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A New Synthesis of Adenine and 4-Aminoimidazole-5-carboxamide

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Numerous methods have been reported for the synthesis of adenine, most of which are modifications of Traube's method (1) via 2-substituted 4,5,6-triaminopyrimidines. One distinctly different route using malonodiamidine as an intermediate was reported by Shaw (2). However, Shaw's synthesis of 4-aminoimidazole-5-carboxamide (I) and 2-aminomalonamidine (3), as well as Heilbron's method (4) for the preparation of 2-aminoacetamide are complicated and the yields are unsatisfactory.

We have found that 1,3-diamino-1,2,3-trioximinopropane (II) produces 4-formylaminoimidazole-5-carboxamide (IV), when refluxed in formic acid in the presence of a reducing agent such as zinc dust or Raney-nickel. When 3-aminofurazane-4-carboxamidoxime (III) was similarly treated with formic acid and a reducing agent, adenine was formed. Liau *et al.*, (5) employed the same approach for the synthesis of 6-hydroxypurines.

When III was refluxed in formic acid for several hours, followed by addition of a reducing agent and an additional 1-2 hours refluxing, IV was obtained instead of adenine and the yields of IV were better than when produced via II. 4-Aminoimidazole-5-carboxamide (I) can be obtained easily from IV by treatment with acids (2). The yields of adenine vary considerably depending upon the reducing agents, Raney-nickel being superior to either zinc dust or hydrogen at high pressures with palladium-charcoal catalyst.

The adenine obtained was identified by direct comparison of its infrared and ultraviolet spectra with those of authentic samples. The structure of IV was confirmed by elemental analysis and the fact that IV was converted into hypoxanthine by heating with dilute potassium bicarbonate solution and into I with dilute hydrochloric acid.

The synthetic procedure to obtain II reported by Longo (6) is troublesome and yields are unsatisfactory since the isolation of isonitrosomalononitrile is inevitably accompanied by considerable decomposition. It has been found that malononitrile is readily nitrosated in aqueous solutions and the subsequent addition of hydroxylamine hydrochloride at *p*H 9-11 gives II without isolation of intermediates.

Compound III was prepared either by heating an aqueous solution of II, (0.1 *N* with respect to sodium hydroxide) or from malononitrile treated first with nitrous acid followed by treatment with hydroxylamine hydrochloride (*p*H adjusted to 10). When compound III was refluxed with formic acid, different products were obtained depending on the periods of refluxing: Refluxing periods of 0.5, 1.0, 6.0 and 16.0 hours produced V (m.p. 136-139°), VI (m.p. 137-138°), VII (m.p. 229-230° dec.) and VIII (m.p. 174-177°) respectively.

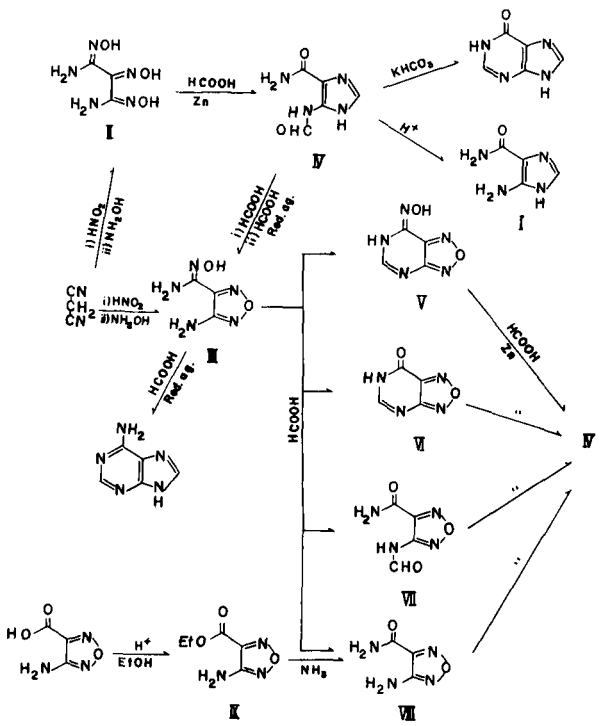
Since V has the empirical formula $C_4H_3N_5O_2$ and showed infrared absorption bands at 3440, 3305 and 3161 cm^{-1} the structure was deduced to be 7-hydroximino-6,7-dihydrofurazano[3,4-d]pyrimidine. The structural formula of VI was confirmed by the determination of its empirical formula ($C_4H_2N_4O_2$) and by its infrared absorption bands at 3320, 3100 and 1698 cm^{-1} and by the absence of bands in the 3000-4000 cm^{-1} region. Compound VIII was found to have the composition $C_3H_4N_4O_2$. Its ultraviolet spectrum resembles that of III and its infrared absorption at 1675 and 1600 cm^{-1} show the presence of a primary amino group. The bands at 794, 1010 and 1199 cm^{-1} are in good agreement with those observed at 792, 992 and 1176 cm^{-1} of II. The facts led to the conclusion that VIII had the structure of 3-aminofurazane-4-carboxamide. This conclusion was further confirmed by the synthesis of VIII from 3-aminofurazane-4-carboxylic acid (6). The acid (3-aminofurazane-4-carboxylic acid) was esterified to give its ethyl ester (IX) which in turn was converted into VIII by treatment with methanolic ammonia.

