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Alchivemycin A, a Bioactive Polycyclic Polyketide with an Unprecedented Skeleton from *Streptomyces* sp.†

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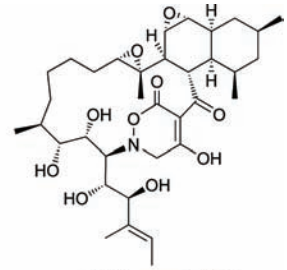
ABSTRACT



Alchivemycin A (**1**)

Alchivemycin A, a novel polycyclic polyketide, was isolated from the culture extract of a plant-derived actinomycete *Streptomyces* sp. The structure and relative configuration were elucidated by spectroscopic analysis and X-ray crystallography, and the absolute configuration was determined by a ^{1}H NMR anisotropy method using MPA ester derivatization. The new compound contains an unprecedented heterocyclic ring system, 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine. Alchivemycin A showed potent antimicrobial activity against *Micrococcus luteus* and inhibitory effects on tumor cell invasion.

Actinomycetes have provided a wide array of bioactive secondary metabolites. In particular, those belonging to the genus *Streptomyces* are the most prolific source of chemically diverse metabolites.[2] Their high capacity of secondary metabolite biosynthesis is largely attributable to the diversity of polyketide synthase (PKS), which gives rise to more complex molecular architectures in combination with non-ribosomal peptide synthase (NRPS).[3,4] In our continuing search for structurally unique metabolites from this genus,[5] alchivemycin A (**1**), a novel metabolite possessing an unprecedented framework presumably derived from PKS-

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(1) Part 35: Igarashi, Y.; Shimasaki, R.; Miyanaga, S.; Oku, N.; Onaka, H.; Sakurai, H.; Saiki, I.; Kitani, S.; Nihira, T.; Wimonsiravude, W.; Panbangred, W. *J. Antibiot.* **2010**, advance online publication June 30, 2010, DOI: 10.1038/ja.2010.70.

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(4) Dewick, P. M. *Medicinal Natural Products−A Biosynthetic Approach*, 3rd ed.; John Wiley & Sons; New York, 2009.
(5) (a) Igarashi, Y.; Iida, T.; Yoshida, R.; Furumai, T. *J. Antibiot.* **2002**, *55*, 764–767. (b) Igarashi, Y.; Futamata, K.; Fujita, T.; Sekine, A.; Senda, H.; Naoki, H.; Furumai, T. *J. Antibiot.* **2003**, *56*, 107–113. (c) Igarashi, Y.; Miura, S.; Fujita, T.; Furumai, T. *J. Antibiot.* **2006**, *59*, 193–195.

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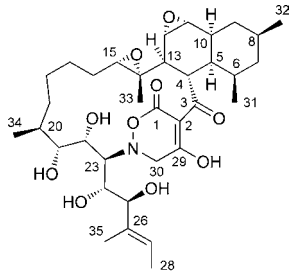
NRPS hybrid biosynthesis, was found from the culture extract of a *Streptomyces* strain collected from a leaf of a Chinese chive (*Allium tuberosum*)[6] by HPLC/UV-based chemical screening. The producing strain TP-A0867 was cultured in A-11 M medium at 30 °C for 6 days, and the whole culture broth was extracted with 1-butanol. UV-guided fractionation using normal- and reversed-phase column chromatographies, followed by HPLC purification on a C18 column, yielded alchivemycin A (**1**) (Figure 1). The structure and the absolute stereochemistry of **1** were elucidated by chemical and spectroscopic methods. The new compound represents the first example of natural products containing the 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine ring system.



Alchivemycin A (**1**)

Alchivemycin A (**1**)[7] was isolated as optically active colorless needles ($[\alpha]_D$ −17, MeOH; mp 150−153 °C). The molecular formula of **1** was determined as $C_{35}H_{53}NO_{10}$ by high-resolution FABMS data that showed a pseudomolecular ion at *m*/*z* 648.3754 $[M + H]^+$. The IR spectrum of **1** displayed absorption bands at 3433 and 1646 cm^{-1}, indicating the presence of hydroxyl and carbonyl functionalities, respectively. The UV spectrum was closely similar to that reported for tenuazonic acid (λ_{max} 227, 284 nm),[8] suggesting the presence of a tetramic acid-like chromophore in which an acyl functionality is substituted at the α-position. In the ^1H and ^{13}C NMR spectra, most of the signals were doubled in the ratio of 1.2:1 in $CDCl_3$ and 4:1 in CD_3OD. These observations suggested that **1** existed as a mixture of tautomeric isomers derived from the above-mentioned conjugated system.[9] To simplify the analysis, the structure elucidation was undertaken for the major isomer present in CD_3OD.

