Computational Prediction and Experimental Validation of a Bridged Cation Intermediate in Akanthomycin Biosynthesis

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Abstract

Here we report a computation-driven chemoenzymatic synthesis and biosynthesis of the natural product deoxyakanthomycin, an atropisomeric pyridone natural product that features a 7-membered carbocycle with five stereocenters, one of which a quaternary center. The one-step synthesis from a biosynthetic precursor is based on computational analysis that predicted a σ-bridged cation mediated cyclization mechanism to form deoxyakanthomycin. The σ-bridged cation rationalizes the observed substrate-controlled selectivity; diastereoselectivity arises from attack of water *anti* to the σ-bridging, as is generally found for σ-bridged cations. Our studies also reveal a unifying biosynthetic strategy for 2-pyridone natural products that derive from a common *o*-quinone methide to create diverse structures.

Chemists and nature leverage inherent substrate reactivity to conduct stereoselective catalyst-free reactions – perhaps most notably exemplified by Stork's synthesis of germine.[1,2] More commonly, chemists and nature rely on catalyst development to control stereoselectivity, regioselectivity, and chemoselectivity in synthesis. In synthesis, a plethora of small molecule catalysts that select the reaction outcomes of complex molecules have been designed and implemented.[3–5] In comparison, nature evolved a variety of enzymes to impose selectivity via noncovalent interactions. One such family is the recently discovered pericyclases that catalyze pericyclic reactions.[6] Such enzymes can selectively generate complex molecular architectures from acyclic intermediates in a single step exemplified by the structural diversity in the 4-hydroxy 2-pyridone family of natural products (Figure 1).[7] Previously, we have characterized the enzyme-catalyzed pericyclic reactions that lead to pyridone natural products.[8–11] Herein, we describe natural products obtained from the exclusion of enzymes that control these reaction pathways and showcase nature's use of inherent substrate reactivity to synthesize challenging-to-make molecular architectures. This work establishes a

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Computational and experimental methods, characterization of key chemical structures, molecular coordinates, and calculated energies of all chemical structures (PDF)

chemoenzymatic synthesis and biosynthesis of the natural product deoxyakanthomycin (**1a** and **1b**).

