The Role of Nocardicin G in Nocardicin A Biosynthesis

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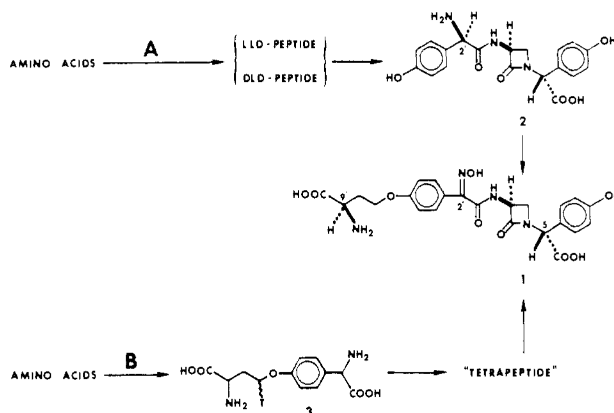
The monocyclic β-lactam antibiotic nocardicin A (**1**) is derived from the L-antipodes of methionine, (*p*-hydroxyphenyl)glycine (PHPG), and serine.[1] The seven known nocardicins differ among themselves by the nature of the substituents at C-2′ (amine, ketone, *syn*- or *anti*-oxime) and the presence or absence of the D-homoseryl ether.[2] Neither the biosynthetic relationships among these compounds nor the identity of a hypothetical peptide precursor[3] are known. Of several potential biosynthetic routes that may be visualized to nocardicin A, two are considered in this communication. The results of experiments described below indicate that nocardicin G (**2**), the simplest member of the family, plays a central role in the pathway.

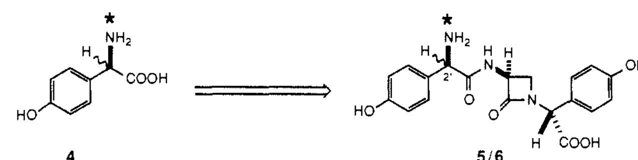
Progress toward understanding monocyclic β-lactam biosynthesis beyond determination of the amino acid building blocks of **1** has been dependent upon the availability of nocardicins A–G from recently completed syntheses.[4] As depicted in Scheme I, two overall biosynthetic routes were considered for nocardicin A formation, each of which was subject, in principle, to experimental test. The first (path A) proposed that serine and PHPG combine to give one of the following tripeptides: L- or D-PHPG-L-Ser-D-PHPG, by analogy to the LLD-tripeptide[5] involved in isopenicillin N formation.[3] Closure to the critical β-lactam ring would give nocardicin G (**2**), whose amino acid configurations have been secured as DLD.[4] In unspecified order, amine oxidation,[6] attachment of the homoserine unit, and epimerization at C-9′ would give nocardicin A. Alternatively (path B), bearing in mind that small, nonribosomal peptides are synthesized from their N-terminus, ether formation between methionine and PHPG could give a "starter" **3** resistant to proteolysis that could then lead on to a "tetrapeptide" capable of cyclizing, e.g., to nocardicin C.[2] Amine oxidation[6] at C-2′ would then yield nocardicin A (**1**).

The aryl–alkyl ether **3** was prepared by a modified Mitsunobu coupling[4] of *N-t*-Boc-PHPG fluorenyl ester and *N-t*-Boc[4-^{3}H]-homoserine *tert*-butyl ester. The latter was synthesized[4,7] in five steps from aspartic acid with the radiolabel being introduced in a sodium borotritide reduction of the γ-carboxyl as its mixed anhydride. Of the four possible stereoisomers of **3**, the LL-diastereomer was chosen initially owing to the amino acid transport properties of the producing *Nocardia*[1] and the assumption[8] that

Scheme I



Scheme II



C-9′ epimerization (in **1**) would be a late step in the biosynthesis. Administration of LL-**3** to growing cultures of *Nocardia uniformis* subsp. *tsuyamanensis* (ATCC 21806) as previously described[1] afforded a sample of nocardicin A (**1**) devoid of radioactivity. Moreover, less than 0.1% of the administered activity was found in the washed, broken cells. On the basis of these observations path B was pursued no further, and attention was turned to a test of path A.

