Synthesis of Purines under Possible Primitive Earth Conditions.
I. Adenine from Hydrogen Cyanide

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Adenine has been synthesized in substantial amounts by heating a solution of hydrogen cyanide (1 to 15 M) in aqueous ammonia for one or several days at moderate temperatures (27° to 100°). The insoluble black polymer of hydrogen cyanide was removed by centrifugation and adenine was isolated from the red-brown supernatant by chromatographic methods. The main ultraviolet absorbing compound of the reaction product was identified as adenine by eight different procedures, among which, ultraviolet spectrophotometry, melting point of its picrate, and the specific method of Gerlach and Döring were used. From a 11.1 M hydrogen cyanide reaction mixture, at 70°, a yield of 110 mg. of adenine per liter of original reaction mixture was obtained, which could be increased to 685 mg. by evaporation of the supernatant to dryness on the steam bath and subsequent treatment of the residue with hydrochloric acid.

Since adenine is an essential building block of nucleic acids and of the most important coenzymes, and since hydrogen cyanide, ammonia and water are common natural constituents of the solar system, these experiments are considered of significance in relation to the problem of the origin of life. In particular, the experiments provide the first demonstration of the spontaneous synthesis of adenine from simple compounds of carbon and nitrogen under conditions presumed to have existed on the primitive earth.

INTRODUCTION

The possibility that hydrogen cyanide has played an important role in the formation of biochemical compounds on the primitive earth was first indicated by the model experiments of Miller (1-4) and of Oró (5) and Oró and co-workers (6, 7) on the synthesis of amino acids and amino acid amides from simple carbon and nitrogen compounds. A common mechanism of synthesis was found in these experiments. The hydrogen cyanide formed in the early phase of the reaction condensed with aldehydes in the presence of ammonia to form a-amino acid nitriles which then underwent progressive hydrolysis into amino acid amides and finally into amino acids. Although not all of the amino acids commonly found in proteins have yet been obtained in experiments of this kind, it is considered by investigators in this field (8, 9) that the problem of the formation of amino acids under the reducing conditions of the primitive earth postulated by Oparin (10), Urey (11), and others (12, 13) has been solved in its essential aspects.

The next two major problems of the many remaining to be solved in order to gain some understanding about the origin of life on the earth are, according to Miller and Urey (8), the synthesis of peptides and the synthesis of purines and pyrimidines. A partial solution to the first of these problems, the formation of peptides at moderate temperatures in the absence of strongly dehy-
drating agents, has been found recently in the polycondensation of certain amino acid amides (14) and amino acids (15) in aqueous ammonia systems.

The synthesis of purines appears, a priori, a more difficult problem because it requires the formation of two fused heterocyclic ring structures, an imidazole and a pyrimidine. However, the unexpected finding has been made recently in our laboratory that adenine and 4-aminoimidazole-5-carboxamide can also be synthesized from hydrogen cyanide in aqueous ammonia systems at temperatures below 100° (16).

We wish to describe now in more detail these experiments. The present paper reports the isolation of adenine in the form of crystalline adenine picrate from the reaction product of hydrogen cyanide; a second paper, concerned with the mechanism of adenine synthesis reports the detection of 4-amino-5-substituted imidazoles and other purine intermediates in the reaction product (17).³

EXPERIMENTAL

CONDITIONS OF SYNTHESIS

Hydrogen cyanide, liquid or gas, was prepared by reaction of sodium cyanide with sulfuric acid. A simplified procedure requires only the introduction of a fine stream of 30% sodium cyanide solution underneath the surface of a 60% sulfuric acid solution. Two types of experiments were carried out, one with relatively low (0.9-3 M) and the other with relatively high (8-15 M) concentrations of hydrogen cyanide.

In a typical experiment of the first type, the hydrogen cyanide gas obtained from 1 mole sodium cyanide was purified by passing it through warm sulfuric acid, and then it was allowed to be absorbed slowly for about 1 hr. in 500 ml. of 3 N ammonium hydroxide contained in a 1-l. flask provided with a water-cooled condenser. The concentration of cyanide ion in the resulting solution of ammonium cyanide was measured by the method of Schilt (18) and found approximately 1.5 M.

