Procedure B. Ethyl *trans*-4-Acetyl-2,5-dimethyl-1-piperazinecarboxylate (V).—A mixture of 18.6 g. (0.1 mole) of ethyl *trans*-2,5-dimethyl-1-piperazinecarboxylate[11] and 25 ml. of acetic anhydride was refluxed for 8 hours. The resulting solution was distilled *in vacuo*. After removal of acetic acid and acetic anhydride two fractions were obtained: (a) 7.2 g. of colorless liquid, b.p. 128–143° (15 mm.) (mainly ethyl *trans*-2,5-dimethyl-1-piperazinecarboxylate); (b) 10.2 g. of nearly colorless viscous liquid, b.p. 120–163° (5 mm.) (mainly b.p. 163°). Fraction b was redistilled and after removal of 3.0 g. of forerun, b.p. 113–163° (5 mm.), 5.1 g. (22%) of V was obtained as a viscous yellow liquid, b.p. 163° (5 mm.). After standing for a long time the material solidified.

Procedure C. *n*-Butyl 4-*n*-Butyryl-1-piperazinecarboxylate (X).—To 37.2 g. (0.2 mole) of *n*-butyl 1-piperazinecarboxylate,[9] 31.6 g. (0.2 mole) of *n*-butyric anhydride was added with mixing and cooling. The resulting solution was heated on a steam-bath for 1 hour and then distilled through a Vigreux column. After removal of the *n*-butyric acid, 40.8 g. (80%) of X was obtained as a colorless liquid, b.p. 155–156° (0.4 mm.), $n^{25}D$ 1.4820.

Procedure D. Benzyl 4-*n*-Butyryl-1-piperazinecarboxylate (XI).—To a stirred mixture of 44 g. (0.2 mole) of benzyl 1-piperazinecarboxylate[10] and ice were added simultaneously and dropwise 32 g. (0.3 mole) of *n*-butyryl chloride and 100 ml. of 4 *N* sodium hydroxide; ice was added as required to maintain an excess. A colorless oil separated. The mixture was kept cold by means of an ice-bath and stirring was continued for 3 hours. The oil was extracted into chloroform and the chloroform extract was washed with 0.5 *N* hydrochloric acid and then with water and dried over magnesium sulfate. The dried extract was evaporated *in vacuo* to remove the chloroform and the residual liquid was distilled *in vacuo* to yield 43.2 g. (75%) of nearly colorless liquid, b.p. 197–203° (0.3 mm.), $n^{25}D$ 1.5353.

Procedure E. Ethyl 4-*p*-Nitrobenozyl-1-piperazinecarboxylate (XXVI).—A mixture of 31.6 g. (0.2 mole) of ethyl 1-piperazinecarboxylate, 37.1 g. (0.2 mole) of *p*-nitrobenzoyl chloride and 33.6 g. (0.4 mole) of sodium bicarbonate in 300 ml. of water was stirred at room temperature for 7.5 hours and then heated on a steam-bath for 30 minutes. The resulting precipitate was removed by filtration and crystallized twice from absolute ethanol (using Norit), yielding 27.5 g. (45%) of very pale yellow crystals, m.p. 91–92°. When recrystallized from absolute ethanol the product had m.p. 91.5–92.5°.

Procedure F. Ethyl 4-Isobutyryl-1-piperazinecarboxylate (XII).—To a cold stirred solution of 150 g. (0.95 mole) of ethyl 1-piperazinecarboxylate in 1 l. of ethyl acetate, 50 g. (0.47 mole) of isobutyryl chloride was added dropwise. After standing for 1.5 hours at room temperature the mixture was filtered to remove 91.9 g. (100%) of colorless crystals of ethyl 1-piperazinecarboxylate hydrochloride.[22] The filtrate was distilled to remove the ethyl acetate and the residual red-brown liquid was distilled *in vacuo*. The product, 89 g. (97%), was obtained as a colorless liquid, b.p. 130–131° (0.5 mm.), $n^{25}D$ 1.4840.

Procedure G. Ethyl 4-(3-Methylvaleryl)-1-piperazinecarboxylate (XVIII).—3-Methylvaleryl chloride (13.5 g., 0.1 mole) was added dropwise to 34.8 g. (0.22 mole) of ethyl 1-piperazinecarboxylate in 250 ml. of ether with cooling. After standing overnight at room temperature the reaction mixture was filtered to remove 19.6 g. (100%) of ethyl 1-piperazinecarboxylate hydrochloride. The filtrate was washed with 1 *N* hydrochloric acid, water and 5% sodium bicarbonate, and dried over magnesium sulfate. The ether was removed on a steam-bath and the residual liquid was distilled *in vacuo* to yield 22.3 g. (87%) of XVIII as a colorless liquid, b.p. 137–140° (0.04 mm.), $n^{25}D$ 1.4840.

