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The Prebiotic Synthesis of Acrolein

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Summary. Acrolein is a central intermediate in the prebiotic synthesis of several amino acids, pentaerythritol, as well as various postulated alternative genetic materials. Acrolein is highly reactive so that its steady-state concentration could not have been very high. The ease of synthesis of acrolein from the aqueous aldol condensation of acetaldehyde and formaldehyde was therefore of interest. It is shown here that acrolein is produced in a low, but significant, steady-state concentration from very dilute solutions of formaldehyde and acetaldehyde under neutral to basic conditions. Acrolein is produced under conditions that are too dilute for the oligomerization of formaldehyde to produce carbohydrates. The implications of these findings for prebiotic chemical evolution are discussed.

Keywords. Acrolein; Aldol reactions; High pressure liquid chromatography; Prebiotic chemistry.

Introduction

Acrolein has been implicated in the prebiotic synthesis of many compounds of possible prebiotic significance, for example methionine, homoserine, glutamic acid, homocysteine, and 2,4-diaminobutyric acid [1] (Scheme 1). It has also been suggested that it may have played a role in the prebiotic synthesis of several alternative primordial genetic polymers [2–6].

The chemistry that produces the above-mentioned amino acids was likely taking place in the pre-solar nebula and this may explain some of the products detected in the *Murchison* meteorite [7–9]. Results from prebiotic simulations using electric discharges on reduced gas mixtures may be good models for the chemistry of the pre-solar nebula, as there is a generally good correlation between the products of these experiments and the organic material found in carbonaceous chondrites [8]. 2,4-Diaminobutyric acid has been produced in 0.02% yield in a spark discharge experiment, which was $\sim 4\%$ the yield of alanine and $\sim 7.6\%$ the yield of glycine produced in the same experiment. 2-Hydroxy-4-aminobutyric acid was synthesized in 0.044% overall yield in this same experiment. The presence of

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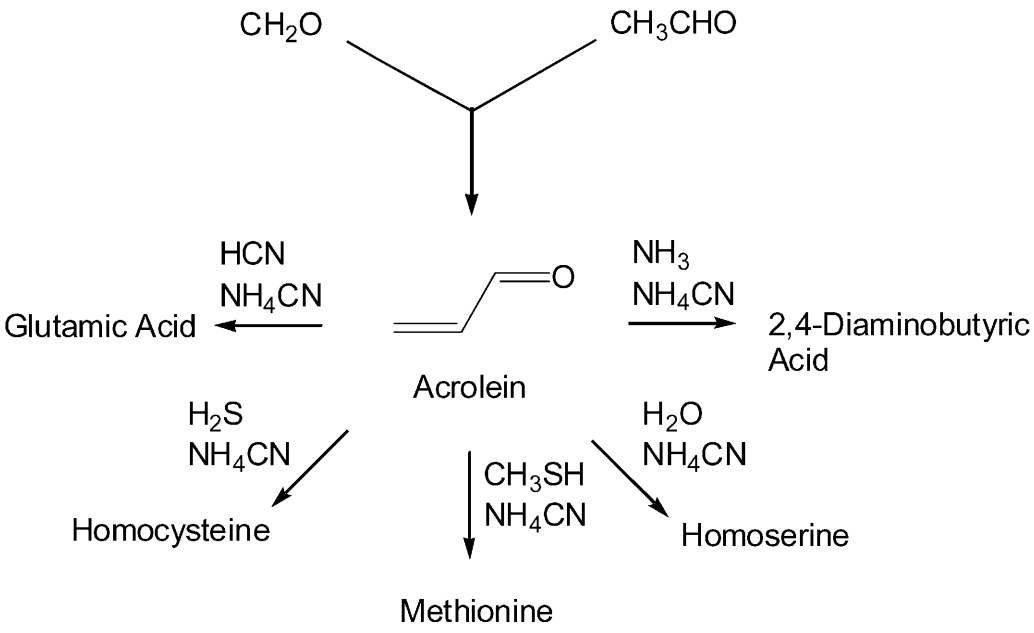
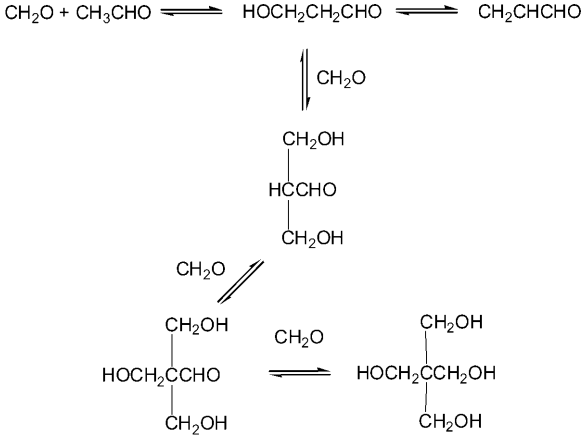


