References and Notes

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Cyanoacetylene in Prebiotic Synthesis

Abstract. *Cyanoacetylene is a major nitrogen-containing product of the action of an electric discharge on a mixture of methane and nitrogen. It reacts with simple inorganic substances in aqueous solution to give products including aspartic acid, asparagine, and cytosine.*

In the synthesis of purines from hydrogen cyanide (*1*) we have shown that 4-aminoimidazole-5-carbonitrile (I) is an intermediate (*2*). This led us to consider β-aminoacrylonitrile (II), and hence cyanoacetylene, as possible prebiotic precursors of the pyrimidines (Fig. 1). The prebiotic synthesis of pyrimidines from β-aminoacrylonitrile has previously been suggested by Oro (*3*). We now report on the synthesis of cyanoacetylene in an electric discharge and on its conversion to biologically important products.

We have studied the effects of an electric discharge on a number of gases and gas mixtures including hydrogen cyanide, cyanogen and acetylene, hydrogen cyanide and acetylene, and methane and nitrogen. In these cases, cyanoacetylene was detected in appreciable amounts; in fact, it was always the most abundant volatile nitrogen-containing product other than hydrogen cyanide. The sparking of a mixture of methane and ammonia resulted in an efficient conversion to hydrogen cyanide. Cyanoacetylene was never present in more than trace amounts; however, it would have been destroyed rapidly in the presence of ammonia. We now describe our experimental work only for the methane-nitrogen system, which we believe to be interesting in the context of prebiotic synthesis.

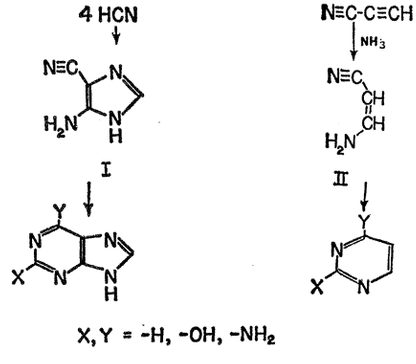

Fig. 1. Proposed pathways for purine and pyrimidine synthesis.

In a typical experiment, a round-bottom flask was charged to atmospheric pressure with 20 cm^3 of methane and 88 cm^3 of nitrogen (each > 99.9 percent pure). A spark was generated across a tungsten electrode gap of 5 mm with an Electro-technic BD-10 high-frequency generator (tesla coil) operating at maximum output. Periodic analyses were made by gas chromatography at 30°C on a 3.6-m column of 1,2,3,-tris(2-cyanoethoxy) propane on Gas-Chrom Q, with a flame-ionization detector.

Gaseous products and their retention times (minutes) were: methane (1.5), acetylene (1.6), various hydrocarbons (2 to 4), diacetylene (6.2), cyanoacetylene (10.4), hydrogen cyanide (12.4), and benzene (15.1). The cyanoacetylene concentration was maximum after about 25 minutes, when its integrated response was 8.4 percent that of the hydrogen cyanide. The cyanoacetylene slowly decreased thereafter, whereas the hydrogen cyanide concentration eventually doubled to an integrated response which was 42 percent that of the original methane.

The identities of the various products were established by comparison of retention times with those of authentic samples, and by collection of the compounds in the individual peaks and comparison of their infrared and ultraviolet spectra with those of authentic samples. The ultraviolet spectrum of cyanoacetylene is highly characteristic and leaves no doubt concerning the identity of this material.

We have not attempted to study the mechanism of cyanoacetylene synthesis. However, it has been shown that this compound is produced by the action of active nitrogen on acetylene (*4*).

Aspartic acid was one of the amino acids obtained by Miller in his classic experiments on the synthesis of amino acid precursors from mixtures of methane, ammonia, and water in an electric discharge (*5*). It was suggested that the aspartic acid was formed by the action of ammonia and hydrogen cyanide on acrolein. However, it seemed possible to us that after part of the ammonia had decomposed to nitrogen, some or all of this aspartic acid might have been formed in the following sequence of reactions.

$$CH_4 + N_2 \xrightarrow{\text{spark}} H\text{-}C{\equiv}CC{\equiv}N$$

$$HC{\equiv}CC{\equiv}N + NH_3 \rightarrow NH_2CH{=}CHCN$$

$$NH_2CH{=}CHCN + CN^- \xrightarrow{H^+} CNCHCH_2CN \quad (NH_2)$$

$$NCCHCH_2 \ (NH_2) \xrightarrow{\text{hydrolysis}} \text{asparagine}$$

$$\xrightarrow{\text{hydrolysis}} \text{aspartic acid}$$

We have now confirmed that a mixture of 0.1*M* cyanoacetylene, 5.0*M* ammonia, and 1*M* HCN heated at 100°C for 24 hours yields aspartic acid (10 to 15 percent) and asparagine (5 to 10 percent).

A number of pyrimidine syntheses starting with propiolic acid or β-aminocrotonic acid derivatives have been described (*6*). It was not surprising, therefore, that under severe conditions we obtained cytosine by fusing cyanoacetylene with urea in a sealed tube; uracil was obtained similarly from propiolamide. Our attempts to obtain cytosine under more plausible prebiotic conditions have met with some success.

When an aqueous solution containing 1.0*M* potassium cyanate and 0.1*M* cyanoacetylene was heated to 100°C for 1 day, a 5 percent yield of cytosine was obtained. When the same mixture was allowed to stand at room temperature for 7 days, cytosine was obtained in 1 percent yield. The cytosine was identified chromatographically by direct comparison in three solvent systems. Furthermore the ultraviolet spectrum of the eluted cytosine was identi-

cal with that of an authentic sample in acidic, basic, and neutral solutions.

