

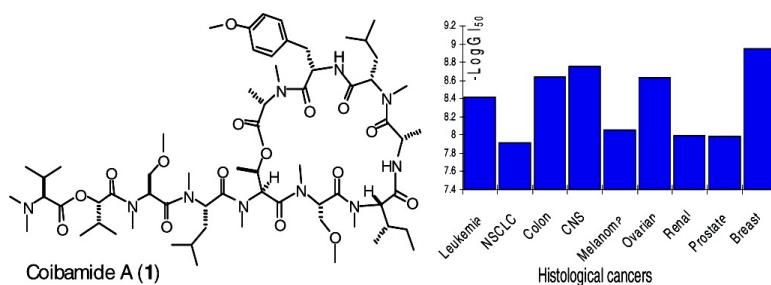
Communication

Coibamide A, a Potent Antiproliferative Cyclic Depsipeptide from the Panamanian Marine Cyanobacterium *Leptolyngbya* sp.

Rebecca A. Medina, Douglas E. Goeger, Patrice Hills, Susan L. Mooberry, Nelson Huang, Luz I. Romero, Eduardo Ortega-Barri#a, William H. Gerwick, and Kerry L. McPhail

J. Am. Chem. Soc., **2008**, 130 (20), 6324-6325 • DOI: 10.1021/ja801383f • Publication Date (Web): 29 April 2008

Downloaded from <http://pubs.acs.org> on February 8, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)

Coibamide A, a Potent Antiproliferative Cyclic Depsipeptide from the Panamanian Marine Cyanobacterium *Leptolyngbya* sp.

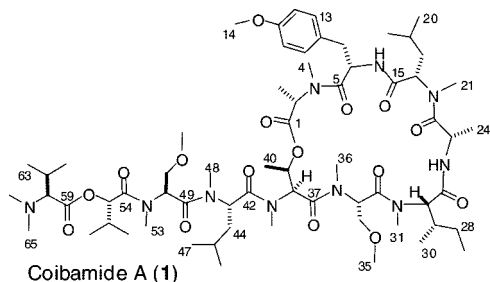
Rebecca A. Medina,[†] Douglas E. Goeger,[†] Patrice Hills,[‡] Susan L. Mooberry,[‡] Nelson Huang,[§] Luz I. Romero,^{||} Eduardo Ortega-Barría,^{||} William H. Gerwick,[⊥] and Kerry L. McPhail^{*,†}

College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, Southwest Foundation for Biomedical Research, San Antonio, Texas 78227, Chemical and Screening Sciences, Wyeth Research, Cambridge, Massachusetts 02140, Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología, Clayton, Panama 5, Republic of Panama, Scripps Institution of Oceanography and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California 92093

Received February 27, 2008; E-mail: kerry.mcphail@oregonstate.edu

Marine organisms continue to yield a diverse array of biologically active molecules, a remarkable number of which are peptide-based cancer cell toxins of putative microbial symbiont biogenesis.¹ Development of these as anticancer drugs has met with some success:² ascidian-derived dihydrodidemnin B (aplidin) has orphan drug status for the treatment of multiple myeloma and acute lymphoblastic leukemia; green algal isolate kahalalide F and TZT-1027, a synthetic analogue of the cyanobacterial metabolite dolastatin 10, reached phase II clinical trials. Other important cyanobacterial peptide leads include the cryptophycins and curacin A,³ and these organisms continue to produce a wealth of anticancer lead compounds.⁴ The high degree of *N*-methylation of many of these cyanobacterial peptides may improve their druggability since *N*-methylation has been shown to improve pharmacological parameters such as lipophilicity, proteolytic stability, and duration of action, properties for which regular peptides are notoriously poor and which limits their bioavailability.⁵

In the context of our International Cooperative Biodiversity Groups program (ICBG) based in Panama, which focuses on drug discovery, biodiversity conservation, and sustainable economic growth, we have isolated a potent cancer cell toxin with an unprecedented selectivity profile in the NCI 60 cell line panel. This cyanobacterial depsipeptide, named coibamide A in tribute to its discovery from the UNESCO World Heritage Site of Coiba National Park,⁶ highlights the importance of conserving pristine, unexplored repositories of diverse marine organisms.