Procedure H. Ethyl 4-Myristoyl-1-piperazinecarboxylate (XXIV).—To a solution of 63.3 g. (0.4 mole) of ethyl 1-piperazinecarboxylate in 350 ml. of ether, 49.4 g. (0.2 mole) of myristoyl chloride was added in portions with shaking and cooling. After standing overnight at room temperature the mixture was filtered to remove 38.4 g. (99%) of ethyl 1-piperazinecarboxylate hydrochloride. The filtrate was washed with 5% sodium bicarbonate and dried over Drierite. The dried solution was heated on a steam-bath to remove the ether, leaving 71.1 g. (96%) of XXIV, m.p. 35–36.5°. Recrystallization from hexane gave colorless crystals, m.p. 36.5–38°.

Procedure I. Ethyl 4-*n*-Caproyl-1-piperazinecarboxylate (XVI).—To a cold solution of 63.3 g. (0.4 mole) of ethyl 1-piperazinecarboxylate in 350 ml. of ether, 26.9 g. (0.2 mole) of *n*-caproyl chloride was added carefully, immediately producing a precipitate of ethyl 1-piperazinecarboxylate hydrochloride. After standing at room temperature for 2 hours 100 ml. of water was added to dissolve the precipitate. The layers were separated and the ether layer was washed successively with 5% hydrochloric acid, water and 5% sodium bicarbonate, and dried over magnesium sulfate. The ether was removed on a steam-bath and the residual pale yellow liquid, 46.1 g. (90%), was distilled *in vacuo*. The product was obtained as a nearly colorless liquid, b.p. 130–136° (0.05–0.08 mm.), $n^{23.8}D$ 1.4835.

Acknowledgment.—The authors are indebted to Mr. W. L. McEwen and associates for synthesis of some intermediates, to Mr. L. M. Brancone and associates for the microanalyses and to Dr. R. W. Cunningham and associates for the pharmacological testing.

PEARL RIVER, N. Y.

(22) K.-R. Jacobi, *Ber.*, **66B**, 113 (1933).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF HARVARD UNIVERSITY AND THE DIVISION OF NUTRITION AND PHYSIOLOGY OF THE PUBLIC HEALTH RESEARCH INSTITUTE OF NEW YORK CITY]

The Synthesis of 4-Amino-2(3H)-oxo-5-imidazolecarboxamide

BY LLOYD H. SMITH, JR.,[1] AND PETER YATES[2]

RECEIVED JUNE 22, 1954

4-Amino-2(3H)-oxo-5-imidazolecarboxamide has been synthesized by the action of base on carboxamidoaminocyanoacetamide. Its structure has been proved by hydrolytic degradation to 2,4-dioxo-5-imidazolecarboxamide and to hydantoin. Biological testing of C^{13}-labeled material gave no evidence of its being a precursor of uric acid.

Introduction

In 1945, Stetten and Fox[3] discovered a new diazotizable amine in cultures of *E. coli* whose growth was inhibited by sulfadiazine or sulfapyridine. The amine was subsequently identified by Shive, *et al.*,[4] as 4-amino-5-imidazolecarboxamide (I). Isotopic studies have demonstrated this compound to be a purine precursor in a number of biological systems including yeast,[5] the pigeon,[6] the rat[7] and man.[8] In man, with a ureotelic nitrogen metabolism, uric

(1) Junior Fellow, Society of Fellows of Harvard University.

(2) Inquiries regarding this article should be addressed to this author at Harvard University.

(3) M. R. Stetten and C. L. Fox, *J. Biol. Chem.*, **161**, 333 (1945).

(4) W. Shive, W. W. Ackermann, M. Gordon, M. E. Getzendaner and R. E. Eakin, THIS JOURNAL, **69**, 725 (1947).

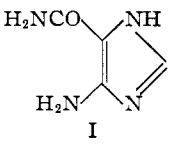
(5) W. J. Williams, *Federation Proc.*, **10**, 270 (1951).

(6) W. P. Schulman, J. M. Buchanan and C. S. Miller, *ibid.*, **9**, 225 (1950).

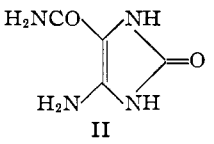
(7) C. S. Miller, S. Gurin and D. W. Wilson, *Science*, **112**, 654 (1950).

(8) J. E. Seegmiller, personal communication.

acid has been thought to originate solely from the catabolism and oxidation of nucleic acid purines. The rapidity and the time curve with which isotopic glycine and 4-amino-5-imidazolecarboxamide appear in urinary uric acid in man indicate that there must be a more direct pathway of uric acid formation, rather than *via* tissue polynucleotides alone.[8] The synthesis of 4-amino-2(3H)-oxo-5-imidazolecarboxamide (II), the oxidized analog of the imidazole I, was undertaken to determine whether it was an effective precursor of uric acid, presumably by such a hypothetical direct pathway of uric acid synthesis.



