

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

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Received November 30, 2000

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-hydroxyptilocaulin from the Brazilian sponge *Monanchora arbuscula*,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 CH₂Cl₂. The CH₂Cl₂-soluble material was subjected to C₁₈ 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, C₁₇H₂₈N₃) calculating for six double bond equivalents. Examination of the ¹³C NMR data for **1** revealed three fully substituted and two disubstituted sp² 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 D₂O 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–¹³C 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–¹³C gHMBC correlation from 1-NH to C-11, together with the ¹H and ¹³C 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 function-

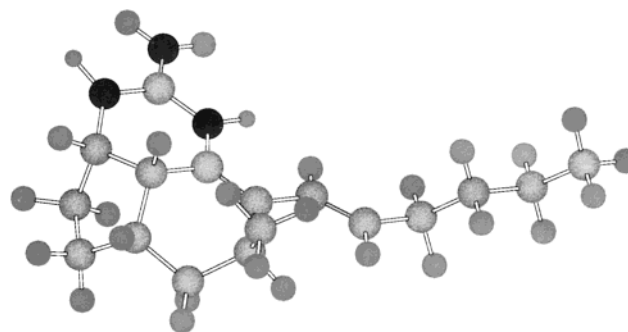
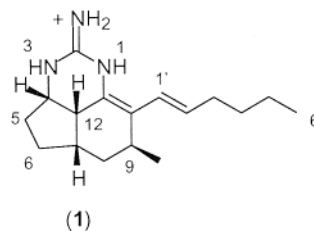


Figure 1. Energy-minimized (Chem3D–MM2) representation of **1**.

ality as indicated. This assignment was confirmed by measurement of a ¹H–¹³C gHMBC correlation from H-4 to C-2, allowing the gross structure for **1** (less stereochemistry) to be assigned as shown.

The *E* stereochemistry about Δ^{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 sp³ 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 ptilocaulin 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 (CDCl₃, 400 MHz) Data for Mirabilin G (1)

no.	¹³ C ppm ^b	¹ H δ (m, JHz) ^a	DQF COSY ¹ H to ¹ H	gHMBC ¹ H to ¹³ C	NOESY ^c ¹ H– ¹ H
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 gHMBC. ^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 subtylole, 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 CH₂Cl₂, MeOH, and H₂O soluble fractions. The CH₂Cl₂-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 C₁₈ HPLC (2 mL/min repeated isocratic elution with 90% then 70% MeOH/H₂O through a Zorbax C₁₈ 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; [α]_D +49° (c, 0.1 in CHCl₃); 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, CDCl₃), see Table 1; ¹³C NMR (100 MHz, CDCl₃), see Table 1; ESI(+)-MS *m/z* 274 (M + H); HRESI(+)-MS *m/z* 274.2284 (calcd for C₁₇H₂₈N₃ (M + H) requires 274.2283).

Acknowledgment. We are very grateful to the CSIRO Division of Oceanography and the crew and scientific staff of the RV Franklin for support in specimen collection. The assistance of L. Goudie in specimen collection and taxonomic classification is also greatly appreciated. This research was funded in part by the Australian Research Council.

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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NP000564G