Scheme 1

2,4-diaminobutyric acid and 2-hydroxy-4-aminobutyric acid suggests that they are produced from a *Strecker* synthesis starting from acrolein. 2,4-Diaminobutyric acid, homoserine, homocysteine, and methionine have not as yet been detected in meteorites. This may be due to the relative instability of these compounds during the long residency of the parent bodies in space, and their exposure to high-energy radiation. Glutamic acid has been detected in carbonaceous meteorites [9], but it may be derived from another mechanism.

Significant quantities of acrolein have been detected in spark discharge experiments [1]. A 0.04% yield based on the methane input was estimated, corresponding to a concentration of $\sim 2.6\,10^{-4}$ M in the final product mixture. It is not known



Scheme 2

how much of the acrolein is synthesized in the gas phase and how much is the product of solution phase synthesis. The ΔG° for the condensation of acetaldehyde and formaldehyde in the gas phase has been estimated as $-18.6\,\mathrm{kJ\,mol^{-1}}$ and the K_{eq} as 1640 [11]. Since alanine and glycine are the major amino acid products of a spark discharge and both are thought to be primarily derived *via* the *Strecker* synthesis of acetaldehyde or formaldehyde [10], it seems possible that the acrolein detected is derived from the reaction of these two products in solution.

The industrial synthesis of pentaerythritol uses concentrated formaldehyde and acetaldehyde in alkaline aqueous solution as starting materials [12]. Acrolein has been determined to be an intermediate in this reaction (Scheme 2).

As the conditions for the industrial synthesis are rather extreme, the limitations of the synthesis of acrolein under more reasonable prebiotic conditions were investigated.

Results and Discussion

The first investigation involved the reaction of 1 M formaldehyde with 1 M acetaldehyde at *pH* 11 and 23°C with or without 10 mM Ca^{+2} or 50 mM Mg^{+2}. The yields of acrolein from these three reactions were $\sim 0.2\%$. The presence of calcium or magnesium had little effect on the yield. Acrolein was rapidly produced in all cases then came to a steady state concentration. This may be due to removal of the starting material to polymerization (for example by the formose reaction [13]), reaction to form of pentaerythritol [12], reduction and oxidation *via Canizarro* reactions, or polymerization of the acrolein produced.

It has been demonstrated that acrolein undergoes a base-catalyzed *Michael*-type polymerization, in which the equilibrium is shifted towards the right with decreasing temperature [14]. In a 1 M solution of acrolein, a pentamer was found to be the dominant species after polymerization had reached equilibrium.

The course of the reaction at *pH* 9 and various concentrations of reactants is shown in Fig. 1.

The percentage yield of acrolein is relatively independent of concentration, from 0.1% to 1.5% over the reactant concentration range of 1 M to 10^{-4} M (Fig. 2).

