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Mirabilin G: A New Alkaloid from a Southern Australian Marine Sponge, *Clathria* Species

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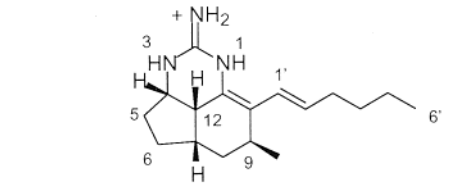
A *Clathria* sp. collected during scientific trawling operations in the Great Australian Bight, Australia, has yielded the new alkaloid mirabilin G (**1**). A structure was secured for **1** by detailed spectroscopic analysis and comparison to known marine alkaloids.

Guanidine-containing alkaloids are known to exhibit a range of biological activities.[1] Examples of bioactive guanidines from marine sponges include the antimicrobial and cytotoxic metabolites ptilocaulin and isoptilocaulin from a Honduran *Ptilocaulis* sp.[2,3] Closely related to the ptilocaulins are the antimicrobial mirabilins A−F from an Australian *Arenochalina miribilis*.[4] Further known examples of this structure class are limited to (+)-8b-hydoxyptilocaulin from the Brazilian sponge *Monanchora arbusula*,[5] as well as 8a,8b-dehydroptilocaulin, 8a,8b-dehydro-8-hydroxyptilocaulin, and 1,8a,8b,3a-didehydro-8-hydroxyptilocaulin from a Caribbean *Batzella* sp.[6] In this report we describe the isolation and structure elucidation of a new antimicrobial alkaloid, mirabilin G (**1**), from a southern Australian *Clathria* sp.

The EtOH extract of a *Clathria* sp. collected during scientific trawling operations in the Great Australian Bight displayed growth inhibitory activity against *Escherichia coli*, *Serratia marcescens*, and *Saccharomyces cerevisiae*. The EtOH extract was decanted and concentrated in vacuo, and the residue was triturated with CH_2Cl_2. The CH_2Cl_2-soluble material was subjected to C_{18} solid phase extraction and HPLC to yield mirabilin G (**1**) as the antimicrobial agent.

The high-resolution ESI(+)MS of **1** displayed a molecular ion consistent with a molecular formula (M + H, $C_{17}H_{28}N_3$) calculating for six double bond equivalents. Examination of the ^{13}C NMR data for **1** revealed three fully substituted and two disubstituted sp^2 carbons, which were attributed to two double bonds (121.0 (s), 123.1 (d), 124.3 (s), and 133.6 (d) ppm) and a guanidino (152.3 ppm) carbon. This analysis required that **1** be tricyclic. The observation of ^1H NMR resonances for 28 protons, four being D_2O exchangeable, was consistent with **1** being a guanidinium salt (the identity of the counterion was not established). Analysis of the 2D NMR data for **1** (see Table 1) revealed a connectivity sequence supportive of the C-1′ to C-6′ hexenyl side chain. A sequence of COSY correlations from H-4 to H-9, and including the 9-Me, was also very informative. These, together with $^1H-^{13}C$ gHMBC correlations from both H-1′ and H-9 to C-10, and from H-12 to C-11, extended connectivity to incorporate all carbons with the exception of the guanidino carbon. A $^1H-^{13}C$ gHMBC correlation from 1-NH to C-11, together with the ^1H and ^{13}C NMR shifts for H-4 (δ 3.79) and C-4 (53.4 ppm) and a TOCSY correlation from 3-NH to H-4, positioned the guanidino functionality as indicated. This assignment was confirmed by measurement of a $^1H-^{13}C$ gHMBC correlation from H-4 to C-2, allowing the gross structure for **1** (less stereochemistry) to be assigned as shown.



(**1**)

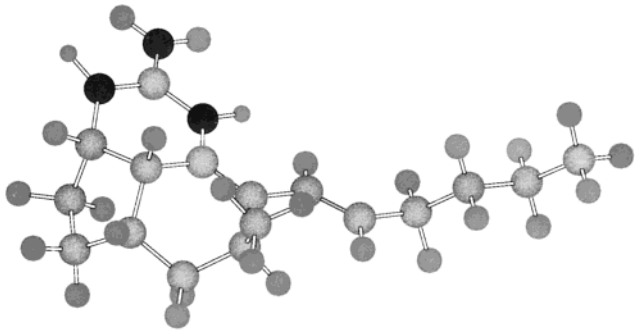


Figure 1. Energy-minimized (Chem3D−MM2) representation of **1**.

The *E* stereochemistry about $\Delta^{1',2'}$ was confirmed from the magnitude of $J_{1',2'}$ (15.6 Hz), while 2D NMR NOESY (and ROESY) correlations between H-4, H-7, 9-Me, and H-12 (Table 1) established the relative stereochemistry about the four asymmetric sp^3 carbons as indicated. Molecular modeling studies (see Figure 1) confirm that the proposed relative stereochemistry orients H-4 and H-8β in an extended coplanar "W conformation", which may explain the observed long-range DQFCOSY correlation between these protons.

Given its close structural relationship with known mirabilins, **1** was attributed the trivial name mirabilin G. As with other examples of this structure class, mirabilin G (**1**) displays modest growth inhibitory activity against the Gram negative bacteria *Escherichia coli* and *Serratia marcescens* and the fungus *Saccharomyces cerevisiae*.