I

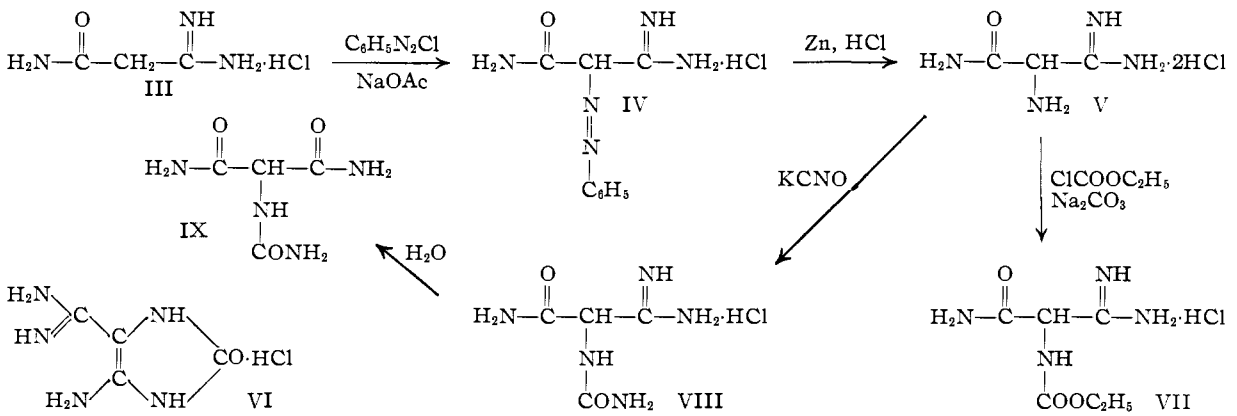


II

Discussion

Several of the methods previously employed for the preparation of imidazolones, imidazolidones and related compounds[9-11] appeared to be adaptable to the problem in hand. Initial efforts were directed to the formation of the imidazolone ring by the condensation of a diamine with a carbonic acid derivative in analogous fashion to the formation of 2-(3H)-imidazolidone from ethylenediamine by the action of diethyl carbonate[12] or phosgene[13] and of 2(3H)-benzimidazolone from *o*-phenylenediamine by reaction with phosgene[14] or urea.[15] The diamine required for this purpose—aminomalonamamidine (V)—has been obtained previously as the hydrochloride by Shaw and Woolley[16] by hydrolysis of formamidomalonamamidine hydrochloride, prepared by reduction of phenylazomalonamamidine hydrochloride (IV) with zinc in 98% formic acid. We were able to obtain V in somewhat higher over-all yield by effecting some changes in the earlier procedure (see Experimental): the phenylazo compound IV was prepared from malonamamidine hydrochloride(III) in 98% yield and the reduction of IV directly to V was accomplished with zinc and 3 *N* hydrochloric acid in 61% yield. However, attempts to convert V to the imidazolone II by fusion with urea were unsuccessful, ammonium chloride being the only product isolated. Also treatment with phosgene in the presence of pyridine led to a gum from which no product could be isolated.

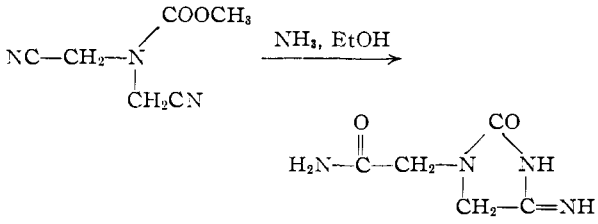
Attention was then turned to the possibility of the elaboration of the imidazolone ring from V by other means. In the course of their interesting work on the synthesis of 2-azaadenine Shaw and Woolley[17] prepared 4-amino-2(3H)-oxo-5-imidazolecarboxamidine hydrochloride (VI or a tautomer) by carbethoxylation of aminomalonamidine hydrochloride followed by closure of the imidazole ring in the presence of a large excess of base; a similar closure was utilized by Traube[18] in his classical work on the synthesis of uric acid. We considered that an analogous ring closure might be effected after carbethoxylation of V. To this end V was converted to carbethoxyaminomalonamamidine hydrochloride (VII) by reaction with ethyl chloroformate in the presence of sodium carbonate. Attempted cyclization of VII by treatment in aqueous solution with one equivalent of base, or with a large excess of base, or by heating slightly above the melting point led in each case to extensive decomposition and no product could be isolated.