Graphical Abstract
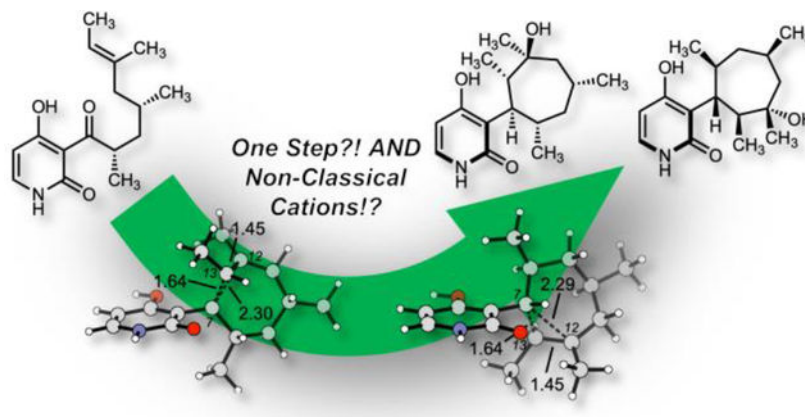
Keywords

Deoxyakanthomycin (**1a**, **1b**), akanthomycin (**2a**, **2b**), cordypyridone A (**3a**), B (**3b**), and C (**4**) are structurally diverse natural products from the 4-hydroxy-2-pyridone natural product family (Figure 1). These natural products share an identical carbon backbone: the 4-hydroxy 2-pyridone connects at C3 to an 8-unit alkyl chain that is methylated at C8 and C10. Structural diversity arises from the various possible intramolecular cyclizations of the alkyl chain. Deoxyakanthomycin (**1a**, **1b**) and akanthomycin (**2a**, **2b**) were originally isolated in 2002 as mixtures of atropisomers (*M* and *P*) and feature a 7-membered carbocycle with 5 stereocenters, one of which at a quaternary carbon C12.[12] Despite multiple attempts, there is no total synthesis of deoxyakanthomycin (**1a**, **1b**) or akanthomycin (**2a**, **2b**).[7,13] The cordypyridones were originally isolated in 2001 and found to have potent anti-malarial activity.[14] Cordypyridone A and B (**3a**, **3b**) are also atropisomeric and feature a vinyl cyclohexyl moiety from a biological Alder-ene reaction. These natural products were synthesized in 2009 in seven steps.[15] In 2020, the Baran lab revamped this synthetic route and completed a total synthesis of (–)-maximiscin, a further functionalized derivative of cordypyridone A and B (**3a**, **3b**) that features an shikimic acid derivative linkage at the pyridone nitrogen.[16] Lastly, cordypyridone C (**4**) is a fused tricyclic structure derived from a hetero-Diels Alder cyclization, and features an *N*-methoxy substituent.[14] Structural diversity among these natural products leads to unique bioactivities ranging from potent anti-bacterial, free radical scavengers, or anti-malarial agents.

We set out to uncover how the unique structures of **1a**, **1b**, **2a**, and **2b** form. Based on our previous work on pyridoxatin (**5a** and **5b**), asperpyridone A (**6**), and related pyridones that are structurally identical except for lacking the methyl group at C12, we predict that the

biosynthesis of the cordypyridones derive from a common *o*-quinone methide intermediate (**7, Figure 1**).[8,9,11] This intermediate undergoes either an enzymatic Alder-ene reaction to form **3a** and **3b** or hetero-Diels–Alder reaction to form **4** and **6** (Figure 1).[8,9,11] Therefore, we propose that **1a**, **1b**, **2a**, and **2b** derive from a common quinone methide intermediate that instead of undergoing a pericyclic reaction, undergoes a cationic cyclization followed by hydration to form the 7-membered carbocycle. Our proposal is different from Clardy's original biosynthetic proposal[12] in which akanthomycin (**2**) is formed by an oxidative ring expansion reaction from cordypyridone A and B (**3a** and **3b**). Of note, the C12 desmethyl derivative of akanthomycin has never been isolated. This indicates that this is likely cation driven as the presence of a C12 methyl group is necessary to form a tertiary carbocation.

We calculated the proposed cationic cyclization pathway to form **1a**, **1b**, **2a**, and **2b** (Figure 2A and S1) from **7** at the ωB97X-D-CPCM(H_2O)/def2-QZVPP//ωB97X-DCPCM(H_2O)/ 6–311+G(d,p) level of theory. These calculations also give evidence to whether the stereochemistries at positions C7 and C12, are substrate-controlled (nonenzymatic) or enzyme-controlled. We employed a protonated model system to best aid in locating transient cationic intermediates. These simplistic model calculations do not account for the barrier to form this protonated intermediate, but such a species should be energetically accessible in a neutral aqueous solution or the enzyme environment. Calculations indicate the protonated (*Z*) *o*-QM **7a** or (*E*) *o*-QM **7b** will readily undergo a cationic cyclization via **TS-1** or **TS-2,** respectively, with a Gibbs free energy of activation ($\Delta G^{\ddagger}$) of 5.3 or 5.1 kcal·mol^{-1} to form the cyclization adducts **8a** and **8b** (Figure 2A). These energetic barriers indicate that if both the protonated (*Z*) and (*E*) *o*-QM **7** are in solution, a mixture of atropisomers of **8** will rapidly form.

