SYNTHESIS OF ADENINE FROM AMMONIUM CYANIDE

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The synthesis of amino acids from simple compounds of carbon and nitrogen under a variety of possible primitive earth conditions has been accomplished in several laboratories. In the experiments of Miller (1957) and of Oró et al. (1959) the mechanism of amino acid synthesis was elucidated and found to depend on the intermediate formation of hydrogen cyanide. The latter investigators also presented evidence for the formation of α-amino acid amides as intermediates in the above synthesis.

According to Miller and Urey (1959) the two next major problems remaining for an understanding of the origin of life are (1) the synthesis of peptides and (2) the synthesis of purines and pyrimidines. A partial answer to the problem of the synthesis of peptides (at temperatures accepted by geochemical evidence and without the use of activating reagents) has been found recently in the direct polycondensation of α-amino acid amides (Oró and Guidry, 1960).

Evidence is now presented for the synthesis of adenine from aqueous solutions of ammonium cyanide at temperatures below 100°.

The important role of adenine as a building block of nucleic acids and coenzymes gives special significance to the present ex-

periments in relation to the problem of the abiogenic formation of biochemical compounds on the primitive earth(Oparin, 1957).

Methods: A 1.5 N solution of ammonium hydroxide was saturated with hydrogen cyanide. The resulting solution of ammonium cyanide was placed in a flask provided with a water cooled condenser and heated at 90° for 24 hours. At the end of the reaction the black polymer of hydrogen cyanide was removed by filtration and 50 ml. of the filtrate was evaporated to dryness at 90° and then hydrolyzed with 6 N hydrochloric acid overnight. The product obtained after evaporation of the hydrochloric acid was suspended in 5 ml. of distilled water and centrifuged. An insoluble brown residue sedimented which was not analyzed. Only the clear supernatant was used in the subsequent analysis.

Samples of about 50 µl. of the supernatant were placed on several sheets of Whatman No. 3 MM filter paper along with 25 µl. of 0.1% standard solutions of adenine and guanine and mixtures of standards and unknown. Three different solvents systems were employed for ascending chromatography: n-butanol-diethylene glycol-water, 4:1:1, (BDW), water saturated n-butanol (BW) and n-butanol-acetic acid-water, 12:3:5, (BAW). After development the papers were dried and the position of the ultraviolet light absorbing compounds was determined by means of a Mineralight lamp emitting at 2537 Å. For the confirmation of the nature of the separated compounds, four different reagents which form colored complexes with purines were applied to the paper chromatograms (see "Results").

Finally, each of the ultraviolet absorbing areas of the chromatograms with Rf corresponding to adenine was cut out in small pieces, placed in a test tube with 5 ml. of 1 N HCl and boiled for a few

seconds. The ultraviolet spectrum of the solution thus obtained was measured in a Beckman DK-1 recording spectrophotometer. The spectrum was also measured in basic pH. For this 2.3 ml. of the solution in 1 N HCl was mixed with 0.7 ml. of 5 N NaOH and the spectrum of the resulting solution in 0.4 NaOH was recorded. The technique thus described was applied in identical manner to the unknown compound, to the standard adenine and to a paper blank.

In addition to purines, the paper chromatograms were also analyzed for amino acids and for diazotizable compounds. For this

