

duced a moderately strong red-violet color. Thus, it appears that on warming to room temperature a large portion of compound **2** in the glass phase lacked the stabilization afforded by the crystal lattice and rearranged to **3** via dissociation to **4**. Thus the conversion to **2** at -40° is probably greater than indicated by the infrared spectrum of the 25° crystallized product.

Thermochromism of 2. Compound **2** (5 mg) was added to 1 ml of chloroform at 25° and dissolved within 30 sec. The intensity of the red-violet color increased as rapidly as the solid dissolved. The colored solution was cooled rapidly to -40° and the color diminished to a light pink within 20 sec (with the major decrease within the first 5 sec) and remained light pink on continued standing. On warming to 25° the solution rapidly restored the deep red-violet color and on recooling the color was diminished once again.

Piezochromism of 2. A solid sample of **2** was ground manually in a mortar with a pestle at 25° . The color of the solid changed from white to a deep purple. An identical change could be effected when the sample was ground under liquid nitrogen. Even the pressure of a typewriter key impinging on a sheet of paper impregnated with finely powdered **2** was sufficient to produce a dark color. The color disappeared after several days at 25° .

Oxidation of Other Imidazoles. The types of imidazoles and the methods of oxidation are presented in Table II. The procedure for oxidation with potassium ferricyanide and product isolation is identical with the procedure for the oxidation of lophine at 5° under oxygen. The imidazoles which were unreactive under these conditions were oxidized by an alternate method. The imidazole (0.01 mole) was heated with lead dioxide (30 g, Eimer and Amend, ACS grade) in 300 ml of benzene at reflux for 15 hr. After filtra-

tion the benzene solution was evaporated to dryness and the residue was recrystallized from ethanol and water. The product from this procedure was the photochromic dimer.

Esr Measurements. All measurements were made on a Varian V-4500 A EPR instrument at 25° . All of the spectra had the same band width, general shape, and no fine structure. The signal from **2** was very weak for the solid, very strong immediately after solution, and weak after the solution was stored in the dark for several minutes. The signal was strong after the solid was ground with a mortar and pestle and diminished on standing. The signal from sample **3** was very weak for the solid, weak when in solution, strong after irradiation of the solution, and weak after the irradiated solution was stored in the dark. In all cases the intensity of the signal was proportional to the color of the substrate.

Infrared Measurements. The infrared spectra were determined with a Beckman IR-7 spectrophotometer. Thoroughly dried, crystalline samples were analyzed in potassium bromide pellets with concentrations of approximately 6 mg/g of potassium bromide.

Ultraviolet Measurements. All measurements were made on a Cary 14 spectrophotometer using 95% ethanol as the solvent or KBr as a matrix. In Table I the spectral data obtained for several arylimidazoles and isoimidazoles are listed.

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Studies in Prebiotic Synthesis. I. Aminomalononitrile and 4-Amino-5-cyanoimidazole^{1,2}

James P. Ferris and L. E. Orgel

*Contribution from The Salk Institute for Biological Studies, La Jolla, California.
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Abstract: The syntheses of aminomalononitrile and 4-amino-5-cyanoimidazole are described. Acid anhydrides react with aminomalononitrile to yield oxazoles. Aminomalononitrile is converted to diaminomaleonitrile by cyanide and to 4-amino-5-cyanoimidazole by formamidine. Adenine results from the reaction of 4-amino-5-cyanoimidazole with formamidine. 4-Cyano-5-aminooxazole is converted to 7-aminooxazolo[5,4-*d*]pyrimidine on treatment with formamidine.

In pioneering experiments Miller³ demonstrated that substantial quantities of amino acids are produced when an electric discharge is passed through a mixture of methane, ammonia, and water. Subsequently, he showed that these amino acids are formed following an initial condensation of hydrogen cyanide with aldehydes formed in the discharge.⁴ When Oro showed that adenine is obtained on refluxing ammoniacal solutions of hydrogen cyanide it became clear that hydrogen cyanide might be a central compound in the prebiotic synthesis of nitrogen compounds.⁵ More recently adenine has been obtained from cyanide in a variety of ways, and several pathways have been dis-

cussed.^{5,6} We have attempted a detailed study of the steps involved in the hope that this might throw light on the prebiotic synthesis.