The reaction mixture was heated for 2 days at 70°. Although higher temperatures could be used, this temperature of 70° was selected because when the reaction mixture was heated at 80° or higher temperatures, ammonium cyanide volatilized partially from the solution and deposited in crystal-line form on the walls of the condenser. A short time after the heating was started, the solution turned first yellow, then red-brown, and finally black. The black color was the result of the formation of polymeric hydrogen cyanide (19) which was collooidally suspended in the alkaline solution. The yellow and red-brown colors were probably caused by intermediates of polymeric hydrogen cyanide (19).

At the end of the reaction, and also during the progress of the reaction, samples (50 ml. or less) of the reaction mixture were withdrawn from the flask. The black polymer of hydrogen cyanide was removed by centrifugation, and the clear yellow-red supernatant was processed for the analysis of adenine.

About 10 experiments were performed using this relatively low concentration of hydrogen cyanide. In all these experiments the concentration of ammonium hydroxide was in excess of that of hydrogen cyanide. In some experiments the reaction was allowed to continue for a period of up to a month.

In a typical experiment of the second type, more than 100 g. of liquid hydrogen cyanide were mixed with 400 ml. of approximately 16 N ammonium hydroxide. Both solutions had been previously cooled to -10° in a salt-ice bath, and the mixing was carried out in the same bath. The temperature of the mixture rose temporarily to 15° upon mixing. The solution became yellow, and crystals of ammonium cyanide were formed which dissolved upon warming the reaction mixture to room temperature. The concentration of cyanide ion was found to be 11.1 M. The solution was then very slowly brought to 70° in order to minimize the volatilization of ammonium cyanide and its crystallization on the walls of the condenser.

It should be noted that the “sublimation” of ammonium cyanide occurs temporarily at temperatures lower than 70° when highly concentrated mixtures, as the above, are used. Therefore, in spite of the fact that this process subsides after the first hours of heating, (presumably due to the transformation of the cyanide ion into condensation products), it is imperative to use an oversized flask and an extra-wide condenser to carry out the reaction, otherwise an explosion may result upon obstruction of the condenser by ammonium cyanide.

The reaction mixture was heated at 70° for 5 days, and each day two samples of 50 ml. each were withdrawn from the reaction flask and kept in a Dry-Ice chest until the time of analysis. At the end of the reaction, each of the daily samples was analyzed for adenine.

More than five experiments with high concentrations of hydrogen cyanide were performed. In
two of these experiments the reaction was carried out at room temperature instead of 70°. One of these two reaction mixtures (14.6 M in HCN and 7 M in NH₂OH) was allowed to stand for 26 hr. in a sealed glass pressure tube. The other mixture (8.25 M in HCN and 13 M in NH₂OH) was allowed to stand for 19 days open in the air. These conditions were used in order to find out if the synthesis of adenine could take place at room temperature and also in order to ascertain if, as to be expected, the reaction was invariant with respect to the presence of oxygen, since it is known that the condensation reactions of hydrogen cyanide are not affected by oxygen (19). In another of these experiments, carried out as usual in a flask, a temperature of about 90° was used.

Determination of Adenine After Acid Treatment of the Product

Although in the original observation by the senior author adenine was detected in the reaction product by direct chromatography and without any acid treatment, it was subsequently found (16) that an acid treatment made the determination much more convenient. In the experiments reported here the presence of adenine in the reaction product has been determined before and after acid treatment.

For the determination of adenine after acid treatment, the supernatant from 50-ml. samples was evaporated to dryness in 25 X 200 mm. digestion tubes on the steam bath overnight. Alternatively, the samples were evaporated to dryness in a vacuum rotating evaporator at 60° in a few minutes. The residue was then dissolved in 50 ml. of 6 N HCl (in one case 50% formic acid was used), and the resulting solution was again evaporated to dryness on the steam bath overnight.

