

FORMATION OF *N*-CARBAMOYLALANINE FROM UREA AND PYRUVIC ACID IN AQUEOUS  
FORMIC ACID<sup>[1]</sup>

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(Abstract)

When an aqueous solution of urea and pyruvic acid was heated under reflux in the presence of formic acid, 2-ureidopropionic acid, that is, *N*-carbamoylalanine, was obtained in the yield of about 35%. This compound was considered to be formed by Leuckart reaction, in which *N*-alkylation is achieved by formic acid reduction of primarily formed Schiff base of a ketone and an amine. It had been believed that Leuckart reaction of amides or urea with formaldehyde does not afford *N*-alkylated products. Although the intended reaction practically failed to proceed below 100 °C, we found that this reaction can be applied to the alkylation of urea at 100 and 120 °C. This study proposed a novel pathway leading to formation of alanine from precursors, which are likely present on the primitive Earth. The formation of *N*-carbamoylalanine is of another significance, since we found in a separate experiment that it undergoes thermal polymerization.

(Keyword)

chemical evolution, urea, pyruvic acid, Leuckart reaction, *N*-carbamoylalanine.

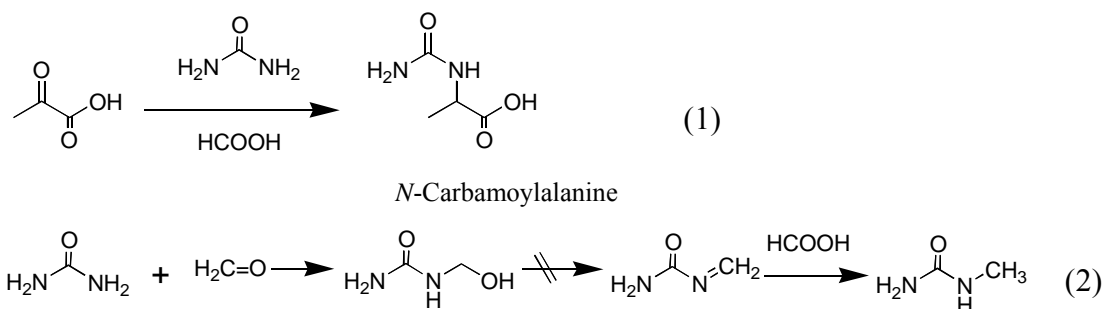
## Introduction

In the course of our studies on the role played by urea in the prebiotic formation of biomolecules on the primitive Earth, we had revealed that molten urea acts as a good nitrogen source and a highly polar reaction medium that facilitates nitrogen-incorporating thermal condensation of maleic and fumaric acids producing polyaspartic acid [2]. Under irradiation with UV lights, urea had proved to add to maleic acid in aqueous solution through radical mechanism to afford 2-isoureidosuccinic acid, which underwent thermal rearrangement to give 2-ureidosuccinic acid, that is, *N*-carbamoylaspartic acid [3]. In the Miller's experiment (1953) [4], urea was generated among other biomolecules from methane, ammonia and water by the action of electric discharge followed by water-phase thermal reaction. As pointed out by Miller and Orgel, urea should have been accumulated to a considerable extent on the primitive Earth, on the basis of its chemical stability and variety of processes that produce urea abiotically, such as hydrolysis of cyanide polymers or dicyan, UV irradiation to ammonium cyanide in the presence of ferrous ions, and so on [5]. Urea and ureido-compounds have been discovered in the meteorites [6-7]. However, the significance of urea has been little appreciated and

rarely been investigated in the standpoint of chemical evolution.

On the other hand, propionic acid had been demonstrated to be formed from methane or graphite in appreciable amounts together with formic and acetic acids by means of ultraviolet lights or electric discharges that simulate the energy sources available on the primitive Earth [8, 9]. These energy sources are also known to bring about dissociation of a water molecule to a hydroxyl radical and a hydrogen atom in water phase [10]. Many simulation experiments have demonstrated that these reactive species can play a role in modifying non-biomolecules into biomolecules, which generally contain many oxidized carbon atoms in the molecules [11-13]. As one of the modification, the stepwise oxidation induced by hydroxyl radicals has been thoroughly investigated and, for instance, propionic acid was transformed into pyruvic acid through lactic acid in aqueous solution [14]. Therefore, formic and pyruvic acids could be present in the primitive hydrosphere.

In this study, we examined abiotic formation of 2-ureidopropionic acid, that is, *N*-carbamoylalanine from urea and pyruvic acid in aqueous formic acid solution (Equation 1 in Scheme 1). This type of reaction is known as Leuckart reaction, which usually employs ammonia or amines as nitrogen sources [15].