Electrical discharges through mixtures of N_2 and hydrocarbons give hydrogen cyanide as the major product (*7*). In a number of cases, the second most abundant nitrogen-containing product is now shown to be cyanoacetylene. Hydrogen cyanide is the starting point for relatively well-understood syntheses of glycine and the purines, and also syntheses for alanine, aspartic acid, and possibly other α-amino acids where the mechanism is less clear.

Miller's work demonstrated that these α-amino acids as well as serine and glutamic acid are produced by the hydrolysis of products formed in discharges through mixtures of methane, ammonia, and water, probably by a Strecker synthesis (*5*). We have now shown that under conditions similar to those used for purine synthesis cytosine may be obtained from cyanoacetylene; under other conditions aspartic acid and asparagine are formed.

These results suggest that a unified, laboratory synthesis of the major nitrogen-containing biochemicals from the products of electrical discharges may be possible, although this is by no means established. Even if this proves to be the case, other proposed modes of prebiotic synthesis would not be implausible, for acetylene and hydrogen cyanide are likely to be major products both of the prolonged photolysis or the thermal cracking of almost any reducing mixture containing carbon, hydrogen, and nitrogen compounds. Thus hydrogen cyanide, cyanogen, and cyanoacetylene are versatile starting materials in the synthesis of biologically important nitrogen-containing organic compounds.

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Retention of the Biological Potency of Human Pituitary Growth Hormone After Reduction and Carbamidomethylation

Abstract. *The reduced alkylated derivative of human pituitary growth hormone was prepared by reacting the native hormone in 8M urea solution, first with mercaptoethanol and then with iodoacetamide. On the basis of amino acid analyses, the product isolated after gel filtration on Sephadex G-100 is completely devoid of unreduced cystine but otherwise has a composition identical to that of the native hormone. When assayed in both the rat tibia test and the pigeon crop-sac assay, it appears that the full potency of the native hormone is retained in the reduced alkylated derivative.*

During the course of determining the sequence of the 188 amino acid residues in the human growth hormone (HGH) molecule (*1*), the reduced-alkylated derivative of the hormone (RA-HGH) was prepared. When RA-HGH was submitted to bioassay for growth-promoting and pigeon crop-sac stimulating activities, it was found that the derivative possesses a potency comparable with that of the native hormone. As far as we are aware, this is the first instance where cystine residues in biologically active proteins can be completely reduced and alkylated without resulting in loss of physiological potency.

For the preparation of RA-HGH, the procedure of Anfinsen and Haber (*2*) was employed. Briefly, the hormone (100 mg) was dissolved in an 8*M* urea solution (5 ml) freshly prepared from recrystallized urea. The solution was adjusted to *p*H 8.5 with 5 percent methylamine solution, freed of air by flushing with nitrogen gas, and allowed to react with mercaptoethanol (0.1 ml) in a stoppered flask for 6 hours at 23°C. After dilution with water to give a urea concentration of 2*M*, a tenfold excess (with respect to mercaptoethanol) of iodoacetamide was added; the *p*H of 8.5 was maintained by the addition of 5 percent methylamine solution and the solution was allowed to sit for 15 minutes. At the end of this period the remaining unreacted alkylating agent in the mixture was removed by reaction with an excess of mercaptoethanol. After 1 hour the products of the reaction were separated from the reagents by gel filtration in 0.005*M* NH_4OH solution on a Sephadex G-50 column.

In order to assess the homogeneity of the reduced alkylated HGH, we submitted it to exclusion chromatography on Sephadex G-100, using 0.01*M* NH_4HCO_3 solution as the eluant. For comparison, a sample of native HGH that had been allowed to stand at room temperature for 6 hours in an 8*M* urea solution of *p*H 8.5 was chromatographed on Sephadex G-100 under identical conditions. In the case of the urea-treated hormone (U-HGH), it was first freed from urea by passage in 0.005*N* NH_4OH solution through the Sephadex G-50 column and recovered by lyophylization before chromatography on the Sephadex G-100.

Figure 1 is a representation of the elution patterns obtained when each of these preparations (HGH, U-HGH, and

Table 1. Effect of various treatments on the biological activities of HGH.

Treatment	Bioassay					
	Tibia test			Pigeon crop-sac stimulation*		
	Total dose (μg)	No. of rats	Tibia width (μ)†	Total dose (μg)	Reaction	Mean
Untreated	20	5	244 ± 5	1	+2, +1, +2, +1, +1, 0, +2, +1	1.3
	60	6	278 ± 6	0.5	+1, +1, +1, +1, +1, 0, +1, +1	0.87
Urea (U-HGH-2)‡	20	6	236 ± 2.2	1	+2, +1, +1, +1, +1, +2, +2, +1	1.4
	60	6	285 ± 1.9	0.5	+1, +1, +1, 0, +1, +1, +1, +1	0.88
Reduced-alkylated (RA-HGH-4)‡	20	6	233 ± 4	1	+2, +1, +1, +1, +1, +1, +1, +1	1.1
	60	6	276 ± 2	0.5	+1, +1, 0, 0, +1, +1, +1, +1	0.75

* No stimulation, 0; moderate stimulation, + 1; good stimulation, + 2. † Mean ± standard error. ‡ See Fig. 1.