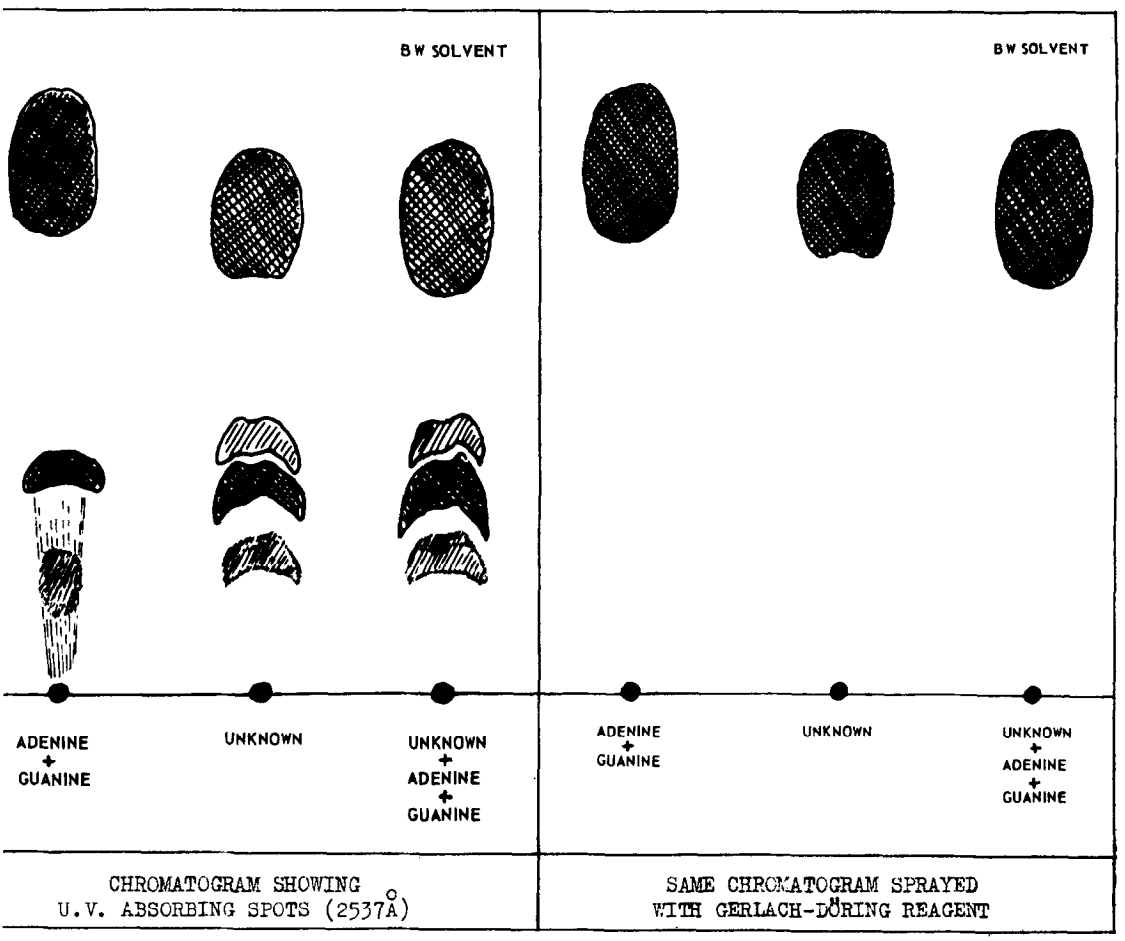

Figure 1. Chromatogram of the reaction products and standard purines

purpose a solution of ninhydrin and a solution of Fast Red Salt GG (Pearl and McCoy, 1960) were respectively used.

Results: Figure 1 shows the reproduction of a photograph of the ultraviolet absorbing spots of a chromatogram in the BW solvent and of a photograph of the same chromatogram after applying the specific adenine reagent described by Gerlach and Döring (1955). A correlation of the areas corresponding to unknown and standard adenine can be observed. The U. V. absorbing area below standard guanine is probably caused by a small impurity of xanthine in guanine and by the trailing of guanine itself as a result of the low solubility of this compound. The second most important absorbing spot in the reaction product appears to have a similar chromatographic behavior to guanine; however, no identification has yet been made.

Additional evidence for the synthesis of adenine was obtained when other chromatograms developed in the BW, BAW and BDW solvents were treated with silver nitrate and ammonium sulfide (Vischer and Chargaff, 1948), mercuric chloride and eosin (Michl, 1953) and silver nitrate and bromophenol blue (Wood, 1955), respectively. The colored derivatives given by the main unknown compound coincided exactly with the position and color of those given by standard adenine.