### Scheme 1

In this reaction, formic acid acts as a reducing agent. The reaction of urea or amides with formaldehyde was reported to give only hydroxymethyl compounds and to fail in obtaining *N*-methylated products (Equation 2 in Scheme 1), but Leuckard reaction of urea with ketocarboxylic acid has not been reported [16].

### Materials and methods

The Leuckart reaction was performed by heating an aqueous formic acid solution (10 ml) of urea and pyruvic acid. Heating at 120 °C was carried out in a degassed sealed tube. The reaction mixture was directly analyzed for *N*-carbamoylalanine with a liquid chromatograph (LC) coupled to a mass spectrometer (MS) (Hewlett Packard series 1100 MSD). The LC was equipped with an amide-80 normal phase column (Tosoh Ltd.), and the elution was effected by MeOH-H<sub>2</sub>O (99:1). The MS was acquired every 0.07 s over *m/z* 50 to 300 in electrospray ionization method by positive mode at 4

kV.

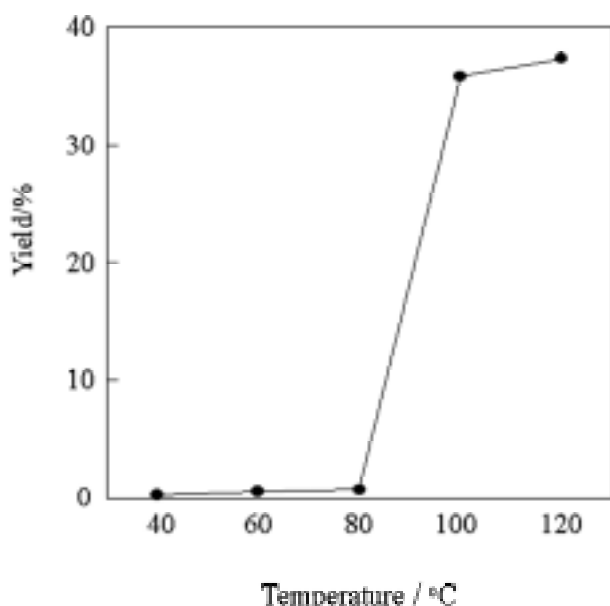
The yield of *N*-carbamoylalanine was determined as that of alanine after hydrolysis of the product. A 5-ml portion of the heated solution was concentrated under a reduced pressure and hydrolyzed with 6M HCl (2 ml) for 48 h at 110 °C in a degassed sealed tube. The hydrolyzate was concentrated under a reduced pressure and analyzed for amino acids with LC. The amino acid analysis was performed by a pre-column derivatization method using *o*-phthalaldehyde/2-mercaptoethanol according to the method of Jones *et al.* [17]. The analysis was carried out by an LC (Shimadzu LC-10) equipped with an ODS-80T column (Tosoh Ltd.), and the elution was effected by Na phosphate buffer-MeCN-MeOH.

The authentic sample of *N*-carbamoylalanine was prepared from L-alanine and potassium isocyanate in a similar manner to that reported [18]. In the isolation of the product, we used a cation-exchange resin (Amberlite IRC 120, H<sup>+</sup> form)

to acidify and desalt the reaction mixture. This method facilitated the purification of the product to give pure crystals (82 %, mp 182 °C (dec with effervesce) (lit. 183 °C [18])) without contamination by the starting material and inorganic salts.

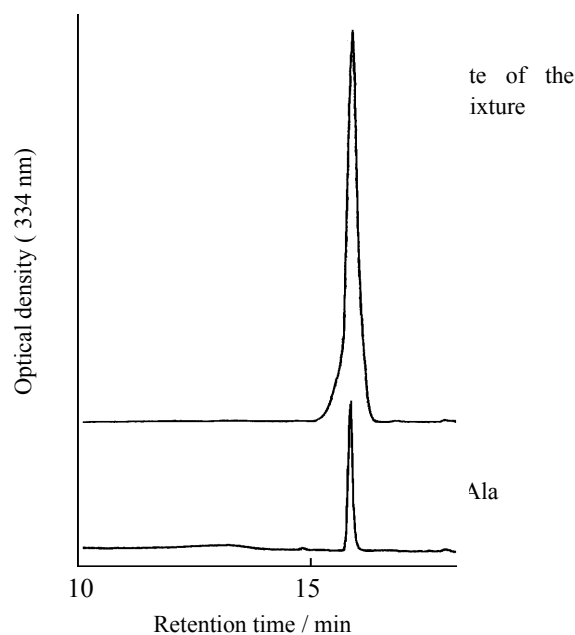
## Results

A mixture of urea (0.4 mol/l) and pyruvic acid (0.2 mol/l) in 10% aqueous formic acid was heated at 40 to 120 °C for 6 h, and the Leuckart reaction proceeded over 100 °C in the yields of about 35%, as shown in Fig. 1. The yield of the reaction was



