Notes

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Teruhisa Noguchi : Studies on the Biosynthesis of Nucleic Acids. IV.[1] Synthesis of Labeled 4-Aminoimidazole-5-carboxamide from Barium Carbonate-^{14}C.

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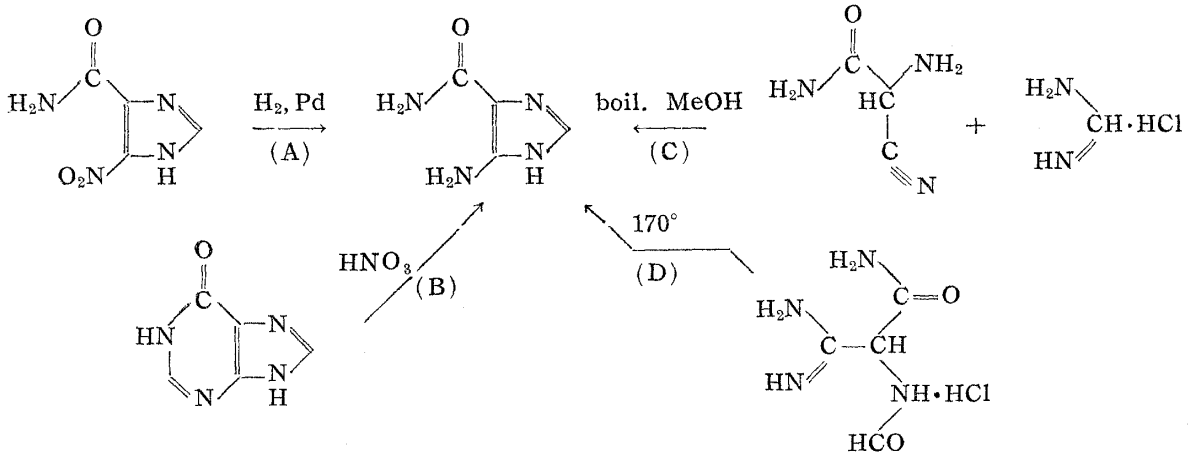
The author attempted the synthesis of labeled 4-aminoimidazole[4-^{14}C]-5-carboxamide (AICA-^{14}C) for use in biological experiments by which AICA-^{14}C will be incorporated into the polynucleotide purines as the ribotide or the riboside *in vitro*[2] and for studies on the effect of antitumor agents, such as antipurines and antifolic acid compounds on the incorporation of the AICA-^{14}C in these biological systems.[3]

It is of considerable importance that the labeled position in the isotopic compound be stable in case it is used in biological precursor experiment. Therefore, it is desirable that the radioactive carbon should be situated in the position 2, 4, or 5 of the imidazole skeleton rather than in the carboxamide residue.

Evidently the method of synthesis differs according to the position of the radioactive carbon in the imidazole ring.

There have been described four different methods for the synthesis of the non-labeled carboxamide as shown in Chart 1.

Chart 1.



Process A was reported by Windaus and Langenbeck[4] with modifications suggested by Allsebrook, *et al.*[5] However, catalytic reduction of 4-nitroimidazole-5-carboxamide, obtained from methylglyoxal, resulted in a poor yield. Process B is a method in which acid degradation of hypoxanthine is considered.[6] However, these methods are not suitable since both make it impossible to label the radioactive carbon in the imidazole ring.