Compound VII ($C_4H_4N_4O_3$) gave VIII upon heating with dilute hydrochloric acid. The infrared absorption bands at 1696, 1602, 1674 and 1520 cm^{-1} show VII to have primary and secondary amide groups. These facts suggested the structure of VII to be 3-formylaminofurazane-4-carboxamide.

When these compounds V, VI, VII and VIII were refluxed with formic acid in the presence of zinc dust, 4-formylaminoimidazole-5-carboxamide (IV) was obtained in each instance with yields of 20, 10, 20 and 60%, respectively (based on quantities of V, VI, VII and VIII).

All attempts to use cyanoacetamide or ethyl cyanoacetate in place of malonodinitrile to prepare IV resulted in failure.

The oximino group in III is unstable in formic acid. When hydrolysis of the carboxamidoxime group takes place, 4-formylaminoimidazole-5-carboxamide IV may be formed, which however, cannot cyclize to give a purine in acidic medium (2), *e.g.*, formic acid. However when the reduction step precedes the hydrolysis step, the cyclization to form the pyrimidine ring becomes possible even in acid solution (2). Therefore under these latter conditions, adenine formation can occur in formic acid. The reduction of the carboxamidoxime group to the amidine group seems to be competitive with the hydrolysis to the carboxamide group, and it is reasonable to expect that the yields may be influenced by the type of reducing agent employed.



EXPERIMENTAL

Paper chromatography.

Toyo filter paper No. 51 was employed in the solvent system A, *n*-butyl alcohol-acetic acid-water (4:1:1, vol./vol.) and solvent system B, *n*-propyl alcohol-*conc.*-aqueous ammonia-water (20:12:3, vol./vol.). The paper chromatogram developed by the ascending method was irradiated by ultraviolet radiation for the detection of the position of the material.

1,3-Diamino-1,2,3-trioximinopropane (II).

To a solution of malononitrile (65 g.) in 1 l. of 2 *N* hydrochloric acid was added an aqueous solution of sodium nitrite (138 g.) with stirring at 15-20° and the mixture was allowed to stand overnight at room temperature. An aqueous solution of hydroxylamine hydrochloride (152 g.) was added to the reaction mixture and the *p*H of the solution was adjusted to 10 by the gradual addition of 10 *N* sodium hydroxide solution keeping the temperature below 20°. The solution was kept at 30° for 1 hour, adjusted to *p*H 7.5 and concentrated under reduced pressure. The solution was cooled in a refrigerator to yield 83 g. of the product, which melted and decomposed at 147-148°. Thirty g. of the product still remained in the mother liquor. The crystalline product was used for the next reaction without further purification. Repeated recrystallization from water gave white crystals melting at 152-153° dec., Rf: 0.21 (solv. A), 0.25 (solv. B).

U. V. λ max (*p*H 1): 276 mμ (ε, 4,000); λ max (*p*H 11): 267 mμ (ε, 12,000).

Anal. Calcd. for $C_3H_7N_5O_3$: C, 22.36; H, 4.36; N, 43.48. Found: C, 22.57; H, 4.61; N, 43.48.