Interpretation of ^1H, ^{13}C, and HSQC NMR data of **1** allowed the assignment of signals for 35 carbons including six methyls, seven methylenes (one is nitrogen- or oxygen-bearing), 16 methines (eight nitrogen- or oxygen-bearing and one olefinic), and six quaternary carbons (one sp^3, two olefinic, and three oxygen-bearing sp^2). The molecular formula indicated that **1** contained 10 double bond equivalents, which by interpretation of NMR data could be attributed to two carbon−carbon double bonds (C-2/C-29 and C-26/C-27), two carbonyls (C-1 and C-3), and six ring systems.

Detailed 2D NMR analysis revealed that **1** was composed of three substructures (Figure 1). From the $^1H-^1H$ COSY spectrum, five small spin systems were established in substructure A: H-6/H_3-31, H-11/H-12, H-5/H-4/H-13, H_3-32/H-8/H_2-9/H-10, and H-15/H_2-16/H_2-17. These fragments were connected on the basis of HMBC correlations, leading to the assembly of the decalin skeleton. The connectivities from C-5 to C-8 were established by HMBC correlations from H_3-31 to C-5 and C-7 and H_3-32 to C-7. Correlations from H-9 to C-5 and C-11, H-4 to C-10, H-12 to C-4, and H-13 to C-11 connected C-10 to C-11, C-5 to C-10, and C-12 to C-13, respectively. Relatively upfield shifts of C-11 and C-12 along with the small coupling constant (3.0 Hz) between the protons attached to these carbons indicated the presence of an epoxide ring at these carbons. Additional small fragment in substructure A could be assembled starting from the methyl group (C-33). H_3-33 showed HMBC correlations to two oxygenated carbons (C-14 and C-15), which were assigned to the carbon atoms of an epoxide ring on the basis of their chemical shifts. This fragment was then connected to the decalin moiety at C-13 on the basis of HMBC correlations of H-12 to C-14 and H_3-33 to C-13, thereby establishing substructure A.

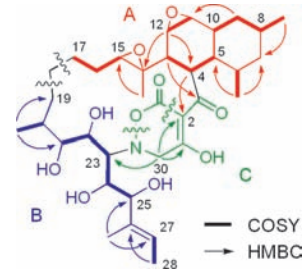


Figure 1. 2D NMR correlations for alchivemycin A (**1**).

In substructure B, three proton-bearing fragments of H-20/H_3-34, H-21/H-22/H-23/H-24/H-25, and H-27/H_3-28 were readily recognized from the $^1H-^1H$ COSY spectrum. These fragments were joined on the basis of HMBC correlations of H_3-34 to C-21, H_3-35 to C-25 and C-27, and H_3-28 to C-26. This unit was expanded to include a methylene carbon C-19 on the basis of an HMBC correlation from H_3-34 to C-19. The carbon chemical shifts suggested the attachment of oxygen atom to C-21, C-22, C-24, and C-25 and nitrogen atom to C-23. The geometry of the double bond between C-26 and C-27 was assigned as *E* on the basis of an NOESY correlation between H-25 and H-27, providing substructure B.

Substructure C was elucidated starting from the methylene protons H_2-30 that showed HMBC correlations to C-29, C-2,

(6) Igarashi, Y.; Iida, T.; Sasaki, T.; Saito, N.; Yoshida, R.; Furumai, T. *Actinomycetologica* **2002**, *16*, 9–13.

(7) Alchivemycin A (**1**): colorless needles; mp 150−153 °C; $[\alpha]^{24}_D$ −17 (*c* 1.0, MeOH); CD (MeOH). $\Delta\varepsilon_{219}$ +12.556, $\Delta\varepsilon_{239}$ +1.470, $\Delta\varepsilon_{245}$ +1.573, $\Delta\varepsilon_{286}$ −0.283; UV (MeOH) λ_{max} (log ε) 222 (3.64), 282 (3.85) nm; (0.01 N HCl−MeOH) 222 (3.76), 284 (3.90); (0.01 N NaOH− MeOH) 205 (3.47), 251 (3.92), 274 (3.92); IR ν_{max} 3433, 1646 cm^{-1}. For ^1H and ^{13}C NMR data, see Table S1 in Supporting Information; HRFABMS $[M + H]^+$ 648.3754 (calcd for $C_{35}H_{54}NO_{10}$, 648.3748).

(8) *Dictionary of Natural Products on DVD-ROM, version 18:1*; Chapman & Hall: London, 2009.