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1) T. Noguchi : Part III : This Bulletin, **4**, 101(1956).
2) T. Noguchi : *Ibid.* **4**, 92(1956).
3) T. Noguchi : *Ibid.* **4**, 97(1956).
4) A. Windaus, W. Langenbeck : Ber., **56**, 683(1923).
5) W. E. Allsebrook, J. M. Gulland, L. F. Story : J. Chem. Soc., **1942**, 232.
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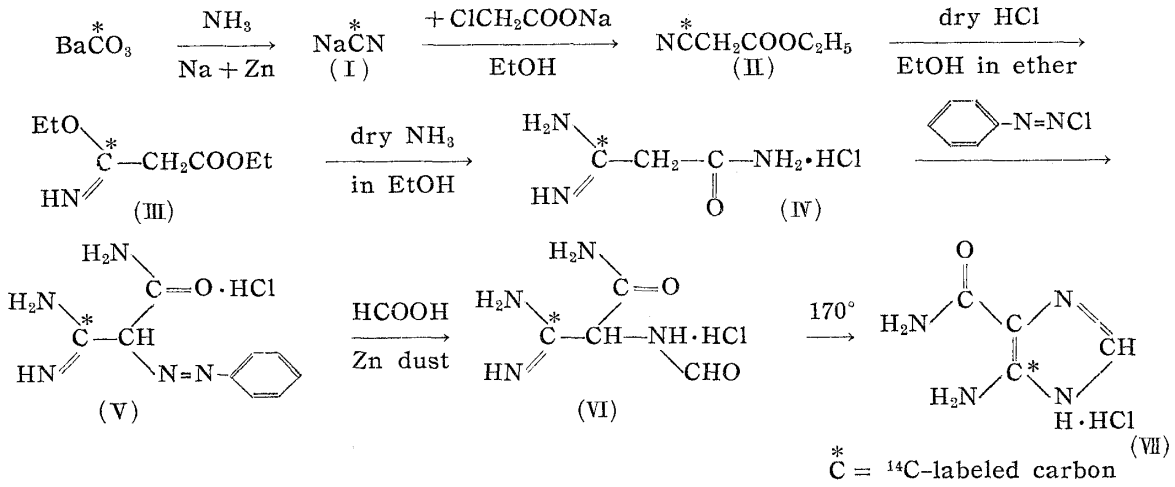
Process C reported by Cook *et al.*[7] involves the interaction of aminocyanoacetamide and formamidine hydrochloride in boiling methanol. The procedure has been applied for isotopic synthesis by Miller *et al.*[8] Process D was reported by Show and Woolley[9] as a convenient method for the non-active carboxamide.

The author considered adapting the last method with minor modifications for the synthesis of AICA-^{14}C labeled in the 4-position. The procedure attempted is shown in Chart 2.

Chart 2.



Sodium cyanide-^{14}C (I) was obtained in one step, in a high yield of 95%, by the modified Jeanes procedure,[10,11] by heating barium carbonate-^{14}C with zinc dust and metallic sodium in a stream of ammonia gas passed over hot iron. Care was taken not to lose radioactivity of crude Na^{14}CN in the solution by exchange with previously dissolved CO_2 in the solution and with atmospheric CO_2, otherwise CO_2-free water and CO_2-free air should be used in this procedure.

In six steps, (I), through malonamamidine[3-^{14}C] hydrochloride (IV) and formamidomalonamamidine[3-^{14}C] hydrochloride hemihydrate (VI), was converted into the desired AICA-^{14}C, in an overall yield of 23% from barium carbonate-^{14}C.

AICA-^{14}C hydrochloride ultimately obtained from the recrystallization exhibited only one radioactive spot (Rf 0.57) by paper chromatography (butanol : ethanol : water solvent[12]). A dark spot was observed under ultraviolet light which colored blue with the Pauly reagent,[13] in contrast to most other imidazoles, which form red dyes on coupling with the reagent.

The ultraviolet absorption spectrum in 0.01*M* phosphate buffer at pH 7.4 indicated a single maximum at 267 mμ and agreed with that reported.[14]

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Experimental*

Sodium Cyanide-^{14}C (I)—0.001 mole of anhyd. $Ba^{14}CO_3$(1.0 mc.) was thoroughly triturated in an agate morter with apporoximately 1 g. of Zn dust and 0.2 g. of metallic Na in small pieces. This paste was transferred to a porcelain combustion boat (100 mm. in length and 10 mm. in depth). The boat was heated electrically at 650° for 4 hrs. in a quartz combustion tube (700 mm. in length and 17 mm. in inside diameter) containig 5 g. of piano wire (0.3 mm. diam.) in NH_3 atmosphere. The boat and its contents were washed with 20 cc. of CO_2-free distilled water. The solution was then acidified with H_2SO_4, and the radioactive $H^{14}CN$ was distilled into 1*N* NaOH in 20% excess of the theoretical. $Na^{14}CN$ (I) solution was then evaporated to dryness *in vacuo*. The specific activity of (I): 0.9 mc./millimole as $Ba^{14}CO_3$. Yield, 95%.

