A NEW SYNTHESIS OF THE PURINES ADENINE, HYPOXANTHINE, XANTHINE, AND ISOGUANINE

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The studies of Emil Fischer (1) on the chemical nature of the bases, adenine and guanine, derived from nucleic acids and of the alkaloids such as caffeine, theobromine, and theophylline obtained from plants showed them to possess a common bicyclic ring structure (I), consisting of a pyrimidine ring fused with an imidazole ring. The first total syntheses from simpler molecules were carried out by Traube (2) who formed first the pyrimidine ring, thereafter completing the imidazole portion of the structure. Subsequent syntheses have, in general, been modifications of Traube's method (3, 4). The alternative possibility of initially preparing the imidazole part of the purine structure has been appreciated by a number of workers who found, with few exceptions (5), that the necessary intermediates were difficult to obtain (6). Renewed interest in the synthesis of purines through imidazole precursors is provided by the implication of such precursors in the metabolic steps leading to purine biosynthesis (7). The recent synthesis of 4-amino-5-imidazolecarboxamide in good yield from cyanoacetic ester as described by Shaw and Woolley (8) overcomes such difficulties of inaccessible intermediates and has now been made the basis of a new total synthesis of purines.

Naturally occurring purines contain either a 6-oxo or 6-amino substituent as shown in generalized formulas II and III (where the 2-substituent, R, may be —H, —OH, —NH$_2$) and may therefore be thought of as derived from either 4-amino-5-imidazolecarboxamide or from 4-amino-5-imidazolecarboxamidine. The formation of purines was studied first with 4-amino-5-imidazolecarboxamide (IV) as the intermediate imidazole. When this base was heated in formamide at 185°, hypoxanthine (VI) was formed. Since the last step in the preparation of 4-amino-5-imidazolecarboxamide (8) was the cyclization of formamidomalonamidine hydrochloride (VII) by heat, the possibility of converting the latter in a single operation to hypoxanthine in hot formamide recommended itself. This transformation was achieved in a yield of 62 per cent. The stepwise course of the reaction was indicated by the isolation, from a solution heated at 150° instead of 185°, of the intermediate 4-formamido-5-imidazolecarboxamide (V). The formamido compound was obtained more con-
veniently, however, from the aminimidazole (IV) through the action of formic acid and acetic anhydride. It is of interest to note that 4-formamido-5-imidazolecarboxamide (V) cyclizes readily to hypoxanthine (VI) in aqueous solution in the presence of so weak an alkali as bicarbonate. In acid solution, hydrolysis of the formyl group takes place and the free aminimidazole is liberated.

\[
\begin{align*}
\text{(I)} & \quad R \quad \text{(II)} & \quad R \\
\text{(III)} & \\
\end{align*}
\]

The synthesis of xanthine (IX) from 4-amino-5-imidazolecarboxamide (IV) was readily accomplished in a yield of 75 per cent by fusion with urea. This reaction had been carried out by Stetten and Fox (9) on the base isolated from *Escherichia coli* before the chemical nature of the bacterial product was understood. In a conversion to xanthine in a stepwise manner, the aminimidazole was treated with ethyl chlorocarbonate and the resultant 4-carbethoxyamido-5-imidazolecarboxamide (VIII) cyclized to xanthine (IX) by means of heat or alkali.

A number of attempts were made to transform 4-amino-5-imidazolecarboxamide (IV) to guanine by means of reagents commonly used to convert amines to substituted guanidines, such as S-methylisothiourea, cyanamide, and guanidine. As interpreted on the basis of paper chromatography in an aqueous quinoline-collidine mixture (12) together with ultraviolet spectroscopy, these reactions lead to the formation not only of
guanine, but also of xanthine and other unidentified insoluble products. A method suitable for the preparation of guanine was not found.