The product thus treated was suspended in 5 ml. of distilled water and, after centrifugation, a 50-μl. aliquot of the clear supernatant was placed on each of several sheets of Whatman No. 3 mm. filter paper side by side with 25 μl. of a 0.1% standard solution of adenine hydrochloride and an equivalent volume of a mixture of the standard and unknown solutions. Four different solvent systems were used for ascending chromatography: water-saturated n-butanol (BW), n-propanol-1 N ammonium hydroxide, 3:1 (PA), n-butanol-acetic acid-water, 4:1:1 (BAW), and n-butanol-diethylene glycol-water, 4:1:1 (BDW). After development, the papers were dried and the ultraviolet light-absorbing compounds were made visible by means of a Mineralight lamp having 90% of its emission at 2537 Å.

The ultraviolet-absorbing areas of the chromatogram with Rₜ corresponding to adenine were each cut in small pieces and placed in a test tube with 5 ml. of 1 N hydrochloric acid. The contents of each test tube were boiled for about 10 sec. and, after cooling, the clear solution was transferred to quartz cells. The ultraviolet spectrum in 1 N acid was obtained by means of either a Beckman DK-1 or a Beckman DB recording spectrophotometer, and the absorbance value at the maximum was measured. The recovery of standard adenine from paper chromatograms by this technique was found essentially quantitative.

Identification and Isolation of Adenine

Several procedures were used for the complete identification of adenine: The specific adenine reaction discovered and studied by Kossel and co-workers (20, 21) and modified for paper chromatography by Gerlach and Dürring (22), the spectrophotometric determination of the ultraviolet spectra in acid and basic solutions (23), and the colorimetric method of Woodhouse (24).

Ordinary chromatographic techniques were also applied. For this, several chromatograms were developed in the above four different solvents and, upon drying, were treated with reagents which form metallic compounds or complexes with purines, such as mercuric nitrate and ammonium sulfide (25), mercuric chloride and eosin (26), and silver nitrate and bromophenol blue (27). Before this treatment with reagents, the papers were usually scanned with ultraviolet light, and a permanent record was obtained by ultraviolet light photography or contact printing on sensitive paper.

Neutral and basic solvents gave excellent chromatograms. Solvents made of organic acids and alcohols were not as satisfactory because they had a tendency to give chromatograms with relatively high ultraviolet light-absorbing backgrounds or bands in some cases (23). When neutral and basic solvents were used, the ultraviolet light scanning and printing methods and the mercuric chloride–eosin method of Michl were found in our hands the most sensitive and practical paper chromatographic methods for the detection of purines among the many described in the literature (23, 28, 29).

For the isolation of adenine, large-scale paper chromatography was used. After evaporation and acid treatment of a 5-day, 50-ml. sample of the second typical experiment (11.1 M in HCN), the residue was dissolved in 15 ml. of 1 N hydrochloric acid and filtered. Exactly 2 ml. of this solution was placed on each of six large sheets of Whatman filter paper (No. 3 mm.) and chromatographed in the BW solvent by descending devel-
development. The bands with the $R_f$ of adenine were located by their ultraviolet absorption and cut out, and the absorbing material from each paper strip was eluted with 1 $N$ hydrochloric acid and collected in small beakers. The eluates in the beakers were evaporated to dryness in a vacuum desiccator over sodium hydroxide. The evaporated material was rechromatographed in the PA solvent, and, after subsequent elution and evaporation, an impure crystalline product was recovered from which a crystalline picrate was prepared (30, 31). A picrate of authentic adenine was also prepared, and the melting points of both derivatives were determined by means of a Fisher-Johns aluminum block apparatus and corrected.

**DETERMINATION AND IDENTIFICATION OF ADENINE IN THE UNTREATED REACTION PRODUCT**

Exactly, 0.5 ml. of the untreated supernatant solution of each of a series of five 50-ml samples, from the second typical experiment, was placed in a small spot on Whatman No. 3 MM. filter paper, and chromatographed in the BW and PS solvents as described above for the isolation of adenine, the only modification being that ascending development was used in this case and that the material was eluted from small spots rather than from long strips of paper.