Percentage yields increase at lower concentrations of formaldehyde and acetaldehyde and are fairly independent of *pH* between *pH* 7 and 11. This can be rationalized by two mechanisms. First, there are side reactions of acrolein. Acrolein may react with a second molecule of acrolein to form a dimer, and this process may continue to form various low molecular weight ionic polymers of acrolein [15]. At higher concentrations of reactants, acrolein may also react with another aldehyde *via* successive aldol condensations or *Michael* additions. These reactions are expected to be faster at higher *pH*. Second, the aldol condensation which produces acrolein is itself expected to be base catalyzed, thus both synthesis and degradation are faster at higher *pH* and the two processes roughly cancel. Thus, while the reaction to form acrolein is robust, its steady state is kept rather low. This may not be significant from a prebiotic standpoint, as acrolein may be generated steadily then react irreversibly *via* a *Strecker* synthesis to give the final amino acid products. The final ratio of product would then depend on the relative concentrations of nucleophiles

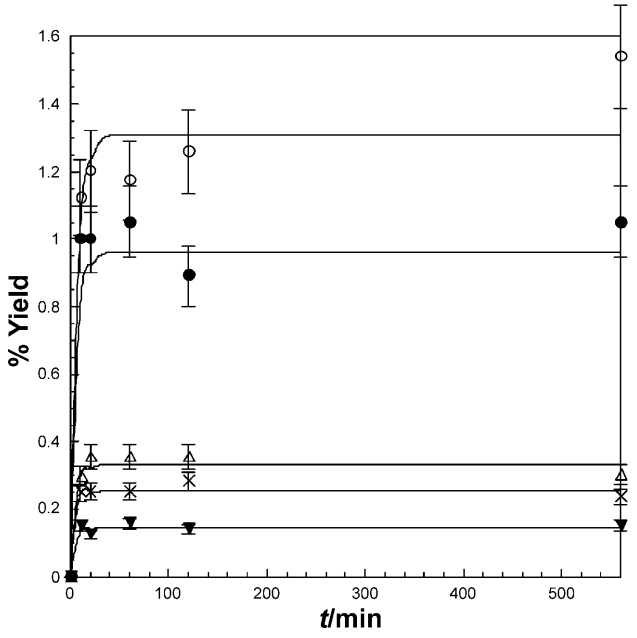


Fig. 1. Percent yield of acrolein *vs.* time for the reaction of equimolar formaldehyde and acetaldehyde in 250 mM *pH* 9 *CAPSO* at 23°C; ○ 10^{-4} M, ● 10^{-3} M, △ 10^{-2} M, × 10^{-1} M, ▼ 1 M

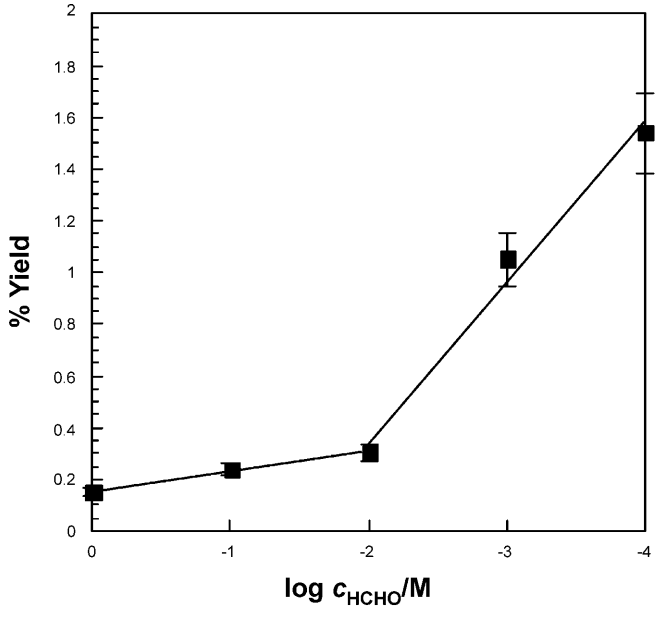


Fig. 2. Percent yield of acrolein *vs.* the log of the concentration of formaldehyde for the reaction of equimolar formaldehyde and acetaldehyde in 250 mM *pH* 9 *CAPSO* at 23°C after 560 minutes

(such as CN^-, SH^-, OH^-, NH_3, glycine, etc.) present in the environment, which in turn depends on the *pH* of the environment [7]. Under dilute conditions it has been determined that only glutamic acid would be produced.

