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Biosynthetic Origin of Alchivemycin A, a New Polyketide from *Streptomyces* and Absolute Configuration of Alchivemycin B

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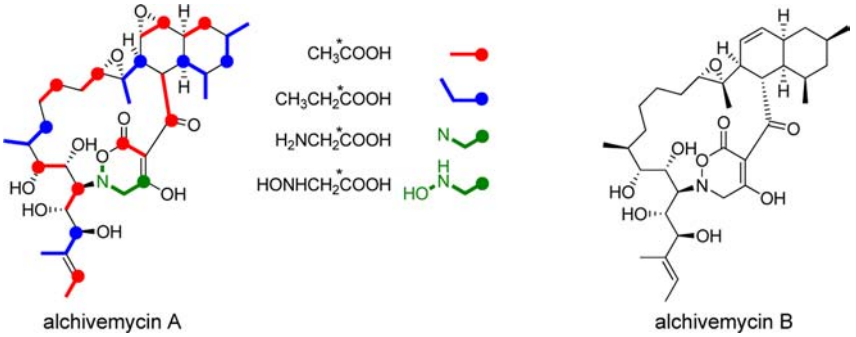
ABSTRACT



Biosynthetic origin of 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine, an unprecedented structural unit first discovered in alchivemycin A (1), was investigated by feeding ^{13}C-labeled precursors. Incorporations of both [1-^{13}C]glycine and [1-^{13}C]-*N*-hydroxyglycine into the carbon at the 4-position of this six-membered ring indicate that the hydrooxazine ring is assembled through a PKS-NRPS hybrid pathway. Additionally, alchivemycin B (2), a deoxygenated analog of 1, was isolated and its relative and absolute configurations were determined by spectroscopic analysis including NMR and CD and X-ray crystallography.

Polyketides are one of the largest classes of secondary metabolites, distributed in a wide range of organisms including bacteria, fungi, and plants. These compounds have served as an invaluable source for drug discovery due to their desirable medicinal properties as well as high structural diversity.[1] Polyketide synthases (PKSs) are multifunctional enzymes responsible for the biosynthesis of polyketides. According to the variation of the chain elongation system, PKSs are largely divided into type I that gives rise to aliphatic compounds such as macrolides and types II and III that essentially afford aromatic compounds such as anthraquinones and flavonoids.[2] Bacterial type I PKSs are multifunctional multienzymes containing all the active sites required for each step of the chain extension, and thus are much larger than fungal type I PKSs in which a single module for chain elongation works repetitively.[3] This difference makes the bacterial type I PKS-derived products larger, more complex with more chiral centers than the fungal counterparts. Nonribosomal peptide synthetases (NRPSs) use a similar strategy to type I PKS for the assembly of amino acid components.[4] Fusion

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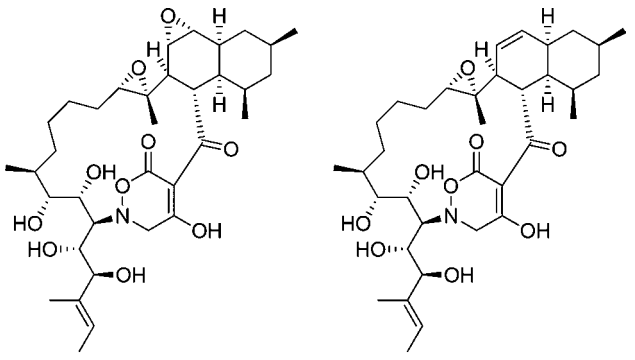
of type I PKS and NRPS enables incorporation of nitrogen atom(s) into the polyketide backbone,[5] providing additional structural variations as exemplified by an immunosuppressant FK506[6] and an antitumor agent epothilone.[7]



alchivemycin A (**1**) alchivemycin B (**2**)

Alchivemycin A (**1**) is a polycyclic polyketide discovered from an extract of *Streptomyces* through our LC/UV-based chemical screening targeted at bacterial type I PKS products.[8] **1** exhibits selective antimicrobial activity against *Micrococcus luteus* with an MIC value of 0.03 μg/mL and inhibits the invasion of murine colon carcinoma 26-L5 cells into Matrigel with an IC_{50} of 0.34 μM without showing cytotoxic effects. Further chemical analysis of the extract of this strain led to the isolation and characterization of alchivemycin B (**2**), a deoxy analog of **1** with more potent antimicrobial activity against *M. luteus* (MIC = 0.004 μg/mL) and a comparable inhibitory effect on tumor cell invasion (IC_{50} = 1.9 μM).