This repeated chromatography was found necessary to remove three compounds, which, using the first solvent, had migrated, along with adenine, to the same place of the paper. The second solvent separated the three compounds conveniently. Two of these compounds showed a slight blue fluorescence. One moved to the front and the other to the back of adenine when using the second solvent. The third of these compounds, which could be detected on the first chromatogram because it formed a colored derivative with diazotized p-nitroaniline (32), decomposed into a purple pigment and moved very little from the origin in the second chromatogram.

The solutions containing the ultraviolet-absorbing compound were analyzed qualitatively and quantitatively for adenine by means of a recording spectrophotometer as described above. As above too, complete identification was secured by the application of the other analytical techniques.

The analysis of adenine in the untreated reaction product was also performed in four additional experiments. Two of these experiments were carried out at room temperature with relatively high concentrations of hydrogen cyanide and two at 70° with relatively dilute reaction mixtures.

In the latter case the supernatant of a 13-day, 50-ml sample, from the product of a 1 $M$ hydrogen cyanide reaction mixture, was concentrated to 10 ml. in a vacuum rotating evaporator and subsequently placed on the top of a 2 × 18 cm. column of Dowex 50 (200-400 mesh, × 8, H⁺ form). The column was first washed with 500 ml. water, and then the material on the column was eluted with 500 ml. of 5 $N$ ammonium hydroxide. The eluate was evaporated in a rotating vacuum evaporator to 3 ml., and aliquots of 0.5 ml. were chromatographed in the BW and PA solvent systems and analyzed as previously described.

**RATE OF ADENINE FORMATION**

Time studies of the formation of adenine under different conditions were also performed. The spectrum of the compound isolated by paper chromatography from each sample was obtained, the absorbance value at the maximum was measured, expressed in milligrams of adenine per liter of original solution and plotted in a graph against the reaction time of the sample.

**RESULTS**

**IDENTIFICATION OF ADENINE IN THE ACID-TREATED PRODUCT**

Table 1 and Figs. 1–3 illustrate the results obtained with relatively low hydrogen cyanide reaction mixtures such as the one described in the first typical experiment. Table I gives the $R_f$ of the unknown and of adenine in four different solvents as obtained by ascending chromatography. In all cases, the unknown showed the position corresponding to the adenine standard. In different chromatograms the unknown was characterized not only by its position and ultraviolet light absorption, but also by the color of the compounds or complexes formed with the mercuric chloride–cosin, silver nitrate–bromophenol blue, and mercuric nitrate–ammonium sulfide reagents.

Figure 1 shows a chromatogram treated with potassium permanganate, chlorine, and potassium hydroxide according to the adenine-specific method of Gerlach and Döring. This chromatogram is compared with a reproduction of an ultraviolet print from the same chromatogram before the treatment. As can be seen, the unknown corresponded exactly to the adenine standard.

In Fig. 2 the ultraviolet spectra of the
Twenty-five microliters of a 0.1% adenine solution and 50 μl of a solution obtained after acid treatment of the hydrogen cyanide reaction product were chromatographed by ascending development in four different solvents. The purine spots were detected by a Minelamp light lamp and by the silver and mercuric complexes.

For the test for solvent composition, diazo derivative obtained from the unknown and from adenine by the method of Woodhouse. An excellent correlation of the two spectra can be observed.

Figure 4 shows the spectrum of the picrate prepared from the ultraviolet light-absorbing compound isolated by large-scale paper chromatography from a 5-day 50-ml sample of the second typical experiment (11.1 M in HCN). The picrate was twice recrystallized in methanol and dissolved in absolute methanol, and the visible and ultraviolet spectra were obtained in a Beckman DB spectrophotometer and compared with that of an authentic sample of adenine picrate. As can be seen, the two spectra are almost indistinguishable. In both cases, the absorption ratio of the maxima at 253 and 355 μm was found identical.

