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ORIGINAL ARTICLE

Macplocimine A, a new 18-membered macrolide isolated from the filamentous sulfur bacteria *Thioploca* sp.

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Macplocimine A (1), a rare naturally occurring 18-membered macrolide, was isolated from the marine-derived filamentous sulfur bacteria *Thioploca* sp. The structure was determined by a combination of spectroscopic techniques, including HRESIMS, 1D and 2D NMR analyses. 1 features a thymine group, which is attached to an aromatic fused 18-membered macrolide ring structure derived from a polyketide synthase biosynthetic pathway.

INTRODUCTION

Natural products can exhibit potent pharmaceutical activity and as a result, are of significant interest for medicinal treatments.[1] They are derived from diverse sources, such as plant, bacteria, fungi and marine invertebrates, and can be found in a broad range of environments. Layers of bacteria combined with sediment, or microbial mats,[2] have proven to be a particularly important source of novel chemical compounds, including polyketides and nonribosomal peptides. These mats develop in various extreme environments, including polar regions, hot springs or hypersaline bodies of water.[3] To date, mats composed of photosynthetic microbes, specifically cyanobacteria, have been the most thoroughly investigated and have been shown to produce a number of biologically active secondary metabolites.[4] The non-photosynthetic bacteria of an individual mat can be composed of sulphur-metabolizing, iron-oxidizing, hydrogen-oxidizing actinobacteria and methantrophic species.[5–7] Of these, one of the most promising components is the sulphur-oxidizing bacteria that are abundant in highly specific marine environments. *Thioploca* and *Beggiatoa* are two genera of these bacteria that are well studied and understood for their sulphur-metabolizing abilities.[8] They tend to develop in sulphur-rich marine environments such as estuaries, deep-sea hydrothermal vents, cold-seeps and continental shelves.[9] These areas include the Bay of Concepción in Chile,[10] the Guaymas Basin,[11] Tokyo Bay,[12] the Monterey Canyon of California[13] and cold seeps in the Atlantic Ocean.[9,14] These genera are morphologically similar, with cells forming filaments that allow them to easily bundle to create mats.[15]

In addition to internally storing elemental sulphur, they have intracellular vacuoles containing high concentrations of nitrate.[16] This is integral to the metabolism of sulphur under anaerobic conditions, as nitrate can be reduced to ammonia, acting as the electron acceptor during sulphide oxidation.[17] Although these species' cycling of sulphur, carbon and nitrogen has been, and continues to be studied, their production of secondary metabolites and the associated enzymology remains unclear.

Recently, a filamentous species of *Thioploca* was obtained from the benthic microbial mat ecosystem on the continental shelf off the coast of Chile. We sought to determine the potential of these sulphur-oxidizing bacteria to generate significant natural products. A small molecule, macplocimine A, was isolated and structurally defined.

RESULTS AND DISCUSSION

Phylogenetic analysis demonstrated that this *Thioploca* sp. can be grouped with several reported *Thioploca* and *Beggiatoa* species deposited in NCBI, and furthermore elucidated the evolutionary distinction of these sulphur-metabolizing bacteria from other bacterial classes (Figure 1, sequences are listed in Supplementary Information). A genome scan of *Beggiatoa* sp. using PKSJ (YP_006629905.1) sequence showed that this group of bacteria is a rich source of secondary metabolites. Detailed chemical investigation led us to the isolation and purification of a novel polyketide, namely Macplocimine A (**1**) (Figure 2). **1** was obtained as a pale yellow solid, with the molecular formula $C_{27}H_{36}O_9N_2$, as deduced from HRESIMS *m/z*: 531.2355 [M-H]$^-$ (calcd 531.2343) (see Supplementary Figure 7),

