed by partitioning with hexane (150 mL × 4). The solution was then diluted to 60% MeOH−H_2O and further partitioned with CH_2Cl_2 (150 mL × 3). The hexane extract was fractionated using vacuum liquid chromatography on Si gel employing a stepwise gradient of hexane−EtOAc with

final elution with acetone and MeOH. Eluted material was monitored by TLC, and similar fractions were combined to yield 10 major fractions.

Following gravity column chromatography of the sixth fraction in 60% $CHCl_3$−hexane, the main portion, fraction 3 (20.3 mg), was subjected to HPLC analysis (Ultracarb, MeCN−H_2O, 8:2). Three main peaks were obtained. Makalikone ester and makalika ester[13] eluted after 20 and 55 min, respectively (flow rate: 4 mL min^{-1}) while a mixture of two compounds eluted after 75 min. Preparative layer TLC of this peak (10% acetone−CH_2Cl_2) yielded four main bands, the first of which was further purified on reversed phase HPLC (Luna, acetonitrile−water, 8:2) to yield malyngamide O (**1**) (0.8 mg). The second band was further purified by reversed-phase HPLC (Luna, MeCN−H_2O−MeOH, 8:1:1) from which malyngamide P (**2**) (1.0 mg) was obtained. Malyngamide A (**3**) was also isolated along with new compounds makalika ester, makalikone ester, and lyngbyatoxin A acetate.[13]

Malyngamide O (1): pale yellow oil (0.8 mg), $[\alpha]_D$ −55.6° (*c* 0.018, MeOH); UV (MeOH) λ_{max} (ϵ) 212 (13 082), 240 (9681); IR (film) ν_{max} 2925, 1708, 1654, 1140 cm^{-1}; LREIMS *m*/*z* (rel int) 471 (3) $[M]^+$, 456 (7), 436 (65), 329 (46), 297 (10), 275 (11), 262 (22), 243 (84), 232 (41), 228 (18), 202 (86), 166 (56), 146 (100), 130 (59), 143 (90); HRFABMS *m*/*z* 472.2830 $[M + H]^+$ (calcd for $C_{25}H_{42}ClNO_5$, 471.2752).

Malyngamide P (2): colorless oil (1.0 mg), $[\alpha]_D$ −75° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (ϵ) 208 (9031), 275 (3086); LREIMS *m*/*z* (rel int) 457 (0.4) $[M]^+$, 442 (1), 422 (2), 390 (3), 315 (9), 279 (13), 225 (13), 167 (13), 143 (44), 127 (13), 111 (48), 97 (14), 69 (100); HREIMS *m*/*z* 457.2644 $[M]^+$, (calcd for $C_{24}H_{40}ClNO_5$, 457.2595).

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