Adenine was formed in only 0.5% yield in Oro's experiment; most of the cyanide formed an intractable polymer. Therefore, we decided to attempt the synthesis of the most plausible intermediates, namely aminomalononitrile (I) and 4-amino-5-cyanoimidazole (II). In this way we hoped to study the key steps in adenine synthesis in a comparatively simple system. In the present paper we discuss the chemistry of these two key intermediates, without particular regard to their reactions under "prebiotic" conditions. In later

(1) For a preliminary account of this work see J. P. Ferris and L. E. Orgel, *J. Am. Chem. Soc.*, **87**, 4976 (1965).

(2) This research was supported by Grant GB-3152 from the National Science Foundation.

(3) S. L. Miller, *J. Am. Chem. Soc.*, **77**, 2351 (1955).

(4) S. L. Miller, *Biochim. Biophys. Acta*, **23**, 480 (1957).

(5) J. Oro and A. P. Kimball, *Arch. Biochem. Biophys.*, **94**, 217 (1961); **96**, 293 (1962); J. Oro and J. S. Kamat, *Nature*, **190**, 442 (1961).

(6) J. Oro, *ibid.*, **197**, 971 (1963); C. U. Lowe, M. W. Rees, and R. Markham, *ibid.*, **199**, 219 (1963); C. Ponnampetuma, R. M. Lemmon, R. Mariner, and M. Calvin, *Proc. Natl. Acad. Sci. U. S.*, **49**, 737 (1963); R. M. Kliss and C. N. Matthews, *ibid.*, **48**, 1300 (1962); M. Calvin, "Chemical Evolution," University of Oregon Press, Eugene, Ore., 1961, p 24; C. Palm and M. Calvin, *J. Am. Chem. Soc.*, **84**, 2115 (1962); for recent reviews, see "The Origins of Prebiological Systems," S. W. Fox, Ed., Academic Press Inc., New York, N. Y., 1965, pp 137-172, 221-242.

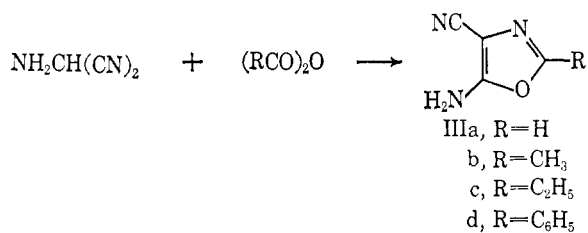
papers of the series we shall concentrate on their aqueous solution chemistry.

The synthesis of aminomalononitrile has been reported on a number of occasions, but it appears that these reports are not correct. It is now clear that the low molecular weight compound formed during the polymerization of hydrogen cyanide is not aminomalononitrile but diaminomaleonitrile (HCN tetramer). The reported procedure for the synthesis of aminomalononitrile from ammonia and bromomalononitrile⁷ yields instead tetracyanoethylene and compounds derived from it.⁸

The reduction of oximinomalononitrile⁹ with aluminum amalgam results in 45–50% yield of aminomalononitrile (I) which may be isolated as the toluenesulfonate. Attempts to isolate the free base led to an oil having the correct infrared spectrum, but which polymerized to a brown solid within 1 hr. The hydrochloride and hydrobromide can also be prepared, but only with some difficulty since the nitrile groups are very susceptible to attack by halogen acids.

Support for the proposed structure is provided by the infrared and nuclear magnetic resonance spectra of the tosylate salt and by the chemistry described below. The infrared spectrum indicates the presence of amino and nitrile groups. The nmr spectrum exhibits, in addition to tosylate absorption, a broad, one-proton peak at τ 3.72 which we attribute to the proton attached to carbon and a resonance at 0.99 for the protons of the ammonium group. The large, low-field shift of the CH proton is due to the two cyano groups and one ammonium group attached to the central carbon atom. In D₂O solution both the CH proton and the ammonium protons exchange rapidly.

