Application of ^{15}N Pulsed Fourier Transform Nuclear Magnetic Resonance Spectroscopy to Biosynthetic Studies; Incorporation of L-[^{15}N]Valine into Penicillin G

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Summary Quantitative ^{15}N Fourier transform n.m.r. studies show that the nitrogen of L-[*U*-^{14}C,^{15}N]valine is incorporated to almost the same extent as the carbon skeleton into penicillin G.

WE have previously established[1] that the carbon skeletons of both D- and L-valine are both very effective precursors of penicillin G when fed to a high-producing strain of *Penicillium chrysogenum*., but in neither case was the α-proton incorporated. An obvious extension of this work was to investigate the incorporation of [^{15}N]valine. Earlier experiments in this area had suggested that considerable deamination occurred before incorporation.[2] Our observations[3] on the conversion of α-labelled cystine into penicillin G had revealed that little deamination of this amino-acid precursor had occurred with our organism.† We now report further studies on the incorporation of L-[^{15}N]valine into penicillin G employing a new quantitative application of ^{15}N pulsed Fourier transform (F.T.) n.m.r. spectroscopy.

Owing to the lack of a long-lived radio-isotope, the study of the metabolism of nitrogen-containing compounds poses considerable difficulties. Studies involving the stable ^{15}N isotope usually require chemical degradation followed by isotope ratio determination using mass spectrometry, a

† 15% of the α-label of cystine is lost and we assume this reflects the extent of deamination before incorporation.

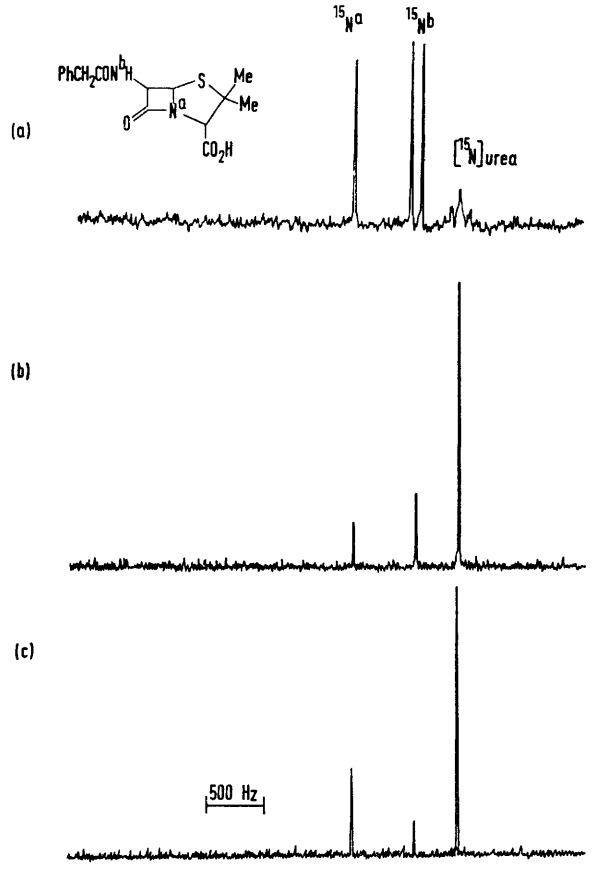

FIGURE 1. ^{15}N-F.T. n.m.r. spectra of penicillin G potassium salt and urea, obtained in H_2O (C_6F_6 lock, coaxial cell) at 10·1367 MHz at 21 °C on a Jeol PS.100 F.T. spectrometer coupled to a Nicolet 1085 computer. The pulse width was 7 μs (13° tip) and free induction decays were sampled over 5000 Hz using 4096 data points. (a) Sample with 33·5 atoms % excess ^{15}N (undecoupled with n.O.e.; phased spectrum; 3600 pulses; repetition 1·5 s). (b) Sample with 0·5 atoms % excess ^{15}N (noise-decoupled; magnitude spectrum; 131,072 pulses; repetition 0·5 s). (c) Sample from valine feed (noise-decoupled, magnitude spectrum; 131,072 pulses; repetition 0·5 s).

method which is tedious and time consuming especially when there is more than one nitrogen atom in the molecule. Pulsed F.T. n.m.r. spectroscopy is potentially a non-degradative method which should in many cases allow diagnostic assignments of different nitrogen signals.