3-Aminofurazane-4-carboxamidoxime (III).

(a) A mixture of II (16.1 g.) and 200 ml. of 0.1 *N* sodium hydroxide solution was refluxed for 1 hour. A paper chromatogram of the reaction mixture showed a single spot when placed under ultraviolet light (Rf: 0.84 (solv. A), 0.84 (solv. B)). Concentration and cooling of the solution gave 13.2 g. of crude crystals melting at 187-188°. Recrystallization from water gave crystals melting at 190-191°.

U. V. λ max (*p*H 1): 280 mμ (ε, 5,300), λ max (*p*H 11): 289 mμ (ε, 6,000).

Anal. Calcd. for $C_3H_5N_5O_2$: C, 25.18; H, 3.52; N, 48.94. Found: C, 25.06; H, 3.70; N, 48.64.

(b) To a solution of malononitrile (65 g.) in 1 l. of 2 *N* hydrochloric acid was added dropwise with stirring at 15-20°, 138 g. of sodium nitrite dissolved in 200 ml. of water. The mixture was then stirred for 1 hour and allowed to stand overnight at room temperature. An aqueous solution of hydroxylamine hydrochloride (152 g.) was added to the mixture and the *p*H of the solution was adjusted to 10 by the addition of 10 *N* sodium hydroxide solution while maintaining the temperature below 20°. After being kept at 30° for 1 hour, the solution was heated to reflux for 2 hours. On cooling 95 g. (64%) of the product separated melting at 186-188° and further concentration and cooling of the mother liquor gave 35 g. (24%) of the product. These crystals were used in the following reactions without further purification. Recrystallization from water gave white crystals, m.p. 190-191°, undepressed on admixture with a sample prepared by the method (a) or by Longo's procedure (6).

Adenine.

(a) To a solution of III (14.3 g.) in 300 ml. of 98% formic acid was added Raney-nickel prepared from 71.5 g. of Ni-Al alloy and the mixture was heated to reflux with stirring for 3 hours. The paper chromatographic analysis showed the presence of 12.1 g. (90% based on III) of adenine in the reaction mixture. The mixture was filtered and the precipitate washed thoroughly with formic acid (98%). The combined filtrate was evaporated to dryness *in vacuo* and the residue dissolved in 500 ml. of water. The *p*H of the aqueous solution was adjusted to 5.5 and hydrogen sulfide was introduced into the solution. The precipitated sulfide was filtered while it was hot and the residue was extracted thoroughly with hot water. The combined solution was concentrated to dryness *in vacuo* and recrystallized from water to give 7.8 g. (55% based on III) of white crystals. The ultraviolet and infrared spectra of this material were both identical with those of an authentic sample of adenine.

Anal. Calcd. for $C_5H_5N_5$: C, 44.44; H, 3.73; N, 51.83. Found: C, 44.18; H, 4.10; N, 52.31.

(b) A mixture of 14.3 g. of III, 65 g. of zinc dust and 300 ml. of 99% formic acid was heated to reflux for 5 hours. After cooling, the mixture was filtered and the residue was washed thoroughly with formic acid (99%) and the combined filtrate was evaporated to dryness. The yield determined by paper chromatographic analysis was 49% based on III. The residue was dissolved in 500 ml. of water and the *p*H of the solution was brought to 5.6 with an aqueous solution of sodium hydroxide. Hydrogen sulfide was introduced into the solution and the mixture was heated and filtered while it was hot. The residue was extracted several times with hot water. The combined solution was taken to dryness under reduced pressure and 5.4 g. of crude adenine (80% purity) was obtained. Recrystallization from water gave pure adenine, the infrared and ultraviolet spectra of which were identical with those of an authentic sample.