Coibamide A (1)

The marine filamentous cyanobacterium *Leptolyngbya* sp. was collected by hand using SCUBA from the Coiba National Park, Panama. A crude organic extract of this material was subjected to bioassay-guided fractionation via normal phase vacuum liquid chromatography (NP-VLC) using a stepped gradient of hexanes to EtOAc to MeOH. In preliminary biological activity screening, the 100% EtOAc eluting fraction was cytotoxic (IC₅₀ 300 ng/mL) to NCI-H460

human lung tumor cells, and was also active against malaria, leishmaniasis, and trypanosomal tropical disease parasites. This VLC fraction was separated by reversed-phase C₁₈ solid phase extraction and isocratic HPLC to yield the optically active colorless oil ([α]_D²⁵ −54.1) coibamide A (**1**, 6.3 mg).

The molecular composition of **1** was established as C₆₅H₁₁₀O₁₆N₁₀ from FT-MS data ([M + H]⁺ *m/z* 1287.8156, Δ −2.4 mmu). The peptidic nature of **1** was evident from its complex ¹H NMR spectra in all solvents (CDCl₃, C₆D₆, DMSO, C₅D₅N). However, *N*-methyl conformations were minimized in CDCl₃ which showed numerous α-proton multiplets (4.75–6.02), overlapped methyl doublets (δ 0.75–1.15), mutually coupled aromatic proton doublets (δ 7.14, 6.76), a broad 2H amide proton signal (6.65), and deshielded singlets integrating to 12 methyl groups attached to heteroatoms (δ 2.34–3.77). The ¹³C NMR spectra for **1** in CDCl₃ and C₆D₆ featured an indeterminate number of resonances, with numerous ester/amide carbonyl ¹³C signals, due to localized symmetry (*O*-Me-Tyr, *N,N*-diMe-Val), steric constraints (*N*-Me-Thr), signal overlap and multiple conformations. These data suggested a high degree of *N*- and *O*-methylation, an observation supported by standard amino acid analysis which yielded only one alanine and one *O*-methyl tyrosine residue. These two amino acids, one hydroxy acid and eight *N*-methylated residues were assigned from 2D experiments (CDCl₃) including COSY, TOCSY, multiplicity-edited HSQC, HSQC-TOCSY, HMBC, H2BC⁷ and ¹H–¹⁵N gHMBC.

Elucidation of seven of the eight *N*-methylated residues began with HMBC correlations from each *N*-methyl singlet to the corresponding α-carbon, the side-chain spin systems of which were delineated by TOCSY experiments to give *N*-methylalanine, two *N*-methylleucines, *N*-methylisoleucine, two *N,O*-dimethylserines, and an *N,N*-dimethylvaline residue. The latter terminal residue was described by a 6H singlet (δ_{H-64/65} 2.34) that was HSQC-correlated to a prominent ¹³C resonance (δ_{C-64/65} 41.3) and ¹⁵N-gHMBC-correlated to a shielded ¹⁵N resonance (δ_N 24.6). Fortunately, nine of ten N atoms in **1** were observed in the latter ¹⁵N-gHMBC⁸ experiment which showed additional correlations from five *N*-methyls to δ_N 105.6, 108.5, 113.4, 117.5, 120.5, two α-methyls (Ala and *N*-Me-Ala) to δ_N 115.1, 122.4, and H₂-7 of *O*-Me-Tyr to δ_N 118.2. Hydroxyisovaleric acid (HIV) was assigned on the basis of TOCSY correlations from deshielded CH-55 (δ_H 5.00, δ_C 74.7) to isopropyl methine (δ_{H-56} 2.21) and methyl (δ_{H3-57/58} 1.06) resonances. At this point, it remained to assign 114 mass units (interpreted as C₅H₈O₂N = *N*-Me-Thr or *N,O*-diMe-Ser), to determine the carboxyl terminus and to establish the sequence of residues in the depsipeptide chain. COSY correlations were observed between an unassigned methyl doublet at δ 1.07 (H₃-40) and an oxygenated methine multiplet at δ 5.50 (H-39). Strong ROESY correlations, but no COSY or TOCSY correlations, were observed between this methyl–oxymethine pair and a very broad, partially obscured signal (δ 2.89, CDCl₃; 3.11 ppm, C₆D₆). Variable temperature experiments in CDCl₃ (298–328K, 700 MHz, 1 mm

[†] Oregon State University.

