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Supplementary Material Available: Listing of positional parameters, general temperature factors, and bond distances and angles for **1-3** (51 pages); listings of structure factor amplitudes for **1-3** (118 pages). Ordering information is given on any current masthead page.

Cell-Free Biosynthesis of Nocardicin A from Nocardicin E and *S*-Adenosylmethionine

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The precursors in primary metabolism of the monocyclic β-lactam antibiotic nocardicin A (**7b**) are the L-isomers of methionine (**1**), serine (**2**), and (*p*-hydroxyphenyl)glycine (**3**, PHPG).[1,2] The possible intermediacy of tripeptide **4** or a related derivative became more likely recently with the discovery that nocardicin G (**5**), the simplest of the seven known nocardicins, gave a remarkably efficient and intact incorporation into nocardicin A,[3] whereas its 2′-epimer suffered only degradation to L-PHPG. The central role of nocardicin G as the first β-lactam-containing intermediate of the pathway and its biosynthetic relation to nocardicin A involves the ordering of an amine oxidation step to generate the C-2′ oxime,[4] the attachment of a homoserine residue from methionine, and an epimerization event in which C-9′ undergoes inversion from the L- to the D-configuration. In this communication we describe the first preparation of a partially purified cell-free system from *Nocardia uniformis* subs. tsuyamanensis (ATCC 21806) and demonstrate its effectiveness in the conversion of nocardicin E (**6**) to isonocardicin A (**7a**) in the presence of *S*-adenosylmethionine (AdoMet) and in the epimerization of the latter to nocardicin A (**7b**).

Compared to transmethylation reactions, 3-amino-3-carboxypropyl transfers from methionine are rare. However, apart from the case of nocardicin A, important examples have been demonstrated or implied in the biosynthesis of, for example, discadenine,[5] X-base,[6] Y-base[7] (modified tRNA bases), diphthamide[8] (EF-2 site of ribosylation by diphtheria toxin), nicotianine,[9] mugineic acid, and related compounds of this series.[10] Recent whole-cell experiments in *N. uniformis* with (2*S*,4*R*)- and (2*S*,4*S*)-[4-^{2}H]methionine have shown that the overall stereochemical course of 3-amino-3-carboxypropyl transfer in **7b** is inversion.[11] This finding paralleled the steric course observed in polyamine biosynthesis[12] from decarboxylated AdoMet and suggested a role for AdoMet itself in nocardicin A formation.

Cell-free extracts of *N. uniformis* were prepared under a variety of conditions. Those obtained from washed cells, harvested after the onset of nocardicin A production, by ultrasonication in the presence of added glycerol and protease inhibitors were found to be sufficiently stable to be processed through nucleic acid and ammonium sulfate precipitation steps and dialysis.[13] Incubation[14] of nocardicin E (**6**), obtained by total synthesis,[15] and AdoMet with this partially purified extract revealed an efficient, time-dependent conversion[16] of **6** to a product that appeared to be nocardicin A (**7b**) on the basis of its HPLC retention time[17] and UV spectrum. However, direct displacement of AdoMet would be expected to give isonocardicin A (**7a**, LLD) as its initial product rather than nocardicin A (**7b**, DLD). A sample of isonocardicin A[18] was found to be indistinguishable from nocardicin A[19] not only by 400 MHz ^{1}H NMR spectroscopy but also by HPLC retention time under a range of conditions. Recalling a similar analytical difficulty in discriminating between penicillin N and its LLD-diastereomer isopenicillin N, **7b** was epimerized in aqueous pyridoxal[20] to a mixture of **7a** and **7b** as evidenced by a control experiment run in deuterium oxide in which the disappearance of H-9′ was monitored by ^{1}H NMR spectroscopy.[2] Derivatization of the reaction products with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)[21] and HPLC analysis of the resulting thioureas as described by Neuss et al.[22] gave two resolved peaks at t_R 20 and 23 min. These were identified as corresponding to the GITC derivatives of **7a** and **7b**, respectively, on comparison with authentic samples. Similar analyses, then, of the partially purified products from the cell-free incubations of **6** and AdoMet revealed a mixture of **7a** and **7b** typically in ratios of 2:1 to 3:2, indicating the presence of an epimerase activity capable of interconverting isonocardicin A and nocardicin A. This epimerase activity was readily confirmed by incubation of pure **7b** with the cell-free system and demonstration of its equilibration to similar mixtures of **7a** and **7b**.

To establish unambiguously the intact conversion of **6** to **7a**/**7b**, a specimen of [2′-^{13}C]nocardicin E (**6**) was prepared from a

