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Mollemycin A: An Antimalarial and Antibacterial Glyco-hexadepsipeptide-polyketide from an Australian Marine-Derived *Streptomyces* sp. (CMB-M0244)

Ritesh Raju,[†,○] Zeinab G. Khalil,[†,○] Andrew M. Piggott,[†] Antje Blumenthal,[‡,§] Donald L. Gardiner,[∥] Tina S. Skinner-Adams,[∥,⊥] and Robert J. Capon*[,†]

[†]Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD 4072, Australia

[‡]The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Woolloongabba, QLD, 4102, Australia

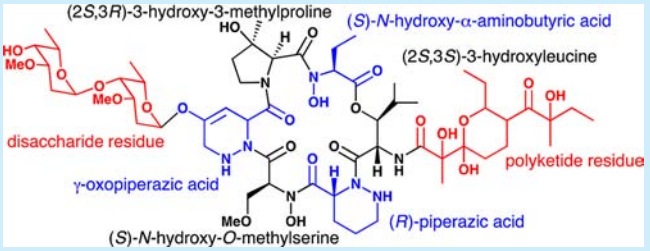
[§]Australian Infectious Diseases Research Centre, The University of Queensland, St. Lucia, QLD 4072, Australia

[∥]Queensland Institute for Medical Research, Herston, QLD, 4006, Australia

[⊥]Eskitis Institute, Griffith University, Nathan, QLD, 4111, Australia

Supporting Information

ABSTRACT: A marine-derived *Streptomyces* sp. (CMB-M0244) isolated from a sediment collected off South Molle Island, Queensland, produced mollemycin A (**1**) as a new first in class glyco-hexadepsipeptide-polyketide. The structure of **1** was assigned by detailed spectroscopic analysis, supported by chemical derivatization and degradation, and C_3 Marfey's analysis. Mollemycin A (**1**) exhibits exceptionally potent and selective growth inhibitory activity against Gram-positive and Gram-negative bacteria (IC_{50} 10−50 nM) and drug-sensitive (3D7; IC_{50} 7 nM) and multidrug-resistant (Dd2; IC_{50} 9 nM) clones of the malaria parasite *Plasmodium falciparum*.



Whether in response to the healthcare threat posed by drug-resistant ESKAPE pathogens (i.e., *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter* species),[1] or for targeting infectious pathogens that kill millions annually (i.e., *Mycobacterium tuberculosis* and *Plasmodium falciparum*), the need to continually invest, discover, and develop new and improved anti-infective therapeutics is compelling. The significance and urgency of this challenge is all the more evident as modern global transportation and mobility of goods and people provide rapidly emerging multidrug-resistant infectious agents with the means to swiftly escape regional boundaries and threaten populations worldwide. In appreciation of the pivotal role played by microbial natural products in delivering the majority of modern anti-infective agents, we turned our attention to Australian marine-derived microbial biodiversity.

During an investigation into structurally diverse bioactive microbial natural products, we acquired a collection of marine-derived bacteria and fungi from Queensland coastal sediments and biota. Utilizing a high-throughput microbioreactor cultivation strategy, we assembled a structurally diverse extract library that was annotated by chemical and biological profiling. A particulary noteworthy observation from these studies was a high molecular weight (MW 1300) secondary metabolite in the antibacterial extract of a *Streptomyces* sp. (CMB-M0244) isolated from a sediment sample collected (−55 m) off South Molle Island, Queensland, Australia. Chemical fractionation of a saline liquid cultivation (3 L), employing solvent partitioning and reversed-phase HPLC fractionation, yielded mollemycin A (**1**) (Figure 1) as a first in class glyco-hexadepsipeptide polyketide. An account of the structure elucidation and biological properties of **1** is detailed below.

HRESI(+)MS analysis of **1** returned an adduct ion $[M + Na]^+$ consistent with a molecular formula ($C_{59}H_{96}N_8O_{24}$, Δmmu −0.9) requiring 16 double bond equivalents (DBE).

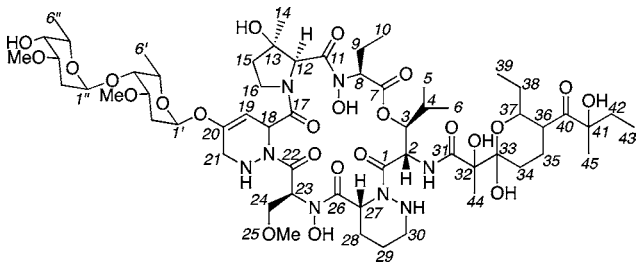


Figure 1. Structure of mollemycin A (**1**).

