A New Biologically Active Malyngamide from a New Zealand Collection of the Sea Hare *Bursatella leachii*

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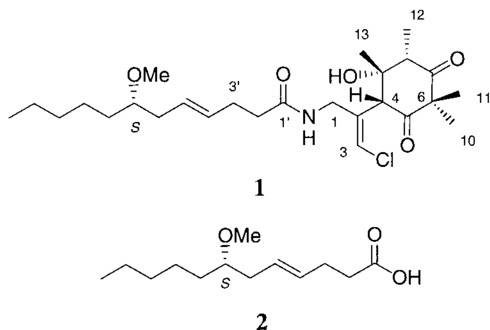
A new malyngamide, S (**1**), was isolated from a New Zealand collection of the sea hare *Bursatella leachii* and structurally characterized by spectroscopic methods and chemical degradation. Malyngamide S exhibited cytotoxicity and antiinflammatory properties.

Opisthobranch molluscs are known to be a rich source of bioactive secondary metabolites,[1−3] the majority of which are sequestered from the algae that make up their diet.[4] We have investigated specimens of *Bursatella leachii* de Blainville, 1817 (order Anaspidea, family Aplysiidae) collected at Eastern Beach, Auckland, New Zealand, and isolated a new malyngamide, S (**1**), which was responsible for the bioactivity observed for the crude extract. Malyngamides A−R have been previously reported,[5] primarily from the marine cyanobacterium *Lyngbya majuscula*. A study by Willan in 1979 has shown that the *B. leachii* in the Auckland, New Zealand region feed on *L. majuscula*.[6] Herein we report full spectroscopic characterization of malyngamide S (**1**) and absolute configuration of the fatty acid portion by hydrolysis to afford the known (−)-7*S*-methoxydodec-4*E*-enoic acid (**2**).[7]



Malyngamide S (**1**) was isolated as a colorless oil from the bioactive organic extract of *B. leachii*. The isotope pattern observed for the molecular ion (FAB) indicated the presence of chlorine, while HRFABMS established a molecular formula of $C_{26}H_{43}ClNO_5$. Inspection of the ^{1}H NMR spectrum indicated doubling, in a 2:1 ratio, of most signals, suggestive of the presence of rotamers in solution. Elevating the temperature to 50 °C resulted in the observation of a single set of signals in the NMR spectra. The ^{13}C NMR spectrum revealed the presence of two ketone resonances at δ 208.3 (C-5) and 209.6 (C-7), while two methyl singlets at ^{1}H δ 1.12 (H-10) and 1.33 (H-11) exhibited HMBC correlations to each other and to both of C-5 and C-7, placing a *gem*-dimethyl group at C-6 (δ 58.5). The proton signal at δ 3.38 (1H, q, J = 6.6 Hz, H-8) and COSY-associated methyl doublet at δ 1.10 (3H, d, J = 6.6 Hz, H-12) were placed at C-8 (δ 50.9) by HMBC correlations from H-8 to C-7, C-9 (δ 73.6), and C-4 (δ 58.2) and from H-12 to C-7, C-8, and C-9. HMBC correlations from the 1H singlet at δ 4.50 (H-4) to C-5, C-8, and C-9 combined with HMBC correlations from the methyl singlet at δ 1.26 (H-13) to C-8, C-9, and C-4 established the C-4/C-9/C-8 fragment of **1**. Final securing of the cyclohexadione ring structure was made by placement of a tertiary hydroxyl functional group at C-9 due to the observation of HMBC correlations from an obscured OH resonance at δ 5.43 to C-8, C-9, and C-13 (δ 22.7). The C-2 (δ 137.2)/C-3 (δ 117.5) double bond was determined as being terminal with the chlorine at C-3 by HMBC correlations from H-4 to C-1 (δ 39.7), C-2, and C-3. An exchangeable proton resonance at δ 8.00 (1H, t) and corresponding amide carbonyl in the ^{13}C NMR spectrum (δ 171.6) linked the ring portion to the fatty acid via an amide group. ^{13}C NMR and 2D-NMR established the fatty acid as a 12-carbon chain with unsaturation at the 4′-position. Placement of the methoxy group (δ_H 3.22 and δ_C 55.4) was made by virtue of an HMBC correlation from the methoxy proton singlet to C-7′ (δ 79.6). Base hydrolysis[8] of malyngamide S yielded the acid portion **2**, which was identical in all respects to that previously reported for (−)-7*S*-methoxydodec-4*E*-enoic acid,[7] thus establishing the planar structure of **1**.

