Fate of [^{15}N]-(*p*-Hydroxyphenyl)glycine in Nocardicin A Biosynthesis

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Incorporation of D,L-[2-^{13}C,^{15}N]-(*p*-hydroxyphenyl)glycine into nocardicin A has revealed that both the β-lactam and the oxime nitrogens are derived from this amino acid precursor.

Biosynthetic experiments in *Nocardia* have established[1] that L-(*p*-hydroxyphenyl)glycine (**3**) (PHPG) is derived from L-tyrosine and serves as the amino acid precursor of the two aryl sites in nocardicin A (**1**). It is noteworthy that the only other appearance of PHPG units among secondary natural products is as a structural element in the glycopeptides,[2] an antibiotic class of clinical importance in recent years. In this paper the fate of nitrogen-labelled PHPG in β-lactam and oxime formation in (**1**) is reported.

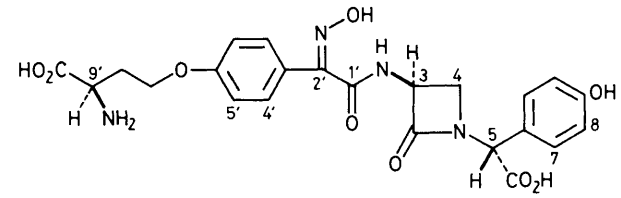
Bycroft in collaboration with workers at Beecham, using a high producing strain of *Penicillium chrysogenum*, investigated the incorporation of L-[U-^{14}C,^{15}N]valine into penicillin G (**2**, R = $PhCH_2CO$–).[3] Relative to the ^{14}C-internal standard, it could be shown that 84 ± 10% of the ^{15}N present in valine was retained through inversion at the valyl α-centre and incorporation into (**2**).[4] This group subsequently re-examined[5] the problem using L-[2-^{13}C,^{15}N]valine (**4**) and found that while ^{15}N incorporation could be demonstrated to have occurred, the hoped for ^{15}N-coupling to C-3 in (**2**) was unfortunately undetectable in the ^{13}C n.m.r. spectrum.[6]

We have performed a parallel experiment with D,L-[2-^{13}C,^{15}N]PHPG‡ to study the metabolic fate of the α-amino function in both β-lactam and oxime formation in nocardicin A (**1**). D,L-[2-^{13}C,^{15}N]PHPG (**3**) was prepared by Vilsmeier reaction[7] of anisole with [^{13}C]dimethylformamide followed by Strecker elaboration [1.0 equiv. $^{15}NH_4Cl$ (99%), 1.1 equiv. NaCN, MeOH, 40 °C, 2.5 h; aq. workup] of the resulting [formyl-^{13}C]anisaldehyde.[8] Hydrolysis of the doubly labelled hydantoin and demethylation to (**3**) [δ (D_2O) 56.5, $^1J_{CN}$ 6.8 Hz] was carried out essentially as before.[1]

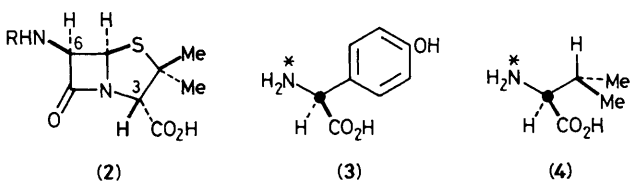
Incorporation of this material by growing cultures of *N. uniformis*[1] gave a sample of (**1**) whose $^{13}C\{^1H\}$ n.m.r. spectrum revealed enrichments at C-5 (δ 61.6) and C-2′ (δ 153.9). Expansions of these immediate regions are shown in Figure 1 as spectra (a) and (c), respectively; and, after resolution enhancement, as (b) and (d). In both instances an upfield-shifted doublet is discernable superimposed on a singlet corresponding to [^{13}C/^{14}N]-labelled material. Consistent with Bycroft's findings noted above, the β-lactam nitrogen survives in large measure (*vide infra*) intact through stereochemical inversion and incorporation into nocardicin A; but, unlike the negligible spin–spin interaction observed at C-3 in (**2**, R = $PhCH_2CO$),[5] coupling was readily detected at C-5 in (**1**) ($^1J_{CN}$ 7.3 Hz).

The oxime function is rarely encountered in natural products and could be visualized to arise by stepwise oxidation of an amine-containing precursor[9] or through reaction of a keto intermediate with an hydroxylamine donor as, for example, hydroxyurea.[10] Inspection of spectra (b) and (d) clearly shows that the former occurs ($^1J_{CN}$ *ca.* 2.9 Hz).

In conclusion, both the oxime and β-lactam nitrogens of nocardicin A (**1**) have their origin in the amino acid precursor, (*p*-hydroxyphenyl)glycine. It is interesting to note that the extent of transamination prior to incorporation at both sites is roughly equivalent, 47 ± 5% as estimated from integration of spectra (a) and (c). This similarity might suggest some unison in the assembly of two PHPG units into an as yet unknown precursor of nocardicin A.



(1)



(2) (3) (4)

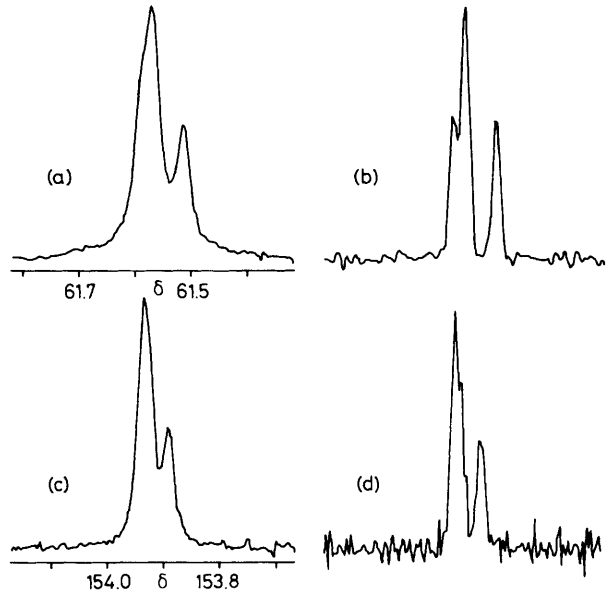


Figure 1. Partial $^{13}C\{^1H\}$ n.m.r. spectra (50 Hz wide) of nocardicin A (**1**) (8 mg in 3.0 ml D_2O) obtained at 100.6 MHz on a Varian XL-400 for enhanced resonances resulting from incorporation of (**3**); spectral width 12 000 Hz, 60 000 points, acquisition time 2.5 s, 60° pulse, 10 000 transients. Spectrum (a): C-5, transform of unweighted free induction decay (FID). Spectrum (b): C-5, resolution enhanced by weighting FID with an exponential function (RE 0.125 s) and a Gaussian apodization function (AF 0.375 s). Spectrum (c): C-2′, transform of unweighted FID. Spectrum (d): C-2′, as (1b) but RE 0.225 s, AF 0.675 s.

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‡ The L-isomer is much more efficiently utilized by whole cells of *N. uniformis* (ATCC 21806) than the D-isomer, ref. 1.

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