Figure 2 shows the spectra of the main unknown compound and standard adenine in 1 N HCl and 0.4 N NaOH. The maxima in acid and basic pH as well as the ratio of the extinction coefficients in acid and base are identical in both cases. Thus it is concluded that the main ultraviolet absorbing compound formed in this synthesis is adenine. The amount of adenine synthesized in the pre-

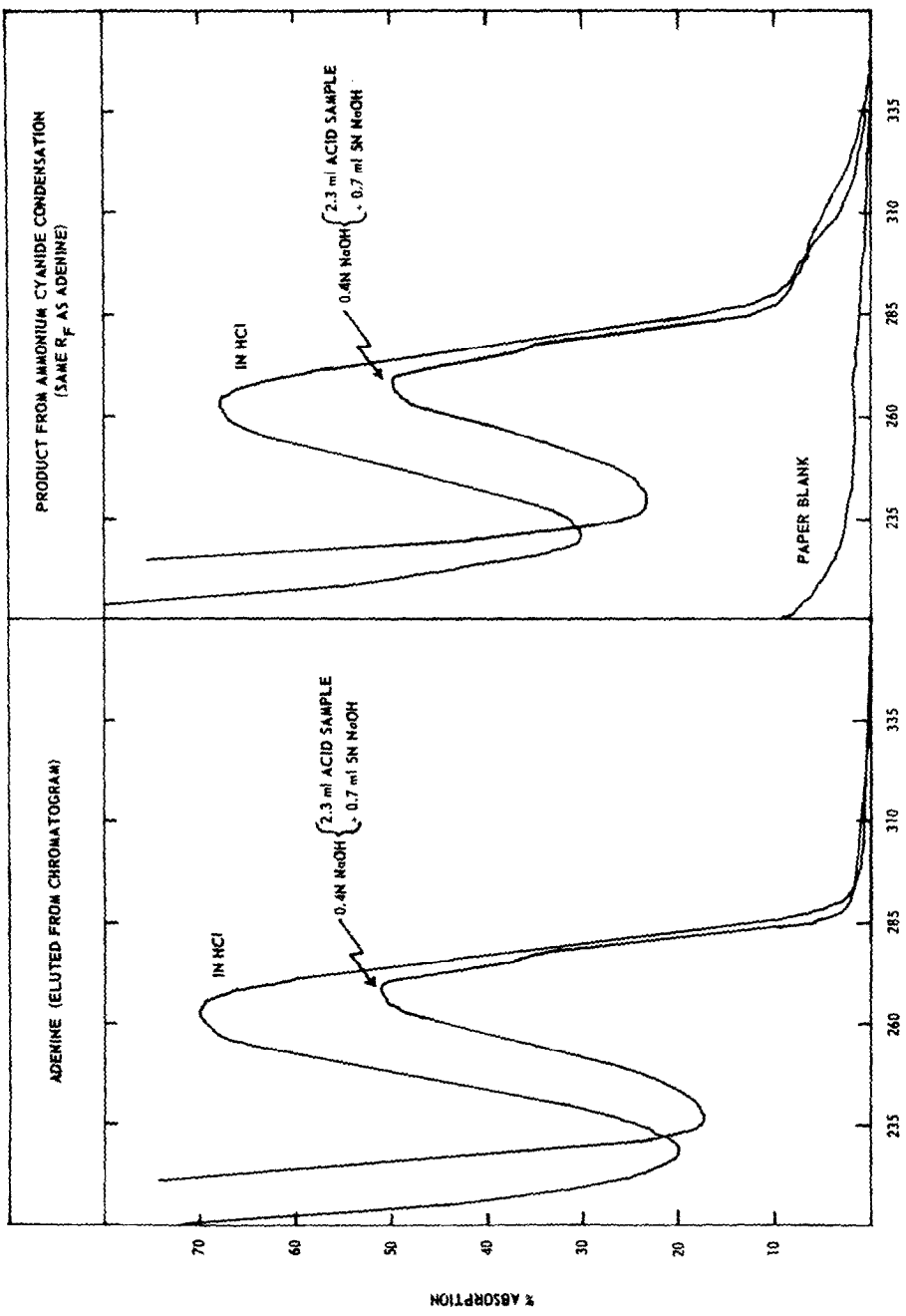

Figure 2. Ultraviolet spectra of standard adenine and unknown compound

sent experiments has been estimated at about 20 mg per liter of original reaction mixture. A consideration of the yield is of no significance here, since most of the ammonium cyanide is probably

used in the formation of the black polymer of hydrogen cyanide (Wadsten and Anderson, 1959).

Preliminary evidence has also been obtained for the formation of glycine and 4-aminoimidazole-5-carboxamide as products of the reaction, indicating that the synthesis of adenine under the present conditions follows a parallelism with the known pathway of biochemical synthesis of purines. Work is in progress for the elucidation of the mechanism of adenine synthesis.

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