Relative configuration around the cyclohexadione ring was established by the observation of strong ROE correlations from H-4 to H-13 (δ 1.26) and H-11, from H-13 to H-8 (δ 3.38), and from the hydroxyl proton at C-9 to H-12 (δ 1.10). ROE correlations observed between the amide NH (δ 8.00) and methylene protons H-1 (δ 4.17, 3.68) to the olefin proton H-3 (δ 6.28) confirmed the Δ^2 bond geometry as *E*.

Malyngamide S is a combination of the shorter 12-carbon fatty acid side chain, common only to malyngamide G,[5] and a unique highly substituted cyclohexane ring with diketo functionality, which is similar to an oxidized ring system of malyngamide D.

The observation of rotamers in solution is regularly reported for structures of the malyngamide series primarily due to the *N*-methyl amide moiety.[5] For **1**, it is most likely caused by restricted rotation about the C2−C4 bond due to the highly substituted cyclohexane ring.

Malyngamide S (**1**) and (−)-7*S*-methoxydodec-4*E*-enoic acid (**2**) were assayed for a range of antiinflammatory, cytotoxicity, and antimicrobial properties. In an antiin-

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flammatory assay, **1** inhibited superoxide production generated in response to the inflammation-promoting agents *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol myristate acetate (PMA) (73% and 83%, respectively, 100 mg/mL) in an activated human peripheral blood neutrophil assay.[9,10] Malyngamide S also inhibited proliferation of human leukemic HL60 cells in a 2-day growth assay (IC_{50} ~6−8 μM) and exhibited P388 murine leukemia (IC_{50} 29 μM) and NCI human tumor activity (panel average values: GI_{50} 16.6 μM, TGI 35.5 μM, and LC_{50} 69.2 μM) and cytotoxicity toward the BSC-1 cell line (excess radius zone size >4.5 mm, 120 μg/disk loading). No antimicrobial activity (*E. coli*, *B. subtilis*, *T. mentagrophytes*, and *C. albicans*, 120 μg/disk loading) or antitubercular activity (*M. tuberculosis*, 0% inhibition at 6.25 μg/mL) was detected. The cleaved acid **2** exhibited no P388 activity (IC_{50} >100 μM) but did show cytotoxicity toward the BSC-1 cell line (excess radius zone size >4.5 mm, 120 μg/disk loading) and antimicrobial activity against *B. subtilis* and *T. mentagrophytes* (excess radius zone sizes 2 and 3 mm, respectively, 120 μg/disk).

Experimental Section

General Experimental Procedures. Analytical reversed-phase HPLC was run on a Waters 600 HPLC photodiode array system using an Alltech C_{18} column (3μ Econosphere Rocket, 7 × 33 mm) and eluting with a linear gradient of H_2O (0.05% trifluoroacetic acid) to MeCN. Semipreparative C_{18} reversed-phase HPLC was performed with H_2O−MeOH solvent mixtures on an Alltech 10μ Econosil column (10 × 250 mm). Details of general procedures[11] and biological assays[9,10,12] have been reported previously.