(9) (a) Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981–2001. (b) Schobert, R.; Schlenk, A. *Bioorg. Med. Chem.* **2008**, *16*, 4203–4221.

and C-23. HMBC correlations were also observed from H-4 and H-13 to C-3 and from H-4 to C-2. These data established the four carbon fragment C-3/C-2/C-29/C-30 which was connected to substructure A between C-3 and C-4 and substructure B through a nitrogen atom between C-30 and C-23. The carbon chemical shifts of C-3 (δ_C 201.3), C-2 (δ_C 105.3), and C-29 (δ_C 192.2) could be assigned to the aforementioned α,β-unsaturated ketone in which the β-position was oxygenated. Although there was no HMBC correlation available to locate the carbonyl carbon C-1 (δ_C 176.6), it seemed reasonable that this carbon was connected to C-2 to extend the conjugation, which was consistent with the UV spectroscopic data. The remaining oxygen atom, mandated from the molecular formula, was allowed to be positioned between C-1 carbonyl carbon and the nitrogen atom at C-23, constructing the six-membered heterocyclic ring system. Finally, the remaining methylene fragment (C-18) was placed between C-17 and C-19, connecting partial structures A and B to complete the planar structure of **1**.

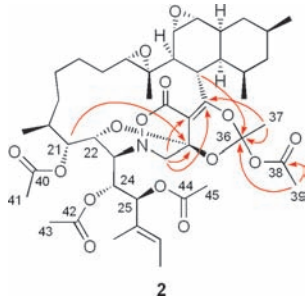


Figure 2. Structure and key HMBC correlations of **2**.

To verify the proposed structure, **1** was treated with acetic anhydride in pyridine to afford a peracetate (**2**). The high resolution FABMS gave an $[M + Na]^+$ peak at *m*/*z* 880.4078 appropriate for $C_{45}H_{63}NO_{15}$ (calcd for $C_{45}H_{63}NO_{15}Na$ *m*/*z* 880.4096), corresponding to the addition of five acetate units. In the ^{1}H NMR spectrum of **2**, resonances for five singlet methyl protons were observed in the region 2.0−2.2 ppm. The oxymethine protons H-21, H-24, and H-25 were shifted downfield and had HMBC correlations to the carbonyl carbons C-40, C-42, and C-44, respectively, to which in turn correlated singlet methyl protons (H_3-41, H_3-43, H_3-45), thereby establishing that three hydroxyl groups (21-OH, 24-OH, 25-OH) were acetylated. The UV spectrum of **2** showed an absorption maximum at 256 nm, which is similar to that for actinobolin,[8] indicating that the conjugated enone system was chemically modified during the acetylation. This was consistent with the ^{13}C NMR data for **2** in which two deshielded carbon signals for C-3 (δ_C 201.3) and C-29 (δ_C 192.2) in **1** were largely shifted upfield to δ_C 171.3 and δ_C 89.8, respectively, while the resonance of C-2 carbon was observed in the similar region. Assignment of these carbons was established on the basis of HMBC correlations from H-13 to C-3, H-4 to C-3 and C-2, and H_2-30 to C-29 and C-2 (Figure 2). Furthermore, a four-bond HMBC correlation was observed from H-21 to C-29, connecting C-29 and C-22 through an oxygen bridge. The remaining methyl protons H_3-37 and H_3-39 were correlated to a quaternary carbon C-36 (δ_C 113.9) and a carbonyl carbon C-38 (δ_C 166.4), respectively, establishing the connectivities for C-36/C-37 and C-38/C-39. Furthermore, four-bond HMBC correlations were shown from H_3-37 to C-3 and H-4 and H_3-39 to C-36. These correlation data, together with its relatively downfield ^{13}C chemical shift, suggested the connectivity of C-36 to C-3 and C-29 through ether linkages and the attachment of an acetoxy group to C-36, constructing an orthoacetate. Formation of this unusual structural unit can be explained by the following reaction sequence: (1) Michael addition of the hydroxyl group at C-22 to C-29 and enolization at C-3 carbonyl carbon, (2) acetylation of either 29-OH or 3-OH, and the following nucleophilic addition of a hydroxyl group to the acetyl carbonyl (C-36), resulting in the formation of orthoester-like structure, (3) acetylation of the tertiary hydroxyl group at C-36.

In the end, because the proposed structure was unusual, **1** was crystallized from a mixture of CH_2Cl_2−MeOH. This provided orthorhombic crystals suitable for X-ray analysis, the result of which confirmed the assigned structure and the relative configuration of **1** (for crystal structure, see Supporting Information).

