Lorneamides A and B: Two New Aromatic Amides from a Southern Australian Marine Actinomycete

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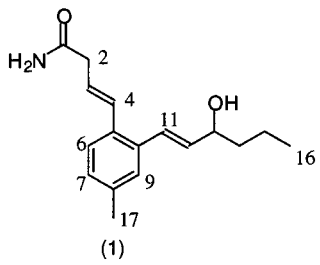
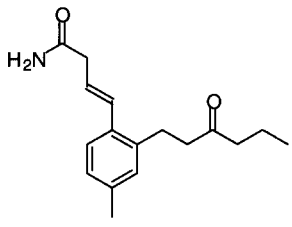
A marine actinomycete (MST-MA190) isolated from a sample of beach sand collected near Lorne on the southwest coast of Victoria, Australia, has yielded two new aromatic amides, lorneamide A (**1**) and lorneamide B (**2**). The lorneamides belong to a novel class of tri-alkyl-substituted benzenes, and their structures were determined by spectroscopic methods.

Marine organisms are a recognized source of novel metabolites across a wide range of structure classes, with some possessing valuable biological properties. Although the marine natural product literature is dominated by the chemistry of macroscopic marine organisms, such as algae and invertebrates, in recent times marine microbes have emerged as a valuable source of molecular diversity.[1,2] Although challenging, the quest for novel bioactive metabolites from marine bacteria, actinomycetes, and fungi offers great potential. In addition to molecular novelty and bioactivity, marine microbes present a secure and renewable supply of targeted metabolites for scientific enquiry and possible commercial exploitation. Thus, we have extended our exploration into the chemistry of southern Australian algae and invertebrates to include marine microbes. In this report we describe the isolation and structure elucidation of two new aromatic amides, lorneamides A (**1**) and B (**2**), from a marine actinomycete (MST-MA190) cultured from beach sand collected near Lorne on the south west coast of Victoria, Australia.



(1)



(2)

The marine actinomycete (MST-MA190) was isolated from a sample of beach sand collected at Lorne, Victoria, using ISP2 (yeast, malt, and glucose) agar. Initial bioassay of a MeOH extract identified antifungal (*Saccharomyces cerevisiae*) and nematocidal (*Haemonchus contortus*) activities. On scaling up production of the organism, additional insecticidal and antibacterial activities were detected. Analytical HPLC/PDA/MS analysis under three sets of chromatographic conditions identified a complex suite of metabolites, none of which correlated with known actinomycetes metabolites (based on reference to inhouse databases). The metabolic diversity of MST-MA190, together with its intriguing bioassay profile, prompted a more detailed investigation. The biomass for chemical analysis was obtained by liquid fermentation at 1-L scale. The freeze-dried liquid culture was extracted with MeOH, after which the MeOH solubles were concentrated in vacuo, triturated with CH_2Cl_2, and the insoluble fraction partitioned between *n*-BuOH and water. The *n*-BuOH-soluble material was then eluted through C_{18} SPE and HPLC to return pure lorneamide A (**1**) (2.9 mg, 0.15%) and lorneamide B (**2**) (2.1 mg, 0.11%). The lorneamides are structurally novel, and lorneamide A (**1**) is responsible for the antibacterial activity exhibited by extracts of MST-MA190.

ESIMS (positive) analysis of lorneamide A (**1**) revealed a pseudo-molecular ion [$(M + Na)^+$, Δmmu −0.7] consistent with a molecular formula ($C_{17}H_{23}NO_2$) requiring seven degrees of unsaturation. The NMR data for **1** (Table 1) revealed a 1,2,4-trisubstituted benzene ring as well as two (*E*)-1,2-disubstituted double bonds, accounting for six degrees of unsaturation. The NMR data also displayed resonances attributed to a 2°−OH (^{1}H: δ 4.22 br dt; ^{13}C: 73.5 d ppm). These observations together with characteristic IR absorptions (3345, 1664 cm^{-1}) required that the remaining heteroatoms (O + N) and degree of unsaturation be accommodated in an amide functionality.

Analysis of the 2D NMR COSY and TOCSY data for **1** (Table 1) identified three isolated spin systems. These included; (a) a 1-substituted (*E*)-3-hydroxy-1-hexene (C-11 to C-16); (b) a 1,3-disubstituted *E*-propene (C-2 to C-4); and (c) a 3,4-disubstituted toluene (C-5 to C-10, plus C-17). This last subunit was confirmed by NOE difference analysis, where irradiation of H_3-17 (δ 2.30) returned enhancements to H-7 (3.5%) and H-9 (2.9%). Complete regiochemistry about the aromatic ring was established by a 2D NMR NOESY experiment, which revealed correlations between H-12 and H-9 and between H-3 and H-6. This required positioning of the amide functionality on the allylic meth-

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Table 1. NMR (MeOH-d_4, 400 MHz) Data for Lorneamide A (**1**)

	^{1}H δ (m, J Hz)	COSY	NOESY	^{13}C (gHMQC)[b]
2	3.14 (dd, 1.5, 7.1)	H-3	H-3, H-4	40.5
3	6.13 (dt, 7.1, 15.6)	H-2, H-4	H-2, H-4, H-6	125.4
4	6.79 (br d, 15.6)[a]	H-3	H-2, H-3	132.1
6	7.32 (d, 7.9)	H-7	H-3, H-7	127.3
7	7.02 (dd, 2.0, 7.9)	H-6, H-9	H-6, H_3−17	129.2
9	7.22 (d, 2.0)	H-7	H-12, H_3−17	127.9
11	6.83 (br d, 15.6)[a]	H-12	H-12, H-13	128.6
12	6.03 (dd, 6.7, 15.6)	H-11, H-13	H-9, H-11, H-13	136.0
13	4.22 (br dt, 6.7, 6.7)[a]	H-12, H-14	H-11, H-12, H-14	73.5
14	1.50−1.62 (m)	H-13, H-15	H-13	40.5
15	1.40−1.50 (m)	H-14, H_3−16		19.5
16	0.97 (t, 7.4)	H-15		14.0
17	2.30 (s)	H-7, H-9	H-7, H-9	21.0