Assignment of absolute stereochemistry to known examples of this structure class has proved challenging, with only pitilocaulin having been secured, by total synthesis.[3] Definitive assignment of absolute stereochemistry across this structure class must presumably await the attention of synthetic chemists.

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Table 1. NMR ($CDCl_3$, 400 MHz) Data for Mirabilin G (**1**)

no.	^{13}C ppm[b]	^1H δ (m, J Hz)[a]	DQFCOSY ^1H to ^1H	gHMBC ^1H to ^{13}C	NOESY[c] $^1H-^1H$
1-NH		9.61 (s)		C-11	
2	152.3				
C2−NH_2		7.50 (s)			
3-NH		8.33 (s)	H-4		
4	53.4	3.79 (dt, 7.6, 6.3)	H-12, H-5, H-8β, 3-NH	C-2, C-5, C-12	H-7, H-12
5	32.3	1.54 (m)	H-4, H-6	C-4	
6	26.3	1.74 (m)	H-5, H-7, H-8α, H-8β	C-5, C-7	H-7, H-9
7	26.3	2.47 (m)	H-6, 9-Me	C-6, C-8, C-12	H-4, H-8β, H-6, 9-Me
8β	31.7	2.02 (m)	H-4, H-6, H-9	C-7, C-9	H-7
8α		1.86 (m)	H-6, H-9	C-7, C-9	H-9
9	27.5	2.73 (dd, 7.2, 7.3)	H-8α, H-8β, 9-Me	C-7, C-8, 9-Me, C-10	H-6, H-8α, 9-Me
9-Me	22.5	1.18 (d, 7.2)	H-7, H-9	C-8, C-9, C-10	H-7, H-9
10	124.3				
11	121.0				
12	37.2	2.57 (dd, 7.1, 7.6)	H-4	C-4, C-7, C 8, C-11	H-4
1′	123.1	6.50 (d, 15.6)	H-2′	C-2′, C-3′, C-9, C-10	
2′	133.6	5.71 (dt, 15.6, 7.2)	H-1′, H-3′	C-1′, C-3′, C-10	
3′	33.1	2.24 (dt, 7.2, 6.3)	H-2′, H-4′	C-2′, C-4′	
4′	31.6	1.40 (tt, 6.1, 6.3)	H-3′, H-5′	C-3′, C-5′, C-6′	
5′	22.3	1.32 (tq, 6.1, 7.2)	H-4′, H-6′	C-3′, C-4′, C-6′	
6′	14.1	0.90 (t, 7.2)	H-4′, H-5′	C-3′, C-4′ C-5′	

[a] Assignments supported by TOCSY. [b] gHMQC. [c] ROESY data.

Experimental Section

General Experimental Procedures. See ref 7.

Animal Material. A sponge specimen, *Clathria* (Isociella) sp. (order, Poecilosclerida; family, Microcionidae; Museum of Victoria Registry Number, F80004) (150 g extracted dry wt), was collected in 1995 by epibenthic sled off the coast of Cape Arid, Western Australia, during a scientific cruise aboard the RV Franklin. A description of the specimen is as follows: growth form macrobenthic, flabelliform (5−10 mm) lamellate; color in life pale orange; color on deck beige; texture firm flexible; surface transparent, rugose honeycomb with irregular longitudinal striations; oscules inconspicuous, sunken; spicules megascleres styles occasionally subtyole, curved (250−280 × 10 μm), straight (230−300 × 5 μm); microscleres palmate isochelae (30 μm); ectosome membranous, hispid with tangential, paratangential, and occasionally plumose auxiliary styles at the surface with points of choanosomal primary spicules protruding through; choanosome a slightly compressed skeleton formed by a renieroid reticulation of thick spongin fibers cored by paucispicular tracts of principal styles. Extra axially the skeleton becomes plumose. Echinating megascleres are absent, and the mesohyl matrix contains abundant pigmented collagen and scattered chelae.

Extraction and Isolation. The sponge was diced, steeped in EtOH, and stored at −18 °C until required. The crude ethanol extract displayed growth inhibitory activity against *Escherichia coli*, *Serratia marcescens*, and *Saccharomyces cerevisiae*. The decanted EtOH extract was concentrated in vacuo and partitioned into CH_2Cl_2, MeOH, and H_2O soluble fractions. The CH_2Cl_2-soluble fraction was subjected to solid phase extraction (10% stepwise gradient elution from hexane to EtOAc through a silica SEP-pak), and the fraction eluting with 90% EtOAc/hexane further purified by C_{18} HPLC (2 mL/min repeated isocratic elution with 90% then 70% MeOH/H_2O through a Zorbax C_{18} eclipse 5 μ 250 × 10 mm column) to yield as the single antimicrobial agent mirabilin G (**1**) (5 mg, 0.03% extracted dry weight).

Mirabilin G (1): pale yellow oil; $[\alpha]_D$ +49° (*c*, 0.1 in $CHCl_3$); IR (film) ν_{max} 3374, 1708, 1610 cm^{-1}; UV (EtOH) λ_{max} 295 nm (ϵ 3.4), 241 (ϵ 3.7); ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; ESI(+)MS *m/z* 274 (M + H); HRESI(+)MS *m/z* 274.2284 (calcd for $C_{17}H_{28}N_3$ (M + H) requires 274.2283).

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Supporting Information Available: Structure diagrams for all known closely related metabolites listed in the introduction. This material is available free of charge via the Internet at http://pubs.acs.org.

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