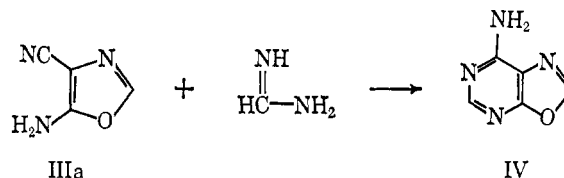
Aminomalononitrile is a useful intermediate in the synthesis of certain heterocyclic systems. With acid anhydrides it is readily converted to 4-cyano-5-aminoxazoles (III). We have confirmed that the compound



formed from benzoic anhydride is identical with a specimen of the 2-phenyloxazole prepared by a literature procedure.¹⁰ Oxazoles are converted to imidazoles and thiazoles by treatment with ammonia and hydrogen sulfide, respectively, so these heterocycles are readily synthesized from aminomalononitrile.¹¹

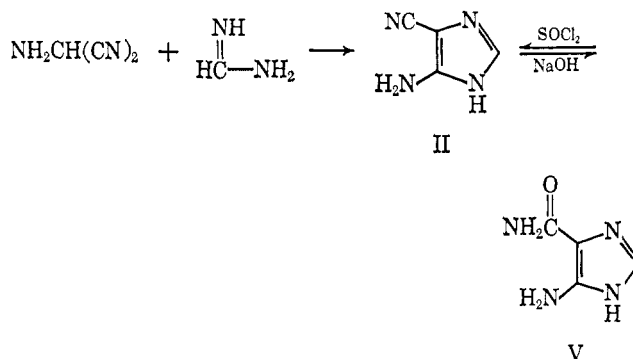
4-Cyano-5-aminoxazole was condensed with formamidine¹² to the adenine analog 7-aminooxazolo[5,4-d]pyrimidine (IV). We believe that a series of related

alkyl "oxyadenines" and "thioadenines" could also be prepared in this way.



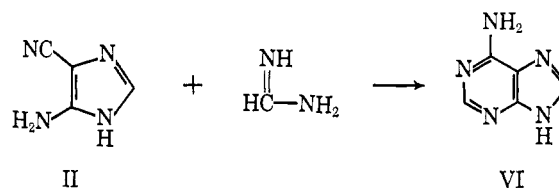
Addition of cyanide to aminomalononitrile gives diaminomaleonitrile, the HCN tetramer. This compound is identical with Oro's compound A formed in the ammonia-hydrogen cyanide reaction.^{5,13} This reaction supports the hypothesis that aminomalononitrile is an intermediate in the polymerization of hydrogen cyanide. We now have strong independent evidence for this conclusion.¹⁴

The second of the intermediates is obtained by the condensation of aminomalononitrile with formamidine acetate. Its structure was proved by hydrolysis to be 4-aminoimidazole-5-carboxamide (V) and by an alterna-



tive synthesis from 4-aminoimidazole-5-carboxamide using thionyl chloride in pyridine. This compound is identical with Oro's compound B as shown by its *R_f* values and the color test it gives with diazotized sulfanilic acid.^{5,13}

Finally, 4-amino-5-cyanoimidazole was converted to adenine (VI) by a further condensation with formamidine acetate.



Experimental Section¹⁵

Aminomalononitrile Tosylate. Oximinomalononitrile silver salt⁹ (51 g, 0.252 mole) was dissolved in 100 ml of 8 *N* HNO₃ and the free oxime was extracted into 300 ml of ether. This ether extract was added dropwise to 14 g (0.52 mole) of amalgamated aluminum

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(12) This approach to the synthesis of purines was developed by E. C. Taylor; e.g., see E. C. Taylor and R. W. Hendess, *J. Am. Chem. Soc.*, **87**, 1995 (1965), and references cited therein.

(13) These identifications have been suggested tentatively: J. Oro, *Proc. Lun. Plan. Exptl. Colloq.*, **3**, 9 (1963).