Earlier experiments with D,L-[2-^{13}C,^{15}N]PHPG (**4**) had demonstrated[6] that this amino acid was incorporated with essentially equal efficiency into both aryl sites of nocardicin A accompanied by a 45–50% loss of ^{15}N at the β-lactam and oxime functions, owing presumably to transamination as the free amino acid prior to utilization in the pathway. The absence, therefore, of randomization of ^{13}C-label from either [2′-^{13}C,^{15}N]nocardicin G (**5**; DLD) or its 2′-diastereomer, [2′-^{13}C,^{15}N]epinocardicin G (**6**; LLD) (Scheme II), into C-2′ and C-5 of nocardicin A (**1**) would constitute proof of intact incorporation. In addition, a high retention of ^{15}N(*) relative to the ^{13}C-reporter nucleus would further militate against degradation of either substrate and the release of free PHPG, which would be subject to the nitrogen exchange process noted above and subsequent incorporation into **1**.

The Grignard reagent of (*p*-benzyloxy)bromobenzene was carbonated[9] (from barium ^{13}C-carbonate), and the resulting acid was esterified with diazomethane. Reduction ($LiAlH_4$) and oxidation (MnO_2) gave [*formyl*-^{13}C](*p*-benzyloxy)benzaldehyde whose bisulfite addition product was converted by a modified Strecker reaction[1] in the presence of ^{15}N-ammonium chloride (*) to D,L-[2-^{13}C,^{15}N]PHPG (**4**, 27% overall yield), after hydrogenolysis. The latter was reacted with di-*tert*-butyldicarbonate and coupled with *tert*-butyl 3-aminonocardicinate as described elsewhere[4] to give upon deprotection a mixture of [2′-^{13}C,^{15}N]nocardicin G (**5**; DLD) and [2′-^{13}C,^{15}N]epinocardicin G (**6**; LLD). The diastereomeric products were separated by preparative HPLC, crystallized,[10] and administered to growing cultures of *N. uniformis*.[1]

The nocardicin A produced in each of these experiments was isolated, crystallized, and analyzed by ^{13}C{^{1}H} NMR spectroscopy

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(10) Preparative HPLC separation of **5** and **6**: Regis VAL-U-PAK 25 × 1 cm, 10 micron ODS, 0.01 N ammonium carbonate adjusted to pH 5.5 with glacial acetic acid, 8 mL/min. Analytical HPLC analyses of **5** and **6** recrystallized from water (pH 5) for **5**: >96% DLD-diastereomer, C-2′ = 57.4 ppm, $^1J_{CN}$ = 6.2 Hz; for **6**: >98% LLD-diastereomer, C-2′ = 58.1 ppm, $^1J_{CN}$ = 5.3 Hz.

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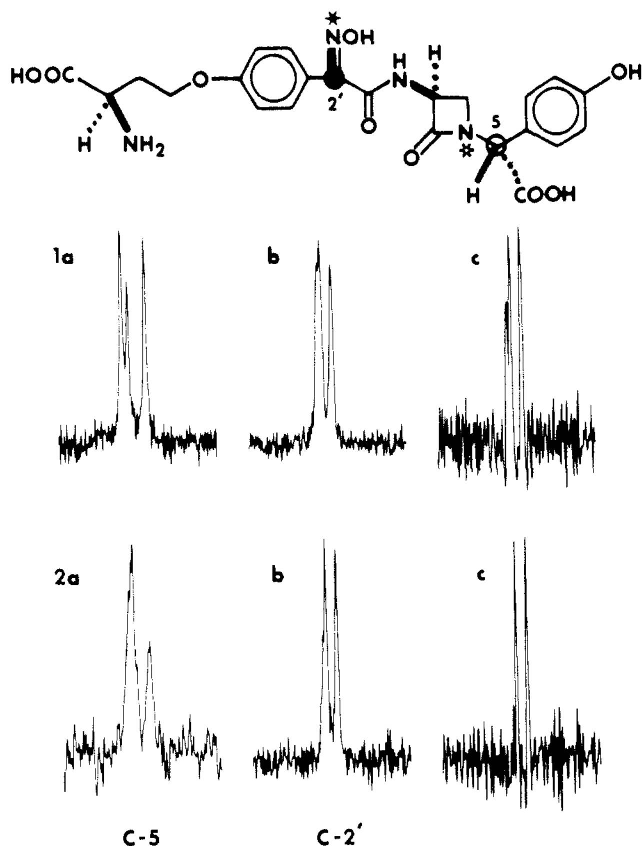

