Communications to the Editor

Salinamides A and B: Anti-Inflammatory Depsipeptides from a Marine Streptomycete

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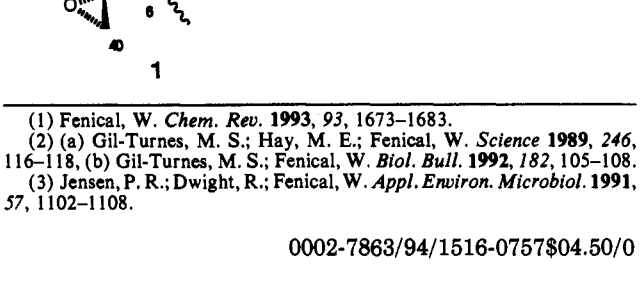
Terrestrial actinomycetes or filamentous bacteria produce a large number of antibiotic and associated compounds. For the past years, we have explored the biosynthetic potential of diverse classes of marine bacteria to determine whether they produce secondary metabolites that extend the molecular diversity of their terrestrial counterparts. Uniquely adapted actinomycetes exist in marine habitats,[1] and these habitats include the surfaces of animals and plants[2] as well as shallow water sediments.[3] Previous reports have documented the production of new antibiotics[4] and antitumor agents[5] from this source. In this Communication, we augment these earlier observations by reporting the structures of the salinamides **1** and **2**, anti-inflammatory cyclic depsipeptides with unusual structures.



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The salinamide-producing actinomycete was isolated from the surface of the jellyfish *Cassiopeia xamachana* collected in the Florida Keys. Fermentation in seawater-based media, followed by double EtOAc extraction of the whole-broth suspension, vacuum-flash chromatography, and reversed-phase HPLC, yielded only salinamide A (**1**) as approximately 9% of the dry extract. Salinamide A (**1**), a pale yellow noncrystalline solid, analyzed for $C_{51}H_{69}N_7O_{15}$ by high-resolution FABMS. A variety of NMR spectral techniques revealed a great deal about the components of **1**, but they did not conclusively lead to a structural formulation. Analyses by both ^1H and ^{13}C NMR showed that **1** contained seven known amino acids and two non-amino acid pieces. Six of the amino acids could be linked into larger fragments: Gly/Ser/Thr and *N*-methyl-Phe/α-(hydroxyphenyl)-Gly/Ile. In addition, the Gly residue formed an amide link to a non-amino acid fragment containing an olefin and an epoxide, and another nine-atom, non-amino acid fragment, C50–C58, was identified.

A major breakthrough in the structural elucidation came when a subsequent fermentation yielded roughly equal portions of salinamide A (**1**) and a new crystalline compound, salinamide B (**2**), $C_{51}H_{70}N_7O_{15}Cl$, mp 239–241 °C. Salinamides A and B are related as epoxide and chlorohydrin. The structure of **2** was established by single crystal X-ray diffraction analysis, and a computer-generated stereo drawing is given in Figure 1.[6] Since the X-ray analysis determined only the relative stereostructure, chiral GC analysis of the hydrolytic fragments (2*R*,3*S*)- (d-) Thr and (2*S*,3*S*)- (l-allo-) Thr was used to set the absolute stereochemistry shown.[7]

The core of salinamide B (**2**) is a bicyclic hexadepsipeptide with two ester links involving serine (Gly to Ser at C1–O28 and Ser to Thr at C25–O24) as well as an aromatic ether link [C8–O9]. The connectivity of **2** severely limits the flexibility of the central core—a feature that complicates the NMR analysis by increasing relaxation times—and a CPK model shows a tightly packed molecular interior. Hydrophobic interactions dominate the left-hand side of **2** (Figure 1). The aromatic ring of the α-(hydroxyphenyl)glycine participates in two important interactions: a π–π stacking with the C5–C6 double bond and a face-to-edge aromatic stack with Phe. The tight turn in this region prevents rotation of the (hydroxyphenyl)glycine ring about the O9–C16 axis. These aromatic protons are barely visible as broad humps in the room temperature ^1H NMR spectrum, but at −30 °C they become broad doublets with a coupling of 8 Hz. Distinct ^{13}C shifts are seen for this ring, and the lack of flexibility can be further appreciated by noting that in a ROESY experiment the proton on C8 correlates only to the C11 proton and not the C15 proton. Intramolecular hydrogen bonds—12 of the 16 total—organize the right-hand side of **2**. A relatively open cleft