When the picrate of the isolated com-

Fig. 1. Reproductions of a paper chromatogram of the reaction product in water-saturated n-butanol. A: Ultraviolet print at 2537 Å; B: Chromatogram treated according to the method of Gerlach and Döring; 1: adenine; 2: unknown; 3: adenine plus unknown.

unknown and of adenine eluted from a chromatogram are recorded. The maxima in acid and basic pH, as well as the ratio of the extinction coefficients at these two pH, were identical for the unknown and for adenine.

Figure 3 records the visible spectra of the

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* See text for solvent composition.
Fig. 2. Ultraviolet spectra of standard adenine and the unknown compound from hydrogen cyanide in acid and basic pH. The standard and unknown compounds with the $R_f$ of adenine were eluted from the paper chromatogram with 1 N hydrochloric acid, and the spectra were recorded as described in the text.

Fig. 3. Visible spectra of the diazo products obtained by the method of Woodhouse with standard adenine and the unknown compound from hydrogen cyanide.
ric acid, from which these two compounds were quantitatively recovered by elution with 1 N hydrochloric acid and methanol, respectively.

IDENTIFICATION OF ADENINE IN THE UNTREATED REACTION PRODUCT

Figure 5 shows the ultraviolet spectra in acid and base of the compound with the $R_f$ of adenine isolated directly from the untreated hydrogen cyanide reaction product by two consecutive chromatographic separations. This reaction was carried out at room temperature, and a Beckman DB spectrophotometer was used to record the spectra.

As can be seen, the spectra show the same absorption maxima and shoulders in the unknown as the standard adenine. Also, the shoulder at about 280 m$\mu$ (0.4 N sodium hydroxide) disappears from both adenine and the unknown when these compounds are placed in a highly protonated solvent (1 N hydrochloric acid). This is precisely what should be expected if the 280-m$\mu$ band of the unknown corresponded to the $n-\pi^*$ transition of adenine (33).

Figures 6 and 7 give chromatographic and spectrophotometric evidence for the identification of the unknown compound obtained from a dilute hydrogen cyanide reaction mixture after its retention in a Dowex 50 column and elution from the column with ammonium hydroxide. The purine spots were detected by means of a Mineralight lamp.
Fig. 7. Ultraviolet spectra in 1 N hydrochloric acid of the compound with the $R_f$ of adenine from the PA chromatogram of Fig. 6. Elution as described in the text.

Fig. 8. Time study of the formation of adenine from hydrogen cyanide after evaporation on the steam bath and acid treatment (upper curve) and after rapid evaporation under vacuum and acid treatment (lower curve).

Fig. 9. Rate of formation of adenine from a concentrated (11.1 $M$) hydrogen cyanide reaction mixture at 70°. Lower curve: untreated product; Upper curve: after evaporation on the steam bath and treatment with 6 N HCl.
time all the reaction mixture had been withdrawn for analysis, the concentration of adenine in the crude reaction product was 110 mg./l. of original solution. For comparison, the concentration of adenine found after the acid treatment of duplicate samples of the same experiment was also measured. At the end of 5 days, the amount of adenine synthesized in the acid-treated samples was 685 mg./l. of original solution.

The larger adenine synthesis observed in the acid-treated samples was caused in part by the prolonged evaporation of the reaction product on the steam bath (see Fig. 8), and in part by the acid treatment as such. It appears that once the condensation of hydrogen cyanide has taken place, the acid hydrolytic conditions favor the synthetic reaction to a greater extent than the basic conditions of the original reaction mixture.

As can be seen in Fig. 9, the synthesis of adenine in the untreated reaction mixture increased linearly with time at the approximate rate of 1 mg./hr., and it may be expected that the synthesis would have continued at this rate as long as there was a sufficient concentration of cyanide or adenine precursors. Thus, in principle, given sufficient time, it should be possible to synthesize as large an amount of adenine under the basic conditions of the reaction mixture as under the acid conditions of the subsequent treatment. This becomes evident from the fact that the yield in the untreated samples increased 175% in 4 days whereas the yield in the acid-treated samples increased only 7% in the same time. At any rate, if one considers that at the end of 5 days much of the hydrogen cyanide has been used in the formation of polymeric hydrogen cyanide (see Figs. 10 and 11), the adenine yields of 110 mg./l. of solution are quite substantial in the context of these experiments. This concentration is, no doubt, several times larger than that found in general in biological fluids.