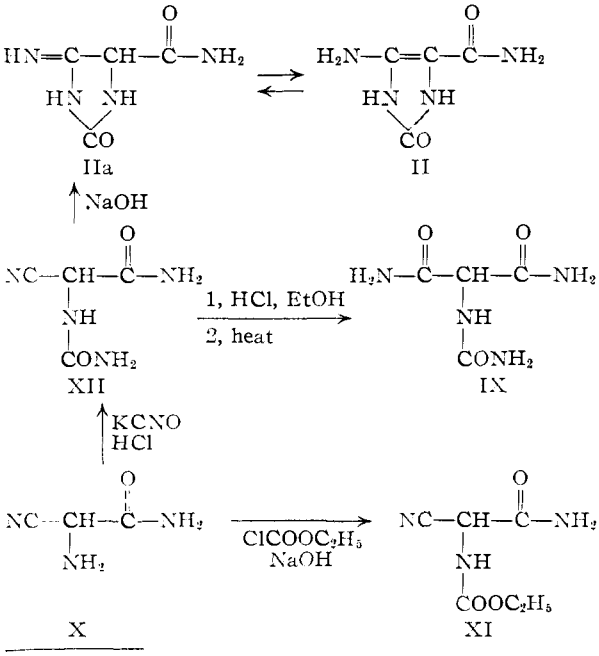
Another avenue of approach was next investigated *via* carboxamidoaminomalonamamidine hydrochloride (VIII), prepared by the reaction of V with potassium cyanate in aqueous solution. It was hoped that an internal alkylation might be effected analogous to the formation of methylurea from methylamine hydrochloride and urea.[19] However, attempted cyclization by heating in aqueous solution alone or with one equivalent of base gave only carboxamidoaminomalonamide (IX), by hydrolysis of the amidine grouping, while heating slightly above the melting point led to extensive decomposition. The identity of IX was confirmed by its preparation from carboxamidoaminocyanoacetamide (*vide infra*) by conversion to the corresponding iminoethyl ether hydrochloride followed by pyrolysis.

(9) K. Hofmann, "Imidazole and its Derivatives," Part 1, Interscience Publishers, Inc., New York, N. Y., 1953, pp. 60, 226, 285.
(10) A. Lespagnol in V. Grignard, "Traité de Chimie Organique," Vol. XX, Masson et Cie, Paris, 1953, p. 849.
(11) T. B. Johnson in H. Gilman, "Organic Chemistry," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1st Edition, 1938, p. 948.
(12) E. Fischer and H. Koch, *Ann.*, **232**, 222 (1885).
(13) N. A. Puschin and R. V. Mitic, *ibid.*, **532**, 300 (1937).
(14) A. Hartmann, *Ber.*, **23**, 1046 (1890).
(15) O. Kym, *J. prakt. Chem.*, [2] **75**, 323 (1907).
(16) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).
(17) E. Shaw and D. W. Woolley, *ibid.*, **194**, 641 (1952).
(18) W. Traube, *Ber.*, **33**, 3035 (1900).
(19) F. Arndt, L. Loewe and S. Avan, *ibid.*, **73**, 606 (1940).

Since these approaches had proved abortive, a new attack on the problem was sought, starting from a compound without an amidine grouping, which was suspected of causing the extensive decomposition in the base-catalyzed reactions. The formation of 6-aminouracil by the action of bases on cyanoacetylurea[20] appeared to be closely analogous to the type of cyclization we sought; further, a similar reaction had been recorded by Jongkees[21] in the imidazole series itself, *i.e.*



The requisite starting material, aminocyanoacetamide (X), has been prepared previously.[7,22] In the present work it was obtained in 59% yield from oximinocyanoacetamide by reduction with aluminum amalgam and water. Carbethoxylation of X with ethyl chloroformate and sodium hydroxide gave carbethoxyaminocyanoacetamide (XI); this was unaffected by aqueous or ethanolic ammonia.[23] Carboxamidoaminocyanoacetamide (XII) was obtained from X by the action of potassium cyanate and hydrochloric acid: two crystalline modifications could be isolated by varying the conditions used for the crystallization of the crude product from water. Short treatment of XII with hot aqueous sodium hydroxide gave the desired product II in 75% yield as matted needles which darkened on heating above 200° but did not melt below 370°.



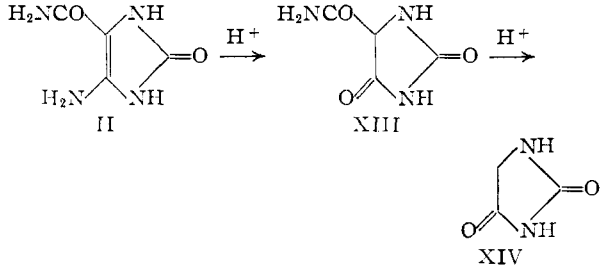
(20) F. Baum, *Ber.*, **41**, 532 (1908).

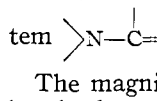
(21) W. J. A. Jongkees, *Rec. trav. chim.*, **27**, 287 (1908).

(22) A. H. Cook, I. Heilbron and E. Smith, *J. Chem. Soc.*, 1440 (1949).

(23) *Cf.* C. S. Miller, S. Gurin and D. W. Wilson, THIS JOURNAL, **74**, 2892 (1952).