For the preparation of 6-aminopurines, such as adenine, 4-amino-5-imidazolecarboxamidine (XIII) was desired. A synthesis of this compound was therefore undertaken by application of the novel ring closure used in the preparation of 4-amino-5-imidazolecarboxamide (8). Malonitrile was converted to the diamidine (X) which readily coupled with benzenediazonium chloride, producing phenylazomalonamidine (XI). In the reduction of the azo compound by means of zinc dust in formic acid, part of the formamidomalonamidine (XII) produced cyclized to the desired imidazole (XIII). Rather than separate the products, the mixture of amidine hydrochlorides (XII and XIII) was thermally converted entirely to 4-amino-5-imidazolecarboxamidine dihydrochloride (XIII) at 170°. The yield was 25 per cent from malonitrile. The amidine has recently been reported as a hydrolytic degradation product of adenine (10).

As in the hypoxanthine synthesis, the aminoimidazole (XIII) was readily formylated in formic acid and acetic anhydride to 4-formamido-5-imidazolecarboxamidine (XIV). In hot bicarbonate solution, the pyrimidine ring closed, completing the synthesis of adenine (XV) in good yield. The tendency for the ring to close in the case of the amidine (XIV) was greater than for the corresponding amide. Recrystallization from water resulted in a considerable conversion to adenine. The cyclization also took place
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in warm hydrochloric acid in contrast to the formamidoamide (V) which lost its formyl group under the same conditions.

When 4-amino-5-imidazolecarboxamidine dihydrochloride was fused with urea, isoguanine (XVI) was obtained.

The spectra in the ultraviolet region of the synthetic purines were measured and found to be in general agreement with published curves (11).

EXPERIMENTAL

Melting points were determined in a copper block and are uncorrected.

Paper Chromatography—For an additional check on the identity of the synthetic purines and for a convenient means of studying the effect of reaction conditions, paper chromatography was of value. The method of Vischer and Chargaff (12) was employed except that upward migration of the solvent boundary was used. The compounds were applied to Whatman No. 1 filter paper along a line 3 cm. above the solvent level. The paper was formed into a cylinder and placed in a mixture of n-butanol (4 parts), diethylene glycol (1 part), and water (1 part). A beaker of N NH₄OH was present during the solvent rise which was allowed to proceed usually for about 16 hours.

Hypoxanthine—A solution of formamidomalonamidine hydrochloride hemihydrate (8) (1.0 gm.) in formamide (15 ml.) was heated for 2½ hours in a flask suspended in an oil bath at about 185°. The flask was then attached to a water pump and heating was continued until crystals began to separate. The cooled suspension was thinned with 95 per cent alcohol and filtered. The resultant dark powder was stirred with cold water (10 ml.) and the insoluble portion taken up in boiling water (60 ml.). The hot solution was filtered to remove colored impurities, concentrated to 25 ml., and allowed to stand at 4°. The resulting crystals were dried at 100° in vacuo, yielding 0.45 gm., 62 per cent. A solution of the product in phosphate buffer at pH 6.5 showed a single maximum absorption at 250 mμ, ε = 11,500. On a paper chromatogram, a single spot, R₇ = 0.40, was obtained, as with authentic hypoxanthine.

C₉H₈ON₄. Calculated, C 44.12, H 2.96; found, C 44.15, H 3.13

When 4-amino-5-imidazolecarboxamide hydrochloride was treated with formamide at the same temperature for 1½ hours, a 63 per cent yield of hypoxanthine was obtained.

4-Formamido-5-imidazolecarboxamide (V)—Formamidomalonamidine hydrochloride hemihydrate (0.50 gm.) was heated in formamide (5 ml.) for 45 minutes in an oil bath at 150°. Most of the formamide was then distilled off in vacuo. The residual syrup was thinned gradually with 95 per cent alcohol until the separating crystals appeared to become gummy,
then filtered. The product, 0.25 gm., was recrystallized from water and dried at 100° in vacuo.

\[ \text{C}_4\text{H}_7\text{O}_2\text{N}_4 \]. Calculated, C 38.97, H 3.92; found, C 38.73, H 3.87

For preparative purposes, the following procedure was more suitable. 4-Amino-5-imidazolecarboxamide hydrochloride (1.63 gm.) and sodium formate (0.74 gm.) were dissolved in 98 per cent formic acid (5 ml.). Acetic anhydride (10 ml.) was added and an exothermic reaction took place, initiated by gentle warming. After 10 minutes, the mixture was heated in a water bath at 70° for an additional \( \frac{1}{2} \) hour, then taken to dryness in vacuo. The residue was stirred with water (15 ml.) to remove starting material and salts and filtered. The insoluble portion, 1.32 gm., 86 per cent, gave a single spot, \( R_F = 0.50 \), on the paper chromatogram and was therefore essentially free of hypoxanthine. Recrystallization of the material from water gave 0.87 gm.