**Fig. 1.** Yield of *N*-carbamoylalanine from a mixture solution of urea (0.4 M) and pyruvic acid (0.2 M) at various temperatures for 6 h in the presence of 10% aqueous formic acid.

estimated by determining alanine in the hydrolyzate of the reaction mixture (Fig. 2). When the reaction mixtures were analyzed after being hydrolyzed for 24



**Fig. 2.** Liquid chromatograms of hydrolyzate of the reaction mixture of urea and pyruvic acid at 100 °C for 6 h in the presence of 10% aqueous formic acid (a) and the authentic sample of alanine (b).

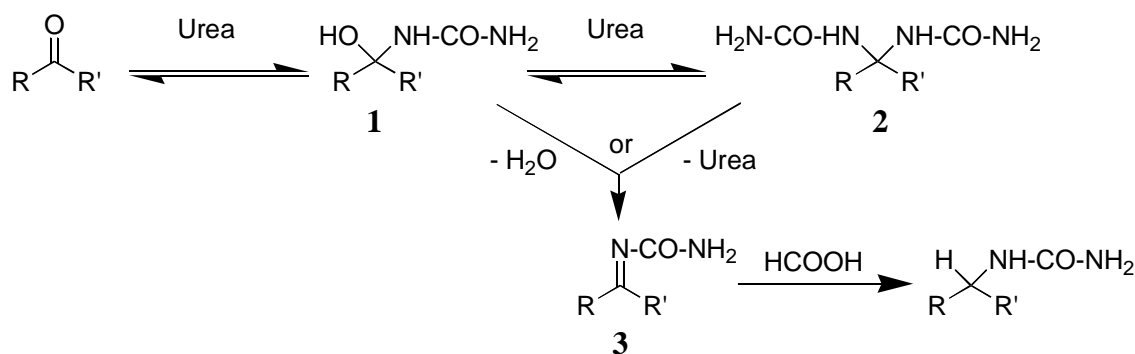
h, only small amounts of alanine were detected. Since urea and ureido-compounds are known to be resistant to acid hydrolysis, we hydrolyzed the products for 48h, and larger amounts of alanine were found in hydrolyzates (data not shown). These results show that the products are mainly composed of ureidocarboxylic acid. The formation of *N*-carbamoylalanine by this reaction was also confirmed on the  $[M + 1]^+$ -mass fragmentogram (Fig. 3) of the reaction mixture. This reaction gave the best yields by using 2 equivalents of urea to pyruvic acid in 5 to 20% aqueous formic acid for more than 5 h, and the yield did not exceed about 35% under all the conditions tested here (data not shown).

### Discussion

Pyruvic acid is rather unstable due to the reactivity of a polarized carbonyl group in its molecule. On account of its polarity, the hemiaminal-type (**1**, Scheme 2) or the aminal-type derivative (**2**) is considered to be formed at first, as in the reaction of urea with glyoxylic acid, which was reported to give a mixture of a hemiaminal and an aminal of the acid at room temperature. In these derivatives, the polarity should be cancelled, and their lifetimes may be longer than that of the ketocarboxylic acid. *N*-Carbamoylalanine is considered to be formed through dehydration of **1** or elimination of urea from **2** followed by formic acid reduction of a C-N double bond of a Schiff base (**3**). We further carried out the reaction of urea with glyoxylic and oxaloacetic acids at 100 °C, expecting formation of *N*-carbamoylglycine and *N*-carbamoylaspartic acid, respectively, but no desired products were detected in the reaction

mixtures. From these results, the Leuckard reaction of urea may be limited to special ketocarboxylic acid.

This study proposed a novel pathway leading to amino acids from precursors in water phase on the primitive Earth. *N*-Carbamoylamino acid may have a potential as an intermediate of polyamino acids. Collet *et al.* showed that reaction of *N*-carbamoylamino acids with a mixture of NO and O<sub>2</sub> affords *N*-carboxyanhydrides, which are known to polymerize under mild conditions [19]. Furthermore, we have recently found that *N*-carbamoylalanine is capable of undergoing thermal polymerization at its melting point [20]. Further researches are under way in order to find out an abiotic formation pathway to other *N*-carbamoylamino acids and to investigate polymerizing and copolymerizing abilities of *N*-carbamoylamino acids.



**Scheme 2**

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