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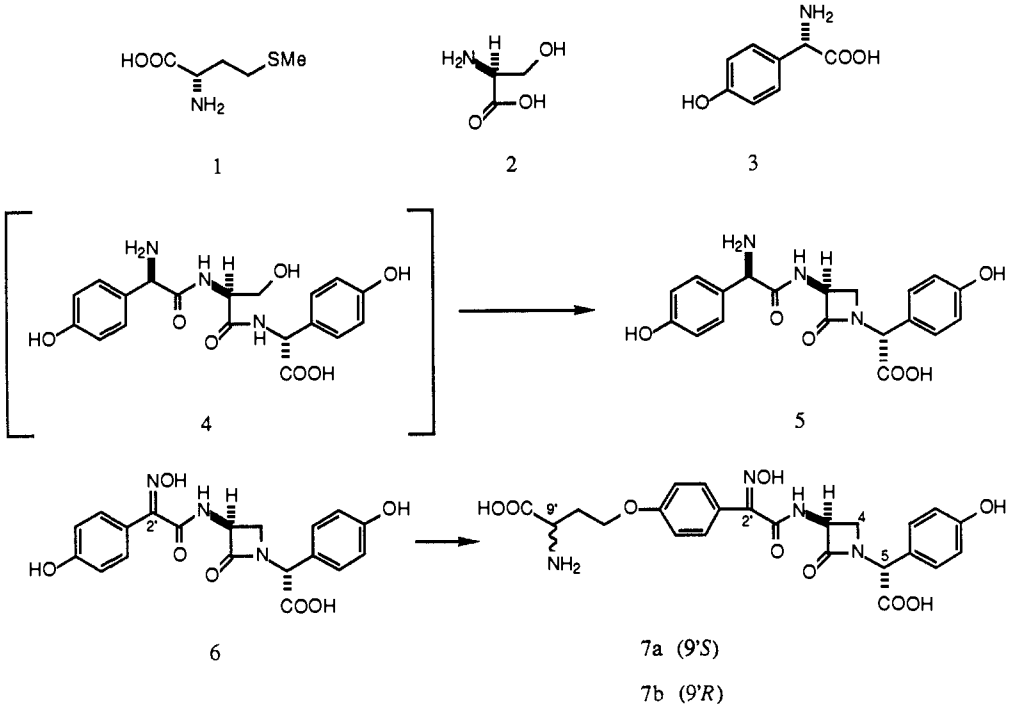
Scheme I



mixture of [2′-^{13}C]nocardicin G and epinocardicin G[3] by selective transformation of the 2′-amino function to the *syn*-oxime of **6**[15] and purified by preparative HPLC. In five large-scale cell-free incubations, [2′-^{13}C]nocardicin E (19.7 mg) was completely converted to a mixture of [2′-^{13}C]isonocardicin A and nocardicin A in the presence of an approximately 2.5 molar excess of AdoMet. Proteins were precipitated by brief heat denaturation, and the mixture of labeled **7a**/**7b** was isolated by ion exchange (DEAE Sephadex A-25) and absorption (Amberlite XAD-4) chromatography followed by crystallization from water (pH 2.5).[1] The intact incorporation of [2′-^{13}C]-**6** into the **7a**/**7b** (20 mg) isolated was demonstrated by the presence of a single enhanced resonance[23] at 153.98 ppm in its ^{13}C{^{1}H} NMR spectrum corresponding to the C-2′ oxime carbon of the product.[2] Addition of a small amount of [2′-^{13}C]-**6** resulted in the appearance of a second signal at 154.37 ppm. Further ^{1}H NMR and HPLC analysis confirmed the identity of the product as **7a**/**7b** (vide supra).

In conclusion, we have obtained a partially purified cell-free system from *N. uniformis* exhibiting two enzymic activities involved in the late stages of nocardicin A biosynthesis. The first, a 3-amino-3-carboxypropyl transferase, carries nocardicin E (**6**) and AdoMet (but not L-methionine and ATP) to isonocardicin A (**7a**), and the second, an epimerase, inverts the C-9′ configuration of **7a** to give nocardicin A (**7b**). Under normal conditions of fermentation the latter is selectively transported out of growing cells to give nocardicin A as the principal metabolite of the pathway. The group-transfer reaction from AdoMet has been shown to proceed with inversion of configuration,[11] paralleling the stereochemical course of polyamine biosynthesis[12] from decarboxylated AdoMet. On the basis of these findings an overall biosynthetic route to nocardicin A, and quite probably the major one, may now be more clearly defined (see Scheme I). Nocardicin G (**5**) is the first β-lactam-containing intermediate of the pathway[3] and originates from the amino acids **2** and **3**, presumably by way of the hypothetical tripeptide **4** or a closely related derivative. Nocardicin G (**5**) is then elaborated to the remaining six members of this antibiotic family,[24] but, specifically, amine oxidation must yield the 2′-*syn*-oxime of nocardicin E (**6**) which serves as the nucleophilic partner to AdoMet, in an S_N2 transfer[11] of a 3-amino-3-carboxypropyl group, to give isonocardicin A (**7a**). An epimerase then acts to convert the latter to nocardicin A (**7b**). Purification of the 3-amino-3-carboxypropyl transferase is in progress, and its detailed role in the biosynthesis of the nocardicins will be reported in due course.

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Metal-Catalyzed Cyclization via Isomerization of α-Dienyl-ω-allyl Acetates

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It can be argued that the most effective approach for construction of organic molecules involves a process where the combined elemental compositions of the substrates are equivalent to that of the final product. In cyclizations, such a process corresponds to an isomerization of an acyclic system to a desired ring. Besides processes such as intramolecular cycloadditions,[1,2] reactions of this type, exemplified by the intramolecular Alder ene reaction[1b,3,4] and transition-metal-catalyzed cyclizations of enynes,[5]

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