When the formyl compound was heated for 15 minutes with methanolic HCl plus sufficient water to effect solution, 4-amino-5-imidazolecarboxamide hydrochloride was recovered from the solution, m.p. 255-256° with decomposition.

The formyl derivative showed a single maximum absorption in the ultraviolet region at 268 \( \text{m} \mu \), \( \varepsilon = 10,900 \) at pH 6. In this respect it resembled the free amino compound. In addition, no neutral solvent mixture was found to distinguish between the base and its formyl derivative by paper chromatography. The formyl compound sublimed unchanged above approximately 220°. The imidazole compounds, unlike purines, are not precipitated by ammoniacal silver nitrate.

**Cyclization of 4-Formamido-5-imidazolecarboxamide to Hypoxanthine under Alkaline Conditions**—The formamido compound (0.75 gm.) was added to a solution of sodium (0.30 gm.) in absolute alcohol (50 ml.) and the suspension refluxed for 6 hours. Uncyclized formyl derivative remaining at this time was hydrolyzed by acidification with alcoholic HCl and continued refluxing for 10 minutes. After the mixture had been taken to dryness, the residue was dissolved in dilute KOH, treated with charcoal, and precipitated with acetic acid. The hypoxanthine thus obtained was recrystallized from water (25 ml.) to yield 0.43 gm., 65 per cent.

\[ \text{CsH}_{16}\text{O}_4\text{N}_4 \]. Calculated, C 44.12, H 2.96; found, C 44.18, H 2.90

4-Formamido-5-imidazolecarboxamide was also readily cyclized to hypoxanthine in dilute aqueous alkali. The formyl compound (93 mg.) was refluxed in 0.05 \( N \) potassium bicarbonate (40 ml.) for 3\( \frac{1}{2} \) hours. The solution was taken to dryness, and the residue stirred with a small volume of water. The insoluble portion was dissolved in \( \text{NH}_4\text{OH} \) and the solution
treated with 5 per cent silver nitrate. The precipitate was washed and decomposed with hydrogen sulfide in the usual manner. The resultant solution showed selective absorption at 240 m\(\mu\), and comparison with dilutions of known hypoxanthine concentrations indicated the formation of 70 mg. of hypoxanthine in the reaction, a conversion of 85 per cent. At 37\(^\circ\), ring closure of the formamido compound to hypoxanthine can also be observed in boric acid solution by the above method.

Xanthine—4-Amino-5-imidazolecarboxamide hydrochloride (0.30 gm.) and urea (0.30 gm.) were fused together for 2 hours in an oil bath at 175\(^\circ\). The cooled melt was ground up with added water. The crude xanthine was filtered and washed. A solution of the product in \(\text{N KOH}\) was decolorized with charcoal, and the xanthine precipitated by the addition of acetic acid. Finally, the product was recrystallized by concentration of a strongly ammoniacal solution to half volume, yielding 0.21 gm., 75 per cent. The sample was dried in \textit{vacuo} at 125\(^\circ\) for 2 hours.

\[\text{C}_9\text{H}_8\text{O}_2\text{N}_4. \quad \text{Calculated, C 39.48, H 2.65; found, C 39.38, H 2.81}\]

A solution of the product in phosphate buffer at pH 6.5 had maximum absorption at 268 m\(\mu\), \(\epsilon = 10,800\). Like an authentic sample of xanthine, this material gave an \(R_f\) value of 0.28 on the paper chromatogram.

