Published in final edited form as:

Org Lett. 2008 February 21; 10(4): 629. doi:10.1021/ol702952n.

The Marinopyrroles, Antibiotics of an Unprecedented Structure Class from a Marine *Streptomyces* sp

Chambers C. Hughes, Alejandra Prieto-Davo, Paul R. Jensen, and William Fenical

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California—San Diego, La Jolla, California 92093-0204

Chambers C. Hughes: ; Alejandra Prieto-Davo: ; Paul R. Jensen: ; William Fenical: wfenical@ucsd.edu

Abstract

(-)-marinopyrrole A (X = H)

(-)-marinopyrrole B (X = Br)

Cultivation of an obligate marine *Streptomyces* strain has furnished the marinopyrroles A and B, densely halogenated, axially chiral metabolites that contain an uncommon bispyrrole structure. X-ray analysis of marinopyrrole B showed that the natural product exists as an atropo-enantiomer with the *M*-configuration. Though configurationally stable at room temperature, *M*-(-)-marinopyrrole A can be racemized at elevated temperatures to yield the non-natural *P*-(+)-atropo-enantiomer. The marinopyrroles possess potent antibiotic activities against methicillin-resistant *Staphylococcus aureus*.

For over half a century, the cultivation of terrestrial actinomycetes has yielded structurally diverse secondary metabolites with remarkable biological activities.¹ Today, studies of these chemically prolific bacteria have been expanded from traditional soil habitats to include strains obtained from marine samples. At a time when a pressing need exists for the discovery of new antibiotics,² the rationale to sample under-explored habitats in search of new microbial resources is great. For these reasons, we have focused our attention on actinomycetes that

Correspondence to: William Fenical, wfenical@ucsd.edu.

Supporting Information **Available:** Isolation procedure for **1** and **2**. Procedure for the synthesis of *P*-**1**. HRMS data, proton and carbon NMR spectra for **1**, **2**, and *P*-**1**. Crystallographic data for **2** (CCDC 645271) in CIF format. This material is available free of charge via the Internet at <http://pubs.acs.org>.

inhabit ocean sediments. Chemical studies of these bacteria, which include new species and genera, have yielded a growing number of unique, bioactive natural products.³

Actinomycete strain CNQ-418, obtained from a marine sediment sample collected near La Jolla, CA, at a depth of 51 m, required seawater for growth and shared 98.1% 16S rRNA gene sequence identity with its nearest neighbor (*Streptomyces sannurensis*). This level of similarity suggests that it may be a new *Streptomyces* species.⁴ Strain CNQ-418, the culture extracts of which displayed significant antibiotic activity, may represent the first strain of a new marine lineage to be examined for its ability to produce biologically active natural products.

Strain CNQ-418 was cultivated in a seawater-based medium (20 × 1 L) for 7 days under vigorous shaking. Solid-phase extraction of the broth using Amberlite resin (XAD-16), filtration through cheesecloth, and elution of the resin with acetone afforded, after solvent removal under vacuum, a gummy extract that was subjected to fractionation on silica gel. Two prominent metabolites, the marinopyrroles A (**1**) and B (**2**), were isolated from a fraction using C8 reversed-phase HPLC. These compounds were analyzed for the molecular formulas C₂₂H₁₂Cl₄N₂O₄ and C₂₂H₁₁BrCl₄N₂O₄, respectively.

Initial progress toward the structural elucidation of these new metabolites began with 2D NMR studies of marinopyrrole A (**1**). Two independent benzoyl groups were identified by interpretation of COSY, HSQC, and HMBC spectral data (Table 1). The rather limited NMR spectral data defining the central core of these molecules, however, made unambiguous structural elucidation difficult if not impossible.

The structure of marinopyrrole A (**1**) was ultimately assigned using X-ray crystallographic techniques. At first, several derivatives of **1** were prepared in order to procure material that would be suitable for crystallographic analysis. The phenolic groups were cleanly esterified to produce the corresponding *O,O'*-diacetyl, di-*p*-bromobenzoyl, and di-*p*-nitrobenzoyl esters. Reaction of **1** with ethereal diazomethane provided an *N*-methyl derivative that was also crystalline. Although these derivatives did not yield X-ray quality crystals, we were able to obtain crystals of marinopyrrole B (**2**) via slow evaporation from toluene. The structure of marinopyrrole B, including its absolute configuration, was then readily determined (Figure 1). Subsequently, the planar structure of marinopyrrole A (**1**) was assigned by comparison of its spectral data with those derived from **2**.

