

## Thermal Fission and Re-formation of the C–N Bond in Formamide as Studied by $^{13}\text{C}$ – $^{15}\text{N}$ Coupling

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*Summary* Thermal fission and re-formation of the C–N bond in formamide was detected by change of  $^{13}\text{C}$ – $^{15}\text{N}$  coupled peaks to an uncoupled peak in the  $^{13}\text{C}$  n.m.r. spectra.

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THE ready formation of the nucleic acid base, purine, simply by heating neat formamide prompted us to investigate the reaction mechanism.<sup>1</sup> Homonuclear and heteronuclear spin-spin coupling in natural products obtained by feeding doubly enriched precursors has been successfully applied to clarify detailed biosynthetic pathways.<sup>2</sup> Although we hoped to clarify the mechanism for the formation of purine from formamide using <sup>13</sup>C-<sup>15</sup>N spin-spin coupling, the purine obtained showed no <sup>13</sup>C-<sup>15</sup>N coupled peaks in its <sup>13</sup>C n.m.r. spectra, and we report now an experiment which may explain why this is so.

Doubly enriched formamide (<sup>13</sup>C, 90%; <sup>15</sup>N, 99%) was diluted 20-fold with formamide of natural isotopic abundance to differentiate the labelled C-N bond from newly formed C-N bonds. The <sup>13</sup>C n.m.r. spectra of the neat formamide in a 10 mm n.m.r. tube with D<sub>2</sub>O in a co-axial 5 mm tube were recorded in the proton-noise decoupled mode on a Bruker SXP 4-100 n.m.r. spectrometer at 22.6357 MHz [spectral width, 6 kHz; data points, 4 K/4 K;

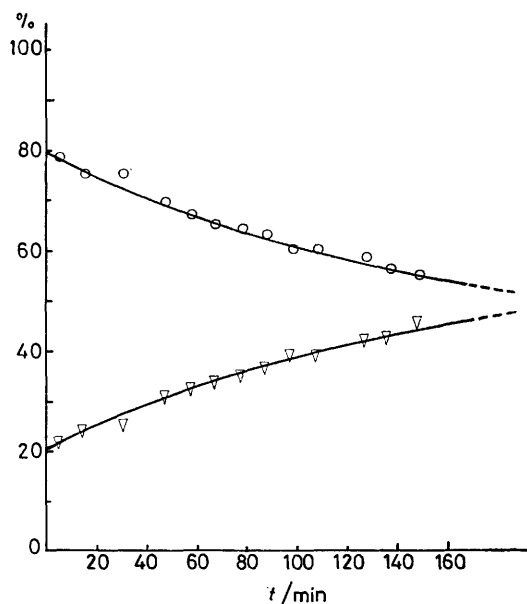


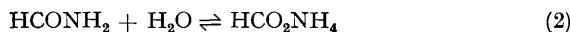
FIGURE. Change in peak height (% of total peak height) with time at 436 K for: O, coupled peaks ( $\text{H}^{13}\text{CO}^{15}\text{NH}_2$ ) and  $\nabla$ , uncoupled peak ( $\text{H}^{13}\text{CO}^{14}\text{NH}_2$ ).

<sup>1</sup> H. Yamada and T. Okamoto, *Yakugaku Zasshi*, 1975, **95**, 493.

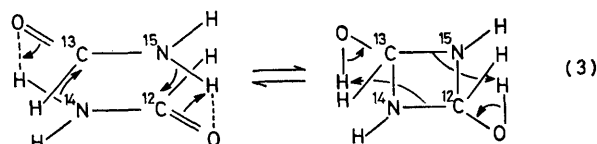
<sup>2</sup> M. Tanabe, in 'Biosynthesis,' vol. 3 (Specialist Periodical Report), ed. T. A. Geissman, The Chemical Society, London, 1974.

flip angle, 37°; 24 pulses (5 s between pulses)]. <sup>13</sup>C-<sup>15</sup>N spin-spin coupled peaks ( $J$  13 Hz) were observed on both sides of the natural abundance peak in a ratio of 2:1 and at a slightly (< 1 Hz) higher field than the natural abundance peak due to the <sup>15</sup>N isotope shift. The sample was heated from 353 to 433 K and no changes in intensities between coupled and uncoupled peaks were recorded (even when kept at 433 K for 50 min) except for the decreased signal to noise ratios due to the higher probe temperature. When the sample was heated at 436 K (slightly lower than the temperature at which the formation of purine starts<sup>1</sup>), the intensities of the coupled peaks started to decrease and that of the uncoupled peak started to increase. A plot of the height of the coupled and the uncoupled peaks against time at 436 K is shown in the Figure. The decrease in amount of doubly labelled molecules (<sup>13</sup>C-<sup>15</sup>N coupled peaks;  $\text{H}^{13}\text{CO}^{15}\text{NH}_2$ ) was accompanied by an equivalent increase in the singly labelled molecules (uncoupled peak,  $\text{H}^{13}\text{CO}^{14}\text{NH}_2$ ). Although no detectable products (as shown by the <sup>13</sup>C n.m.r. spectra) were formed at this temperature and the starting formamide remained as formamide, this result indicated that fission and re-formation of its C-N bond formamide had occurred.

The fission and re-formation of the C-N bond can be explained by the reversible formation of ammonium formate as shown by the equilibria (1) and (2). Another possibility



is a switch of the C-N bond between two molecules *via* a stacked structure through intermolecular hydrogen bonding as shown in equilibrium (3).



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