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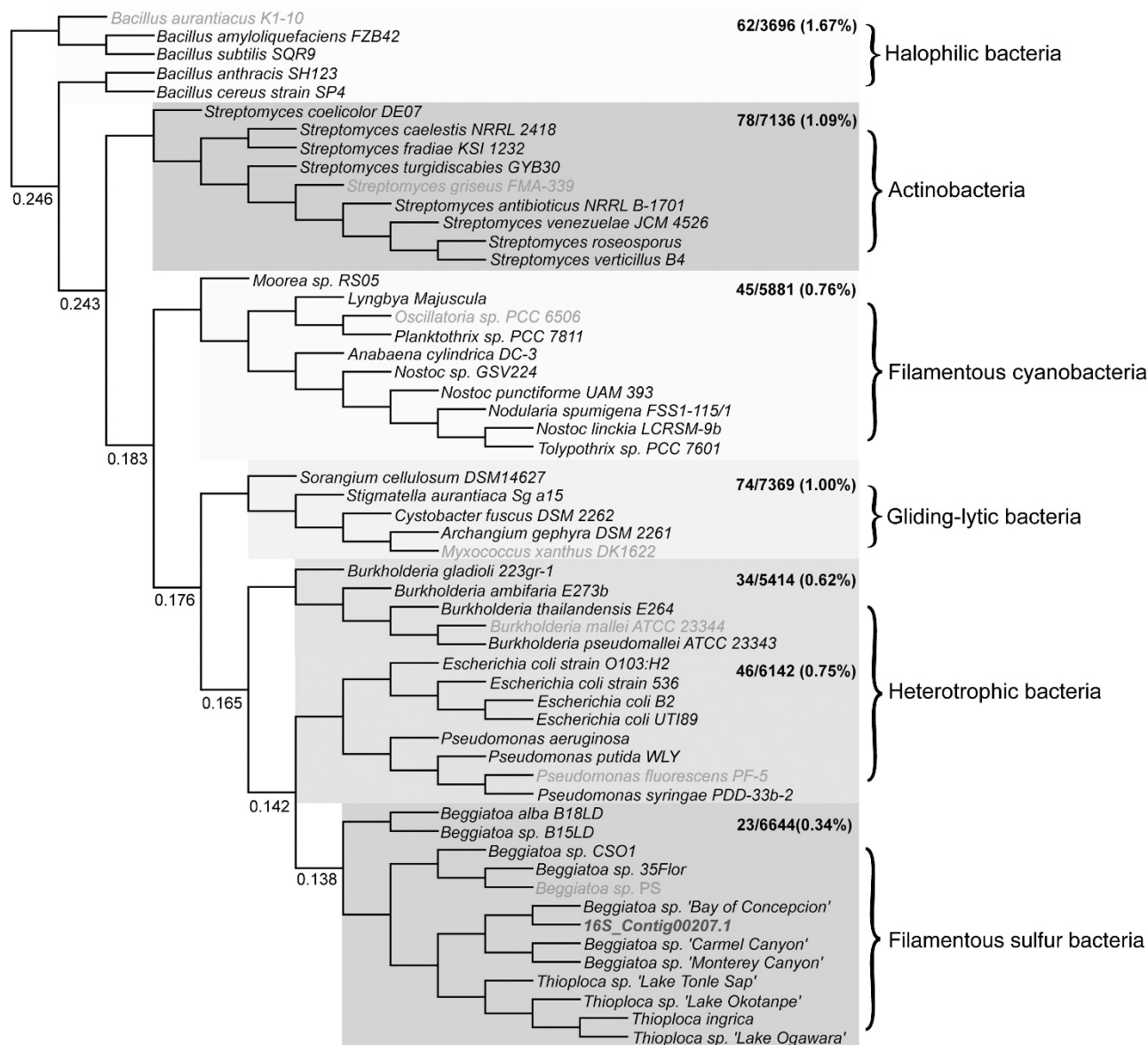
Figure 1 Phylogenetic analysis of 16S sequence of *Thioploca sp.* Six groups were assigned based on the phylogenetic analysis: halophilic bacteria, actinobacteria, filamentous cyanobacteria, gliding-lytic bacteria, heterotrophic bacteria and proteobacteria. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

requiring eleven sites of unsaturation. The UV–vis spectrum exhibited absorption maxima at 224, 264 and 302 nm (MeOH). Analysis of the ^1H NMR spectrum of **1** (see Supplementary Figure 1) in combination with the DEPT-Q (see Supplementary Figure 2) and HSQC (see Supplementary Figure 4) spectra indicated the presence of one heterocyclic proton (δ_H 7.95), two aromatic protons (δ_H 6.11 and 6.17), two olefinic protons (δ_H 5.37 and 5.43), one *N*-methine (δ_H 5.92), three oxygenated methines (δ_H 4.39, 3.80, and 3.46), one methine (δ_H 1.52), seven methylenes (δ_H 2.73–0.94), as well as two methyl (δ_H 2.39 and 0.80) protons. The ^1H and ^{13}C NMR chemical shifts were all adequately assigned by detailed analysis of ^1H-^1H COSY (see Supplementary Figure 3), HSQC, and HMBC (see Supplementary Figure 5) spectroscopic data. The connectivity from C-2 to C-15 was confirmed by the continuous array of COSY couplings, thus the chain system was established (Figure 3), in which the double bond was assigned to C-6 (δ_C 137.1) and C-7 (δ_C 123.8) accordingly.