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Table 1. ^{1}H (900 MHz) and ^{13}C (225 MHz) NMR Data for Mollemycin A (1) in $CDCl_3$[a]

pos.	δ_H, mult (J in Hz)	δ_C
	(2*S*,3*S*)-3-hydroxyleucine	
1		171.1
2	4.90, dd (*10.4*, *10.3*)	56.1
3	5.38, dd (*10.3*, *1.9*)	76.8
4	1.87, m[b]	29.2
5	0.94, d (*6.9*)	15.5
6	0.86, d (*6.9*)	19.8
2*N*-H	8.23, d (*10.4*)	
	(*S*)-*N*-hydroxy-α-aminobutyric acid	
7		170.6
8	5.16, dd (*11.9*, *4.0*)	59.1
9a	2.02, m[b]	22.4
9b	1.90, m[b]	
10	0.94, t (*6.6*)	10.9
8*N*-OH	7.88, s	
	(2*S*,3*R*)-3-hydroxy-3-methylproline	
11		166.1
12	4.79, s	67.9
13		78.2
14	1.47, s	27.2
15a	2.35, m	37.4
15b	1.87, m[b]	
16a	4.86, m[b]	44.9
16b	3.23, m	
13-OH	5.46, br s	
	γ-oxopiperazic acid (enol glycoside)	
17		168.8
18	5.67, d (*4.7*)	48.7
19	4.96, d (*4.7*)	92.1
20		152.6
21a	3.43, m	47.8
21b	3.27, dd (*16.7*, *3.3*)	
21*N*-H	4.85[b]	
	(*S*)-*O*-methylserine	
22		175.3
23	6.13, dd (*9.1*, *3.6*)	55.6
24a	3.94, dd (*10.5*, *9.1*)	68.8
24b	3.63, dd (*10.5*, *3.6*)	
25	3.41, s	59.0
23*N*-OH	9.88, s	
	(*R*)-piperazic acid	
26		169.0
27	5.14, m	49.7
28a	2.28, m	24.9
28b	1.78, m[b]	
29	1.61, m[b]	20.6
	(*R*)-piperazic acid	
30a	3.13, br d (*13.1*)	46.8
30b	2.81, m[b]	
30*N*-H	4.94, d (*13.1*)	
	polyketide residue	
31		177.0
32		76.6
33		99.1
34a	1.79, m[b]	27.0
34b	1.73, ddd (*13.6*, *4.7*, *2.1*)	
35a	1.97, m	24.5
35b	1.90, m[b]	
36	2.80, m[b]	47.5
37	4.23, ddd (*10.6*, *9.0*, *2.4*)	72.4
38a	1.35, m	26.7
38b	1.20, m	
39	0.80, t (*7.2*)	9.5
40		215.8
41		79.4
42a	1.67, m	31.5
42b	1.62, m[b]	
43	0.91, t (*6.6*)	7.4
44	1.38, s	20.7
45	1.34, s	23.7
32-OH	2.96, s	
33-OH	6.69, d (*2.1*)	
41-OH	*c*	
	disaccharide residue	
1′	5.52, d (*2.9*)	95.4
2′a	2.00, m[b]	30.7
2′b	1.87, m[b]	
3′	3.59, ddd (*12.3*, *4.5*, *2.9*)	74.8
4′	3.83, d (*2.9*)	73.8
5′	3.75, q (*6.5*)	68.0
6′	1.21, d (*6.5*)	17.4
3′-OMe	3.42, s	56.5
1″	4.97, d (*3.7*)	99.4
2″a	2.03, m	30.4
2″b	1.87, m[b]	
3″	3.68, ddd (*11.9*, *5.0*, *3.0*)	74.5
4″	3.79, br d (*3.0*)	67.5
5″	4.31, q (*6.6*)	65.8
6″	1.28, d (*6.6*)	16.9
3″-OMe	3.39, s	55.6

[a] ^{13}C NMR assignments supported by gHSQC and gHMBC data. [b] Overlapping signals. [c] Not observed.