[‡] Southwest Foundation for Biomedical Research.

[§] Wyeth Research.

^{||} INDICASAT.

[⊥] Scripps Institution of Oceanography and UCSD.

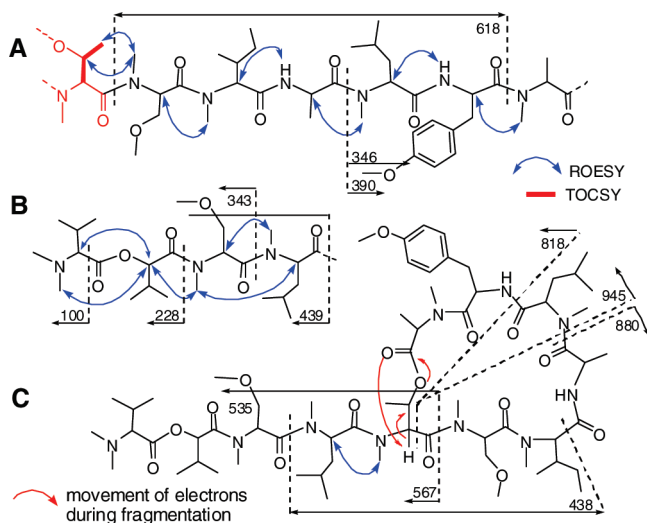


Figure 1. Partial structures **A** and **B** assembled from key ROESY correlations and mass fragments; structure **C** shows the key mass spectrometric fragments supporting the position of *N*-Me-Thr and cyclization of **1**.

cryoprobe) resolved this broad peak into a 3H singlet which was HSQC-correlated to an *N*-methyl resonance (δ_{C-41} 29.7). Furthermore, careful examination of C_6D_6 HSQC data revealed an additional heteroatom-substituted methine (δ_{H-38} 6.72, δ_{C-38} 56.6), which showed weak TOCSY correlations to the above-described methyl–oxymethine pair. Hence, the remaining residue was assigned as *N*-Me-Thr.

Two partial structures (Figure 1, **A** and **B**) could be assembled based on a combination of mass spectrometric data and ROESY correlations between each *N*-methyl and the α -proton of the adjacent residue. Additionally, a ROESY correlation between *N*-CH₃-36 and the *N*-Me-Thr β - and γ -protons (H-39, H₃-40) positioned this residue at the *N* terminus of partial structure **A**.

A ROESY correlation between γ -H₃-40 of the *N*-Me-Thr and α -H-43 (*N*-Me Leu) in combination with MS fragments of *m/z* 535 and 567 (Figure 1, **C**) oriented *N*-Me-Thr as the fifth residue in the depsipeptide backbone, thus linking partial structures **A** and **B**. This sequence of residues was also consistent with MS² fragments observed by LC–MS of the base hydrolysate of **1**, which comprised four major linear products (**2–5**, pS30). Finally, an HMBC correlation from H-39 to carbonyl C-1 (δ_C 170.4) indicated an ester linkage from *N*-Me-Thr to the C-terminal *N*-Me-Ala to complete the planar structure of coibamide A (**1**).

Acid hydrolysis of **1** followed by various HPLC–MS and GC–MS methodologies was used to determine the absolute configuration of coibamide A. While some standards were commercially available (*N*-Me-Leu, *N*-Me-Ile, *N*-Me-Ala, Ala, HIV and *O*-Me-Tyr), others required laboratory synthesis by standard methods (*N*-Me-Thr, *N,N*-diMe-Val, and *N,O*-diMe-Ser). Chiral HPLC (Phenomenex Chirex phase 3126 (D), 4.6 × 250 mm) established the presence of *O*-Me-L-Tyr, two *N,O*-diMe-L-Ser residues, *N,N*-diMe-L-Val, and L-Ala, while chiral GC–MS (CyclosilB, 30.0 m × 250 μ m × 0.25 μ m) of methylated standards and the natural product hydrolysate identified L-HIV. Treatment of the acid hydrolysate of **1** with Marfey's reagent, followed by C₁₈ HPLC established the presence of *N*-Me-L-Ile, *N*-Me-L-Leu,

