vious studies have suggested (*17*). The possible role of virus infections and of thyroid autoimmunity in this population should be investigated.

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Prebiotic Synthesis of Propiolaldehyde and Nicotinamide

Abstract. *We have identified propiololdehyde as a product of the action of an electric discharge on mixtures of methane and water or methane, nitrogen, and water. The aldehyde reacts with cyanoacetaldehyde and ammonia (other "prebiological molecules") to yield nicotinonitrile. This substance can be hydrolyzed to nicotinamide and nicotinic acid.*

Highly unsaturated molecules that can readily be obtained from simple gas mixtures by strong heating or by the action of an electric discharge are the starting materials in many potentially prebiotic syntheses (*1*). Recently we have shown that cyanoacetylene is formed in an electric discharge and that it reacts with cyanogen or cyanamide to give cytosine. We noted that cyanoacetylene also reacts with ammonia to give 6-aminonicotinonitrile. This led us to consider cyanoacetylene as a possible precursor of the vitamin nicotinamide (*2*).

Although 6-aminonicotinonitrile is formed in high yields from cyanoacetylene and ammonia, we have been unable to convert it to nicotinonitrile under potentially prebiotic conditions. Direct syntheses of nicotinonitrile from one molecule of cyanoacetylene and a three-carbon source at a more reduced level were therefore attempted.

Nicotinonitrile is not formed under mild conditions from cyanoacetylene, ammonia, and acrylonitrile nor from cyanoacetylene, ammonia, and compounds such as glyceraldehyde or malonaldehyde. Attempts to synthesize dihydronicotinonitrile—for example, from acrylonitrile and ammonia—were also unsuccessful. This led us to investigate propiolaldehyde as a three-carbon source. Here we describe the identification of propiolaldehyde as a product of the action of a discharge on certain simple gas mixtures, and its utilization in a plausible prebiotic synthesis of nicotinonitrile, nicotinamide, nicotinic acid, and corresponding *N*-alkyl derivatives.

Triatomic carbon (C_3) reacts with alcohols at low temperatures to give the diacetals of propiolaldehyde (*3*).

$$:C=C=C: + ROH \rightarrow HC \equiv C - CH(OR)_2$$

The corresponding reaction with water should give propiolaldehyde.

$$C_3 + 2\,H_2O \rightarrow HC \equiv C - CH(OH)_2 \rightleftharpoons HC \equiv C - CHO + H_2O$$

Since C_3 is formed by the action of an electric discharge on hydrocarbons such as methane (*4*) we investigated the products accumulating in liquid water when an electric discharge is passed through an atmosphere of pure methane above it (*5*).

Propiolaldehyde was first detected in the aqueous mixture by its color reaction with thiobarbituric acid (TBA), which is a specific test for propiolaldehyde, malonaldehyde, and certain of their derivatives (*3*, *6*), and by the formation of an adduct with HS^- with maximum absorption at 328 nm at *p*H's 7 to 9 (not formed by malonaldehyde).

In further experiments the propiolaldehyde was concentrated, first by distillation at reduced pressure and then by careful freezing of the distillate to crystallize out most of the water. The concentrate contained a complex mixture of organic compounds. We could not resolve propiolaldehyde completely by gas chromatography even though a number of different columns were used. The identification was therefore confirmed as follows.

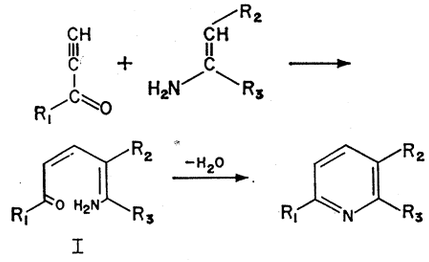
1) A standard solution of authentic propiolaldehyde (*7*) was injected at 150°C onto a copper column (6 m by 0.3 cm) packed with Porapak Q, and the aldehyde peak was collected. A portion of the reaction product was then injected, and the material with the retention time of propiolaldehyde was collected. Both eluates gave identical TBA color reactions (maximum absorption at 532 nm; shoulder at 500 nm). By comparing the color intensities given in the TBA test by the solutions before injection and by the fractions collected from the column, we estimated a 10 percent recovery both for the standard and for the reaction product.

2) Chromatography at 95°C through a copper column (6 m by 0.9 cm) packed with 5 percent Carbowax 1500 on Chromosorb P showed the authentic aldehyde to have a retention time corresponding to a shoulder on the emerging side of a major peak of the product mixture (probably toluene). The area with the same retention time as propiolaldehyde was collected. This eluate gave an intense TBA test identical to that given by authentic propiolaldehyde. A collection was then made of the total eluate excluding the propiolaldehyde region. Although this eluate gave a TBA test, its intensity was only 5 percent of that given by the propiolaldehyde fraction.

The maximum yield of propiolaldehyde obtained in these experiments was about 0.1 percent based on the methane destroyed. When a mixture of nitrogen and methane (90:10) was used the yield of propiolaldehyde (as judged by the TBA test carried out on the total aqueous phase) was increased up to 0.37 percent.

The formation of pyridines by the condensation of enamines with acetylenic ketones or aldehydes and subsequent cyclization of the adducts has

been described by Bohlmann (*8*). He suggests the following reaction mechanism on the basis of the isolation of intermediates to which he ascribed the structure I.



I

This suggested pathway A (Fig. 1) as a possible route to nicotinonitrile in aqueous solution.