Strikingly, the cyclization adducts **8a** and **8b** feature a non-classical σ-bridged cation in which two electrons are delocalized over three carbon atoms (C7, C12, and C13). Such a bonding motif was first proposed by Winstein in his studies of the norbornyl cation.[17] In such structures, σ-bridging leads to cation stabilization through hyperconjugation. This is the first instance of such a structure in natural product biosynthesis outside of terpene biosynthesis.[2,18–20] Structurally, we see elongation of the C7–C13 σ-bond from a typical C–C bond length of 1.54 Å to 1.64 Å and σ-bridging at the adjacent carbon with a C7–C12 distance of ~ 2.30 Å. The C7–C12 distance is well within the cutoff for van der Waals interactions (~ 2.30 Å versus 3.40 Å). The C7–C12 σ-bridging interaction is elongated when compared to the C7–C13 σ-bond due to the asymmetrical substitution pattern. Formally, C12 has an extra electron-donating alkyl group compared to C13 with a hydrogen substituent. This indicates that the C7–C13 interaction is to be stronger than the C7–C12 interaction to best stabilize the bridged cation. The best evidence to validate the computational result proposing a σ-bridged cation is the stereochemistry of **1** and **2**. These natural products **1** and **2** have only been isolated with C12 in the (*S*)-configuration. If this reaction were to proceed via this proposed σ-bridged cation, then one would expect diastereoselectivity in the hydration of the cation. This diastereoselectivity arises from attack of water *anti* to the σ-bridging, as is generally found for σ-bridged cations. Based on the original isolation paper, akanthomycin was isolated as a single diastereomer which validates the proposal of σ-bridged cations.

To validate that this is substrate controlled stereoselectivity, we calculated the *anti-* and *syn-* hydration reaction transition states (**TS-3** and **TS-4** versus **TS-5** and **TS-6**, Figure 2A and S1). The *anti*-hydration transition states are low in energy and preferred over the *syn*-hydration transition states by >1 kcal·mol^{-1} (Figure S1). **TS-3** and **TS-4** lead to atropisomeric deoxyakanthomycin hydrates (**1a'** and **1b'**), which upon deprotonation form **1a** and **1b**. The atropisomeric deoxyakanthomycin hydrates are unable to interconvert between each species due to the hindered rotation C3–C7 bond; the calculated barrier of rotation is greater than 35 kcal·mol^{-1} (Figure S2). The computational studies predict that akanthomycin and deoxyakanthomycin can form non-enzymatically from the common biosynthetic quinone methide intermediate. This proposed reaction cascade forms three stereocenters, one of which is a quaternary carbon, all in one-pot without the need of a catalyst.

To identify the biosynthetic gene cluster of **1** and verify the proposed reaction scheme, we searched and screened the homologous biosynthetic gene clusters of pyridoxatin in the National Center for Biotechnology Information (NCBI) fungal genome database as reported in our previous study[11] as the original producing strain was unavailable. This led us to identify the putative biosynthetic gene cluster (*Sdx*) of **1** in the genome of *Sarocladium oryzae*, which encodes a polyketide synthase-nonribosomal peptide synthetase (PKS–NRPS) (SdxC), a partnering enoylreductase (ER) (SdxD), a ring-expansion P450 (SdxA), a putative *N*-hydroxylation P450 (SdxB), a short-chain dehydrogenase/reductase (SDR) (SdxG) and a putative *O*-methyltransferase (OMT) (SdxI) (Figure 2B). We then heterologously expressed the PKS–NRPS SdxC, the ER SdxD, and the ring expansion P450 SdxA in *Aspergillus nidulans* 1145 ΔEMΔST,[21] which led to the production of ketone **12** (2 mg·L^{-1}). Compound **12** is structurally confirmed to be the dephenylated 2-pyridone ketone (Table S3 and Figures S3-7). To probe the proposed mechanism of the formation of **1** from the quinone methide **7**, we purified soluble SDR homologs to SdxG, including PdxG, AdxG and EpiG and performed the *in vitro* reaction using **12** as the substrate with NADPH as the reducing cofactor. In good agreement with the calculation data, deoxyakanthomycin **1a** and **1b** predominantly formed after overnight incubation and proceeded to completion within 72 h (Figure 2C, iii, and Figure S8, 9). To exclude the participation of the SDR in the carbocycle formation, we performed reduction of **12** with excess NaBH$_4$ and quenched the reaction mixture with excess water. This nonenzymatic reaction similarly formed deoxyakanthomycin **1a** and **1b** (Figure 2C, iv). In trace *iv*, a peak with MS corresponding to the alcohol species **13** is present, which was also present in the enzymatic *in vitro* reactions with shorter reaction times (Figure S9). In summary, deoxyakanthomycin **1a** and **1b** form upon incubation of ketone **12** with a ketone-reducing enzyme or via chemical reaction with NaBH$_4$.

