Flame-Induced Addition of Urea to Unsaturated Carboxylic Acids[1]

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When a hydrogen-oxygen flame was blown against an aqueous solution of urea and maleic acid, *N*-carbamoylaspartic acid was generated. This compound seemed to be formed by the addition of an amidinoxyl radical to a C–C double bond of the acid, followed by a thermal rearrangement of an amidinoxy to a ureido group.

We had found that a hydrogen–oxygen flame, when kept in contact with an aqueous solution, can supply a hydroxyl radical and a hydrogen atom into the solution.[2,3] The flame-induced reaction is unique in view of the fact that radicals generated in a gas phase react directly with compounds in a water phase, and, therefore, this technique provides a clean reaction system without using any radical-generating reagents. Taking into account the short lifetimes of the radicals, the reaction zone in a solution must be limited to the vicinity of the solution surface exposed to the flame. However, the flame supplies radicals continuously into the solution and affords an effective radical reaction system. Furthermore, the flame-induced reaction has the advantage of permitting suppression of vigorous oxidation due to a hydroxyl radical by using hydrogen-rich flames, in contrast to the previously reported methods of generating radicals in the water phase by means of an electric discharge[4] or an arc plasma.[5]

In the course of our study on the role played by urea in the prebiotic formation of biomolecules on the primitive Earth, we revealed an excellent feature of urea as a nitrogen source in the nitrogen-incorporating thermal condensation of unsaturated dicarboxylic acids producing polyaspartic acid.[6] Our present interest is aimed at an elucidation of the radical reaction of urea leading to the formation of amino acids or related compounds in water solution. We therefore performed a flame-induced reaction of urea with unsaturated carboxylic acids in this study. Although *N*-halogenated amides and urethanes had been revealed to add to olefins with the aid of UV light to afford their *N*-(2-haloethyl) derivatives,[7,8] no reports had appeared which discuss radical reactions of urea with olefins.

A hydrogen–oxygen flame (3.2 l min^{-1} of H_2, 0.8 l min^{-1} of O_2) was blown against the surface of an aqueous solution (200 mL) of urea (0.005 M) and maleic acid (0.05 M) by using a previously reported apparatus.[2] The temperature of the reaction mixture was maintained at 60 °C by circulating cold water through the jacket of the reaction vessel. At 30-min intervals, a 1-mL aliquot of the solution was withdrawn and analyzed by LC-MS.

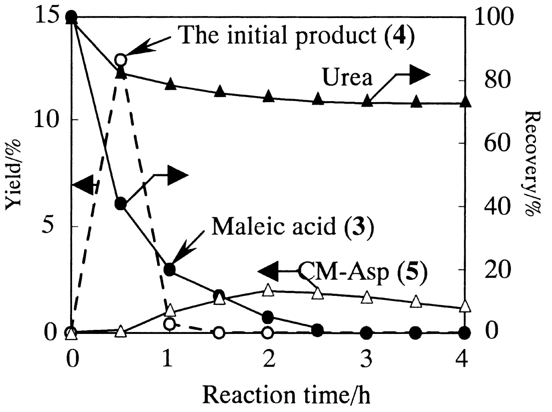


Fig. 1. Time-course plots for flame-induced reaction of urea with maleic acid. The yield of the initial product was tentatively estimated by using mol/area ratio of *N*-carbamoylaspartic acid (CM-Asp) in LC–MS analysis.

Figure 1 shows time-course plots for the flame-induced reaction. The concentration of urea decreased very slowly, indicating its low reactivity to a hydroxyl radical, while maleic acid was decomposed almost completely within 3 h, possibly due to the high radical reactivity of a C–C double bond, which is known to be oxidatively decomposed by a hydroxyl radical. The expected product, *N*-carbamoylaspartic acid (CM-Asp (**5**)), was detected, as shown in Fig. 2. Since CM-Asp was not detected in the aqueous solution of urea and maleic acid after refluxing for 2 h, this amino acid derivative seemed to be formed through a radical reaction of the starting materials. On the other hand, another product, which was proved to have the

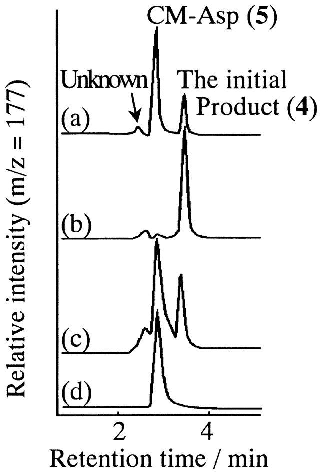
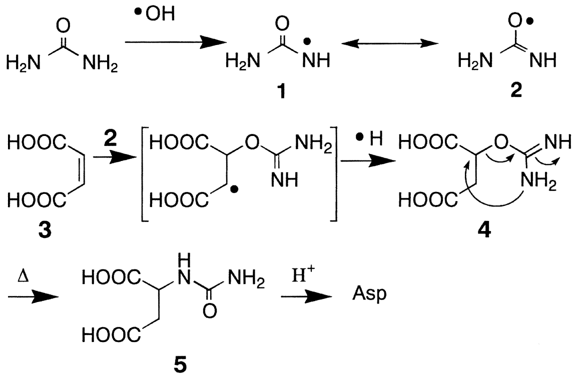
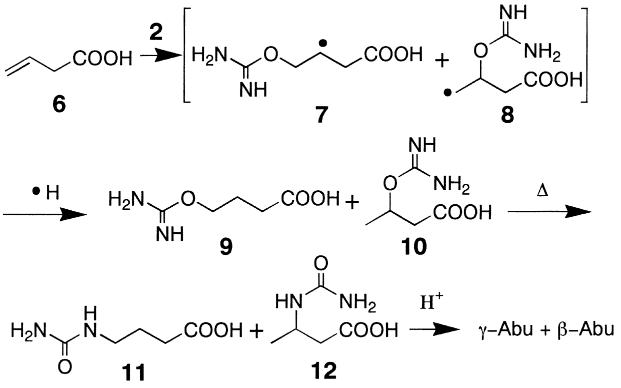