Analysis of the NMR ($CDCl_3$) data for **1** (Table 1) revealed resonances for a ketone (δ_C 215.8), seven ester/amide carbonyls (δ_C 166.1−177.0), and two sp^2 carbons (δ_C 92.1, 152.6), accounting for nine DBE and requiring that **1** incorporate seven rings. Further analysis of the 2D NMR data (Figure 2) revealed correlations suggestive of a hexadepsipeptide comprised of a 3-hydroxyleucine (C-1 to C-6) bearing a lactone linkage to an *N*-hydroxy-α-aminobutyric acid (C-7 to C-10), with sequential amide linkages through a 3-hydroxy-3-methylproline (C-11 to C-16), an enolized γ-oxopiperazic acid (C-17 to C-21), an *N*-hydroxy-*O*-methylserine (C-22 to C-25), and a piperazic acid (C-26 to C-30). A C_3 Marfey's analysis[2] was performed on **1** to confirm and assign absolute configurations to the amino acid residues. To support this analysis we obtained an authentic sample of synthetic (2*S*,3*R*)-3-hydroxy-3-methylproline and performed an acid-catalyzed hydrolysis on commercially available aurantimycin A to access standards for (2*S*,3*S*)-3-hydroxyleucine, (*S*)-*N*-hydroxy-*O*-methylserine, and (*S*)- and (*R*)-piperazic acids (Note: the structure of aurantimycin A including the absolute configuration was previously established by single crystal X-ray analysis).[3] As we lacked a standard for *N*-hydroxy-α-aminobutyric acid we subjected a sample of **1** to chemical reduction[4] prior to acid hydrolysis, releasing (*S*)-α-aminobutyric acid that

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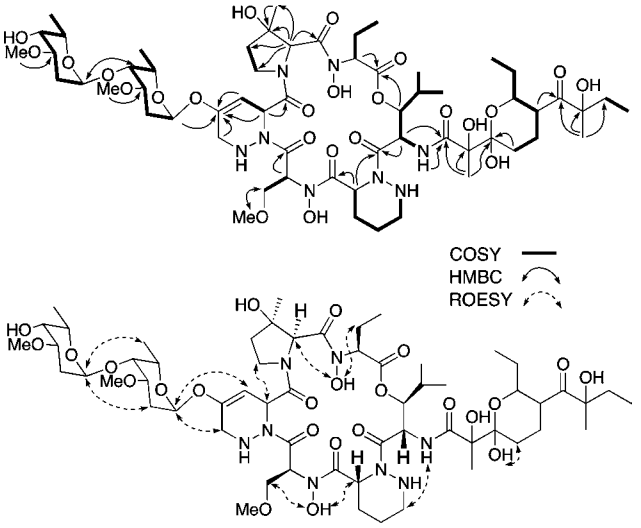

Figure 2. Selected 2D NMR correlations for mollemycin A (**1**).

was identified by comparison (C_3 Marfey's analysis) with a commercial standard.

To be confident that the C_3 Marfey's method could resolve the diastereomeric derivatives of all the target amino acids, we prepared and compared the HPLC-DAD-ESI(+)MS profiles for both L-FDAA and D-FDAA Marfey's amides. Analysis of the Marfey's derivatives obtained from **1** and the standards, including coelution, confirmed the presence of (2*S*,3*S*)-3-hydroxyleucine, (*S*)-*N*-hydroxyl-α-aminobutyric acid, (2*S*,3*R*)-3-hydroxy-3-methylproline, (*S*)-*N*-hydroxy-*O*-methylserine, and (*R*)-piperazic acid residues in **1**. As the γ-oxopiperazic acid residue was not detected in the C_3 Marfey's analysis its absolute configuration remains unassigned.