From these computational and experimental results, we propose that the biosynthesis of akanthomycin (**2a**/**2b**) occurs by an enzymatic reduction of the ketone **12** to the corresponding alcohol **13 (Figure 2D)**. This alcohol **13** can slowly dehydrate in solution to form a mixture of the (*E*) and (*Z*) *o*-quinone methide species **7**. Once the quinone methide **7** forms, it can rehydrate to reform the alcohol **13** or cyclize to form the σ-bridged cation **8a** and **8b** which is selectively hydrated to exclusively form atropisomeric

deoxyakanthomycin **1a** and **1b**. Lastly, we predict that deoxyakanthomycin **1a** and **1b** can be *N*-hydroxylated by the putative enzyme SdxB to form akanthomycin **2a** and **2b**. These results showcase how nature can construct a variety of natural product scaffolds from a common intermediate. When nature needs to construct a pyran or cyclohexane, a pericyclase is used. Otherwise, substrate-controlled reactivity is utilized to create difficult-to-synthesize 7-membered rings. This discovery refines our understanding of 2-pyridone natural product biosynthesis and unifies all known biosynthetic routes to go through a reactive *o*-quinone methide intermediate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ABBREVIATIONS

QM	quinone methide
TS	transition state
PKS–NRPS	polyketide synthase-nonribosomal peptide synthetase
ER	enoylreductase
SDR	short-chain dehydrogenase/reductase
OMT	O-methyltransferase