Ethyl Cyanoacetate[3-^{14}C] (II)—(I) (0.163 mole; 0.5 mc. of ^{14}C) and sodium chloroacetate (0.17 mole) were heated at 70~80° for 10 mins. and esterified (4 hrs.). (II) $b.p_{20}$ 104°. Yield, 85%.

Iminoethyl[3-^{14}C] Ether Hydrochloride (III)—Dry HCl gas was passed through a mixture of (II) (0.062 mole), dehyd. EtOH (0.069 mole) and dehyd. ether (3.2 g.) chilled in ice-salt bath until the increase in weight became 7.5 g. (III) m.p. 99~100°. Yield, 92%.

Malonamamidine[3-^{14}C] Hydrochloride (IV)—A mixture of (III) (0.057 mole) suspended in 110 cc. dehyd. EtOH, previously saturated with NH_3, was occasionally shaken while NH_3 gas was bubbled during 20 mins. The suspension was kept in a stoppered flask for 5 days at room temperature. Theradioactive amidine, m.p. 174~175°, was obtained in 87% yield.

Phenylazomalonamamidine[3-^{14}C] Hydrochloride (V)—(IV) (mole 0.051 mole) was coupled with benzenediazonium chloride by the method of Show and Woolley.[9] The reaction mixture was maintainedat pH 4 with conc. AcONa solution. Recrystallization of the product from EtOH gave yellow needles, m.p. 199~200°. Yield, 90.5%.

Formamidomalonamamidine[3-^{14}C] Hydrochloride Hemihydrate (VI)—(V) (0.046 mole) was reduced with Zn dust in 93% HCOOH at 45~48° until the reaction mixture became colorless via light green and pale yellow. Recrystallization from EtOH and dehyd. ether, through a gummy substance, afforded (VI), m.p. 93~95°. Yield, 60.5%.

4-Aminoimidazole[4-^{14}C]-5-carboxamide Hydrochloride (VII)—(VI) (0.027 mole) was melted at 100° and crystallized at 170° in an oil bath. The massive pale green product, the impure and crude (VII), was dissolved in a small amount of hyd. EtOH, decolorized with activated charcoal, and its picrate was recrystallized from water. It was converted to the hydrochloride by the method of Miller *et al.*[15] For the removal of a trace of picric acid and radioactive contaminants, (VII) was recrystallized with ethereal solution of dry HCl to (VII), m.p. 255~256°. Yield, 65.2%. Specific radioactivity, 14,500 cpm/micromole (Q-gas flow counter). Picrate, m.p. 237~238°. *Anal.* Calcd. for $C_4H_7ON_4Cl$ (diluted with carrier): C, 29.6; H, 4.3; N, 34.4, Cl, 21.8. Found: C, 29.8; H, 4.3; N, 34.0; Cl, 21.7.

Summary

4-Aminoimidazole[4-^{14}C]-5-carboxamide hydrochloride (AICA-^{14}C) was synthesized in order to use it as the radioactive biological tracer to follow the biosynthetic mechanism of polynucleotide purines and the behavior of antimetabolites expected to act during the course of such biosysnthesis. The synthetic route started with barium carbonate-^{14}C and went through 7 steps including sodium cyanide-^{14}C, malonamamidine[3-^{14}C] hydrochloride, and formamidomalonamamidine[3-^{14}C] hydrochloride hemihydrate, with a final yield of 23% of AICA-^{14}C.

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* All melting points are uncorrected.

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