(14) R. Sanchez, J. P. Ferris, L. E. Orgel, *Science*, **153**, 72 (1966).

(15) Infrared spectra were determined in KBr using a Perkin-Elmer 237 B spectrophotometer, ultraviolet spectra were measured in alcohol using a Cary 14, and nmr spectra were measured on a Varian HR-60 in dimethyl sulfoxide-*d*₆ using TMS as internal standard. Paper chromatography was done on Whatman 3MM paper using butyl alcohol saturated with water as solvent. Products were identified on the paper by ultraviolet absorption (253 mμ) and by spraying with diazotized sulfanilic acid reagent.

foil covered with 250 ml of tetrahydrofuran. The mixture was stirred and an ice bath was used as needed to maintain the temperature just below reflux. After completion of the addition the mixture was stirred for an additional 15 min and then 25 ml (1.4 moles) of water was added slowly. After all the water had been added the mixture was maintained at a reflux temperature for 1 hr. The mixture was filtered and the aluminum salts were washed with ether and tetrahydrofuran. The filtrate was concentrated to 200 ml and to it was added 40 g (0.232 mole) of toluenesulfonic acid suspended in 300 ml of ether. Sufficient ether was added to make a total volume of 1 l. before cooling and filtering the product. A yield of 31.4 g (49.5%), mp 170–173°, was obtained. An analytical sample was obtained by charcoal treatment and recrystallization from acetonitrile, mp 175–176°; infrared (CN) 2270 cm^{-1} (weak); nmr (CH_3) τ 7.63, aromatic H, 2.53 (quartet), (CH) 3.72¹⁶ (broad multiplet), (N^+H_3) 0.99.¹⁶

Anal. Calcd for $\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_5\text{S}$: C, 47.41; H, 4.38; N, 16.59. Found: C, 47.20; H, 4.39; N, 16.52.

4-Amino-5-cyanoimidazole Tosylate. A. From Aminomalononitrile. To a solution of 33.2 g (0.32 mole) of formamidine acetate in 700 ml of absolute ethyl alcohol at reflux was added 20.24 g (0.08 mole) of aminomalononitrile tosylate as a slurry in 240 ml of absolute alcohol. After addition was complete the mixture was maintained at reflux for 1 hr. The solution was concentrated to near dryness under aspirator vacuum, taken up in 50 ml of H_2O , treated with charcoal, and chromatographed on a column of 200 g of Dowex 1 (200–400 mesh, hydroxide form). The product was detected in the eluates (orange diazotized sulfanilic acid test) after about 400 ml of water had passed through the column. The product was found in the next liter of eluate from the column. The aqueous solution was concentrated *in vacuo* and finally by freeze drying to yield 4.2 g of crude 4-amino-5-cyanoimidazole. This was dissolved in 100 ml of acetonitrile (some insolubles), treated with charcoal, and filtered. Ether (200 ml) was added to the filtrate, the mixture was warmed, and to it was added 7.4 g of toluenesulfonic acid monohydrate in 10 ml of warm acetonitrile. The product precipitated (oil and/or crystals) and was then crystallized from an acetonitrile-ether mixture to yield 8.0 g (35.8%) of product, mp 168–169°; infrared (CN) 2225 and 2230 cm^{-1} ; ultraviolet λ_{max} 246 $\text{m}\mu$ (ϵ 12,300) and 220 $\text{m}\mu$ (ϵ 25,500); nmr (CH_3) τ 7.62, aromatic H, 2.40 (quartet), (N^+H_3) 2.50,¹⁶ (C_2H) 1.30.

Anal. Calcd for $\text{C}_{11}\text{H}_{12}\text{N}_4\text{O}_5\text{S}$: C, 47.13; H, 4.31; N, 20.00. Found: C, 46.90; H, 4.54; N, 19.62.