Figure 10 illustrates the rate of disappearance of hydrogen cyanide as measured in the first typical experiment carried out at 70°. When higher temperatures were used the disappearance of hydrogen cyanide was much faster. This was observed, for instance, in the reaction illustrated in Fig. 11. In this reaction, which was carried out at about 90°, the concentration of hydrogen cyanide dropped almost to zero after the first day. A good correlation between the

![Fig. 10. Rate of disappearance of hydrogen cyanide from a dilute (1.5 M) reaction mixture at 70°.](image)

![Fig. 11. Lower curve: rate of disappearance of hydrogen cyanide from a concentrated (9.9 M) reaction mixture at about 90°; Upper curve: rate of formation of adenine in the same experiment. The adenine was isolated directly from the untreated product by two consecutive chromatographic separations as described in the text.](image)
curves for the disappearance of hydrogen cyanide and formation of adenine was observed in this case. The relatively fast disappearance of hydrogen cyanide can be explained by the autocatalytic nature of the initial hydrogen cyanide condensation reactions (19, 34), which result in the formation of intermediates (35, 36) which eventually lead to the synthesis of polymeric hydrogen cyanide on one hand (19) and adenine on the other.

DISCUSSION

The synthesis of uric acid from glycine, and of purine from formamide, were carried out a long time ago at high temperatures by Horbaczewski (37) and by Bredereck et al. (38, 39), respectively. However, none of the biochemical purines found in nucleic acids were isolated or identified in these experiments.

Interest in studying the reactions of hydrogen cyanide in aqueous ammonia arose from the observation that polymeric hydrogen cyanide was formed in the experiments of Miller (1) on the synthesis of amino acids by electric discharges and in experiments from this laboratory (5, 6) on the synthesis of amino acids and amino acid amides by reaction of formaldehyde and hydroxylamine. This interest was further stimulated by the numerous studies on the controversial nature of one of the hydrogen cyanide oligomers (19, 35, 36) and by the report that crude polymeric hydrogen cyanide yielded amino acids upon acid hydrolysis.

The first experiments on the condensation of hydrogen cyanide carried out by the senior author in this laboratory more than 1 year ago were undertaken for the purpose of studying the formation of amino acids (7). When a compound with the Rf of adenine giving a positive Michl’s test was detected for the first time on the paper chromatograms, the finding was so inexplicable that the results were not immediately accepted. However, upon reinvestigation, the results were confirmed (16).

and, as has been shown here, adenine has been identified not only in the acid-treated reaction product of hydrogen cyanide, but also in the crude reaction product which had not undergone any acid treatment at all. These experiments have been repeated many times in this laboratory, and adenine has been found each time that the reaction product has been adequately analyzed for it.

The likelihood that similar conditions to the ones used here did exist on the primitive earth is discussed in some detail in a recent paper by one of us (13), where a cometary origin of biochemical compounds is postulated in line with the ideas proposed some time ago by Oparin (10) and Urey (11). In essence, this concept is based on the facts that the collision of comets with the earth has a finite probability (40) and that three of the major constituents of comets, as deduced from spectroscopic observations (41), are hydrogen cyanide, ammonia, and water in addition to methane and other hydrocarbons (42).

However, the significance of these experiments does not depend on considerations of this or any other specific model for the primitive earth’s environment, since hydrogen cyanide has been demonstrated or considered to be an intermediate in most of the experiments in which amino acids have been synthesized from simple carbon and nitrogen compounds (5–8, 43–45).

The significance of present experiments in relation to the general biopoietic problem resides mainly on the fact that they constitute the first demonstration of the spontaneous synthesis of adenine, a compound which is an essential building block of nucleic acids and of the most important coenzymes such as ATP, NAD, FAD, CoA, and the amino acid-activating s-RNA.

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*Also, F. L. Whipple, private communication.
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