Figure 1. Partial $^{13}C\{^1H\}$ NMR spectra (expansions 50 Hz wide) obtained at 100.54 MHz on a Varian XL-400 spectrometer. Spectra 1(a–c) are of nocardicin A (20.4 mg/3.0 mL D_2O) derived from incorporation of [2′-^{13}C,^{15}N]epinocardicin G (**6**): (a) 500 transients, C-5 singlet 61.6 ppm, doublet 1.75 Hz upfield, $^1J_{CN}$ = 7.5 Hz, (b) 4000 transients, C-2′ singlet 153.8 ppm, doublet 2.6 Hz upfield, $^1J_{CN}$ = 3.4 Hz, (c) 4000 transients, C-2′ resolution enhanced RE = 0.225, AP = 0.675. Spectra 2(a–c) are of nocardicin A (7.1 mg/3.0 mL D_2O) derived from incorporation of [2′-^{13}C,^{15}N]nocardicin G (**5**): (a) as above, 50 000 trasients, (b) as above, 40 000 transients, (c) as above, 40 000 trasients.

as shown in Figure 1. Spectral expansions from the incorporation of the LLD-diastereomer **6** are represented in 1(a–c) and those from the DLD-diastereomer **5** are shown in spectra 2(a–c). The data are arrayed in each instance as (a) C-5 (61.6 ppm), (b) C-2′ (153.8 ppm), and (c) C-2′ after resolution enhancement. For [2′-^{13}C,^{15}N]epinocardicin G (**6**) carbon label was found to randomize totally into C-5 and C-2′ of nocardicin A, 22% and 20%, respectively, accompanied by 25–30% loss of ^{15}N (*). It is quite apparent that this substrate was efficiently degraded to doubly-labeled PHPG prior to utilization. However, in sharp contrast [2′-^{13}C,^{15}N]nocardicin G (**5**) gave a 21% incorporation of label selectively at C-2′ [spectrum 2(b), a remarkably high value for a whole-cell experiment] with negligible attendant ^{15}N-exchange (<5%). Amplification of the C-5 region [spectrum 2(a)] revealed an approximately 2.5% incorporation of carbon label at this locus accompanied by significant loss of ^{15}N, presumably owing to limited degradation of the precursor or possibly of a diastereomeric impurity.[10]

In conclusion, we have prepared a doubly-labeled sample of nocardicin G (**5**), the structurally simplest member of the nocardicin family, and have demonstrated its intact incorporation into nocardicin A (**1**). This finding establishes the central intermediate of the pathway and suggests the existence of an at present unknown precursor composed of most probably two D-PHPG units and L-serine. As evidenced by the rapid degradation of epinocardicin G (**6**), the presence of D-PHPG residues may confer proteolytic stability to both this hypothetical precursor and to nocardicin G to allow elaboration of the latter by amine oxidation and reaction with methionine to the other members of this antibiotic group.

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Preparation of Polysilanes in the Presence of Ultrasound

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Sonochemical synthesis of organometallic compounds has recently received considerable amount of attention.[1] Not only have higher yields been reported but also new compounds have also been prepared. Sonochemistry is based on the implosive collapse of cavities with very high pressures and temperatures existing locally for a short time. Sonochemical reductive coupling of chlorosilanes with lithium leads to the formation of disilenes and cyclotrisilanes.[2] We have used a similar method for the preparation of high molecular weight polysilanes.[3] The latter materials have exciting photochemical and photophysical properties which confirmed earlier theoretical predictions concerning conjugation of the catenated Si-Si bonds in linear polymers.[4] The properties of polysilanes depend on the degree of polymerization[5] and structure-properties relationships should be established for well-defined species with controlled molecular weight and low polydispersities.

Polysilanes are typically prepared by reductive coupling with molten sodium in boiling toluene or xylene.[5] Polymers formed in this process have polymodal molecular weight distributions and, in addition to cyclics (Si_5 or Si_6), a low molecular weight polymer ($\bar{M}_n \approx 10^3$) and a high molecular weight polymer ($\bar{M}_n > 10^5$) are found. The polymer is separated from cycles by precipitation in, e.g., isopropyl alcohol, but polymer fractionation is usually difficult. Formation of a bimodal polysilane has been explained by diffusion phenomena;[4e] however, the alternative explanation could be based on multiple mechanisms of polymerization which operate simultaneously. For example, reactive intermediates involved in

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