B. By Dehydration of 4-Aminoimidazole-5-carboxamide. To a suspension of 1.6 g (0.01 mole) of 4-aminoimidazole-5-carboxamide hydrochloride in 25 ml of pyridine cooled to 5° was added 5 g (0.042 mole) of thionyl chloride. This mixture was allowed to warm to room temperature and after 30 min was poured into ice. The mixture was freeze dried and the residue was chromatographed on 100 g of Dowex 1 in the hydroxide form. On washing with water the bulk of the product was eluted in a 200-ml fraction after the initial 100 ml of eluent passed through the column. The product was freeze dried and dissolved in acetonitrile, and an ether solution of toluenesulfonic acid was added. Yellow crystals formed, 0.35 g (13%), which were purified by recrystallization from acetonitrile, mp 167–168°. This material was identical in melting point, mixture melting point, R_f value, and infrared spectrum with a sample prepared from aminomalononitrile and formamidine.

4-Cyano-5-aminoimidazole. An aqueous solution of 0.5 g of 4-cyano-5-aminoimidazole tosylate was passed through 10 g of Dowex 1 ion-exchange resin in the hydroxide form. The aqueous eluate was freeze dried to yield 78 mg of product, λ_{max} 246 $\text{m}\mu$. Alternatively, 1.0 g of the toluenesulfonate was dissolved in 200 ml of warm tetrahydrofuran and ammonia was bubbled through the solution. The mixture was cooled in an ice bath and the ammonium tosylate was filtered. The filtrate was concentrated *in vacuo* at room temperature and crystallized from a tetrahydrofuran-chloroform mixture to yield 0.24 g (70%), mp 124–128°. The product was recrystallized twice from tetrahydrofuran-chloroform, mp 123–125°; infrared (CN) 2225 and 2230 cm^{-1} ; ultraviolet λ_{max} 246 $\text{m}\mu$ (ϵ 11,000).

Anal. Calcd for $\text{C}_4\text{H}_4\text{N}_4$: C, 44.44; H, 3.73; N, 51.39. Found: C, 44.14; H, 4.09; N, 51.39.

Hydrolysis of 4-Amino-5-cyanoimidazole Tosylate. A 10^{-4} M solution of the 4-amino-5-cyanoimidazole tosylate was boiled for 15 hr with 0.1 M sodium hydroxide. The resulting solution had

λ_{max} 277 $\text{m}\mu$ (4-aminoimidazole-5-carboxamide has λ_{max} 277 $\text{m}\mu$ in base) and a yield of 100% was estimated from the extinction coefficient. The reaction product gives the same R_f and diazotized sulfanilic acid color as an authentic sample of 4-aminoimidazole-5-carboxamide.

Preparation of Oxazoles. A. From Aminomalononitrile. To an ether solution of the aminomalononitrile taken directly from the aluminum amalgam reduction was added 1 molar equiv of the anhydride and the mixture was allowed to stand at room temperature for 4 hr. The mixture was concentrated *in vacuo* and the residue was crystallized from ether.

2-Methyl-4-cyano-5-aminooxazole had mp 152.5–154.5°; infrared (CN) 2225 cm^{-1} ; ultraviolet λ_{max} 244 $\text{m}\mu$ (ϵ 13,400); nmr (CH_3) τ 7.69, (NH_2) 2.42.¹⁶

Anal. Calcd for $\text{C}_5\text{H}_5\text{N}_3\text{O}$: C, 48.78; H, 4.09; N, 34.13. Found: C, 48.77; H, 4.35; N, 33.91.

2-Ethyl-4-cyano-5-aminooxazole had mp 148–149°; infrared (CN) 2230 cm^{-1} ; ultraviolet λ_{max} 245 $\text{m}\mu$ (ϵ 16,200); nmr (CH_3) τ 8.83 (triplet), (CH_2) 2.39 (quartet), and (NH_2) 2.44.¹⁶

Anal. Calcd for $\text{C}_7\text{H}_7\text{N}_3\text{O}$: C, 52.55; H, 5.14; N, 30.64. Found: C, 52.32; H, 5.29; N, 30.55.