N-Me-L-Ala, and either *N*-Me-L-Thr or *N*-Me-L-*allo*-Thr. The presence of *N*-Me-L-Thr is proposed from computational models⁹ of the two possible coibamide structures, constrained by ROESY correlations between *N*-CH₃-4 and CH₃-40, and between α -H-2 and *N*-CH₃-48.

Coibamide A displayed potent cytotoxicity to NCI-H460 lung cancer cells and mouse neuro-2a cells (LC₅₀ < 23 nM), but did not interfere with tubulin or actin in cytoskeletal assays. Flow cytometric studies showed that **1** caused a significant dose-dependent increase in the number of cells in the G₁ phase of the cell cycle with little change in G₂/M and a loss of cells in S phase (see Supporting Information). Coibamide A was evaluated against the NCI's in vitro panel of 60 cancer cell lines and produced mean cytostatic (GI₅₀ and TGI with range) and cytotoxic (LC₅₀ and range) parameters as follows: log GI₅₀, −8.04 (2.96); log TGI, −5.85 (3.43); log LC₅₀, −5.11 (2.66). These log mean values of < −4 with log range values of > 2 indicate both potency and histological selectivity. Coibamide A showed highest potency (GI₅₀) to MDA-MB-231 (2.8 nM), LOX IMVI (7.4 nM), HL-60(TB) (7.4 nM), and SNB-75 (7.6 nM) and good histological selectivity for breast, CNS, colon, and ovarian cancer cells (see Supporting Information). Coibamide A was COMPARE negative,¹⁰ indicating that it likely inhibits cancer cell proliferation through a novel mechanism.

In summary, coibamide A (**1**) is a promising lead agent in cancer drug discovery, with a potentially new mechanism of action. Further investigation of the molecule is being pursued via chemical synthesis, since the producing organism has not yet been cultured successfully in the laboratory.

Acknowledgment. Financial support from the NIH Fogarty International Center (ICBG Grant TW006634) and NCI (CA52955), and from the William Randolph Hearst Foundation (to S.L.M.) is gratefully acknowledged. We thank the Smithsonian Tropical Research Institute, crew of R/V Urraca, T. Capson, C. Guevara, and R. Thacker for collection of the cyanobacterium and Autoridad Nacional del Ambiente (ANAM), Panama, for permission to make this collection, OSU Biochemistry NMR Facility for 600 MHz spectrometer time, J. Morre and the OSU EIHS Center for MS data acquisition (NIEHS P30 ES00210), Prof. F. D. Horgen for *N,O*-diMeSer standards, and C. Anklin (Bruker Biospin) for 700 MHz NMR data.

Supporting Information Available: Experimental section and analytical data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Simmons, T. L.; Coates, R. C.; Clark, B. R.; Engene, N.; Gonzalez, D.; Esquenazi, E.; Dorrestein, P. C.; Gerwick, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4587–4594.
- (2) Rawat, D. S.; Joshi, M. C.; Joshi, P.; Atheaya, H. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 33–40.
- (3) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. *Alkaloids* **2001**, *57*, 75–184.
- (4) Tan, L. T. *Phytochemistry* **2007**, *68*, 954–979.
- (5) (a) Loffert, A. J. *Pept. Sci.* **2002**, *8*, 1–7. (b) Morishita, M.; Peppas, N. A. *Drug Discovery Today* **2006**, *11*, 905–910.
- (6) Coiba National Park, <http://whc.unesco.org/en/list/1138>.
- (7) Nyberg, N. T.; Duus, J. Ø.; Sørensen, O. W. *J. Am. Chem. Soc.* **2005**, *127*, 6154–6155.
- (8) Martin, G. E.; Hadden, C. E. *J. Nat. Prod.* **2000**, *63*, 543–585.
- (9) Macromodel 9.1, see Supporting Information, pS6.
- (10) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088–1092.

JA801383F