4-Carbethoxyamido-5-imidazolecarboxamide—4-Amino-5-imidazolecarboxamide hydrochloride (0.82 gm.) was stirred at 0\(^\circ\) with a solution of potassium bicarbonate (0.60 gm.) in water (15 ml.). At 10 minute intervals, three 0.2 ml. portions of ethyl chlorocarbonate were added with additional potassium bicarbonate (0.2 gm. per 2 ml.). Stirring was continued for 1 hour after the last addition. The separated crystals were filtered and combined with an additional small crop obtained on treating the filtrate with acetic acid, yielding 0.36 gm., 36 per cent. The product was recrystallized from water.

\[\text{C}_{15}\text{H}_{16}\text{O}_2\text{N}_4. \quad \text{Calculated. C 42.42, H 5.08, N 28.27} \quad \text{Found. C 42.27, H 5.21, N 28.54}\]

The product melted at about 175\(^\circ\), with foaming and resolidification, due to the formation of xanthine. Analytically pure xanthine was obtained when the carbethoxyamido compound was refluxed in nitrobenzene for 10 minutes, followed by recrystallization. The same change was brought about by heating in concentrated \(\text{NH}_2\text{OH}\) at 100\(^\circ\). The identity of the products was checked by paper chromatography and spectroscopy.

\textit{Phenylazomalonamidine Dihydrochloride—Malonitrile and 2 molar equivalents of absolute ethanol in dioxane solution were saturated at 0\(^\circ\) with hydrogen chloride as described by McElvain and Schroeder (13). The resultant imino diethyl ether dihydrochloride on treatment with alcoholic}
ammonia gave malonamidine dihydrochloride (14) in a yield of 60 per cent for the two steps.

Aniline (9.6 ml.) dissolved in 6 N hydrochloric acid (60 ml.) was diazotized below 5° by slow addition of a solution of sodium nitrite (8 gm.) in water (20 ml.). 15 minutes after the addition had been completed, the solution was mixed with malonamidine dihydrochloride (17.3 gm.) in water (75 ml.). The pH was adjusted to about 4 by the slow addition of powdered potassium bicarbonate (20 gm.) followed by sufficient concentrated sodium acetate. The reaction mixture was left overnight at room temperature, subsequently freed of some dark gum by filtration, and concentrated in vacuo until thickened by crystallization. The fine, yellow needles of azo compound were filtered with suction and washed twice with 6 N HCl. A second crop of azo compound may be obtained by the slow addition of concentrated HCl to the filtrate. Such material is contaminated with inorganic chlorides and should be recrystallized from a small volume of water. The product is quite soluble in water. Dried to constant weight in vacuo over NaOH, the yield of azo compound is very nearly quantitative. For analysis, a sample was recrystallized several times from water and dried for 2 hours in vacuo at 78°. The results indicate hydration.

\[
\text{C}_8\text{H}_{13}\text{N}_4\cdot2\text{HCl}\cdot\frac{1}{2}\text{H}_2\text{O}. \quad \text{Calculated. C 37.77, H 5.28, Cl}^- 24.8
\]

\[
\text{Found. C 37.65, H 5.35, Cl}^- 24.4
\]

4-Aminodimidazolecarboxamidine Dihydrochloride—Phenylazomalondimidine dihydrochloride (10 gm.) in 98 per cent formic acid (100 ml.) was treated gradually with zinc dust (10 gm.) at 40°-45°. The colorless reaction mixture was filtered. The filtrate, combined with formic acid washings of the insoluble material, was concentrated to a syrup in vacuo. After the addition of water, the concentration was repeated to complete the removal of formic acid. An aqueous solution of the residue was freed of zinc by means of hydrogen sulfide, and the filtrate taken once more to a syrup in vacuo. The crude reaction product was now precipitated by dissolving the syrup in methanol (25 ml.) and adding anhydrous ether (200 ml.). The crude dihydrochloride, dried over sodium hydroxide in a vacuum desiccator, was heated for 15 minutes in an oil bath at 170°-180°. The melt was dissolved in a minimum amount of water and treated with absolute alcohol and ether. The initial precipitates contained most of the pigmented impurities and were discarded. Continued precipitation yielded, finally, 4.2 gm. of crystals, m.p. 238°-242°. Recrystallization gave 3.3 gm., m.p. 242°-244°, 45 per cent.

\[
\text{C}_8\text{H}_{14}\text{N}_3\text{Cl}_2. \quad \text{Calculated. C 24.25, H 4.58, Cl}^- 35.8
\]

\[
\text{Found. C 24.08, H 4.46, Cl}^- 36.3
\]

