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REACTION MECHANISM FOR PURINE RING FORMATION AS STUDIED BY ^{13}C-^{15}N COUPLING

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The one-step synthesis of purine by heating neat formamide prompted us to investigate the reaction mechanism.[1, 2] Heteronuclear spin-spin coupled peaks between ^{13}C and ^{15}N in the product, purine, derived from doubly enriched formamide were not observed and the result was explained by thermal fission and reformation of the C-N bond in formamide.[3] The introduction of hydrogen cyanide gas (an assumed product in the one-step synthesis of purine from heating formamide above 160° C) to the reaction vessel lowered the reaction temperature and gave purine as a minor product along with adenine, a major product.[4] A ^{13}C nmr study of adenine prepared either from doubly enriched hydrogen cyanide or formamide indicated that the adenine ring is constituted from three molecules of hydrogen cyanide and two molecules of formamide for the employed conditions.[5]

The present study was intended to clarify the reaction mechanism for purine ring formation from hydrogen cyanide and formamide using doubly enriched material and analyzing with ^{13}C nmr and mass spectrometry.

Doubly enriched potassium cyanide (^{13}C, 90.5 % ; ^{15}N, 99.2 %) was diluted twentyfold with potassium cyanide of natural isotopic abundance (3 g) to differentiate newly formed C-N bonds from the labeled C-N bond. Hydrogen cyanide which was generated by acidifying the potassium cyanide with concentrated sulfuric acid was introduced into formamide (5 g) under ice cooling. The mixture

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was sealed and heated at 160° C for 5 hr. Unreacted formamide was removed under reduced pressure and the residue was mixed with charcoal in methanol to remove adenine, a major product. The ^{13}C nmr spectrum of the purine obtained from the solution and dissolved in deuterium oxide (6 mg / 0.5 ml) was recorded on a JEOL FX-100 NMR Spectrometer at 25.05 MHz (data points, 4 K : spectral width 1 KHz : flip angle 36° : 3 sec between pulses : 62000 pulses) in the proton-noise decoupled mode. The ^{13}C nmr spectrum of non-labeled purine (10 mg / 0.5 ml) was also recorded for the same conditions to compare the relative peak heights. The mass spectra were recorded on a JEOL JMS-D 300 Mass Spectrometer (ionization energy, 70 eV).

The relative intensities of carbon peaks in labeled and non-labeled purine were shown in Table 1 and indicated that C_4 and C_5 peaks were enriched without ^{13}C-^{15}N coupled peaks. On the other hand, the ^{13}C nmr spectrum of adenine obtained for the same conditions (using doubly labeled hydrogen cyanide of fifty-fold dilution instead of twentyfold dilution in the present study) showed the

Table 1. Relative intensities of carbon peaks in labeled and non-labeled purine

	C_2	C_4	C_5	C_6	C_8
chemical shift[a]	152.2	155.6	128.9	145.2	148.0
labeled purine	100	29.6	41.1	92.0	49.6
non-labeled purine	100	14.5	21.6	91.3	55.5

a. ppm downfield with respect to Me_4Si (dioxane as internal reference $\delta_{TMS} = \delta_{dioxane} + 67.4$)

three coupled peaks, namely C_4, C_5, and C_6.[5] The absence of ^{13}C-^{15}N coupled peaks in the purine in spite of the presence of coupled peaks in the adenine obtained from the same reaction conditions indicated that C-N bond fission occurred and only carbon atoms of the labeled hydrogen cyanide molecule were introduced into the purine ring. Table 2 shows the relative intensities of mass spectral peaks of labeled and non-labeled purine and adenine. The result indicated that only carbon atoms of labeled hydrogen cyanide were incorporated into purine ring and confirmed the result of ^{13}C nmr spectrum.

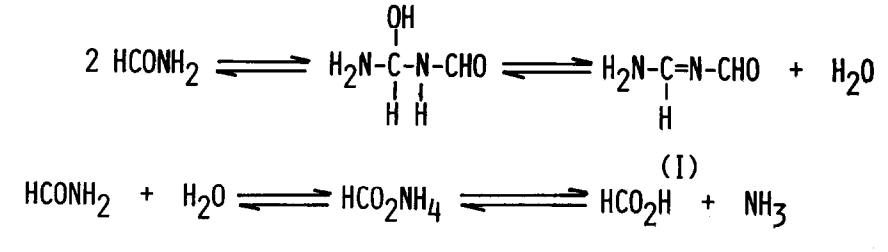
The thermal fission and reformation of the C-N bond in formamide during

Table 2. Relative intensities of M^+ peaks in purine (m/e 120) and adenine (m/e 135)[a]

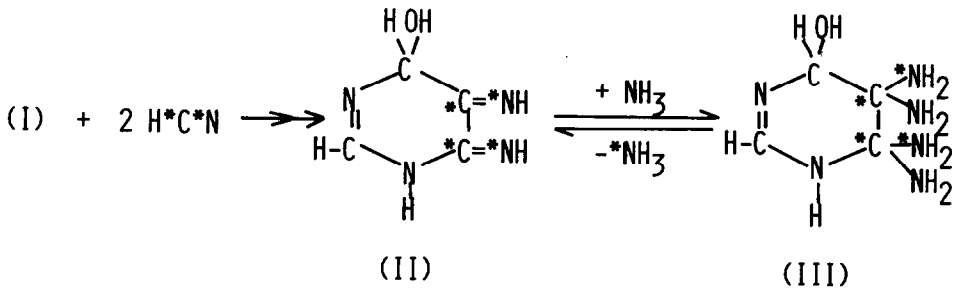
	purine[b]		adenine[c]	
	labeled	non-labeled	labeled	non-labeled
M^+	100	100	100	100
$M^+ + 1$	22.7	7.4	19.8	9.0
$M^+ + 2$	2.4	0.6	8.4	0.4

a. relative intensities with respect to M^+ peak (100).
b. prepared from doubly enriched hydrogen cyanide which was diluted *twentyfold* with hydrogen cyanide of natural isotopic abundance.
c. prepared from doubly enriched hydrogen cyanide which was diluted *fiftyfold* with hydrogen cyanide of natural isotopic abundance.

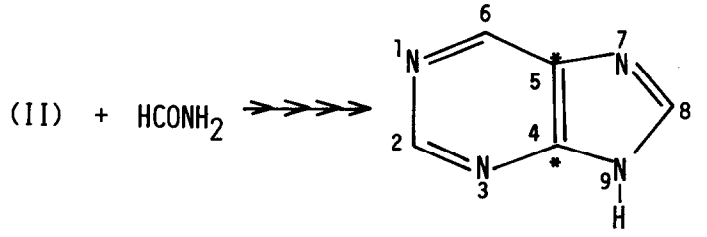
the reaction prevented the use of the ^{13}C-^{15}N coupling method for detection of reaction units and gave only information about the origin of carbon atoms.[3,5]



(I)



(II) (III)



On the other hand, fission of the C-N bond in hydrogen cyanide and incorporation of the carbon atoms in the present study, however, must be explained since fission of the C-N bond in hydrogen cyanide did not occur during the reaction (as evidenced in the case of adenine[5]) but at a later atage. To explain the present data, we propose the following reaction pathways for the formation of the purine ring from hydrogen cyanide and formamide. The fission of the ^{13}C-^{15}N bond and incorporation of the labeled carbon atoms can be explained by an equilibrium between the diimino intermediate (II) and the ammonia adduct (III).

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