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# $C^{14}$ Labeled 4(5)-Amino-5(4)-Imidazolecarboxamide in the Biosynthesis of Purines[1]

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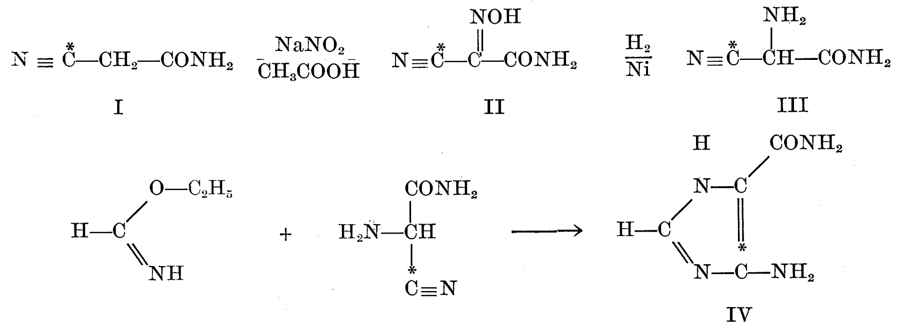
In a recent report from this laboratory (*1*), it has been shown that pigeon liver homogenates can convert 4(5)-amino-5(4)-imidazolecarboxamide (IV) to hypoxanthine, whereas the intact pigeon transforms the substance into uric acid. As part of a general program to study the biosynthesis of purines from labeled precursors, we have developed a convenient synthesis of 4(5)-amino-5(4)-imidazolecarboxamide containing $C^{14}$ in the 4(5) position. Recently two new syntheses of this compound (nonlabeled) have been reported (*2, 3*).



Sodium cyanide (6 g) containing 0.6 mc of radioactivity as $C^{14}$ and chloroacetic acid (12.7 g) were converted to cyanacetamide (I) by a modification of procedures described in *Organic Syntheses* (*4, 5*). The resulting labeled cyanacetamide was diluted with nonlabeled cyanacetamide (total, 16.75 g) and subsequently nitrosated with sodium nitrite in dilute acetic acid at 10°–15° to give labeled nitrosocyanacetamide (II) (17.4 g). The nitrosocyanacetamide was reduced with hydrogen and Raney nickel catalyst in methanol solution to the corresponding unstable aminocyanacetamide (III), which was used immediately in the next step without isolation. The addition of ethyl formimino ether in 20% excess to the cold methanol solution of aminocyanacetamide gave the desired 4(5)-amino-5(4)-imidazolecarboxamide (IV) which was isolated and purified as the picrate (9.5 g pure); mp, 239°–240° C decomposition (cor.) when inserted in a bath preheated to 227°.

*Analysis* calculated for $C_{10}H_9O_8N_7$: C, 33.81; H, 2.56; N, 27.58. Found: C, 34.00; H, 2.51; N, 27.68.[2]

The picrate was conveniently converted to the hydrochloride by suspending it in dry acetone and rapidly saturating with dry HCl while maintaining the temperature constant; mp, 262.5°–263.5° decomposition (cor.) when inserted in a bath preheated to 245°.

*Analysis* calculated for $C_4H_7ON_4Cl$: C, 29.53; H, 4.34; N, 34.46; Cl, 21.83. Found: C, 29.78; H, 4.39; N. 34.30; Cl, 21.78.[2]

The picrate and hydrochloride respectively were found to be identical with samples of picrate and hydrochloride[3] prepared by the method of Windaus and Langenbeck (*6*).

A sample of the picrate was oxidized to carbon dioxide and assayed as $BaCO_3$ in the Geiger counter to give a value of 400 cpm/mg of carbon (calculated to infinite thickness) for the picrate. This corresponds to 1,000 cpm/mg of carbon for the free base.

To 5 rats of 155 g average weight was administered subcutaneously a total of 0.92 g of the 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride in 45 ml of solution half isotonic with respect to sodium chloride. Each rat received 1 ml of the solution three times a day for 3 days. The rats were kept in individual metabolism cages, and 24-hr collections of urine were pooled and kept in the cold room. The respiratory $CO_2$ contained negligible radioactivity.