If the crude hydrochloride from the zinc dust reduction were subjected to
crystallization, instead of thermal conversion to the imidazole as described, crystals could be obtained which appeared to consist of formamidomalonamidine (XII) mixed with imidazole (XIII). Thus, when the gummy product was triturated with a small volume of methanol, crystallization slowly proceeded and was completed by the slow addition of ether, yielding 4.3 gm., m.p. about 240°. However, if the sample was inserted into a preheated block at 150°, foaming at about 165° was observed with recrystallization of the melt and eventual final melting at 240°. The pure imidazole amidine undergoes no preliminary change at 165° observed in the same manner. When taken in the usual way, the melting point observed for formamidomalonamidine is that of the imidazole formed during the heating. That some imidazole was present at the start was indicated by some selective absorption at 285 m.µ.

4-Amino-5-imidazolecarboxamidine dihydrochloride obtained in the above synthesis gave a single maximum absorption at 285 m.µ, ε = 11,300, pH 6.5.

Adenine—4-Amino-5-imidazolecarboxamidine dihydrochloride (0.99 gm.) and sodium formate (0.70 gm.) were dissolved in 98 per cent formic acid (10 ml.) and treated with acetic anhydride (10 ml.). After a lag of 10 minutes, an exothermic reaction occurred, bringing the mixture to the boiling point. When this had subsided, the solution was warmed at 70° for an additional 15 minutes, then taken to a syrup in vacuo. The residue crystallized when stirred with water and was filtered, yielding 0.70 gm. of 4-formamido-5-imidazolecarboxamidine. The base showed a single maximum in the ultraviolet at 272 m.µ; a slight contamination with adenine was suggested by the shape of the curve.

4-Formamido-5-imidazolecarboxamidine (0.20 gm.) was refluxed for 1 hour in 0.5 N potassium bicarbonate (10 ml.). The solution was neutralized, concentrated to 2 ml., and left at 4° to crystallize. The filtered crystals, washed with water and dried in air at 100°, weighed 0.155 gm., a yield of 80 per cent for the two steps from 4-amino-5-imidazolecarboxamidine dihydrochloride. For analysis, a sample recrystallized from water was dried to constant weight in vacuo at 125°.

C₃H₆N₆. Calculated, C 44.45, H 3.73; found, C 44.26, H 3.82

The base formed a picrate, m.p. 286–287°, undepressed by admixture with the picrate of an authentic sample. On a paper chromatogram, both synthetic and natural bases gave Rf = 0.58. In phosphate buffer at pH 6.5, the synthetic adenine showed a single maximum absorption in the ultraviolet at 261 m.µ, ε = 13,600.

Isoquinine—4-Amino-5-imidazolecarboxamidine dihydrochloride (0.30 gm.) and urea (0.30 gm.) were mixed and heated for 2 hours in an oil bath
at 160°. The initial melt gradually solidified. After cooling, the mixture was thoroughly triturated with water, washed, and dried. The product, 0.22 gm., was recrystallized by concentrating a solution of the base in strong ammonium hydroxide at the boiling point until crystals began to form. The material which separated from the chilled solution was washed and dried in vacuo at 125° for 3 hours.

C₁₈H₂₄N₄O₅. Calculated, C 39.15, H 3.34; found, C 39.67, H 3.30

In phosphate buffer at pH 6.5, the synthetic isoguanine exhibited two maxima in the ultraviolet: at 240 mμ, ε = 8000, and at 286 mμ, ε = 9850.

**SUMMARY**

The preparation of imidazole intermediates and the conversion of these to purine bases have been carried out easily and with high yields by new methods which offer a practical route of purine synthesis. The synthesis of 4-amino-5-imidazolecarboxamide in an over-all yield of 25 per cent from malonitrile has been accomplished. This imidazole, and the corresponding carboxamide, have each been condensed with formic acid and the resulting 4-formamido-5-imidazolecarboxamide and carboxamide cyclized to adenine and hypoxanthine, respectively. When the aminoimidazoles were heated with urea, the amidine lead to isoguanine; the amide, to xanthine.

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**BIBLIOGRAPHY**

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