Naphthyridinomycin Biosynthesis. The Involvement of Ornithine and the Origin of the Oxazolidine Nitrogen[1]

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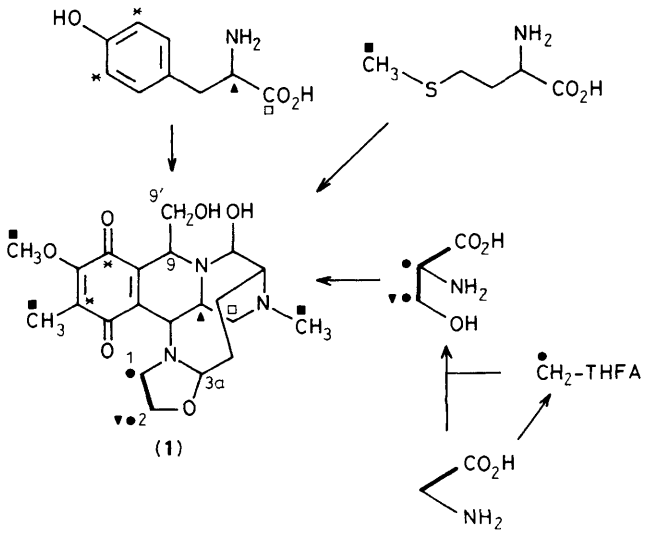
Ornithine is a direct biosynthetic precursor to naphthyridinomycin, and the oxazolidine nitrogen is derived intact from serine.

The biosynthesis of naphthyridinomycin (**1**), an antibiotic produced by *Streptomyces lusitanus*,[2,3] has been found to occur as shown in Scheme 1.[4,5] Tyrosine contributes nine nuclear carbon atoms while methionine provides the three methyl groups, and serine provides the two-carbon unit of the oxazolidine moiety. The origin of the two-carbon unit C-9–C-9′ remains obscure, but ^{14}C labelling studies indicated that ornithine (**2**) was the probable precursor for the remaining five-carbon section. Were this to prove correct, generation of the oxazolidine moiety would require loss of either the α-amino group of serine or the δ-amino group of ornithine. In this report, we describe the role of ornithine in the biosynthesis of (**1**) as well as the origin of the nitrogen atom at C-1.

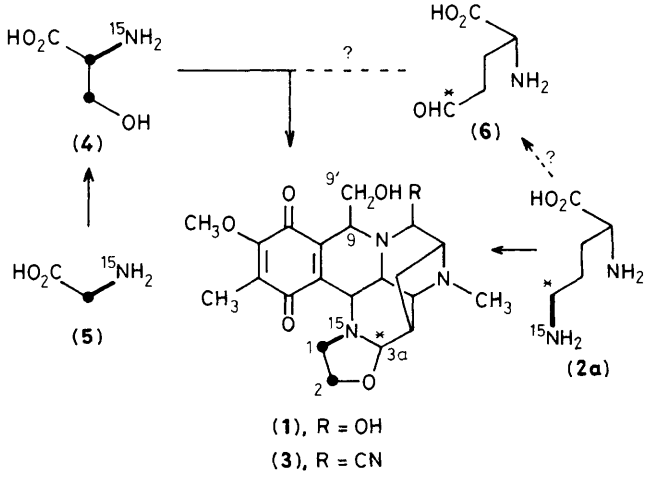
Whereas DL-(1-^{14}C)- and DL-(5-^{14}C)-glutamic acid failed to label (**1**), DL-(1-^{14}C)- and DL-(5-^{14}C)-ornithine each were incorporated at very significant levels (0.6, 2.3, and 1.5% with the former and 3.5% with the latter). DL[5-^{13}C,5-^{15}N]ornithine (**2a**) was then synthesized from sodium [^{13}C,^{15}N]cyanide.[6] This material (35 mg, 99 atom % ^{13}C, 99 atom % ^{15}N) mixed with (5-^{14}C)ornithine (6.63 μCi) and dissolved in H_2O (10 ml), was added under sterile conditions to 4 1 litre Erlenmayer flasks, each containing 200 ml of broth,[7] 57 h after they had been inoculated with a 48 h old seed culture. After an additional 39 h the fermentations were worked up as previously described[7] to yield 12 mg of pure cyanonaphthyridinomycin (**3a**) (0.9% incorporation of ^{14}C, 5.8% calculated enrichment in ^{13}C). The 100.61 MHz ^{13}C n.m.r. spectrum ($CDCl_3$) showed a symmetrical resonance for C-3a, 6.0% enriched over natural abundance, thus confirming the direct involvement of (**2**) in naphthyridinomycin biosynthesis. However, the spectrum was totally devoid of ^{13}C–^{15}N satellites, and the origin of the oxazolidine nitrogen was still elusive.