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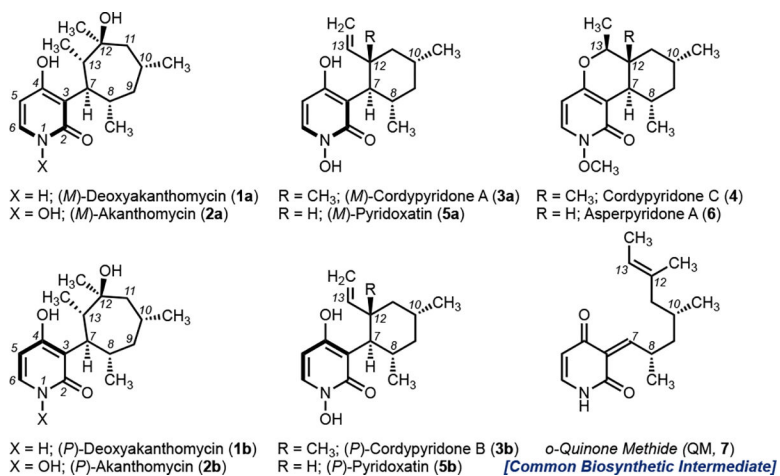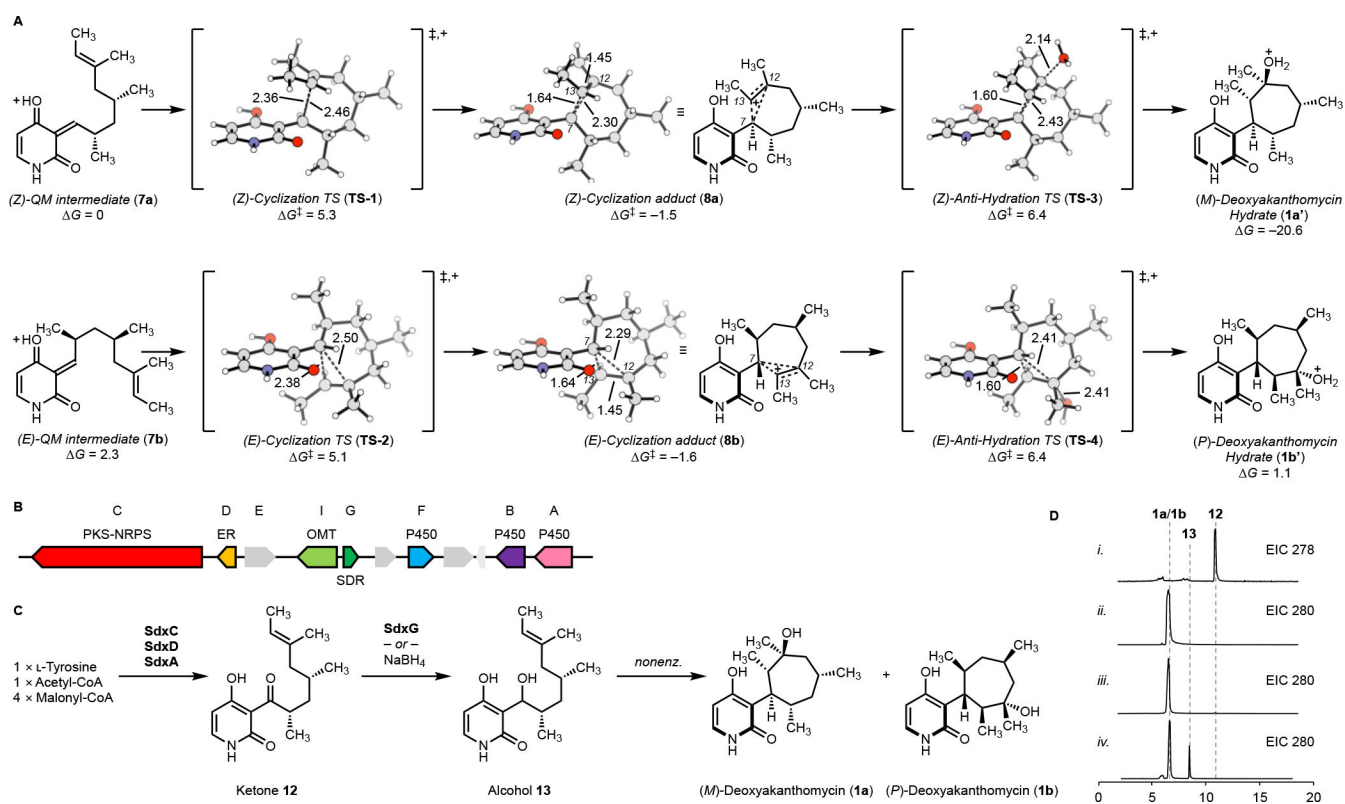
X = H; (M)-Deoxyakanthomycin (1a)
X = OH; (M)-Akanthomycin (2a)

R = CH₃; (M)-Cordypyridone A (3a)
R = H; (M)-Pyridoxatin (5a)

R = CH₃; Cordypyridone C (4)
R = H; Asperpyridone A (6)

X = H; (P)-Deoxyakanthomycin (1b)
X = OH; (P)-Akanthomycin (2b)

R = CH₃; (P)-Cordypyridone B (3b)
R = H; (P)-Pyridoxatin (5b)

o-Quinone Methide (QM, 7)
[Common Biosynthetic Intermediate]

Figure 1.
4-hydroxy 2-pyridone natural products (**1-6**) with identical carbon backbones and divergent molecular architectures all derive from a common biosynthetic intermediate, QM **7**.

Figure 2.
A. Calculated reaction scheme to form atropisomeric deoxyakanthomycin hydrates (**1a'** and **1b'**). **B.** *sdx* gene cluster from *Sarocladium oryzae*. **C.** Biosynthetic pathway to form deoxyakanthomycin **1**. **D.** Standards of **12** and **2a/2b** (*i, ii*) and enzymatic reaction of **12** with a SDR (*iii*) and chemical reaction of **12** with NaBH₄ (*iv*) to form deoxyakanthomycin.