Down-field chemical shifts of H-4 (δ_H 4.39), H-8 (δ_H 3.80) and H-12 (δ_H 3.46) indicated that C-4, C-8 and C-12 were oxygenated. A tetra-substituted aromatic ring was established by HMBCs between H-17 (δ_H 6.17) and C-19 (δ_C 100.5), C-18 (δ_C 161.7) and C-21 (δ_C 146.2), and between H-19 and C-17 (δ_C 109.4), C-18, C-20 (δ_C 161.4) and C-21 (δ_C 146.2). The thymine structure was established by HMBCs between methyl H-27 (δ_H 2.39) and C-24 (δ_C 161.5), C-25 (δ_C 136.8) and C-26 (δ_C 138.1), and H-26 (δ_H 7.95) and C-23 (δ_C 156.3), C-24 and C-25. The thymine group was linked to C-2 (δ_C 66.2) based on HMBCs between H-26 and C-2 and H-2 (δ_H 5.92) and C-23 and C-26. The HMBCs between H-15 (δ_H 2.53, 2.73) and C-17 and C-21, and between H-17 and C-15 indicated that one side of the chain was attached to C-6; and the HMBCs between H-2 and C-21 and C-1 (δ_C 169.1) suggested that the other side of the chain was bridged by a lactone to C-21. ^1H and ^{13}C chemical shift indicated the presence of *Z*-geometry double bond
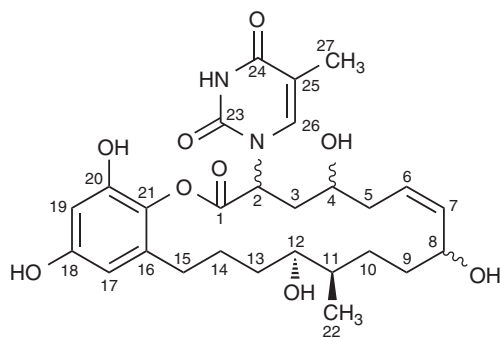
Figure 2 Structure of macplocimine A.

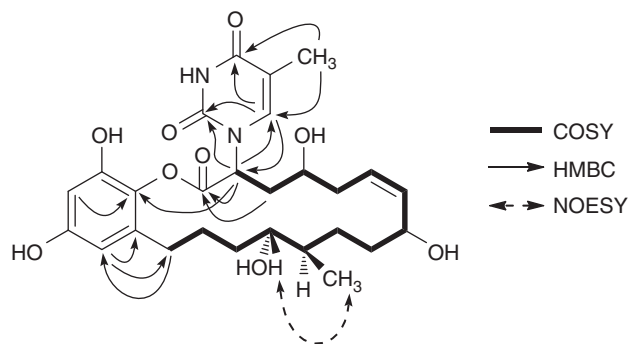Figure 3 Key ^{1}H-^{13}C HMBC, ^{1}H-^{1}H COSY and NOESY (DMSO-d_6) correlations of macplocimine A.

($J_{6,7}$ = 5.7 Hz) between C-6 (δ_H 5.37; δ_C 137.1) and C-7 (δ_H 5.43; δ_C 123.8). Thus, the planar structure of **1** was determined as shown in Figure 2. The stereochemistry was not completely assigned due to the failure in crystallization and Mosher reaction attempts. The relative stereochemistry between C-11 and C-12 was, however, assigned as 11*S* and 12*R* based on NOESY (see Supplementary Figure 6) correlations between H-12 (δ_H 3.46) and H-22 (δ_H 0.80).

Macplocimine A was found to be structurally close to resorcylic acid lactones (RAL), a series of bioactive compounds showing interesting activities.[18] For example, the 14-member ring radicicol is a potent and selective HSP90 inhibitor and hypothemycin, LL-Z1640-2 and LL-783, 277 were found to be potent kinase inhibitors.[19,20] It will be interesting to test macplocimine A for either HSP90 or kinase inhibition activities. The recent characterization of the biosynthetic radicicol clustered from the endophytic fungus *Chaetomium chiversii* indicated that the type I polyketide synthases involved in RAL biosynthesis harbored large multidomain enzymes that iteratively catalyze the condensation of nine units of thioacetates or malonates.[21] On the basis of the structural similarity, we propose that macplocimine A could also be derived from a polyketide synthase biosynthetic pathway.