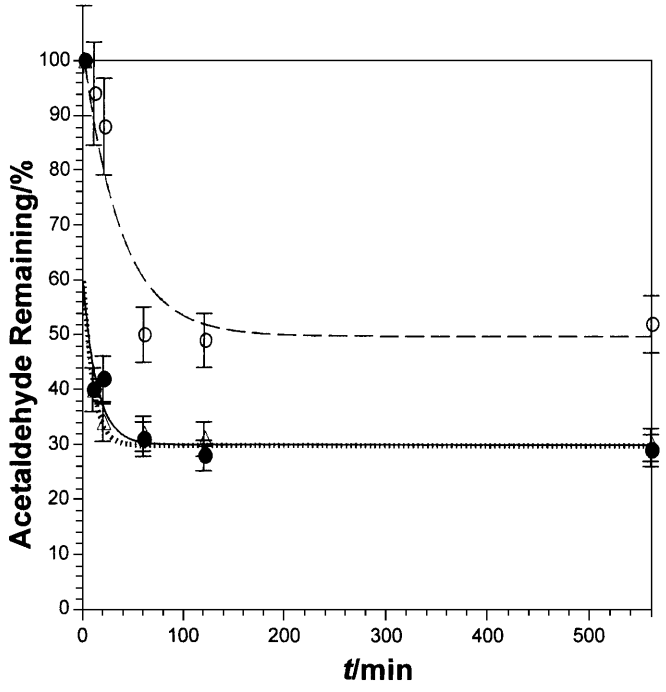


Fig. 3. Percent acetaldehyde remaining *vs.* time from the equimolar reaction of various concentrations of acetaldehyde and formaldehyde in 250 mM *pH* 9 *CAPSO* at 23°C; ○ 10^{-4} M, ● 10^{-3} M, △ 10^{-2} M

It was possible to determine the concentrations of formaldehyde and acetaldehyde *via* derivitization with 2,4-dinitrophenylhydrazine (2,4-*DNPH*). Yields of acetaldehyde and formaldehyde versus time for the experiments shown in Fig. 1 are shown in Figs. 3 and 4.

It was found that the reactions had come to equilibrium from 10^{-2} M reactant concentration and below while at higher reactant concentration, almost all of the starting material was consumed ($>99\%$ after 20 minutes).

The homodimers of formaldehyde and acetaldehyde, glycolaldehyde and acetoin, respectively, as well as glyceraldehydes, could be detected reliably *via* derivatization with 2,4-*DNPH*. Dihydroxyacetone, ribose, and deoxyribose could not be detected in these reactions by this method.

Interestingly, the syntheses of glycolaldehyde, acetoin, and glyceraldehyde were found to be much more concentration dependent than that of acrolein. At 10^{-3} M initial reactant concentration and below no glycolaldehyde, acetoin, or glyceraldehyde was detectable. The same is presumably true of the higher sugars, since their synthesis depends on the synthesis of glycolaldehyde. This may be significant if the reaction were conducted in the presence of other nucleophiles, such as CN^-, ammonia, or glycine, which would have limited the concentrations of the aldehydes. Further, it was found that higher concentrations of acrolein oligomerize more rapidly than lower concentrations, as would be expected. From initial concentrations of acrolein of 10^{-2} or 10^{-3} M approximately 15% of the initial acrolein was detectable after ten hours at *pH* 9 and 23°C.