The identity of the product was established on the basis of the following data. The compound analyzes for $C_4H_6N_4O_2$ and is, therefore, isomeric with carboxamidoaminocyanoacetamide (XII). However, unlike both forms of XII, it is soluble in cold, dilute hydrochloric acid.[24] The infrared spectrum of II differs from those of the two forms of XII, *inter alia*, in the absence of the weak nitrile band at 4.44 μ and the presence of a band at 5.80 μ, characteristic of an amide grouping in a five-membered cycle. When a solution of II in water containing one equivalent of hydrochloric acid was warmed on the steam-bath, a crystalline product XIII, m.p. 254–255° dec., was obtained; the infrared spectrum of this product was identical with that of 2,4-dioxo-5-imidazolecarboxamide, obtained by the action of base on carbethoxyaminomalonamide,[25] and its melting point was undepressed on admixture with the latter compound. More vigorous hydrolysis of II with excess hot, concentrated hydrochloric acid gave hydantoin (XIV), identified by infrared spectrum and mixed melting point.



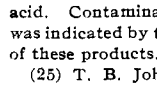
The ultraviolet spectrum of II is of considerable interest: in both aqueous and ethanolic solution the spectrum was found to be time-dependent (see Table I).[26] Clearly, complex changes occur in solution, most probably involving tautomerization, ionization or hydrolysis. The maxima at 287–288 mμ observed for the freshly prepared solutions in both water and ethanol may be assigned to the system  .[27]

The magnitude of the initial intensities of these bands shows that the compound exists in solution only partly as the tautomer II and the rapid decrease in these intensities with simultaneous increased intensities of the bands at 223–226 mμ indicates that tautomerization is occurring. Tautomerization, involving a prototropic change, would be expected to be more rapid in water than in ethanol, as observed. The source of the band at 223–226 mμ may perhaps be the imino tautomer IIa,

(24) Attempts to isolate the hydrochloride of II gave only impure products, due to the very ready hydrolysis of II in the presence of acid. Contamination with XIII, the hydrolysis product from II, was indicated by the presence of a band at 5.60 μ in the infrared spectra of these products.

(25) T. B. Johnson and B. H. Nicolet, THIS JOURNAL, **36**, 355 (1914).

(26) We are indebted to M. B. G. Christensen for these measurements.

(27) *Cf.* ethyl β-aminocrotonate with λ_{max}^{EtOH} 274 mμ (log ϵ 4.31)[28]; *cf.* also VI, with the system  , which has λ_{max} 309–310 mμ (log ϵ 4.13).[17]

(28) S. A. Glickman and A. C. Cope, THIS JOURNAL, **67**, 1017 (1945).

TABLE I

ULTRAVIOLET SPECTRUM OF II

Time after soln. effected,[a] hr.	λmax, mμ (log ε) Water	95% Ethanol
0	223 (3.90), 287 (3.28)	222[b] (3.59), 287 (3.69)
0.5	223 (3.95), 287 (2.84)	223 (3.67), 288 (3.71)
1.0	223 (3.96), 287 (2.64)	225 (3.73), 287 (3.58)
1.5	223 (3.96), 287 (2.42)	226 (3.68), 288 (3.46)
2.0		226 (3.79), 288 (3.32)
2.5		226 (3.78), 288 (3.19)
14.5	222[b] (3.82), 287[b] (2.15)	226 (3.71), 287 (2.88)
66.5		226 (3.69), 286 (2.83)
72.0	224[b] (3.69)	

[a] Solution was effected in 2 minutes with the aqueous solution and in 5 minutes with the ethanolic solution. [b] Points of inflection.

which might well have a maximum at this low wave length since 2,4-dioxo-5-imidazolecarboxamide (XIII) shows only end-absorption (log ε 3.53 at 210 mμ in water). Such an interpretation must remain provisional owing to the possibility of the presence of other tautomers involving the carbonyl group at position 2. That no *considerable* deep-seated changes involving hydrolysis occur in the *early stages* may be deduced from the facts that: (i) II may be recrystallized from water and (ii) the intensity of the band at 223–226 mμ increases with time initially, whereas neither of the two hydrolysis products—XIII and hydantoin—have a maximum above 218 mμ.[29] The further changes occurring over longer periods of time in aqueous solution resulting in the disappearance of the maximum at 287–288 mμ and the replacement of the maximum at 223 mμ by a point of inflection bespeak the slow hydrolysis of II.

For the purposes of biological testing II was prepared labeled with C^{13} at position 2 by the use of C^{13}-labeled potassium cyanate in the preparation of carboxamidoaminocyanoacetamide. Testing of the labeled II in pigeons and in man demonstrated that, in spite of structural analogies, this compound is not a precursor of uric acid.