METHODS

General

NMR spectra were recorded on a Bruker Avance 500 MHz NMR spectrometer (Fremont, CA, USA) with a 5 mm inverse detection probe. ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak for DMSO-d_6 (Cambridge Isotope Laboratories, Andover, MA, USA) at δ_H 2.49 and δ_C 39.5 ppm, respectively. The Silica gel (200−300 mesh) used for column chromatography, and silica gel GF254 (10−40 μ) used for TLC, were supplied by Sigma-Aldrich

Table 1 ^{1}H and ^{13}C NMR spectral data of macplocimine A (1) in DMSO-d_6

C/H	^{1}H NMR	^{13}C NMR	C/H	^{1}H NMR	^{13}C NMR
1	—	169.1 (C)	**13**	1.24 (m)	31.3 (CH$_2$)
2	5.92 (dd, 3.78, 11.6)	66.2 (CH)	**14**	1.54 (m)	28.9 (CH$_2$)
3a	2.03 (m)	35.2 (CH$_2$)	**15a**	2.53 (m)	35.1 (CH$_2$)
3b	2.42 (m)	—	**15b**	2.73 (m)	—
4	4.39 (m)	68.7 (CH)	**16**	—	106.5 (C)
5a	2.18 (m)	36.3 (CH$_2$)	**17**	6.17 (s)	109.4 (CH)
5b	2.26 (m)	—	**18**	—	161.7 (C)
6	5.37 (td, 5.7, 10.7)	137.1 (CH)	**19**	6.11 (s)	100.5 (CH)
7	5.43 (d, 5.7)	123.8 (CH)	**20**	—	161.4 (C)
8	3.80 (m)	72.2 (CH)	**21**	—	146.2 (C)
9a	1.30 (m)	35.7 (CH$_2$)	**22**	0.80 (d, 6.62)	15.0 (CH$_3$)
9b	1.54 (m)	—	**23**	—	156.3 (C)
10a	0.94 (m)	27.9 (CH$_2$)	**24**	—	161.5 (C)
10b	1.25 (m)	—	**25**	—	136.8 (C)
11	1.52 (m)	38.5 (CH)	**26**	7.95 (s)	138.1 (CH)
12	3.46 (m)	72.7 (CH)	**27**	2.39 (s)	13.6 (CH$_3$)

(St Louis, MO, USA). Spots were detected on TLC under UV light, or by heating after spraying with 5% H_2SO_4 in EtOH (v/v). HR– MS spectra were collected on a Thermo LTQ OrbiTrap XL mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) with an (ESI) and using collision-induced dissociation with helium for fragmentation. Preparative HPLC was conducted with Waters Alliance 2695 RP-HPLC separations module (Milford, MA, USA), equipped with a Waters 2998 photodiode array and a Luna 5 μm C$_{18}$ column (250 mm × 10.0 mm, *Phenomenex*). The water used for chromatography, UV, and MS was Millipore Milli-Q PF filtered and all of the solvents were HPLC grade.

Sequence alignment and phylogenetic analysis

Nucleotide sequences were aligned using the Geneious R6 program (Auckland, New Zealand), with default parameter settings (gap-opening penalty, 10; gap extension penalty, 0.05; gap separation penalty range, 8; identity for alignment delay, 40%). The phylogenetic tree was constructed using the neighbor-joining algorithm with MEGA version 5 (Tempe, AZ, USA) with 1000 bootstrap trials performed.

Extraction and isolation

Thioploca sp. was obtained from the benthic microbial mat ecosystem on the continental shelf off the coast of Chile. The wet material was extracted with 95% EtOH at room temperature for one week. The solvent was evaporated under reduced pressure to obtain an extract ($\sim$100 g), which was suspended in H_2O and then extracted with EtOAc. The EtOAc-soluble portion was subjected to vacuum liquid chromatography over silica gel eluted with gradient CHCl$_3$:MeOH (9:1, 5:1 and 1:1) to yield three fractions. Fraction 2 was separated on a Sephadex LH-20 column (Björkgatan, Uppsala, Sweden) and the subfraction 2-2 was subject to silica gel combiflash column, which was eluted with gradient hexane and EtOAc to give five fractions. Macplocimine A (**1**, 1.5 mg) was purified from fraction 2-2-1 using preparative HPLC with a linear gradient of methanol (from 50% to 90%) for 35 min at a flow rate of 3 mlmin^{-1}.

Macplocimine A (**1**): pale yellow solid; UV (MeOH) λ_{max} 224, 264, 302 nm; ^{1}H NMR and ^{13}C NMR data (DMSO-d_6, 500 MHz), see Table 1; HRESIMS *m/z* 531.2355 [M–H]$^-$ (calcd for C$_{27}$H$_{35}$O$_9$N$_2$, 531.2343).

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