Collection, Extraction, and Isolation Procedures. Ten specimens (collection number 2001MS2-1) of *B. leachii* were collected in April 2001 at Eastern Beach, Auckland, New Zealand, and identified by David Todd, School of Biological Sciences, The University of Auckland. A voucher specimen (2001MS2-1) is held in the Department of Chemistry, The University of Auckland. Animal tissue was freeze-dried (dry weight 9.81 g), combined, and extracted with MeOH (3 × 100 mL) and then CH_2Cl_2 (2 × 100 mL). Solvents were removed in vacuo to give a green extract (4.43 g). A portion of extract (2.11 g) was fractionated on a C_{18} reversed-phase column using a steep gradient from MeOH−H_2O (50:50) to MeOH. The compound of interest eluted at 75% MeOH. Purification was achieved using semipreparative HPLC (C_{18}, MeOH−H_2O, 85:15, 5 mL/min) followed by SiO_2 flash column chromatography eluting with CH_2Cl_2, yielding **1** (14.9 mg, 0.32% dry weight).

Malyngamide S (1): colorless oil: $[\alpha]^{20}_D$ −8° (*c* 1.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.25), 275 (2.30); IR (smear) ν_{max} 3309, 2931, 1729, 1697, 1642, 1630, 1544, 1462, 1380, 1096 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz, 323 K) δ 8.00 (1H, bt, *J* = 6.0 Hz, N*H*), 6.28 (1H, t, *J* = 1.5 Hz, H-3), 5.43 (1H, obsc, O*H*), 5.43 (2H, m, H-4′, H-5′), 4.50 (1H, s, H-4), 4.17 (1H, ddd, *J* = 16.8, 6.6, 1.3 Hz, H-1), 3.68 (1H, ddd, *J* = 17.0, 5.4, 1.8 Hz, H-1), 3.38 (1H, q, *J* = 6.6 Hz, H-8), 3.22 (3H, s, OC*H*3), 3.12 (1H, p, *J* = 5.7 Hz, H-7′), 2.22 (4H, m, H-2′, H-3′), 2.14 (2H, m, H-6′), 1.37 (2H, m, H-8′), 1.33 (3H, s, H-11), 1.30 (4H, m, H-9′, H-11′), 1.28 (2H, m, H-10′), 1.26 (3H, s, H-13), 1.12 (3H, s, H-10), 1.10 (3H, d, *J* = 6.6 Hz, H-12), 0.86 (3H, t, *J* = 6.8 Hz, H-12′); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz, 323 K) δ 209.6 (C-7), 208.3 (C-5), 171.6 (C-1′), 137.2 (C-2), 130.7 (C-4′), 126.5 (C-5′), 117.5 (C-3), 79.6 (C-7′), 73.6 (C-9), 58.5 (C-6), 58.2 (C-4), 55.4 (O*C*H3), 50.9 (C-8), 39.7 (C-1), 35.7 (C-6′), 34.9 (C-2′), 32.4 (C-8′), 31.1 (C-10′), 27.8 (C-3′), 26.5 (C-11), 24.0 (C-9′), 22.7 (C-13), 21.7 (C-11′), 18.5 (C-10), 13.5 (C-12′), 7.9 (C-12); FABMS *m*/*z* 486/484 $[M + H]^+$; HRFABMS *m*/*z* 486.2814 (calcd for $C_{26}H_{43}{}^{37}ClNO_5$ 486.2800), 484.2821 (calcd for $C_{26}H_{43}{}^{35}ClNO_5$ 484.2830).

(−)-7*S*-Methoxydodec-4*E*-enoic acid (2). **1** (10 mg) was refluxed in 10% KOH−ethanediol (2 mL) for 4.5 h. After cooling, the solution was acidified with 3 N HCl. The solution was extracted with ether (3 × 5 mL), and then the organic fraction was dried with anhydrous $MgSO_4$. SiO_2 flash column chromatography eluting with CH_2Cl_2−MeOH (4:1) yielded **2** (3.4 mg, 72%) as a colorless oil: $[\alpha]^{20}_D$ −9° (*c* 0.43, MeOH) (lit.[7] $[\alpha]^{20}_D$ −8° (*c* 1.8, $CHCl_3$). All other spectroscopic data were consistent with those previously reported;[7] CIMS *m*/*z* 229 $[M + H]^+$; HRCIMS *m*/*z* 229.1801 (calcd for $C_{13}H_{25}O_3$ 229.1804).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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