

# UREA-ACETYLENE DICARBOXYLIC ACID REACTION: A LIKELY PATHWAY FOR PREBIOTIC URACIL FORMATION

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**Abstract.** A number of routes have been suggested for the prebiotic synthesis of uracil involving the reaction of urea with malic acid, propiolic acid, cyanoacetylene and others. Cyanoacetylene has been detected in the interstellar medium as well as simulated prebiotic experiments. It is therefore plausible that dicyanoacetylene and its hydrolytic product acetylene dicarboxylic acid (ADCA) may have played a role in chemical evolution. This aspect has been examined in the present work for the synthesis of uracil from ADCA and urea reaction.

It was found that when ADCA reacted with urea, uracil was formed only in the presence of phosphoric acid and phosphates. Ammonium phosphates gave higher yields of uracil than other phosphates. In the absence of phosphoric acid or phosphates no uracil formation took place. This type of synthesis could have taken place in prebiotic oceans which contained ammonium phosphates and other salts.

## 1. Introduction

The routes suggested for the prebiotic synthesis of uracil include the reactions of urea, with malic acid (Fox and Harada, 1961); acrylonitrile (Oro, 1963, 1965); maleic acid (Takemoto and Yamamoto, 1971); propiolic acid (Harada and Suzuki, 1976) and  $\beta$ -alanine (Chittenden and Schwartz, 1976). The reaction of cyanoacetylene with cyanate to give cytosine (Sanchez *et al.*, 1966; Ferris *et al.*, 1968) and that of cyanoacetaldehyde with guanidine to give 2,4-diaminopyrimidine that can be hydrolyzed to cytosine and uracil (Ferris *et al.*, 1974), are the other pathways by which uracil has been obtained. Recently Ferris and Joshi (1979) have reported uracil formation by photo-decarboxylation of orotic acid.

In this paper, the formation of uracil by the reaction of urea with acetylene dicarboxylic acid or its potassium salt in presence of inorganic phosphates, has been examined and the relevance of this reaction to prebiotic chemistry has been considered.

## 2. Materials and Methods

Acetylene dicarboxylic acid monopotassium salt (ADCA-K-salt) was obtained from Aldrich Chemical Co. Inc. U.S.A., urea from M & B Ltd., England, diammonium hydrogen phosphate from E. Merck Germany, ammonium dihydrogen phosphate from B.D.H., England while phosphoric and polyphosphoric acids were from Riedel AG Germany. Polyphosphoric acid was also made in the laboratory by known procedure (Fieser and Fieser, 1967).

The IR spectra were recorded on a Perkin Elmer Infracord 137-B, the UV spectra on

a Carl Zeiss Model RPQ 20A spectrophotometer and the mass spectra on a CEC-Model 21-110B Mass spectrometer.

Urea, ADCA or its K-salt, and appropriate phosphate/phosphoric acid/polyphosphoric acid were dissolved in water and heated at 100° for 8 hours in a petri dish (10 cm diameter). The details are given in Table I. Almost all the water in the reaction system had evaporated at the end of 8 hours.

In some experiments a slurry of the above reactants in water was made with adsorbents such as tricalcium phosphate, silica gel, alumina, bentonite, montmorillonite and illite (3 g in each case), spread as a thin layer on glass plates and heated at 100° for 8 hours.

When no adsorbent was used the reaction mixture was treated with hot dilute alcohol. The solid residue was found to be mainly inorganic. The filtrate on concentration gave more of this residue. The remaining solution was examined by paper chromatography on whatman 1 using butanol: acetic acid : water (100:22:50). Two very close spots were detected corresponding to uracil and urea. The presence of urea was inferred by spraying with Ehrlich reagent, while uracil was identified by its characteristic UV absorption. Separation of uracil was carried out by employing cellulose column chromatography, but it was difficult to remove last traces of urea from uracil, either by cellulose column chromatography or by paper chromatography. However, when the mixture was passed through a sephadex-G-10 column and eluted with water collecting 1 ml fractions, it was