Aminoacrylonitrile was obtained as described (*2*). To a 0.1*M* solution we added an equivalent amount of propiolaldehyde. After about 2 hours at 24°C and *p*H 8, the formation of structure I ($R_1 = R_3 = H$, $R_2 = CN$), as judged by the absorption around 350 nm, was maximum. One portion of the reaction mixture was cyclized with glacial acetic acid following Bohlmann's procedure to give a 7 percent yield of nicotinonitrile. A second portion was cyclized with 1*M* ammonia at 100°C for 5 hours to give 3 percent nicotinamide and 3 percent nicotinic acid. The products were identified by gas chromatography (for the nitrile), paper chromatography, and paper electrophoresis at *p*H's of 2.7 and 7.4. The identification of nicotinic acid and nicotinamide was further confirmed from the ultraviolet spectra at *p*H's 2, 7, and 11 of samples eluted from paper chromatograms and paper electrophoretograms.

A more detailed study of scheme A showed that it was not easily modified to give better yields of products, at least under plausibly prebiotic conditions. However, an almost equivalent sequence of reactions was discovered which is satisfactory (Fig. 1, pathway B).

Cyanoacetylene undergoes hydrolysis in dilute aqueous solution at *p*H 8.5 to give cyanoacetylaldehyde in about a 95 percent yield (*2*). Cyanoacetaldehyde adds to propiolaldehyde to give two major ultraviolet absorbing products, 2-cyanoglutaconic dialdehyde (II) and III. Structure II has a strong absorption band at 346 nm ($\varepsilon = 40{,}500$) and a much weaker band at 260 nm ($\varepsilon = 4000$). Structure III absorbs at 442 nm ($\varepsilon = 67{,}400$). Both structures were confirmed by nuclear magnetic resonance spectroscopy and in the case

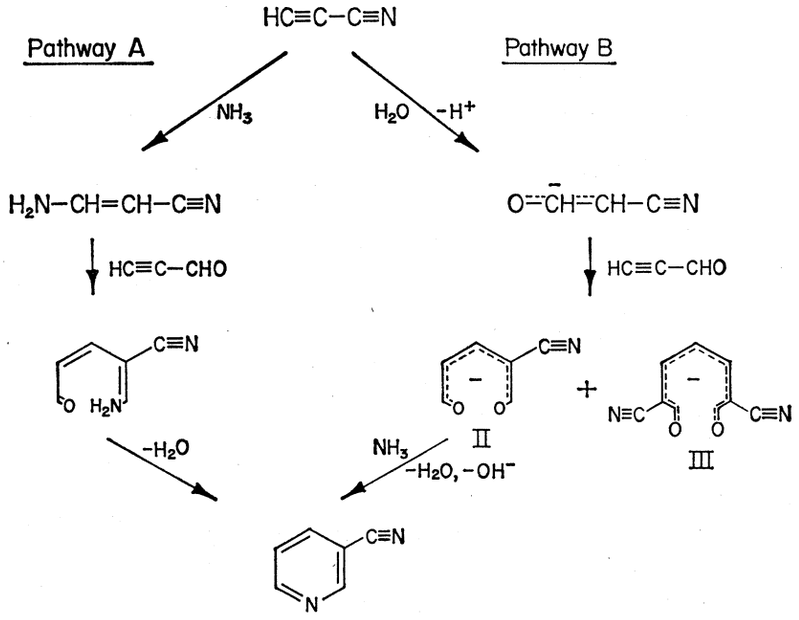


Fig. 1. Pathways in nicotinonitrile synthesis.

of the intermediate II by elemental analysis.

The central intermediate II is obtained in greater than 40 percent yield from equimolar mixtures of reactants at *p*H 8.5 and room temperature over the concentration range 0.1 to 0.00005*M*. We have also detected II and III in low yield from the reaction of a 0.001*M* solution of cyanoacetaldehyde and the concentrated aqueous product from a discharge reaction.

The reversible cyclization of glutaconic dialdehydes and amines to pyridine derivatives has been studied extensively (*9*). We have obtained yields of up to 30 percent of nicotinonitrile from 0.1*M* cyanoacetylaldehyde, 0.1*M* propiolaldehyde, and 1.0*M* ammonia held at 100°C for 5 hours. More significantly we have carried out the cyclization of II at 60°C and *p*H 8.5. After 5 days the yields of nicotinic acid derivatives using 1, 0.2, and 0.04*M* ammonia were 70, 22, and 4 percent, respectively.

The cyclization reaction proceeds much more rapidly with methylamine or cyclohexylamine to give the *N*-alkyl nicotinonitriles. When intermediate II was allowed to react at room temperature and *p*H 8.5 for 3 weeks with a solution 1.0*M* in ribose and ammonia, a 9.3 percent yield of a compound carrying a single positive charge at *p*H 7.4 was obtained. On the basis of its ultraviolet spectrum and the spectrum of an adduct which it forms with cyanide ion we believe this compound to be a nicotinonitrile riboside, presumably formed from ribosylamine and II. The feasibility of this nucleoside synthesis under potentially prebiotic conditions needs further investigation (*10*).

We believe that these results give further support to the hypothesis that unsaturated molecules formed in the primitive reducing atmosphere of the earth could have reacted in the atmosphere or in aqueous solution to give many of the organic molecules which are most important in contemporary biochemistry.

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10. *Note added in proof:* We have isolated the nucleoside in 0.8 percent yield from a partially frozen solution initially 0.001*M* in II, 0.01*M* in ribose and NH_3 at *p*H 8.5 maintained at −10°C for 1 month.

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