2-Phenyl-4-cyano-5-aminooxazole. The reaction mixture was concentrated, dissolved in dilute sodium bicarbonate, and extracted into ether. After the ether was concentrated the product was crystallized from *n*-butyl alcohol to yield white crystals, mp 241–243°. Mixture melting point with an authentic sample¹⁰ melting at 237–238.5° was 238–240°; infrared (CN) 2230 cm^{-1} ; ultraviolet λ_{max} 309 $\text{m}\mu$ (ϵ 20,000), 258 (12,300), and 227 (17,300), respectively.

Anal. Calcd for $\text{C}_{10}\text{H}_7\text{N}_3\text{O}$: C, 64.80; H, 3.81; N, 22.69. Found: C, 64.49; H, 3.88; N, 22.84.

B. From Aminomalononitrile Tosylate. **4-Cyano-5-aminooxazole.** To 20.24 g (0.08 mole) of aminomalononitrile tosylate in 400 ml of formic acid at 5–10° was added 100 ml of acetic anhydride over a 2.5-hr period while maintaining the temperature at 5–10°. The mixture was allowed to warm to room temperature over a 2-hr period, ice was added, and the mixture was concentrated to near dryness. Crystallization of the residue from water yielded 4.9 g (43%) of product, mp 185–186°. The product was recrystallized from water for analysis, mp 184–186°; infrared (CN) 2225 cm^{-1} ; ultraviolet λ_{max} 244 $\text{m}\mu$ (ϵ 15,100); nmr (CH) τ 2.20, (NH_2) 6.46.¹⁶

Anal. Calcd for $\text{C}_4\text{H}_3\text{N}_3\text{O}$: C, 44.04; H, 2.77; N, 38.52. Found: C, 43.99; H, 2.93; N, 38.58.

Adenine. To a solution of 1.40 g (0.005 mole) of 4-amino-5-cyanoimidazole toluenesulfonate in 100 ml of Methyl Cellosolve was added 2.1 g (0.02 mole) of formamidine acetate. The mixture was heated at reflux for 1 hr and concentrated *in vacuo*, and 20 ml of concentrated ammonium hydroxide and 80 ml of water were added. The solution was freeze dried to a solid which was digested in methyl alcohol to yield 0.46 g (68%) of adenine, mp 357–360°. This material was identical in melting point, R_f value, infrared spectrum, and ultraviolet spectrum with an authentic sample of adenine.

7-Amino-oxazolo[5,4-*d*]pyrimidine. A solution of 31.2 g (0.3 mole) of formamidine acetate in 600 ml of dimethylformamide was heated to 100° and a stream of nitrogen was bubbled through it. To this was added a solution 5.0 g (0.042 mole) of 4-cyano-5-aminooxazole in 200 ml of dimethylformamide. After addition was complete the mixture was heated at 100° for 10 min. The solvent was removed *in vacuo*, water was added, and the solution again was concentrated to near dryness to crystallize. The crude product was dissolved in ethanol, treated with charcoal, concentrated, and recrystallized twice from water to yield 1.5 g (26%), mp 239°, which resolidified and remelted at 405–410°; ultraviolet λ_{max} 252 $\text{m}\mu$ (ϵ 15,300); nmr (NH_2) τ 1.87,¹⁶ (C_2 , C_5H) 1.26, 0.96.

Diaminomaleonitrile. Aminomalononitrile tosylate (10 g, 0.039 mole) was dissolved in 60 ml of water and was added to 10 g (0.2 mole) of sodium cyanide in 30 ml of water. A solid precipitate formed rapidly and was filtered and washed with ice-water and dried to yield 2 g (47%) of product, mp 177–178°, which had an infrared spectrum identical with the published spectrum for diaminomaleonitrile.⁷ Sublimation *in vacuo* yielded white crystals, mp 182–183°.

Acknowledgment. We wish to thank D. Trentham for determining the nmr spectra and for a number of valuable suggestions, R. Sanchez for an improved procedure for the preparation of HCN tetramer, and R. Mancuso for technical assistance.

(16) This peak exchanged rapidly with D_2O .