Experimental

Phenylazomalonamamidine Hydrochloride (IV).—Malonamamidine hydrochloride[16] (8.7 g.) was coupled with benzenediazonium chloride by the method of Shaw and Woolley.[16] For maximum yields it was found necessary to maintain the *p*H of the reaction mixture at 4–5 by further additions of sodium acetate during the first two to three hours of reaction. Recrystallization of the product from ethanol gave yellow needles, m.p. 199–200°, yield 14.6 g. (98%).

Aminomalonamamidine Dihydrochloride (V).—Phenylazomalonamamidine hydrochloride (12.0 g.) was reduced with zinc dust (7.5 g.) in 3 *N* hydrochloric acid (80 ml.) using a procedure similar to that used by Shaw and Woolley[17] for the reduction of phenylazomalondiamidine dihydrochloride. Pyridine was added to bring the *p*H to 5 before the passage of hydrogen sulfide: there resulted a heavy, white precipitate of zinc chloride–pyridine hydrochloride complex which was removed by filtration. The product was recrystallized from an aqueous ethanolic solution with addition of ether and vigorous scratching. The recrystallized material had m.p. 209–210°, yield 5.7 g. (61%).

Anal. Calcd. for $C_3H_{10}N_4OCl_2$: N, 29.64. Found: N, 29.32.

Reaction of Aminomalonamamidine Dihydrochloride with (i) Phosgene and (ii) Urea.—(i) Finely ground V (2.0 g.) was suspended in anhydrous ether (30 ml.) to which pyridine (3.4 g.) was added. Phosgene was passed through the suspension for one hour. From the resulting sticky brown gum no product could be isolated; a small amount of starting material only could be recovered. (ii) Urea (0.25 g.) and V (0.5 g.) were fused together both at 110 and 160°. In each experiment a dark brown paste resulted from which ammonium chloride was the only product which could be isolated.

Carbethoxyaminomalonamamidine Hydrochloride (VII).—Aminomalonamamidine dihydrochloride (1.89 g., 0.01 mole) was dissolved in water (10 ml.) and to the solution was added slowly with shaking sodium carbonate (0.53 g., 0.005 mole). To this solution was added ethyl chloroformate (1.10 g., 0.01 mole) and a solution of sodium carbonate (0.53 g., 0.005 mole) in water (5 ml.); the mixture was effected by alternate additions of a few drops of the ethyl chloroformate and of the carbonate solution and shaking until the globules of ethyl chloroformate disappeared. After addition was complete, the solution was allowed to stand for one hour and was then taken to dryness at 40° under 20 mm. pressure. The residue was extracted with three 30-ml. portions of boiling ethanol. The combined ethanolic extracts were concentrated at 40° and 20 mm. to *ca.* 10 ml. A few drops of water were added to the ethanolic solution followed by careful addition of ether until a sirup began to separate. Intensive scratching led to the solidification of the deposit. More ether was then added and after standing overnight the solid was filtered off. Two crystallizations from ethanol with charcoal treatment gave colorless crystals, m.p. 177–178.5° dec.

Anal. Calcd. for $C_6H_{13}O_3N_4Cl$: C, 32.08; H, 5.83; N, 24.94. Found: C, 32.23; H, 5.98; N, 24.60.

Attempted cyclization of carbethoxymalonamamidine hydrochloride by heating at 190° or by treatment in aqueous solution with one equivalent of base or with a large excess of concentrated base led to extensive decomposition and no product could be isolated.

Carboxamidoaminomalonamamidine Hydrochloride (VIII).—Aminomalonamamidine dihydrochloride (1.89 g., 0.01 mole) was dissolved in water (5 ml.) and the solution was cooled in an ice-bath. Potassium cyanate (0.85 g., 0.0105 mole) dissolved in water (2 ml.) was added slowly with stirring to the cooled solution over a period of 15 minutes; after addition was complete, the reaction mixture was allowed to stand for one hour. Most of the potassium chloride was precipitated by the addition of ethanol (75 ml.) and was filtered off. The filtrate was taken to dryness at 40° and 20 mm. and the product was crystallized from the residue by dissolving it in the minimum quantity of water, adding ethanol until the solution was faintly cloudy and then adding a small amount of ether and scratching vigorously; yield 1.8 g. (92%). Three recrystallizations from water–ethanol–ether as before gave colorless crystals, m.p. 190–191° dec.

Anal. Calcd. for $C_4H_{10}N_6O_2Cl$: C, 24.55; H, 5.15; N, 35.80. Found: C, 24.59; H, 5.47; N, 36.03.

Carboxamidoaminomalonamamide (IX).—(i) Carboxamidoaminomalonamamidine hydrochloride (0.2 g.) was dissolved in water (5 ml.) and the solution was boiled gently under reflux for 2 hours. Most of the water was removed and the residual aqueous solution was treated with ethanol. The solid separating was recrystallized from aqueous ethanol giving colorless crystals, m.p. 216–219° dec.[30] A similar product was obtained when one equivalent of sodium hydroxide was added to the aqueous reaction mixture.