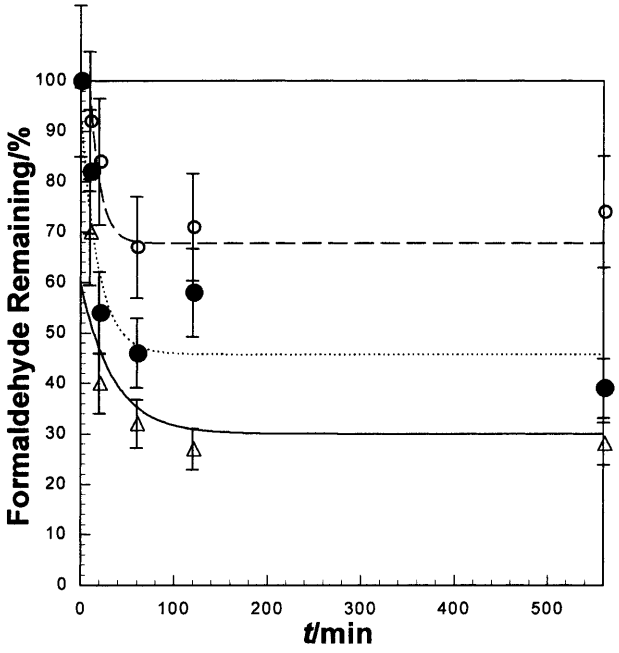


Fig. 4. Percent formaldehyde remaining *vs.* time from the equimolar reaction of various concentrations of acetaldehyde and formaldehyde in 250 mM *pH* 9 *CAPSO* at 23°C; ○ 10^{-4} M, ● 10^{-3} M, △ 10^{-2} M

The effect of the buffer on the disappearance of acrolein was investigated. Little buffer catalysis was observed for 250 mM *pH* 7 Bis-Tris over pure water. The rate of acrolein disappearance was found to be dependent on hydroxide concentration. At *pH* 9 the rate of disappearance was approximately four times greater than at *pH* 7 for a starting concentration of 10^{-3} M acrolein.

An equilibrium value for the hydration of acrolein at 23°C of 19.6 was estimated using the data of *Hall* and *Stern* [16]. Approximately 95% of the acrolein produced is hydrated at equilibrium. The reversible hydration of acrolein to 3-hydroxypropionaldehyde has been measured [17, 18]. An equilibrium constant of 21.2 was measured and a first order rate constant for the dehydration of 3-hydroxypropionaldehyde $1.5 \pm 1\,10^{-3}\,\mathrm{day}^{-1}$ was determined. The forward rate constant was measured at $3.21 \pm 0.13\,10^{-2}\,\mathrm{day}^{-1}$. This gives an approximate half-life for hydration of 22 days. Since the concentrations of acrolein measured *via* the chromatographic analytical method used herein were much lower than this, it is suggested that the loss is not due to hydration, but rather to dimerization and oligomerization and that these products do not react with *m*-aminophenol.

Based on the early work of *Butlerov* [13], the self-condensation of formaldehyde has been investigated as a potential prebiotic source of sugars [19–21]. It has been found that UV irradiation of dilute formaldehyde solutions produces high yields of pentaerythritol and acetaldehyde [22]. Since it is known that acrolein is an intermediate in the synthesis of pentaerythritol [15], this reaction could be a prebiotic source of acrolein. The photochemical synthesis of formaldehyde is quite robust [23]. The vapor phase photochemical decomposition of acrolein has been investigated [24]. Acrolein was found to be resistant to destruction at 313 nm. It

had been determined earlier that acrolein is extremely resistant to photo-oxidation [25]. The prebiological photochemical production rate of formaldehyde has been calculated [23]. These data were used to estimate a best-case scenario for the concentration of formaldehyde in the primitive oceans after 10 million years of $\sim 4 \cdot 10^{-4}$ M [26]. It is of course possible that the photochemical reactions of formaldehyde that produce acetaldehyde [21] could have occurred in the vapor phase, thus acrolein could have been generated in the atmosphere. This would have depended on the *pH* of the rain generated in the early atmosphere, which could have been quite acidic due to the presence of volcanically outgassed HCl and SO_2. Alternatively, acrolein could have been generated in the primitive hydrosphere from the chemistry described herein. There are also direct atmospheric syntheses of acrolein possible from hydration of C_3. Thus it seems likely that formaldehyde, acetaldehyde, and acrolein could all been produced robustly on the primitive earth even from a relatively non-reducing atmosphere.

Concentrations of $MgCl_2$ and $CaCl_2$ were used as those of the present ocean [26]. Although these may have changed over the course of time with variations of the atmospheric CO_2 levels and the *pH* of the oceans, these seem fairly reasonable concentrations. Much higher concentrations of these salts could perhaps have been achieved in evaporating basins, however, the concentrations could also have been limited by phosphate and carbonate. The fact that the reaction proceeds in the absence of these metals suggests that they are not required, however, and that they function merely to raise the *pH* of the solution.

The rapid reaction of acrolein with nucleophiles such as amines and thiols would have severely limited the concentrations of acrolein which could have built up. However, a continuous supply of this compound could have been generated from atmospheric and solution phase syntheses.