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(2) (a) Gil-Turnes, M. S.; Hay, M. E.; Fenical, W. *Science* **1989**, *246*, 116–118, (b) Gil-Turnes, M. S.; Fenical, W. *Biol. Bull.* **1992**, *182*, 105–108.
(3) Jensen, P. R.; Dwight, R.; Fenical, W. *Appl. Environ. Microbiol.* **1991**, *57*, 1102–1108.
(4) (a) Pathirana, C.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1993**, *33*, 7663–7666, (b) Pathirana, C.; Jensen, P. R.; Dwight, R.; Fenical, W. *J. Org. Chem.* **1992**, *57*, 740–742, (c) Okami, Y.; Hoptta, K.; Yoshida, M.; Ikeda, D.; Kondo, S.; Umezawa, H. *J. Antibiot.* **1979**, *32*, 964–96 6.
(5) (a) Tapiolas, D. M.; Roman, M.; Fenical, W.; Stout, T. J.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 4682–4683, (b) Takahashi, A.; Kurosawa, S.; Ikeda, D.; Okami, Y.; Takeuchi, T. *J. Antibiot.* **1989**, *42*, 1556–1561.
(6) Crystals of **2** grown from vapor diffusion of water into methanol solutions belonged to space group $P2_12_12_1$ with $a = 8.801(4)$, $b = 22.595(7)$, and $c = 27.776(5)$ Å and $C_{51}H_{70}N_7O_{15}Cl\cdot H_2O$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 115°$ were collected using $\theta/2\theta$ scans of variable speed. Of the 4232 reflections measured, 2709 (64%) were judged observed. The structure was solved and refined with the SHELXTL series of programs. The final model with anisotropic heavy atoms, isotropic riding hydrogens, and anomalous scattering corrections refined to a conventional crystallographic residual of 0.0687. Additional crystallographic material is included in the supplementary material, and archival data have been deposited with the Cambridge Crystallographic Data Centre.
(7) Charles, R.; Beitler, B.; Gil-Av, E. J. *J. Chromatogr.* **1978**, *112*, 121–133.

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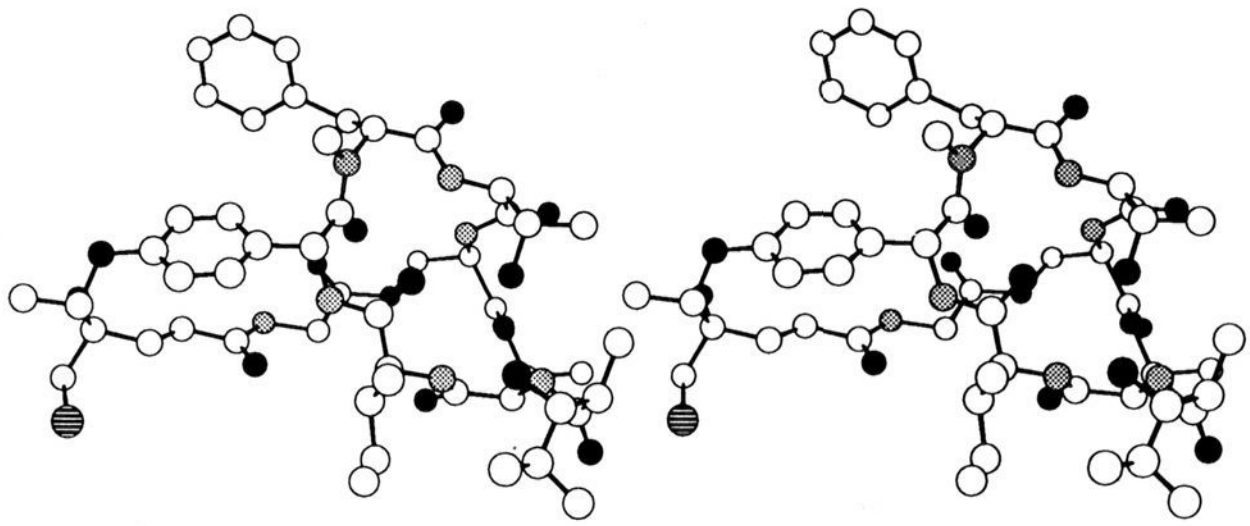

Figure 1. Computer-generated stereo drawing of the final X-ray model of salinamide B (**2**). Hydrogens are omitted for clarity.

runs between the α-(hydroxyphenyl)glycine ring and the adjacent glycine loop. Computer modeling studies show that this cleft could accommodate two residues of a peptide such as D-Ala-D-Ala, and this raises the interesting possibility that salinamide's selectivity as a Gram-positive antibiotic is related to vancomycin's well studied mode of action.[8] A fragment bound in this cleft would also be in close proximity to a potential alkylating group.

With the structure of salinamide B (**2**) firmly in hand, we turned to salinamide A (**1**). Upon treatment with HCl, salinamide A (**1**) converts to a halohydrin that is identical in all respects to salinamide B (**2**), indicating that **1** and **2** are related as shown. The salinamides represent a new class of bicyclic depsipeptides, and no related structures have been reported from terrestrial actinomycetes. The salinamides possess structural components, the α-phenylglycine residue and the non-peptide portions, which are either unknown or of limited distribution in other naturally-occurring compounds.

Salinamides A and B exhibit moderate antibiotic activity against Gram-positive bacteria. The most potent *in vitro* activity is against *Streptococcus pneumoniae* and *Staphylococcus pyrogenes* with MIC values of 4 μg/mL for salinamide A and 4 and 2 μg/mL for B. More importantly, salinamides A and B show potent topical anti-inflammatory activity using the phorbol ester-induced mouse ear edema assay. Salinamide A shows 84% inhibition of edema and salinamide B shows 83% inhibition at the standard testing dose of 50 μg/ear.

(8) (a) Williams, D. H.; Waltho, J. P. *Biochem.* Pharmacol. **1988**, *37*, 133–141, (b) Williamson, M. P.; Williams, D. H. *Eur. J. Biochem.* **1984**, *138*, 343–346. (c) Waltho, J.; Williams, D. H. *J. Am. Chem. Soc.* **1989**, *111*, 2475–2480.

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Supplementary Material Available: X-ray data for salinamide B (**2**) and spectral data for salinamides A and B (17 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.