Further examination of the NMR data for **1** revealed diagnostic correlations for pendant polyketide (C-31 to C-45) and disaccharide (C-1′ to C-6′ and C-1″ to C-6″) moieties. Positioning of the polyketide chain via an amide linkage to C-2 was achieved by HMBC correlations from H-2 to C-31. Although a large $J_{36,37}$ (10.6 Hz) and a ROESY correlation between H-37 and 33-OH was suggestive of *trans*-1,2-diaxial H-36 and H-37, and a *cis*-1,3 diaxial H-37 and 33-OH relative configuration, the full configurational assignment for the polyketide residue remains incomplete. Positioning of the disaccharide residue via a glycoside linkage to a $\Delta^{19,20}$ enolized γ-oxopiperazic residue was achieved by COSY correlations from H-18 to H-19, HMBC correlations from H-18 to C-20 (δ_C 152.6) and from H-21a to C-19 (δ_C 92.1), and ROESY correlations between the anomeric methine H-1′ and the piperazic acid protons H-19 and H-21b. The small scalar couplings for H-1′ (d, $J_{1',2b'}$ 2.9 Hz) and H-1″ (d, $J_{1'',2b''}$ 3.7 Hz) precluded a 1,2-diaxial relationship, necessitating equatorial anomeric protons and β-glycoside linkages. Likewise, small scalar couplings to H-4′ (d, $J_{3',4'}$ 2.9 Hz) and H-4″ (d, $J_{3'',4''}$ 3.0 Hz) precluded diaxial relationships to flanking methines and, together with multiplicities for H-3′ (ddd, $J_{2'a,3'}$ 12.3 Hz; $J_{2'b,3'}$ 4.5 Hz; $J_{3',4'}$ 2.9 Hz) and H-3″ (ddd, $J_{2''a,3''}$ 11.9 Hz; $J_{2''b,3''}$ 5.0 Hz; $J_{3'',4''}$ 3.0 Hz), required that 3′-OMe, 3″-OMe, C-6′, and C-6″ be equatorially disposed, while 4′-OH and 4″-OH were axial. These considerations permitted assignment of the relative configuration of the disaccharide moiety in **1** as indicated, being a C-4′/C-4″ diastereomer of the disaccharide residue found in avermectin B_{1a}. A comparison of the key disaccharide resonances evident in the NMR spectra of **1** and avermectin B_{1a} (Supporting Information, Table S2) provided additional support for this assignment. The absolute configuration of the disaccharide residue remains unassigned.

Mollemycin A (**1**) was determined to be the antibacterial principal detected in the crude *Streptomyces* sp. (CMB-M0244) extract. Antibacterial assays revealed exceptionally potent growth inhibitory activity against the Gram-positive bacteria *Staphylococcus aureus* ATCC 25293 (IC_{50} 50 nM) and ATCC 9144 (IC_{50} 10 nM), *Staphylococcus epidermidis* ATCC 12228 (IC_{50} 50 nM), and *Bacillus subtilis* ATCC 6051 (IC_{50} 10 nM) and ATCC 6633 (IC_{50} 10 nM), as well the Gram-negative bacteria *Escherichia coli* ATCC 25922 (IC_{50} 10 nM) and *Pseudomonas aeruginosa* ATCC 27853 (IC_{50} 50 nM). Mollemycin A (**1**) exhibited moderate activity against *Mycobacterium bovis* (BCG) with IC_{50} 3.2 μM, while it did not exhibit antifungal activity against *Candida albicans* ATCC 90028 (Supporting Information, Figures S3−S5). Mollemycin A (**1**) was proportionately less cytotoxic toward human neonatal foreskin fibroblast cells (NFF, IC_{50} 198 nM) (Supporting Information, Figure S2). Most importantly, **1** exhibited exceptionally potent antimalarial properties against drug sensitive (3D7; IC_{50} 9 nM) and multidrug-resistant (Dd2; IC_{50} 7 nM) *Plasmodium falciparum* clones (Supporting Information, Figure S6), far greater than those exhibited by chloroquine (3D7; 13 nM and Dd2; 130 nM), and similar to those of artemisinin.[5,6]

In conclusion, our investigations reveal that a marine-derived *Streptomyces* sp. (CMB-M0244), isolated from a sediment collected off South Molle Island, Queensland, produced mollemycin A (**1**) as a first in class glyco-hexadepsipeptide-polyketide. The structure of **1** was assigned by detailed spectroscopic analysis, supported by chemical derivatization and degradation, and C_3 Marfey's analysis. Mollemycin A (**1**) exhibits exceptionally potent growth inhibitory activity against Gram-positive and Gram-negative bacteria (IC_{50} 10−50 nM) and drug sensitive (3D7; IC_{50} 9 nM) and multidrug resistant (Dd2; IC_{50} 7 nM) *Plasmodium falciparum*, with significantly lower (>20-fold) cytotoxicity against a mammalian cell line. Notwithstanding that artemisinin combination therapies (ACTs) remain the recommended first-line treatment for *P. falciparum* malaria worldwide, emerging resistance[7,8] demands an urgent commitment to the discovery and development of new antimalarial therapeutics. Further development of the mollemycin A (**1**) pharmacophore requires knowledge of structure−activity relationships and the mechanism of action, to engineer improved selectivity while retaining its remarkable antibacterial/antimalarial potency.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details, NMR spectra, tabulated 2D NMR data, and bioassay results. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: r.capon@uq.edu.au.

Author Contributions

○R.R. and Z.G.K. contributed equally.

Notes

The authors declare no competing financial interest.

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