The absolute configuration of **1** was determined by the Trost's method using α-methoxyphenylacetic acid (MPA).[10,11] Coupling of **1** with 1equiv of (*R*)- and (*S*)-MPA using diisopropylcarbodiimide gave the mono-(*R*)- and (*S*)-MPA esters (**3** and **4**) as the major products. The esters were purified by RP-HPLC, and the site of esterification was confirmed by analysis of ^{1}H, COSY, and HMBC NMR data. In the ^{1}H NMR spectra of **3** and **4**,[12] positive $\Delta\delta_{R-S}$ ($\delta_R - \delta_S$) values were observed for H-21, H-22, H-23, H_2-30, and H_3-34, while negative $\Delta\delta_{R-S}$ values were observed for H-27, H_3-28, and H_3-35 (Figure 3). These data allowed assignment of the absolute configuration of C-25 as 25*S*.

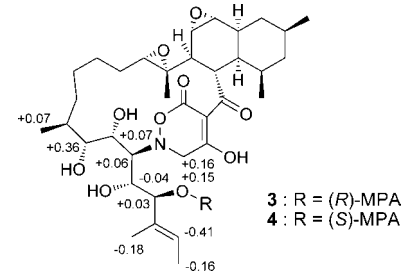


Figure 3. $\Delta\delta_{R-S}$ values for mono-MPA derivatives (**3** and **4**) of alchivemycin A.

Bioactivity of **1** was evaluated in our standard bioassays including antimicrobial activity, cytotoxicity, and anti-

(10) (a) Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* **1986**, *51*, 2370–2374. (b) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–117.

invasive activity.[13] Compound **1** exhibited a selective antimicrobial activity against *Micrococcus luteus* with an MIC value of 50 nM, whereas it was inactive toward *Bacillus subtilis*, *Escherichia coli*, or *Candida albicans*. In addition, **1** inhibited the invasion of murine colon carcinoma 26-L5 cells into Matrigel with an IC_{50} of 0.34 μM without showing cytotoxic effects and lacked significant cytotoxity against murine renal carcinoma Renca cells and human umbilical vein endothelial cells (HUVECs).

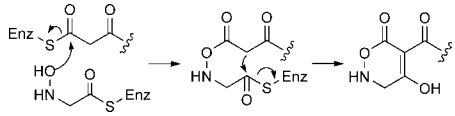
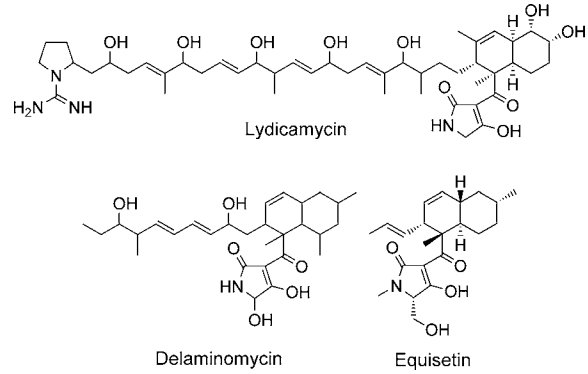


Figure 4. Plausible biogenesis of 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine ring system.

The most structurally intriguing part of **1** is the 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine ring system, which has never been described in either synthetic or natural product chemistry. We assume that this unique heterocyclic structure is assembled through a biosynthetic pathway similar to that for tetramic acid (Figure 4). In polyketide biosynthesis the tetramic acid moiety is constructed by the condensation of an α-amino acid with a growing polyketide chain and the following Dieckmann condensation along with the release of enzyme complex.[9b] Similarly, if *N*-hydroxyglycine, which may be derived from *N*-hydroxylation of glycine,[14] is incorporated onto the polyketide chain tail, the Dieckmann-type cyclization provides the 2*H*-tetrahydro-4,6-dioxo-1,2-oxazine ring. The 2*H*-tetrahydro-1,2-oxazine ring system has been found in natural products from actinomycetes, fungi, and plants, but none of them are of polyketide origin.[15]



The biosynthetically closest compounds to **1** are the tetramic acid antibiotics represented by lydicamycins and delaminomycins from actinomycetes and equisetin-related fungal metabolites which comprise a decalin unit with a linear side chain and a tetramic acid unit.[16] The most significant difference of **1** from these compounds is the additional ring formation between the side chain and the 2*H*-tetrahydro-1,2-oxazine ring through a C−N linkage. The biosynthetic origin of **1** and the structure elucidation of minor congeners are currently under investigation.

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Supporting Information Available: Experimental details; NMR data and 1D/2D NMR spectra of **1** and **2**; ^{1}H NMR spectra of **3** and **4**; ORTEP drawing and a CD spectrum of **1**; X-ray crystallographic file in CIF format for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Mono-(*R*)-MTPA ester of **1** was obtained by treatment with (*S*)-MTPA-Cl, but treatment of **1** with (*R*)-MTPA-Cl did not afford the (*S*)-MTPA ester presumably due to the steric hindrance.

(12) A small amounts of D_2O were added to the acetone-d_6 solution to improve the signal broadening due to the keto-enol tautomerization.

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