Serine has been shown to be the most direct precursor to C-1 and C-2.[5] DL-[2-^{13}C,2-^{15}N]serine (**4**) was synthesized,[8] and 25 mg diluted with 75 mg of unlabelled serine (overall, 24 atom % ^{13}C and 25 atom % ^{15}N; total volume 10 ml) were added under sterile conditions to 10 flasks each containing 50 ml of broth. Work-up yielded 21 mg of (**3b**). A doublet (0.65% ^{13}C enrichment, J_{CN} 2.4 Hz†) was discernible, superimposed on the natural abundance C-1 resonance at δ 50.16 in the 100.61 MHz ^{13}C n.m.r. spectrum (Figure 1B); a slight upfield shift (0.02 p.p.m.) due to the ^{15}N isotope effect caused overlap of the downfield satellite by the natural abundance signal. Thus, it appeared that serine provided the nitrogen atom of the oxazolidine ring (Scheme 2).

This conclusion was confirmed by the incorporation of [2-^{13}C,2-^{15}N]glycine (**5**). Compound (**5**) (60 mg), mixed with unlabelled glycine (140 mg) and dissolved in 10 ml of water, was added to 13 flasks each containing 50 ml of broth, and eventual harvesting yielded 19 mg of (**3c**). Once again (Figure 1C), a spin-coupled satellite could easily be discerned on the upfield side of the natural abundance C-1 resonance; however, in addition, a small quartet was observed (J_{CC} 31.4, J_{CN} 2.4 Hz) flanking these signals. Apparently, even with the initial dilution with unlabelled glycine, the internal metabolic pools were sufficiently small to generate *de novo* some [2,3-$^{13}C_2$,2-^{15}N]serine.[5] As would therefore be expected, the C-2 resonance (δ 61.4) was also flanked by a small doublet (J_{CC} 31.4 Hz).



Scheme 1 THFA = tetrahydrofolic acid.



Scheme 2

† The magnitude of this coupling constant is precisely what would be expected for sp^3 nitrogen and sp^3 carbon, based on numerous examples from our laboratory and others.