Anal. Calcd. for $C_4H_8N_4O_3$: C, 30.00; H, 5.03; N, 34.99. Found: C, 30.18; H, 5.11; N, 34.82.

(ii) Finely powdered carboxamidoaminocyanoacetamide (1.5 g.; *vide infra*) was suspended in a solution of ethanol (0.5 g.) in anhydrous ether (40 ml.) in a flask fitted with a gas delivery tube and drying tube. The mixture was cooled in an ice–salt-bath and dry hydrogen chloride was passed for 4 hours. The flask was stoppered and kept at 4° for 2 days. The crude iminoethyl ether hydrochloride was filtered off rapidly, washed several times with anhydrous ether and transferred to a flask fitted with a drying tube. The flask was heated at 130° for 15 minutes, when gas evolution was complete. The residue was crystallized from aqueous

(29) R. E. Stuckey, *J. Chem. Soc.*, 331 (1947).

(30) T. B. Johnson and B. H. Nicolet give m.p. 200–225° for this compound, prepared from aminomalonamide hydrochloride and potassium cyanate; *cf.* ref. 25.

ethanol giving a product m.p. 214–217° dec., undepressed by admixture with the product obtained from carboxamidoaminomalonamamidine hydrochloride; yield 1.5 g. (89%).

Attempted cyclization of carboxamidoaminomalonamamidine hydrochloride by heating at 190–200° led to extensive decomposition and no product could be isolated.

Aminocyanoacetamide (X).—Aluminum amalgam[31] (17.5 g.) was prepared in a 1-l. flask fitted with a dropping funnel and a Hershberg stirrer. The flask was cooled in an ice-bath and oximinocyanoacetamide[23] (55 g.) in methanol (250 ml.) was added slowly with stirring followed by water (40 ml.). The rate of addition was adjusted so that the temperature of the reaction mixture was maintained at 30–35°. The pale yellow methanolic solution was separated by filtration. The residual aluminum hydroxide was extracted three times with 75-ml. portions of methanol at 40°. The combined filtrate and washings were cooled at −5° overnight and the product separating was filtered off. Additional product was obtained by concentrating the mother liquor at 35° and 20 mm., total yield 28.1 g. (59%). Recrystallization from methanol or ethyl acetate gave pure aminocyanoacetamide, m.p. 122–123° (Cook, *et al.*,[22] give m.p. 121°). It was found to be stable for several months when stored in an evacuated desiccator at −5°.

Carbethoxyaminocyanoacetamide (XI).—Aminocyanoacetamide (1.98 g., 0.02 mole) was dissolved in a solution of concentrated hydrochloric acid (1.7 ml.) in water (15 ml.). The solution was cooled in an ice-bath and stirred mechanically. To the stirred solution were added alternately in small portions at a time ethyl chloroformate (2.28 g., 0.021 mole) and a solution of sodium hydroxide (0.85 g., 0.021 mole) in water (3 ml.). These additions were carried out over a period of 30 minutes; a further solution of sodium hydroxide (0.85 g.) in water (3 ml.) was added slowly during the following 30 minutes. Stirring in the cold was continued for another hour. The product was filtered off and washed with a small amount of cold water; yield 2.7 g. (79%). On repeated recrystallization from water or absolute ethanol the pure product was obtained as colorless needles, m.p. 145–146°.

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.23; H, 5.43; N, 24.37.

Treatment of carbethoxyaminocyanoacetamide with excess concentrated aqueous or ethanolic ammonia led to no reaction and the starting material was recovered unchanged.

Carboxamidoaminocyanoacetamide (XII).—A solution of aminocyanoacetamide (9.9 g., 0.1 mole) in water (30 ml.) and concentrated hydrochloric acid (10.5 g.) was cooled in an ice-bath and stirred mechanically. To the stirred solution was added in small portions at a time potassium cyanate (8.3 g., 0.103 mole) over a period of 30 minutes and the stirring was continued for a further 20 minutes. The product was separated by filtration; yield 12.6 g. (89%). Recrystallization from water gave fine, colorless needles, m.p. 200–201° dec. In some runs it was observed that the once-recrystallized material contained prismatic crystals in addition to the needles. By recrystallization from a very concentrated solution (*ca.* 15%) and seeding with a hand-picked prismatic crystal, a product could be obtained consisting exclusively of prisms, m.p. 202–203° dec. The prisms could be reconverted to needles by recrystallization from dilute aqueous solution.

Anal. Calcd. for $C_4H_6N_4O_2$: C, 33.80; H, 4.26; N, 39.42. Found (i) needles: C, 33.88; H, 4.30; N, 39.82; (ii) prisms: C, 33.86; H, 4.37; N, 39.61.