TABLE I  
Reaction of urea with acetylene dicarboxylic acid for the formation of uracil  
(at 100° for 8 hr)

Expt. No.	Urea mmole	Acetylene dicarboxylic acid monopotassium salt mmoles	Catalyst used and quantity in mgs.	Uracil yield % (based on urea)
1	1.0	0.5	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> 200	1.2
2	1.0	1.0	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> 200	3.2
3	1.0	1.5	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> 200	4.5
4	1.0	1.5	NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> 200	4.4
5	1.0	2.0	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> 200	6.3
6	1.0	2.0	NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> 200	6.1
7	1.0	2.0	Nil	0.0
8	1.0	2.0	Na <sub>2</sub> HPO <sub>4</sub> 200	0.1
9	1.0	2.0	KH <sub>2</sub> PO <sub>4</sub> 200	0.4
*10	1.0	2.0	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> 200	2.3
11	1.0	2.0	Nil	0.0
12	1.0	2.0	H <sub>3</sub> PO <sub>4</sub> 200	1.2
13	1.0	2.0	NH <sub>4</sub> Cl 200	1.3

Urea 1 mmole 60 mg; ADCA-K-salt 1 mmole 152 mg; (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> 200 mg. The reactants were dissolved in 5 ml of water.

\* Free ADCA was prepared after passing through Dowex-50H<sup>+</sup>.

possible to collect a number of fractions containing only uracil (checked by paper chromatography). These fractions were combined and concentrated under vacuum, and the material obtained was crystallized from dilute alcohol. The IR, UV and mass spectra of this compound and that of uracil standard were comparable.

When slurry of the reaction mixture with adsorbents was heated, the products were extracted with hot water and dilute acetic acid. The extracted material was evaporated to dryness, dissolved in dilute alcohol and separated as before.

In a set of experiments a slurry of the reactants and appropriate adsorbent support in water, spread as thin layer on glass plates, was irradiated with a UV lamp (250 watts) rich in 257 Å wavelength at a distance of 8 cm for 15 and 24 hours. The products formed were extracted and worked up as described above.

### 3. Results

The results presented in Table I show that urea reacts with ADCA or its K-salt in presence of  $(\text{NH}_4)_2\text{HPO}_4$  or  $\text{NH}_4\text{H}_2\text{PO}_4$  to give uracil; uracil yield being better with the K-salt. In the absence of  $(\text{NH}_4)_2\text{HPO}_4$  or  $\text{NH}_4\text{H}_2\text{PO}_4$ , uracil formation was not detected either with ADCA or its K-salt. In presence of  $\text{H}_3\text{PO}_4$  uracil formation was 1.2% while in presence of  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  it was less than 0.5%. In the presence of polyphosphoric acid, no uracil could be detected by HPLC or by paper chromatography.\* This is very surprising as polyphosphoric acid has been used for the condensation of urea or 1,4-dimethyluria with propiolic acid to give uracil or 1,4-dimethyluracil (Harada and Suzuki, 1976), and ADCA K-salt on heating in aqueous solution is known to get converted to propiolic acid (Alder and Stein, 1936). Initially it was thought that ADCA K-salt on heating in aqueous solution would get converted to corresponding propiolic acid salt that could react with urea in presence of polyphosphoric acid as suggested by Harada and Suzuki. In presence of  $\text{NH}_4\text{OH}$  or  $\text{HCl}$ , uracil was formed in 0.2 and 0.3% yield respectively but in presence of  $\text{NH}_4\text{Cl}$ , uracil was obtained in 1.3% yield. It would appear that ammonium salts are good condensing agents and phosphates in particular give appreciable yields of uracil.

We tried to explore the role of ammonium phosphates in these reactions by heating separately urea- $(\text{NH}_4)_2\text{HPO}_4$  and ADCA-K-salt- $(\text{NH}_4)_2\text{HPO}_4$  mixtures and examining the products formed by paper chromatography (Choughuley *et al.*, 1972 and the references cited therein). We could not detect significant amounts of higher phosphates or any ureido derivatives. It is likely that propiolic acid is an intermediate in the synthesis of uracil in our experiments, although the mechanism of the reaction is not yet clear.