The most intriguing structural feature present in alchivemycins is the 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine ring that is unprecedented in nature. This nitrogen- and oxygen-containing six-membered ring apparently looks like a hybrid of tetramic acid and tetronic acid. In polyketides, the tetramic acid ring is assembled by condensation of a polyketide unit and an α-amino acid and the following Dieckman cyclization,[9] while a three-carbon unit, probably 1,3-bisphosphoglyceric acid derived from the glycolytic pathway, is proposed to be a precursor for the tetronic acid ring assembly.[10] According to the structural analogy to tetramic acid, we hypothesized that the tetrahydrooxazine is constructed by condensation of a polyketide chain and *N*-hydroxyglycine which could be derived from the oxidation of glycine. To explore the validity of this hypothesis, incorporation experiments with plausible ^{13}C-labeled precursors were carried out. Herein, we describe the results from the ^{13}C-labeling experiments of alchivemycin A (**1**) and the isolation and structure determination of alchivemycin B (**2**).

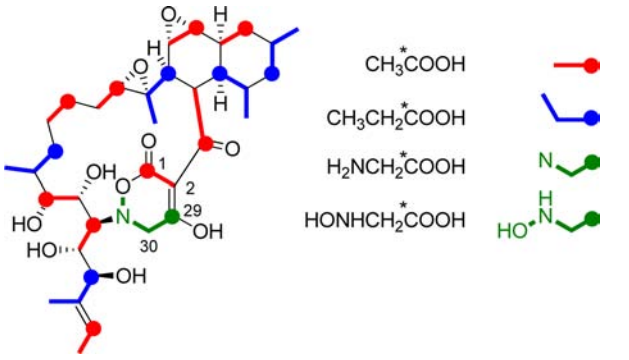


Figure 1. Incorporation of ^{13}C-labeled precursors into **1**.

In order to elucidate the biosynthetic origin of the tetrahydrooxazine ring, incorporation of plausible ^{13}C-labeled precursors into **1** was investigated (Figure 1). First, [1-^{13}C]acetate and [1-^{13}C]propionate were fed to the cultures to confirm the extender units of the polyketide backbone (Table 1). High levels of enrichments were observed at C-1, C-3, C-9, C-11, C-15, C-17, C-21, C-23, and C-27 in [1-^{13}C]acetate-labeled **1**, while C-5, C-7, C-13, C-19, and C-25 were equally enriched in [1-^{13}C]-propionate-labeled **1**. Two carbons C-29 and C-30 in the tetrahydrooxazine ring were not labeled by these precursors, indicating that these carbons are not derived from the malonate pathway. As expected, [1-^{13}C]glycine was incorporated into **1** with high efficiency to give enrichment only at C-29. Next, we examined the incorporation of [1-^{13}C]-*N*-hydroxyglycine which was prepared by the treatment of [1-^{13}C]bromoacetic acid with *N*-hydroxylamine in methanol.[11] C-29 was again enriched by [1-^{13}C]-*N*-hydroxyglycine at a significant incorporation level. These results provided the convincing evidence that the tetrahydrooxazine ring was assembled from a polyketide and an amino acid component.

Based on the labeling experiments, we propose the biosynthetic route to the tetrahydrooxazine ring through a PKS-NRPS hybrid pathway as illustrated in Figure 2. Glycine might be a direct substrate for NRPS giving an enzyme-bound glycine which would be hydroxylated later and condensed with the polyketide moiety. Alternatively,