It is significant that acrolein should be so robustly produced despite the apparent low yields, as it has been demonstrated that acrolein is one of the few compounds with which the nucleobases of RNA/DNA will readily react [2]. The nucleobases do not react with ribose or deoxyribose in solution at low temperatures [27, 28]. The reaction of the nucleobases with formaldehyde or acetaldehyde is freely reversible [29, 30] and it has been demonstrated by this author that neither inhibits the reaction of the nucleobases with acrolein. Thus, acrolein could have been an important sink for the nucleobases in the prebiotic environment, and a significant hindrance to the start of a RNA or DNA world.

It was also demonstrated here that the synthesis of acrolein is much less dependent on the concentration of formaldehyde and acetaldehyde than is the formose reaction or the respective dimerization of acetaldehyde. This, coupled with the reactivity of nucleophiles with acrolein, represents a major source of prebiotic chemical selectivity. The selectivity of this synthesis is further suggested by the relative yields of 2,4-diaminobutyric acid *versus* alanine and glycine in reactions containing acetaldehyde, formaldehyde, and ammonium cyanide [1].

Experimental

$MgSO_4 \cdot 7H_2O$, $CaCl_2$, 37% *w/w* formaldehyde solution, and acetonitrile (HPLC grade) were purchased from Fisher. Chromotropic acid, 2,4-dinitrophenylhydrazine, trifluoroacetic acid, acrolein,

hydroxylamine hydrochloride, ribose, acetoin, deoxyribose, glycolaldehyde, *DL*-glyceraldehyde and *m*-aminophenol were purchased from Aldrich. 7-Hydroxyquinoline was purchased from Acros. Acetaldehyde was purchased from Mallinckrodt.

Reactions were conducted at constant appropriate temperature in a water bath, in 1.5 cm^3 snap-cap *Eppendorf* tubes. Reactions were buffered with 250 mM *CAPS* (*pH* 11), *CAPSO* (*pH* 9), or Bis-tris (*pH* 7) to avoid the precipitation of carbonate or phosphate salts of the alkali metals. Solutions of acetaldehyde and formaldehyde were added immediately prior to starting the reactions. Aliquots of the reactions were removed periodically and flash frozen on dry ice. The analytical procedure generally proved to be faster than the attainment of equilibrium of the reaction system upon thawing.

Analysis of acrolein was performed by conversion of acrolein to 7-hydroxyquinoline (*7HQ*) by the method of *Alarcon* [31]. *7HQ* was measured by RP-HPLC eluted with 0.1% (v/v) aqueous *TFA* containing 10% (v/v) acetonitrile using two Beckman 110B pumps and a YMC ODS-AQ S5 120 Å 4.6 × 150 mm column. Product was detected at 244 nm using a Kratos Spectraflow 757 absorbance detector.

The 2,4-dinitrophenylhydrazones of acetaldehyde, formaldehyde, and various sugars were prepared as standards *via* modification of the method of *Ma* and *Klemm* [32]. Reactions were monitored by RP-HPLC on a YMC ODS-AQ S5 120 Å 4.6×150 mm column eluted with 50% (v/v) aqueous acetonitrile. Products were detected at 360 nm.

7-Hydroxyquinoline was determined to be the only product of the *Skraup* reaction of *m*-aminophenol and acrolein. It was found that the analytical system was saturated with respect to reagent under the experimental conditions used. The UV spectra of the standard *7HQ* and the *7HQ* isolated from experimental runs were identical in acid and base (λ_{max} (*7HQ*) standard in 0.1% *TFA*: 244 nm, 304 nm, 348 nm; experimental: 244 nm, 304 nm, 348 nm; λ_{max} (*7HQ*) standard in 0.1 N NaOH: 244 nm, 278 nm, 360 nm; experimental: 244 nm, 278 nm, 360 nm). The two compounds had identical retention times under the chromatographic conditions used and coinjected precisely.

Formaldehyde was also determined by reaction with chromotropic acid as described by *West* and *Sen* [33]. UVvis spectra were recorded using a Hewlett-Packard 8452A diode array spectrophotometer using 1 cm path length quartz cuvettes.

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