The marinopyrroles were isolated as single atropo-enantiomers, suggesting that the pyrrole–pyrrole coupling is a genuine enzyme-catalyzed process. Similar optical rotations of –69 (*c* = 0.39, MeOH) and –72 (*c* = 0.20, MeOH) were observed for **1** and **2**, respectively. An X-ray Flack parameter, refined to 0.03(1), allowed us to assign an *M*-configuration to **2**. By comparison of CD spectra, marinopyrrole A (**1**) was also assigned an *M*-configuration (Figure 2). Both spectra showed negative first and positive second Cotton effects [**1**: Δε = –6.2 (372 nm) and Δε = +6.8 (315 nm); **2**: Δε = –2.9 (373 nm) and Δε = +2.0 (314 nm)], in agreement with the negative exciton chirality between the two salicyloyl substituents.⁵

Though these metabolites are configurationally stable at room temperature, we sought to determine whether the molecules could be racemized at elevated temperatures. Accordingly, marinopyrrole A (**1**) was heated in toluene at 120 °C. The resulting atropo-enantiomers could be separated on an analytical chiral HPLC column (Chiracel OD-H). Acylation of the racemic mixture with *R*-Mosher's chloride produced a mixture of diastereomers (*S-M-S* and *S-P-S*) that was separated on silica gel. The non-natural atropo-enantiomer of **1**, [α]_D = +110 (*c* = 0.12, MeOH), was then isolated upon saponification of the appropriate bisester. The CD spectrum of *P*-**1** displayed split Cotton effects of opposite sign, as expected [Δε = +6.5 (370 nm) and Δε = –7.0 (310 nm)] (see Figure 3).

The marinopyrroles, a component of which is reminiscent of the *Pseudomonas* metabolite pyoluteorin,⁶ have several novel structural features. Foremost, the *N,C2*-linked bispyrrole motif has not been previously described in the natural products literature.⁷ Though their synthesis via the Paal-Knorr reaction was first detailed in 1957,⁸ bispyrroles of this sort have received little attention in the synthetic community.⁹ The unusual biaryl bond, flanked by four *ortho* substituents, establishes an axis of chirality that further distinguishes these interesting natural products. The biosynthesis of the marinopyrroles would appear to involve a critical pyrrole coupling event that has not been observed in the past.

The compounds display noteworthy activity in antimicrobial bioassays (Table 2). Against methicillin-resistant *Staphylococcus aureus*, **1** and **2** showed minimum inhibitory concentrations (MIC₉₀) of less than 2 μ M. For each of the compounds, cytotoxicity against a human cancer cell line (HCT-116) was less pronounced. Interestingly, in these assays, the configuration about the biaryl axis of **1** appeared to be of little consequence, as the non-natural atropo-enantiomer showed potency similar to that of the natural product.

Derived from what appears to represent a new, obligate marine *Streptomyces* sp., the marinopyrroles are the first examples of natural products containing a *N,C2*-linked bispyrrole structure. These bioactive molecules may encompass a new pharmacophore that could prove useful in the treatment of drug-resistant bacterial pathogens. Studies are now in progress to examine the mechanism of action of these novel antibiotics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support was provided by the National Cancer Institute, NIH (under grant CA44848). We thank Drs. Arnold L. Rheingold and Antonio G. D'Pasquale (UCSD) for assistance with X-ray diffraction experiments. We also acknowledge Sara Kelly (SIO) for performing the bioassays.

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Figure 1.
The marinopyrroles A (**1**) and B (**2**).

Figure 2. X-ray drawing of **2**, depicting its absolute configuration. The hydrogen bond between the perhydroxyl group and the carbonyl is apparent.

Figure 3.
CD spectra of **1**, **2**, and non-natural *P-1* in acetonitrile.

Table 1¹H, ¹³C, and HMBC NMR Spectral Data for Marinopyrrole A (**1**) (CDCl₃)

C nos.	δ _C ^a	δ _H , mult. (J, Hz) ^b	HMBC ^b
1-4,1',2',4' ^c			
5	185.9		
6	118.9		
7	161.1		
8	117.8	6.92, m ^d	5,7
9	136.2	7.35, t (7.8)	7,11
10	118.7	6.52, t (7.8)	6-9,11
11	130.3	7.48, d (7.8)	5,7,9
3'	120.3	6.72, s	5'
5'	186.8		
6'	118.9		
7'	162.5		
8'	118.4	7.03, d (7.8)	5',7',10'
9'	136.3	7.51, t (7.8)	7',11'
10'	119.1	6.90, m ^d	8'
11'	131.9	7.58, d (7.8)	5',7',9'
-OH, -NH		11.18, 10.42, 9.73, br s	

^a 75 MHz.^b 500 MHz.^c δ_C = 128.8, 124.1, 123.8, 123.1, 120.4, 112.7, 110.7^d Overlapping signals.

Table 2Bioassay Results (μM)

	(-)-1	(+)-1	(-)-2
MRSA (MIC ₉₀) ^a	0.61	0.31	1.1
HCT-116 (IC ₅₀) ^b	8.8	9.4	9.0

^aMRSA = methicillin-resistant *Staphylococcus aureus*. Positive control: vancomycin (MIC₉₀ = 0.14–0.27 μM) and penicillin G (MIC₉₀ = 18–34 μM).

^bHCT-116 is a human colon cancer cell line. Positive control: etoposide (IC₅₀ = 0.49–4.9 μM).