In order to evaluate this method, standards of penicillin G potassium salt containing different concentrations of ^{15}N were prepared in the following way. *P.chrysogenum* was grown on a defined medium of which the only nitrogen source was $Na^{15}NO_3$ (33·5 atoms % excess). The penicillin G salt from this experiment was diluted to give three standards (0·50, 1·00, and 1·50 atoms % excess). A fixed amount of [^{15}N]urea,‡ to act as internal standard, was added to each sample and the pulsed F.T. n.m.r. spectra recorded under defined conditions. The noise-decoupled spectrum of the 0·50% standard is shown in Figure 1b. The signals were assigned on the basis of the undecoupled spectrum (Figure 1a) of an undiluted [^{15}N]penicillin G sample (33·5 atoms % excess). Interestingly, both nitrogen atoms of the penicillin suffered nuclear Overhauser enhancement (n.O.e.). The ratio of the integral of each of the penicillin signals to that of the urea signal was shown to be linearly dependent on the ^{15}N content of the penicillin (see Figure 2).

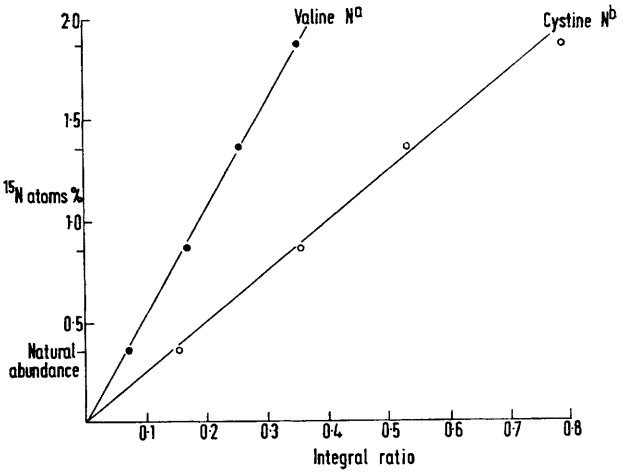

FIGURE 2. Natural abundance ^{15}N was taken as 0·366 atoms %. Standards were prepared from [^{15}N]penicillin G (33·6 atoms % excess) by dilution with natural isotope penicillin G. All spectra were run on the potassium salt (563 mg) made up to 1·0 ml with H_2O containing [^{15}N]urea (1·53 mg; 33·3 atoms % excess) as internal standard. Integration ratio is defined as the ratio of the integral of $^{15}N^a$ or $^{15}N^b$ to the integral of ^{15}N-urea, obtained from the magnitude spectra.

L-[*U*-^{14}C,^{15}N]Valine (8 × 5 mg; 57 μCi/mmol; 99·0 atoms % excess) was fed to the high-producing strain of *P. chrysogenum* as previously described.[1] The spectrum of the penicillin G potassium salt (0·88 μCi/mmol, 23·2% incorporation) isolated from this experiment is shown in Figure 1c. From the initial calibration it was calculated that N^a contained 1·29 ± 0·15 atoms % excess whereas N^b appeared to have no observable enhancement above natural abundance.§ Thus the incorporation of L-[^{15}N]valine into the valine portion of penicillin G was 84 ± 10% of that of the carbon skeleton which is equivalent to 19·0% of the ^{15}N precursor. This demonstrates that, with the high-producing organism, little deamination of L-valine occurs before incorporation, and confirms that the inversion of the α-centre of L-valine on transformation into penicillins does not involve the loss of the nitrogen atom.

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‡ Initial experiments were conducted on penicillin G [^{15}N]ammonium salt since this afforded a fixed internal standard relative to the penicillin nitrogens, but the intensity of the ammonium signal proved to be sensitive to slight pH variation.

§ The average enhancement for both N^a and N^b, as determined by thermal emission spectroscopy on the molecular nitrogen from a Dumas degradation of penicillin G, is 0·584 atom % excess. This corresponds to 1·17 atom % excess for N^a assuming no incorporation in N^b. We are indebted to Mr. C. P. Lloyd-Jones of the Long Ashton Research Station, Bristol, for this determination.

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