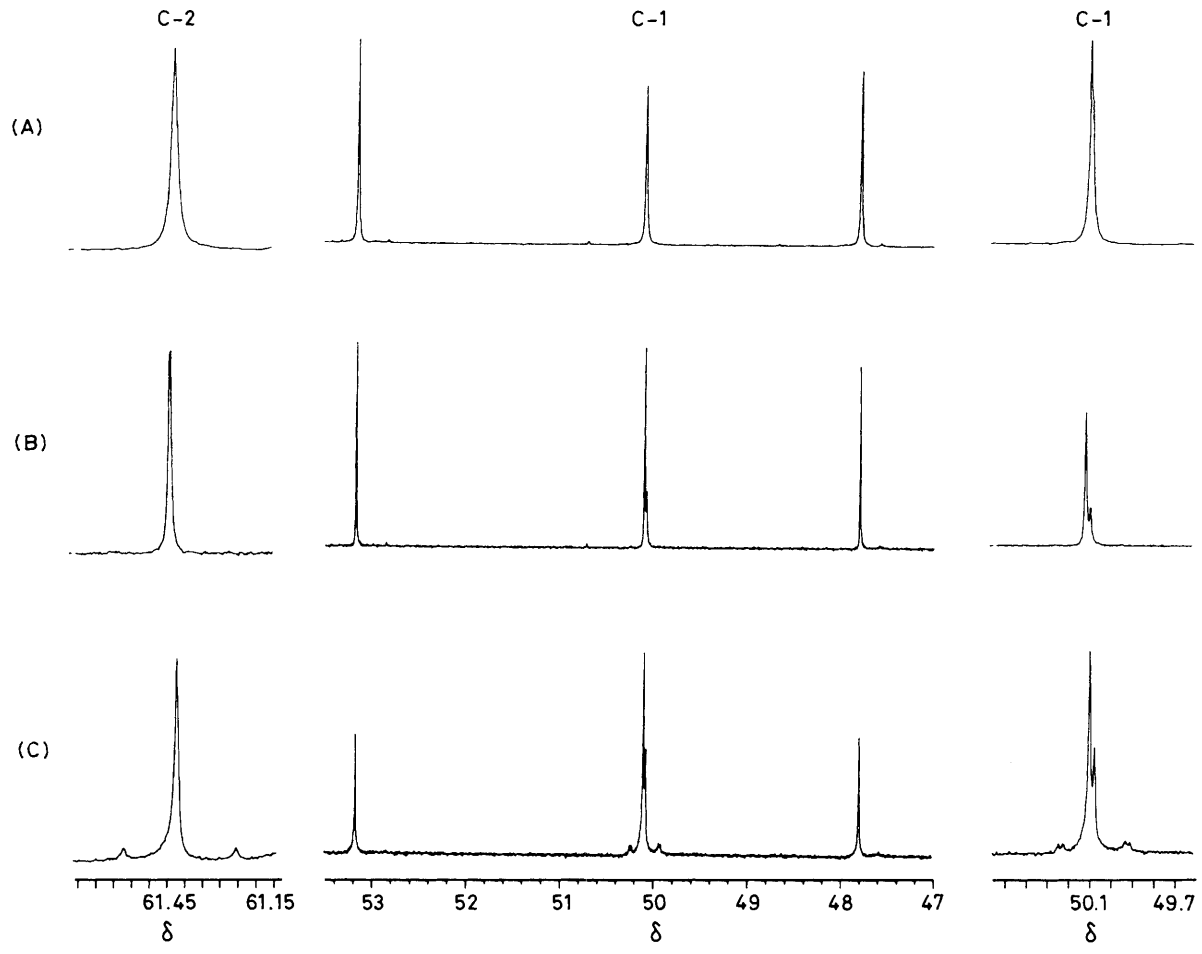


Figure 1. 100.6 MHz ^{13}C n.m.r. spectra of cyanonaphthyridinomycin in $CDCl_3$ taken on a Bruker AM 400 n.m.r. spectrometer. (A) Natural abundance (**3**) (25 349 scans); (B) (**3b**) (24 000 scans); (C) (**3c**) (11 368 scans). Acquisition parameters were: AQ = 1.5 s, PW = 34°, TD = 64 K, SI = 128 K.

On the basis of these results it would be logical to conclude that ornithine is oxidized to glutamate semi-aldehyde (**6**), and this condenses with a serine metabolite.‡ The lack of glutamate incorporation is surprising, since (**6**) is an intermediate between it and ornithine. However, since the convergence of a number of sub-pathways is involved in the formation of (**1**), this may only reflect the timing of these feedings relative to the biosynthesis sequence.

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‡ The precise metabolism of serine has not yet been defined. (^{14}C)Ethanolamine did not label naphthyridinomycin (M.J.Z., unpublished results).

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