The role of clays and inorganic supports has been suggested for a number of prebiotic reactions (Bernal, 1951; Paecht-Horowitz, 1974; Schwartz and Chittenden, 1977) and for this reason we tried to explore the role of various inorganic supports. It was observed that

\* There are three UV absorbing compounds formed in this reaction. These will be discussed elsewhere.

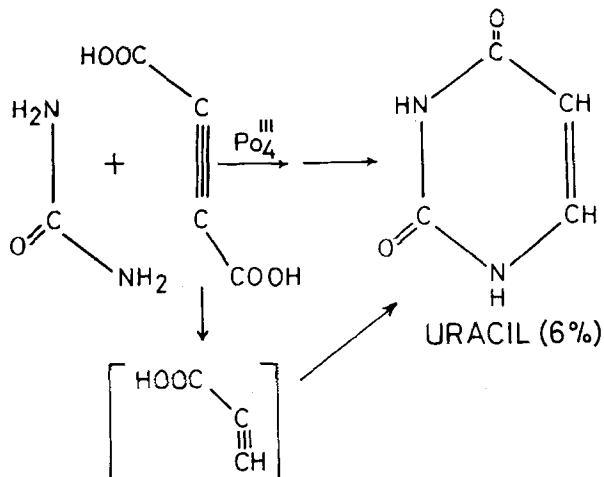


Fig. 1. Possible reaction mechanism for the synthesis of uracil.

only montmorillonite and illite, in presence of  $(\text{NH}_4)_2\text{HPO}_4$  gave uracil in 0.2% and 1.7% yield respectively. In absence of  $(\text{NH}_4)_2\text{HPO}_4$  no uracil was formed.

The experiments in which UV radiation was used indicated that only illite gave 0.3% uracil if  $(\text{NH}_4)_2\text{HPO}_4$  was present in the slurry. In its absence no uracil could be detected.

#### 4. Discussion and Conclusion

In the present work two main points emerge. One is the role of ammonium phosphates, and the other is the reaction of urea with acetylenedicarboxylic acid.

The role of inorganic phosphates in chemical evolution, has been discussed by several authors. The emphasis is on the part played by pyrophosphates (Miller and Parris, 1964) and linear and cyclic polyphosphates (Rabinowitz *et al.*, 1968, 1969; Rabinowitz, 1970; Hulshof and Ponnamparuma, 1976). Lohrmann and Orgel (1971) described urea-inorganic phosphate mixture as a phosphorylating agent, while Flores and Leckie (1973) used orthophosphates and cyanate for the formation of diglycine and triglycine from glycine. Beck *et al.* (1967) suggested that  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{Ca H}_2(\text{PO}_4)_2$  may have played an effective role in prebiotic phosphorylation reactions and Choughuley *et al.* (1972) showed that  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{NH}_4\text{H}_2\text{PO}_4$  gave excellent yields of polyglycine when heated with glycine in concentrated solutions. In the present work also, these two phosphates seem to help the formation of uracil (best yield 6.3%).

Urea is a product of several simulated prebiotic experiments (Lohrman and Orgel, 1971 and references cited therein). Similarly cyanoacetylene is formed by the action of electric discharge on a mixture of methane and nitrogen (Sanchez *et al.*, 1966). Also cyanoacetylene, methylcyanoacetylene, cyanotriacetylene and cyanotetraacetylene have been detected in the interstellar space (Watson, 1977; Schwartz, 1979). It is, therefore, conceivable that dicyanoacetylene might be one such molecule which has yet to be

detected in a simulated prebiotic experiment or the interstellar medium. On hydrolysis, dicyanoacetylene should give ADCA which may get decarboxylated to propiolic acid (Alder and Stein, 1936). It is therefore likely that ADCA or its salt and urea may have reacted together in presence of  $(\text{NH}_4)_2\text{HPO}_4$  to give uracil under prebiotic conditions.

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