(c) To a solution of III (1.43 g.) in 50 ml. of formic acid (99%) was added 1 g. of palladium-charcoal (2%) and the mixture was shaken at 105° for 3 hours in an autoclave under the initial hydrogen pressure of 130 kg./cm^2. After cooling, the catalyst was filtered off and the filtrate was analyzed by paper chromatography. The yield was 59%. The filtrate was taken to dryness and the residue was dissolved in a small amount of water and the solution was neutralized with an aqueous solution of sodium hydroxide. On concentrating the solution, white crystals of adenine separated, which were recrystallized from water. The infrared and the ultraviolet spectra were identical with those of an authentic sample.

4-Forymylaminoimidazole-5-carboxamide (IV).

(a) To 50 ml. of formic acid (98%) were added 1.6 g. of I and 6.5 g. of zinc dust and the mixture was heated to reflux for 1.5 hours. The paper chromatogram of the reaction mixture showed a single spot (Rf: 0.39 (solv. A) and 0.44 (solv. B)) under ultraviolet light. The yield of IV was found to be 43% based on I by the measurement of optical density at 257 mμ of the extracted solution from the excised spot. The reaction mixture was filtered and the residue was thoroughly washed with formic acid and the combined filtrate evaporated to dryness. The residue was dissolved in 100 ml. of water and the *p*H of the solution was brought to 5, then hydrogen sulfide was passed into the solution. The precipitate was filtered off while it was hot and the residue washed several times with hot water. Concentration of the combined solution and recrystallization of the residue obtained from water gave pale red crystals (0.3 g.), m.p. 247-249°.

U. V. λ max (*p*H 1): 257 mμ (ε, 8,600).

Anal. Calcd. for $C_6H_6N_4O_2$: C, 38.96; H, 3.92; N, 36.35. Found: C, 39.01; H, 4.01; N, 36.03.

A 50 mg. portion of the crystalline product was heated to reflux with 0.05 *N* potassium bicarbonate and the reaction mixture was submitted to paper chromatographic analysis. The paper chromatographic behavior in solvent A and B and the ultraviolet spectra at *p*H 1 and 11 of the specimens extracted from the excised paper chromatographic spots were identical with those of hypoxanthine. Another 50 mg. portion of the crystals was quantitatively deformylated by heating with 5 ml. of 0.1 *N* hydrochloric acid for 15 minutes to give 4-aminoimidazole-5-carboxamide (I), which was confirmed by the paper chromatographic behavior (Rf values: 0.43 (solv. A), 0.58 (solv. B)) and comparison of the ultraviolet spectra at *p*H 1 and 11 with those of an authentic sample.

(b) A mixture of 1.4 g. of III and 50 ml. of formic acid (98%) was heated to reflux for 7 hours and cooled. After zinc dust (6.5 g.) was added, the mixture was further refluxed for 1.5 hours. Paper chromatographic analysis indicated the formation of IV in 60% yield based on III, and crystals obtained by the similar method described in (a) melted at 247-249° and showed no melting point depression on admixture with a specimen prepared by the method (a).

(c) A sample of each V, VI, VII and VIII (50 mg.) was dissolved in 10 ml. of formic acid (98%) and zinc dust (300 mg.) was added and the mixture was heated to reflux for 1.5 hours. The residue was filtered off and washed thoroughly with formic acid (98%). The combined filtrate was concentrated under reduced pressure and the volume was adjusted to 10 ml. with formic acid (98%). Analysis by paper chromatography in solvents A and B showed the formation of IV (Rf value and ultraviolet spectra) in 20, 10, 20 and 60% yield based on V, VI, VII and VIII, respectively.

Ethyl 3-aminofurazane-4-carboxylate (IX).

A solution of 3-aminofurazane-4-carboxylic acid (5 g.) (7) in 200 ml. of ethanol was saturated with dry hydrogen chloride while cooling with an ice-bath. A few drops of concentrated sulfuric acid was added and the mixture was heated to reflux for 24 hours. The solvent was removed under reduced pressure and the residue was recrystallized from ethanol, m.p. 100-103°, yield: 2.3 g.

Anal. Calcd. for $C_5H_7N_3O_3$: C, 38.22; H, 4.49; N, 26.74. Found: C, 38.22; H, 4.67; N, 26.98.

3-Aminofurazane-4-carboxamide (VIII).