The rats were killed on the fourth day (15 hr after the last injection), and the free nucleotides and the nucleic acids isolated by the procedure used in this laboratory. Adenine from the nucleotides and the 2 purines from the nucleic acids were isolated as the picrates, recrystallized, and converted to the hydrochlorides by suspending in acetone and saturating rapidly with dry HCl. Each fraction was separately purified by the chromatographic procedure of Cohn (*7*). The fractions were followed spectrophotometrically by comparing ratios of readings at 262 mμ and 248 mμ. A middle fraction containing a

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[2] We are indebted to K. B. Streeter, Edward A. McFadden, and Joyce L. Pyett, of the Organic Research Laboratory of Sharp & Dohme, Inc., for these analyses.

[3] We are indebted to Everett M. Schultz, of the Organic Research Laboratory of Sharp & Dohme, Inc., for a sample of the hydrochloride prepared by the method of Windaus and Langenbeck.

mixture of adenine and guanine was run through the column a second time. The fractions thus obtained were neutralized with concentrated NaOH and precipitated with $CuSO_4$ and $NaHSO_3$. The separated fractions were converted to picrates and purified to constant radioactivity by repeated recrystallization from 25% acetic acid. The counts were corrected to infinite thinness and are recorded in the table.

We found 39% of the administered 4(5)-amino-5(4)-imidazolecarboxamide in the pooled urines, using the colorimetric procedure of Bratton and Marshall (*8*). Of this amount, at least 13% was present in a nondiazotizable form, which was freed on mild HCl hydrolysis.

Isolation and separation of the allantoin and 4(5)-amino-5(4)-imidazolecarboxamide from the urine were effected by the allantoin procedure of Valentine, Gurin, and Wilson (*9*), involving $AgNO_3$ and $NH_4OH$ precipitation (the imidazole compound precipitated first) followed by mercuric acetate precipitation of each of the fractions. There was obtained 0.69 g of the crude imidazole picrate which, on purification, showed no significant difference in radioactivity from a sample of the picrate of the administered material. The allantoin (0.274 g) was purified to constant radioactivity after treating with Dowex 50 to remove any basic material. The radioactivities are shown in Table 1.

TABLE 1

|  | Compound | Cpm/mg comp. inf. thinness |
|---|---|---|
| 1 | 4(5)-amino-5(4)-imidazolecarboxamide picrate | 1,160 |
| 2 | 4(5)-amino-5(4)-imidazolecarboxamide picrate from urine | 1,110 |
| 3 | Nucleic acid guanine picrate | 103 |
| 4 | Nucleic acid adenine picrate | 134 |
| 5 | "Nucleotide" adenine picrate | 122 |
| 6 | Allantoin | 482 |

The carbon of the 4(5) position of 4(5)-amino-5(4)-imidazolecarboxamide appeared in the adenine of the nucleotides and the adenine and guanine of the nucleic acids, as well as in the allantoin of the urine. Some of the unchanged imidazole appeared in the urine with its radioactivity undiluted. This indicates there is no appreciable pool of this material in the body of the rat and suggests that the imidazole compound is not formed as a normal intermediate.

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## An Improved Water Separator

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Many reactions in chemistry involve the removal of water from a reaction mixture through azeotropic distillation with a solvent lighter than water such as benzene or toluene. The water separates from the condensate as it cools. Most of the devices used for this purpose, such as the Dean-Stark receiver, return the condensate through hot vapors to a reservoir where the water separates. We have found that cooling the material in the reservoir greatly hastens the collection of water, and therefore have modified the condenser so that part of the cooling surface is in contact with the condensate, keeping it cold through the help of convection currents. The built-in spiral condenser (see figure) is more compact than the ordinary type. The reservoir, which is made from a graduated centrifuge tube, has a stopcock that permits the use of the apparatus for automatic separation of large quantities of water when a large-scale condensation is done, since an inverted L of rubber or glass tubing attached at the stopcock can be arranged as an automatic water takeoff. A ground joint above the spiral gives a place for an additional condenser if a very rapid reflux is needed.