Fig. 2. $[M + 1]^+$ Mass fragmentograms (*m*/*z* = 177) of flame-induced reaction products from urea and maleic acid after exposure to H_2–O_2 flame for 1 h (a) and for 30 min (b), heating product of 30-min flame-induced reaction mixture (c), and the authentic sample of *N*-carbamoylaspartic acid (d). LC column: TSK gel Amide-80 (Tosoh Ltd.), eluent: MeOH–H_2O (99 : 1).



Scheme 1. Asp: aspartic acid.



Scheme 2. *β*- and *γ*-Abu: *β*- and *γ*-aminobutyric acids.

same molecular weight as that of CM-Asp (Fig. 2a), was detected only at the initial stage of the reaction. Because this product was rather unstable, we failed to isolate it from the reaction mixture. Therefore, it was possible to consider that this compound was the initial product, which was further transformed into CM-Asp by the action of radicals or heat. To confirm this, we heated the reaction mixture under reflux for 1 h after exposure to the flame for 30 min, and observed a decrease in the initial product, along with an increase of CM-Asp (Figs. 2b and 2c). We further hydrolyzed the reaction mixtures before and after heating, and analyzed the amino acids in the hydrolyzates. An evidently larger amount of aspartic acid was found in the hydrolyzate after heating.

From these results, we postulated the mechanism of the flame-induced reaction to be as follows: a hydroxyl radical from the flame abstracts a hydrogen atom from urea to give a ureido (**1**) and an amidinoxyl radical (**2**) (Scheme 1), and then radical **2** adds to maleic acid to form an isourea derivative, whose structure was assumed to be **4** based on its molecular weight. Compound **4** underwent a rearrangement by the action of heat, producing CM-Asp (**5**). An amidinoxy group is known to be a good leaving group in substitution reaction, as exemplified by the fact that heating of *O*-methylisourea hydrochloride affords urea and methyl chloride. We also confirmed a conversion of *O*-methylisourea into *N*-methylurea by refluxing an aqueous solution of commercial *O*-methylisourea sulfate for 1 h in the presence of triethylamine. The conversion of **4** into **5** is likely to be an intramolecular reaction, since it proceeded in a very dilute solution. In a separate experiment, in which the same starting mixture as that used in this study was irradiated with 172-nm UV light at 25 °C, only compound 4 was generated, supporting the idea that a direct addition of radical **1** to **3** does not occur.

In order to confirm the radical mechanism of the reaction of urea, we next carried out a flame-induced reaction of urea with 3-butenoic acid (**6**, Scheme 2) under the same conditions as mentioned above, since compound **6** does not suffer a Michael addition of urea. Although every effort failed to separate the expected products **9** to **12** from each other with LC–MS for their determination in the reaction mixture, *γ*- and *β*-aminobutyric acids were successfully detected in the acid-hydrolyzate of the reaction mixture after refluxing for 1 h in yields of 2.3 and 0.31%, respectively. These amino acids were absent in the hydrolyzate of the unheated reaction mixture, indicating that the heating produced derivatives of these amino acids. *α*-Aminobutyric acid was not detected in the hydrolyzate, indicating that migration of the C–C double bond of compound **6** to the 2, 3-position did not occur during the flame-induced reaction. The predominance of *γ*-aminobutyric acid in the product seemed to be reasonable in view of the higher stability of postulated intermediate radical **7** than that of **8**, considering hyperconjugation of the radicals. These results are consistent with the proposed radical mechanism of the reaction of urea with unsaturated carboxylic acids, including the addition of **2** to a C–C double bond as well as a thermal rearrangement of an isourea to a urea compound.

In conclusion, we found a new feature of the reactivity of urea to olefin. Urea had been probably present on the primitive Earth,[9,10] and unsaturated carboxylic acids have been found in meteorites[11] as well as in the products of electric discharge experiments using methane[9] as a carbon source. This study proposes a novel pathway leading to formation of amino acids from non-amino acid precursors, possibly effected by energy sources, such as solar wind, UV-light, lightning, and so forth, on the primitive Earth. The formation of ureidocarboxylic acids is of another significance, since they are capable of undergoing thermal polymerization,[6] resulting in the formation of polyamino acids.

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1 A part II of a series of studies entitled "on the role of urea in chemical evolution of biomolecules."

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