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

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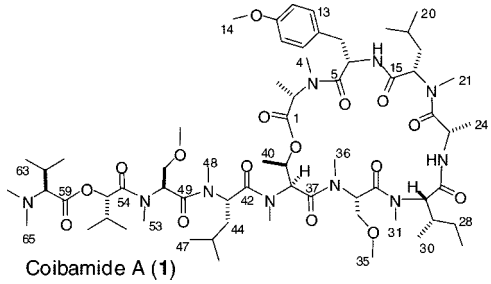
Marine organisms continue to yield a diverse array of biologically active molecules, a remarkable number of which are peptide-based cancer cell toxins of putative microbial symbiont biogenesis.[1] Development of these as anticancer drugs has met with some success:[2] ascidian-derived dihydrodidemnin 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 (IC_{50} 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 C_{18} solid phase extraction and isocratic HPLC to yield the optically active colorless oil ($[\alpha]_D$ −54.1) coibamide A (**1**, 6.3 mg).

The molecular composition of **1** was established as $C_{65}H_{110}O_{16}N_{10}$ from FT-MS data ($[M + H]^+$ *m/z* 1287.8156, Δ −2.4 mmu). The peptidic nature of **1** was evident from its complex ^{1}H NMR spectra in all solvents ($CDCl_3$, C_6D_6, DMSO, C_5D_5N). However, *N*-methyl conformations were minimized in $CDCl_3$ 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 ^{13}C NMR spectra for **1** in $CDCl_3$ and C_6D_6 featured an indeterminate number of resonances, with numerous ester/amide carbonyl ^{13}C 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 ($CDCl_3$) including COSY, TOCSY, multiplicity-edited HSQC, HSQC-TOCSY, HMBC, H2BC[7] and ^{1}H−^{15}N 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 ($\delta_{H\text{-}64/65}$ 2.34) that was HSQC-correlated to a prominent ^{13}C resonance ($\delta_{C\text{-}64/65}$ 41.3) and ^{15}N-gHMBC-correlated to a shielded ^{15}N resonance (δ_N 24.6). Fortunately, nine of ten N atoms in **1** were observed in the latter ^{15}N-gHMBC[8] 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 H_2-7 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 ($\delta_{H\text{-}56}$ 2.21) and methyl ($\delta_{H3-57/58}$ 1.06) resonances. At this point, it remained to assign 114 mass units (interpreted as $C_5H_8O_2N$ = *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 (H_3-40) 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, $CDCl_3$; 3.11 ppm, C_6D_6). Variable temperature experiments in $CDCl_3$ (298−328K, 700 MHz, 1 mm

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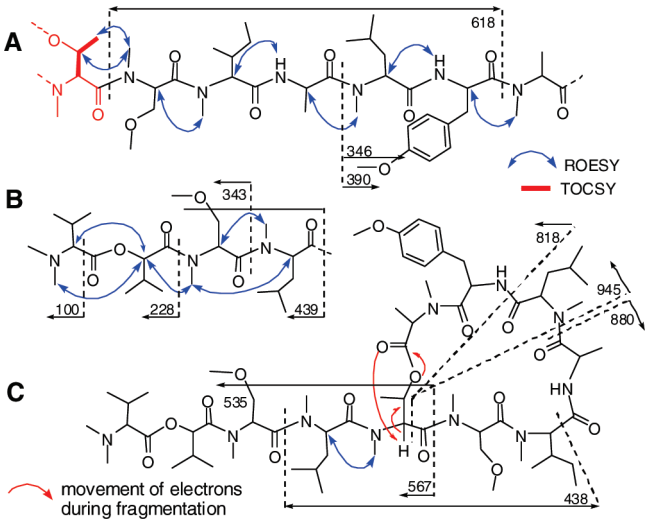

Figure 1. Partial structures **A** and **B** assembled from key ROESY correlations and mass fragments; structure **C** shows the key mass spectrometric fragments supporting the position of *N*-Me-Thr and cyclization of **1**.

cryoprobe) resolved this broad peak into a 3H singlet which was HSQC-correlated to an *N*-methyl resonance ($\delta_{C\text{-}41}$ 29.7). Furthermore, careful examination of C_6D_6 HSQC data revealed an additional heteroatom-substituted methine ($\delta_{H\text{-}38}$ 6.72, $\delta_{C\text{-}38}$ 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*-CH_3-36 and the *N*-Me-Thr β- and γ-protons (H-39, H_3-40) positioned this residue at the *N* terminus of partial structure **A**.

A ROESY correlation between γ-H_3-40 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 MS^2 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 μm × 0.25 μm) of methylated standards and the natural product hydrolysate identified L-HIV. Treatment of the acid hydrolysate of **1** with Marfey's reagent, followed by C_{18} 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 models[9] of the two possible coibamide structures, constrained by ROESY correlations between *N*-CH_3-4 and CH_3-40, and between α-H-2 and *N*-CH_3-48.

Coibamide A displayed potent cytotoxicity to NCI-H460 lung cancer cells and mouse neuro-2a cells (LC_{50} < 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 G_1 phase of the cell cycle with little change in G_2/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 (GI_{50} and TGI with range) and cytotoxic (LC_{50} and range) parameters as follows: log GI_{50}, −8.04 (2.96); log TGI, −5.85 (3.43); log LC_{50}, −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 (GI_{50}) 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.

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

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