(a) A solution of 1 g. of IX in 100 ml. of anhydrous methanol was saturated with ammonia at 20°. After being kept overnight at room temperature, the solution was evaporated to dryness under reduced pressure and the residue recrystallized from water, m.p. 174-176°, yield: 0.7 g.

U. V. λ max (*p*H 1): 287 mμ (ε, 2,100); λ max (*p*H 11): 284 mμ (ε, 2,100).

Anal. Calcd. for $C_3H_4N_4O_2$: C, 28.13; H, 3.15; N, 43.74. Found: C, 28.03; H, 3.32; N, 43.73.

(b) A mixture of 10 g. of III and 100 ml. of formic acid (98%) was heated to reflux with stirring for 16 hours and then evaporated to dryness under reduced pressure. Recrystallization of the residue from water gave 8 g. of crystals, m.p. 174-177°, undepressed on admixture with material obtained by the above method (a).

3-Formylaminofurazane-4-carboxamide (VII).

A mixture of 10 g. of III and 100 ml. of formic acid (98%) was heated to reflux for 6 hours. Formic acid was removed under reduced pressure, water was added to the residue and then evaporated to dryness. Repeated recrystallization from water gave white crystals (4.2 g.) melting at 229-230° dec.

U. V. λ max (*p*H 1) < 220 mμ; λ max (*p*H 11): 234 mμ (measured immediately after the dissolution of the crystal).

Anal. Calcd. for $C_4H_4N_4O_3$: C, 30.78; H, 2.58; N, 35.89. Found: C, 30.72; H, 2.95; N, 35.86.

The crystals (1 g.) which were obtained were added to 100 ml. of 0.2 *N* hydrochloric acid and the mixture was heated to reflux for one hour. The solution was neutralized with sodium hydroxide solution, evaporated to dryness under reduced pressure and the residue recrystallized from water. The crystals melted at 174-176°, undepressed on admixture with VIII.

7-Hydroximino-6,7-dihydrofurazano-(3,4-d)-pyrimidine (V).

A solution of 2 g. of III in 20 ml. of formic acid (98%) was heated to reflux for 30 minutes. Formic acid was removed under reduced pressure, water was added to the residue and then evaporated to dryness. Repeated fractional recrystallization from water gave white crystals (0.2 g.) melting at 136-139° dec.

U. V. λ max (*p*H 1): 286 mμ (ε, 2,500); λ max (*p*H 11): 287 mμ (ε, 2,200).

Infrared absorption bands: 3440, 3305, 3161, 1694, 1640, 1620 cm^{-1}.

Anal. Calcd. for $C_4H_3N_5O_2 \cdot {}^1/_4H_2O$: C, 30.48; H, 2.23; N, 44.44. Found: C, 30.08; H, 2.49; N, 44.76.

7-Oxo-6,7-dihydrofurazano-(3,4-d)-pyrimidine (VI).

A solution of 5 g. of III in 50 ml. of formic acid (98%) was heated to reflux for 1 hour. Formic acid was removed under reduced pressure and 100 ml. of water added and the mixture taken to dryness under reduced pressure. The residue was repeatedly recrystallized from water and 0.8 g. of white crystals melting at 137-138° was obtained.

U. V. λ max (*p*H 1): 257 mμ; λ max (*p*H 11): 243, 290 mμ (shoulder).

Anal. Calcd. for $C_4H_2N_4O_2 \cdot {}^1/_2H_2O$: C, 32.64; H, 2.04; N, 38.08. Found: C, 32.83; H, 2.10; N, 39.27.

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REFERENCES

(1) W. Traube, *Ann.*, 331, 64 (1904).
(2) E. Shaw, *J. Biol. Chem.*, 185, 439 (1950).
(3) E. Shaw, E. W. Woolley, *ibid.*, 181, 89 (1949).
(4) A. H. Cook, I. Heilbron, E. Smith, *J. Chem. Soc.*, 1440 (1949); C. S. Miller, S. Gurin, D. W. Wilson, *J. Am. Chem. Soc.*, 74, 2892 (1952).
(5) C. Liau, K. Yamashita, M. Matsui, *Agr. Biol. Chem.*, 26, 624 (1962).
(6) G. Longo, *Gazz. Chim. Ital.*, 61, 575 (1931).

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