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Table 1. Incorporation of ^{13}C-Labeled Precursors into **1**

		relative enrichments[a]			
	δ_C	[1-^{13}C]-acetate	[1-^{13}C]-propionate	[1-^{13}C]-glycine	[1-^{13}C]-*N*-hydroxy-glycine
1	176.3	**2.26**	0.85	0.68	0.86
2	104.8	0.79	0.58	1.05	1.00
3	201.6	**3.69**	0.96	1.24	1.19
4	38.0	0.92	0.67	1.07	1.00
5	43.0	1.45	**7.47**	1.11	1.04
6	39.3	0.93	0.87	1.04	0.99
7	39.5	1.17	**7.59**	0.94	1.15
8	34.5	1.72	1.83	1.28	0.87
9	39.4	**2.81**	1.04	1.17	1.05
10	40.8	0.88	0.85	0.96	0.85
11	134.4	**2.74**	0.90	1.00	1.00
12	126.0	0.96	0.88	0.94	1.04
13	54.3	1.52	**7.58**	1.14	1.11
14	61.5	0.88	0.82	1.11	1.02
15	64.4	**3.03**	0.87	1.12	1.05
16	25.2	0.88	0.87	1.01	1.00
17	27.2	**2.88**	0.85	1.16	1.08
18	27.4	1.56	1.02	1.25	1.26
19	34.3	0.95	**6.40**	1.03	0.89
20	36.1	0.81	0.64	0.85	0.91
21	79.7	**3.00**	1.09	1.41	1.19
22	74.8	1.10	1.02	1.22	1.18
23	62.2	**3.00**	0.63	1.00	1.03
24	71.3	1.01	0.95	1.17	1.12
25	79.9	1.14	**6.41**	1.04	0.90
26	136.9	0.97	1.02	1.04	1.07
27	123.6	**2.46**	0.71	0.84	0.83
28	13.3	1.00	1.03	1.08	1.05
29	192.1	1.03	0.95	**13.35**	**2.41**
30	63.5	0.72	0.84	1.00	1.00
31	21.6	0.98	0.80	1.07	0.96
32	22.8	1.00	1.00	1.16	1.12
33	13.8	1.15	0.94	1.23	1.34
34	11.9	1.00	0.90	1.00	1.09
35	11.7	0.94	0.88	1.00	1.01

[a] ^{13}C signal intensity of each peak in the labeled alchivemycin A divided by that of the corresponding signal in the unlabeled, normalized to give an enrichment ratio of alchivemycin A for enriched peak (C-28 for [1-^{13}C]acetate, C-32 for [1-^{13}C]propionate, and C-30 for [1-^{13}C]-glycine labeling). The numbers in bold type indicate ^{13}C-enriched atoms from ^{13}C-labeled precursors.

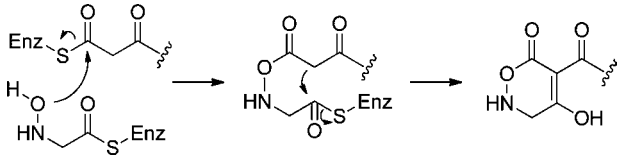

Figure 2. Proposed biosynthetic pathway for tetrahydrooxazine ring.

glycine could be hydroxylated early to give *N*-hydroxy-glycine which is then utilized as a substrate for NRPS. An extremely high incorporation ratio of glycine into **1** likely suggests that glycine is the direct substrate for NRPS; while it is possible that NRPS could accept both glycine and *N*-hydroxyglycine as substrates, glycine is preferred.[12]

In the course of this study, a minor peak showing a UV spectrum (λ_{max} 224, 285 nm) almost identical to that of **1** was detected. The 1-butanol extract of the whole culture broth was successively subjected to silica gel and ODS column chromatographies and reversed-phase HPLC purification using an ODS column to yield alchivemycin B (**2**) as optically active colorless needles. The IR spectrum of **2** displayed absorption bands for the hydroxy and carbonyl groups at 3417 and 1647 cm^{-1}, respectively. The molecular formula of **2** was determined as $C_{35}H_{53}NO_9$ (obsd $[M-H]^-$ at *m*/*z* 630.3641, calcd $[M-H]^-$ at *m*/*z* 630.3642) on the basis of the high-resolution ESITOFMS data, corresponding to the loss of one oxygen atom from **1**.