[a] On reprocessing to enhance resolution a $J_{11,13}$ of 1.3 Hz was observed, as well as H-4 resolving to a dt (J = 1.5, 15.6 Hz). [b] ^{13}C NMR shifts were determined and assigned from the gHMQC data.

ylene C-2, consistent with its observed downfield chemical shift of δ 3.14. Lack of material prevented the direct acquisition of a ^{13}C NMR spectrum; however, ^{13}C chemical shift data for proton-bearing carbons was obtained by analysis of the gHMQC data (see Table 1). Lack of material also precluded a determination of absolute stereochemistry about C-13. The structure assigned to lorneamide A (**1**) is as shown.

ESIMS (positive) analysis [$(M + Na)^+$, Δmmu −0.5] established lorneamide B (**2**) as being isomeric with **1**. The ^{1}H NMR spectrum of **2** differed from that of **1** in that the resonances for the $\Delta^{11,12}$ double-bond olefinic methines were absent, as was the H-13 hydroxymethine resonance. In place of these, the ^{1}H NMR spectrum of **2** revealed two new methylene resonances (δ 2.67 and 2.87). The IR data for **2** exhibited no hydroxyl absorbance but did display an additional carbonyl absorbance (1700 cm^{-1}). These observations suggested that **2** was structurally related to the co-metabolite **1** via reduction of the $\Delta^{11,12}$ double bond and oxidation of the adjacent 2-OH. Thus, the structure assigned to lorneamide B (**2**) is as shown.

Lorneamide A (**1**) was active against *Bacillus subtilitis*, exhibiting a LD_{99} of 50 μg/mL. Although only modest antibacterial agents, the lorneamides are novel natural products with carbon skeletons not previously reported in the chemical literature. The closest known structure is a degradation product of the everninomicin antibiotic, SCH 49088, isolated from a *Micromonospora* fermentation.[3] Bioassay-directed isolation of the antifungal and anthelmintic principals in MST-MA190 is ongoing, and will be reported in due course.

Experimental Section

General Experimental Procedures. For procedures see Ovenden and Capon.[4]

Collection and Fermentation. The actinomycete species (Microbial Screening Technologies Registry Number MST-MA190) was isolated using ISP2 agar (International Streptomyces Project, media 2) from a mixed microbial community present in a sample of beach sand collected near Lorne on the southwest coast of Victoria, Australia. The culture demonstrated aerial mycelia and growth characteristics typical of actinomycetiales belonging to the *Streptomyces* and related genera. Further characterization of the culture is in progress and will be reported elsewhere. Large-scale production of biomass was achieved by 1-L fermentation with MST Soytone media at 28 °C for 5 days.

Extraction and Isolation. The concentrated MeOH extract (1.99 g) of the freeze-dried liquid fermentation culture was subjected to bioassay and found to exhibit antibacterial, antifungal, and anthelmintic properties. This material was triturated with CH_2Cl_2, and the insoluble material was partitioned between *n*-BuOH and water. The *n*-BuOH-soluble portion (398 mg, 20.0%) was fractionated by C_{18} SPE (20% stepwise gradient from 80% H_2O in MeOH to 100% MeOH), and the fraction eluting with 40% H_2O in MeOH (38 mg) was subjected to C_{18} HPLC (2.0 mL/min 30% H_2O in MeOH, Eclipse XDB−C_{18} 9.4 × 250 mm column) to give, in order of elution, lorneamide A (**1**) (2.9 mg, 0.15%) and lorneamide B (**2**) (2.1 mg, 0.11%).

Lorneamide A (1): a colorless glass; $[\alpha]^{19}_D$ −7.2° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (4.09), 263 (3.96) nm; IR (film) υ_{max} 3345, 1665, 1555, 1400, 965 cm^{-1}; NMR data, see Table 1; ESIMS (positive) *m*/*z* 296 $[M + Na]^+$ (28), 256 (100); HRESIMS (positive) *m*/*z* 296.1620 (calcd for $C_{17}H_{23}NO_2Na$, 296.1627).

Lorneamide B (2): a colorless glass; UV (MeOH) λ_{max} (log ϵ) 209 (4.00), 253 (3.80) nm; IR (film) υ_{max} 1700, 1645, 1565, 1415 cm^{-1};^{1}H NMR (MeOH-d_4, 400 MHz) δ 7.34 (d, J = 7.8 Hz, H-6), 6.96 (m, H-7 and H-9), 6.75 (d, J = 15.4 Hz, H-4), 6.15 (dt, J = 7.1, 15.4 Hz, H-3), 3.14 (dd, J = 1.4, 7.1 Hz, H-2), 2.87 (t, J = 7.8 Hz, H-11 or H-12), 2.67 (t, J = 7.8 Hz, H-12 or H-11), 2.38 (t, J = 7.5 Hz, H-14), 2.26 (s, H-17), 1.54 (tq, J = 7.5 Hz, H-15), 0.86 (t, J = 7.5 Hz, H-16); ESIMS (positive) *m*/*z* 296 $[M + Na]^+$ (31), 274 $[M + H]^+$ (100); HRESIMS (positive) *m*/*z* 296.1622 (calcd for $C_{17}H_{23}NO_2Na$, 296.1627).

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