4-Amino-2(3H)-oxo-5-imidazolecarboxamide (II).—Carboxamidoaminocyanoacetamide (1.0 g.) was dissolved in boiling water (10 ml.) and the solution was maintained at 85° in a water-bath. To this solution was added a 5% solution of sodium hydroxide (1.2 ml.) and heating was continued for 10 minutes. The solution was allowed to cool slowly and then held at 5° overnight. The product crystallized from the reaction solution as matted, small needles; yield 0.75 g. (75%). Two recrystallizations from water gave a product which was completely soluble in cold, dilute hydrochloric acid; it darkened on heating above 200° but did not melt below 370°.

Anal. Calcd. for $C_4H_6N_4O_2$: C, 33.80; H, 4.26; N, 39.42. Found: C, 33.54; H, 4.54; N, 39.49.

Hydrolysis of II. (i) **2,4-Dioxo-5-imidazolecarboxamide (XIII).**—A solution of *II* (0.1 g.) in water (1 ml.) containing an equivalent amount of hydrochloric acid was warmed gently on the steam-bath for 15 minutes. On cooling the reaction solution in an ice-bath, a crystalline product was obtained which on recrystallization from aqueous ethanol had m.p. 254–255° dec. This m.p. was undepressed on admixture of the product with 2,4-dioxo-5-imidazolecarboxamide, prepared by treatment of carbethoxyaminomalonamide with aqueous potassium hydroxide.[25] The infrared spectra (Nujol mull) of the two samples were identical.

(ii) **Hydantoin.**—A solution of II (0.1 g.) in concentrated hydrochloric acid (5 ml.) was taken to dryness on the steam-bath. This procedure was repeated with two further 5-ml. portions of hydrochloric acid. The residue was extracted with boiling ethanol; concentration of the ethanolic solution and treatment with a small amount of ether gave a crystalline product. This on recrystallization from ethanol–ether had m.p. 222–224°, undepressed by admixture with an authentic sample of hydantoin of the same m.p. Its infrared spectrum (Nujol mull) was identical with that of hydantoin.

C^{13}-Labeled Intermediates.—C^{13} was available as potassium cyanide and was introduced into carboxamidoaminocyanoacetamide *via* potassium cyanate without isolation of the latter as follows.[32] Potassium cyanide (8.44 g.) was dissolved in water (130 ml.) and to the solution was added well-washed cupric hydroxide obtained by the reaction of cupric sulfate (17 g.) with potassium hydroxide. The mixture was cooled in an ice-bath and potassium permanganate (15.8 g.) in water (250 ml.) was added with mechanical stirring over a period of 2.5 hours. The excess permanganate was destroyed with ethanol and the manganese dioxide was filtered off and washed 4 times with 10-ml. portions of water. The combined filtrate and washings were concentrated at 40° and 20 mm. to a volume of 125 ml. This solution was added slowly during 30 minutes to a cold (5°), mechanically-stirred solution of aminocyanoacetamide (20 g.) in 6 *N* hydrochloric acid (30 ml.) Stirring was continued for a further 15 minutes and the reaction mixture was cooled at 5° overnight. The yield of carboxamidoaminocyanoacetamide was 12.7 g. (69% based on KCN).

Biological.—4-Amine-2(3H)-oxo-5-imidazolecarboxamide containing 13.90 atom % excess C^{13} at position 2 was injected intraperitoneally into a pigeon in a dose of 48.8 mg. (0.001 mmole/g. of pigeon) in 3 ml. of saline. A second pigeon received 41.5 mg. (0.001 mmole/g. of pigeon) of carboxamidoaminocyanoacetamide containing the same concentration of C^{13}. As a control, a third pigeon received 41.5 mg. (0.001 mmole/g. of pigeon) of 4-amino-5-imidazolecarboxamide containing 12.70 atom % excess C^{13} at position 4.[33] Feces were collected daily for three days and uric acid was isolated by the St. John method.[34] There was no detectable C^{13} enrichment (<0.02 atom % excess) in the uric acid obtained from the pigeons receiving 4-amino-2(3H)-oxo-5-imidazolecarboxamide or carboxamidoaminocyanoacetamide. The uric acid from the pigeon receiving 4-amino-5-imidazolecarboxamide contained 0.20 atom % excess C^{13} during day 1, 0.04 atom % excess for day 2 and 0.02 atom % excess for day 3.

Toxicology studies were carried out by giving 4-amino-2-(3H)-oxo-5-imidazolecarboxamide to rats *via* stomach tube at a dose of 0.01 mmole/g. weight. There was no detectable histological damage. One hundred mg. of the C^{13}-labeled compound in a gelatin capsule was then given orally to a man and two 24-hour urine collections obtained. There was no detectable C^{13} enrichment in the isolated urinary uric acid.

Acknowledgment.—The authors wish to express their indebtedness to Dr. De Witt Stetten, Jr., for his suggestions and help in formulating the problem and in carrying out the biological testing.

CAMBRIDGE, MASS.
NEW YORK, N. Y.

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