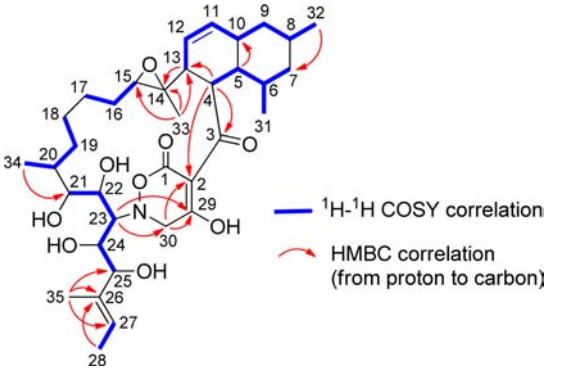

Figure 3. 2D NMR correlations for **2**.

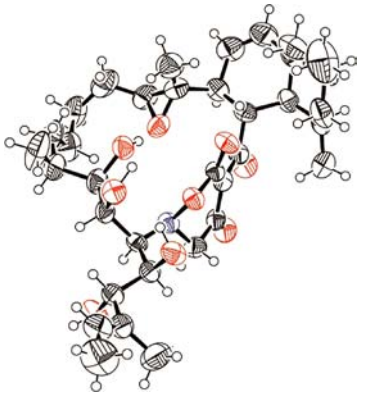

Figure 4. ORTEP drawing of crystal structure of **2**.

1D- and 2D-NMR analysis revealed that most of the proton and carbon chemical shifts of **2** showed high similarity to **1** except for the resonances at C-11 and

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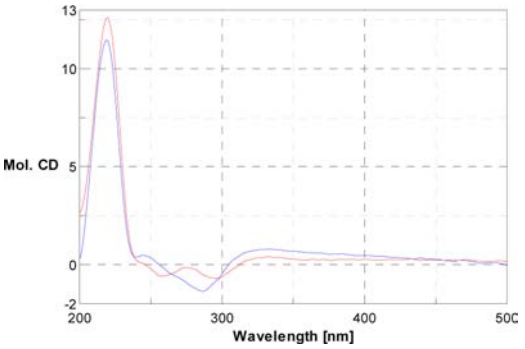

Figure 5. CD spectra of alchivemycins in MeOH (blue: **1**; red: **2**).

C-12. ^{1}H NMR signals of H-11 (δ 5.86) and H-12 (δ 5.39) of **2** were resonated downfield relative to those of **1** (H-11, δ 3.11; H-12, δ 3.05), suggesting that **2** had a double bond between C-11 and C-12 instead of an epoxy functionality. This was consistent with the olefinic ^{13}C NMR resonances for C-11 (δ 134.4) and C-12 (δ 126.0). The remaining part of the planar structure of **2** was readily assigned by comparing the NMR data with **1** (Figure 3, Table S1).

Recrystallization of **2** from a mixture of dichloromethane and methanol afforded colorless prisms suitable for X-ray crystallographic analysis (CDCC accession No. 739981, Figure 4). Relative configurations of all the chiral centers present in **2** were identical with those in **1**. Although **2** showed positive optical rotation ($[\alpha]_D^{22}$ +35 (*c* 1.0, MeOH)), opposite to that of **1** ($[\alpha]_D^{23}$ −17 (*c* 1.0, MeOH)), circular dichroism (CD) spectra of **1** and **2** displayed nearly identical CD curves with a strong positive Cotton effect around 218 nm (Figure 5). In view of the biosynthetic relationship, it is most likely that **1** and **2** possess the same absolute configuration.[13]

In summary, we have proposed a biosynthetic pathway for tetrahydrooxazine ring formation in alchivemycins on the basis of ^{13}C-labeling experiments. Future investigations will be focus on biosynthetic gene analysis to identify the enzymatic reactions responsible for tetrahydrooxazine ring formation. Concomitantly, alchivemycin B (**2**), a new minor congener of alchivemycin A (**1**), was isolated and its absolute configuration was elucidated by spectroscopic and X-ray crystallographic analyses.

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Supporting Information Available. Experimental details; NMR data, 1D/2D NMR spectra, UV and IR spectra of **2**; ^{13}C NMR spectra of **1** derived from ^{13}C-labeled precursors. This material is available free of charge via the Internet at http://pubs.acs.org.

(13) Chemical derivatization with chiral anisotropic reagents (e.g., MTPA, MPA) failed, giving complex mixture of esters difficult to be separated.

The authors declare no competing financial interest.