# An Experimental Approach to the Prebiotic Synthesis of α-Amino Acids under UV Irradiation in Aqueous Medium

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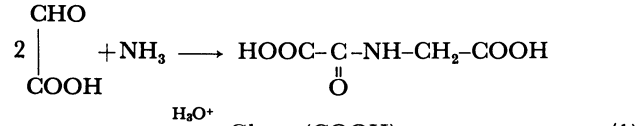
**Synopsis.** *N*-Oxaloglycine obtained from a glyoxylic acid and ammonia mixture was allowed to produce various α-amino acids under UV irradiation using an alkylating agent such as an olefin in the presence of acetone as the initiator. Aspartic acid, norvaline, valine, leucine, phenylalanine, and tyrosine were identified by TLC, amino acid analysis, GC, and GCMS techniques.

On the basis of a generally accepted working hypothesis that life originated in the primeval sea, research at our laboratory has been directed toward the synthesis of biomolecules in modified sea medium from primitive compounds such as formaldehyde and hydroxylamine.[1] Particular attention has been paid to synthetic pathways of amino acid formation in the primeval sea. An understanding of these pathways of amino acid formation may possibly provide a clue to the origin of genetic code, in view of the relation between amino acid and nucleic bases. Our purpose has been to find possible synthetic routes of amino acids prebiotically in a neutral aqueous medium. Besides the well-known Strecker synthesis,[2] we found a new route through *N*-acyl amino acid from α-oxo acid and ammonia.[3] *N*-Oxaloglycine is synthesized from a glyoxylic acid and ammonia mixture simply by combining it with a neutral or weak acidic aqueous solution to obtain a yield of about 20% even at room temperature. The reactions involved are as follows:



$$2\,\begin{matrix}\mathrm{CHO}\\ \mathrm{COOH}\end{matrix} + \mathrm{NH_3} \longrightarrow \mathrm{HOOC\text{-}\underset{\underset{\mathrm{O}}{\|}}{\mathrm{C}}\text{-}NH\text{-}CH_2\text{-}COOH} \xrightarrow{H_3O^+} \mathrm{Gly} + (\mathrm{COOH})_2. \quad (1)$$

This general reaction scheme was demonstrated in the synthesis of sarcosine, alanine, glutamic acid, and phenylalanine.[3,4] In this paper, our effort has been directed to the conversion of *N*-oxaloglycine into other α-amino acids by introducing $C_n$ moieties on the α-carbon of the glycine residue photochemically.

## Experimental

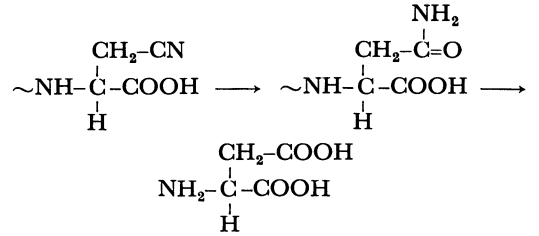
*Materials.* Disodium salt of *N*-oxaloglycine was prepared by two methods. By one method, this compound was isolated from a reaction mixture of glyoxylic acid and ammonia.[3] The other method was the traditional organic synthesis.[5] The purity was determined by elemental analysis, UV, IR, and NMR. And the results were as follows; Calcd for $C_4H_3N_1O_5Na_2 \cdot 1/2\ H_2O$: C, 24.16; H, 1.78; N, 6.74%. Found: C, 23.80; H, 1.55; N, 6.46%. $\lambda_{max}$ ($\varepsilon$)=214 nm ($4.5 \times 10^3$ $cm^{-1}$ $M^{-1}$ (1 M=1 mol $dm^{-3}$)) in 0.6 M HCl. 1680—1600, 1550 $cm^{-1}$. $\delta$=4.29 (s). Research grade propylene, isobutylene and 2-butene (*cis* and *trans*) were purchased from Seitetsu Kagaku Co. Ltd. *N,N*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Pierce Chemical Co. Acetonitrile was refluxed with phosphorus pentaoxide and distilled.

*Method.* An aqueous solution containing 20—40 mg of disodium salt of *N*-oxaloglycine, 10—20% (v/v) of acetone and an alkylating agent was made up, deaerated by charging it with nitrogen gas using the syringe technique and irradiated with a high pressure Hg-lamp (Ushio electric Co. UM-102, 100 W) through a Pyrex-filter. When using a gas as an alkylating agent, it was made to pass through the reaction mixtures under irradiation. After 20 h irradiation, the reaction mixture was evaporated and hydrolyzed with 6 M HCl at 105 °C in Dri-block DB-3H (M & S instrument) over night. A hydrolysate was used to determine what amino acids were present. The spots on silica gel TLC (ethanol : water=6 : 4, 1-butanol: acetic acid : water=4 : 1 : 1, and 1-propanol : ammonia= 3 : 1) were investigated with UV light and ninhydrin spraying. Amino acid analysis was performed with a Hitachi KLA-5 automatic analyzer.

*Analysis of Trimethylsililated Amino Acid (TMS-Amino Acid).* An aqueous solution of hydrolysate was treated with an appropriate amount of $CaCl_2$ to remove calcium oxalate, and the supernatant was lyophilized and dried at 80 °C under vacuum. A 5—10 mg of residue was heated with 0.5 ml distilled acetonitrile and 0.5 ml of BSTFA at 140 °C for 2 h. After concentrated under vacuum, a 0.5—5 μl sample was charged on a GC (Shimadzu GC-4M) and GCMS (Shimadzu 7000) using an OV-11 (10%) column. The technical data obtained were as follows. I) Reaction with acetonitrile: GC, col. temp 195 °C, inj. temp 250 °C. Retention time was 4.51 min (TMS-aspartic acid). GCMS *m/e* 349 ($M^+$), 232, 218. II) Reaction with propylene: GC, col. temp 80—200 °C at 5 °C/min, inj. temp 220 °C. Peaks appeared at 14.56 (TMS-valine) and 15.43 min (TMS-norvaline). GCMS *m/e* 218, 144 for both peaks. III) Reaction with isobutylene: GC, col. temp 80—200 °C at 5 °C/min, inj. temp 220 °C. Retention time was 17.55 min (TMS-leucine). GCMS *m/e* 218, 158. IV) Reaction with toluene: GC, col. temp 190 °C, inj. temp 250 °C. A peak corresponding to TMS-phenylalanine appeared at 5.50 min. GCMS *m/e* 218, 192, 91. V) Reaction with *p*-cresol: GC, col. temp 240 °C, inj. temp 280 °C. A peak of 5.05 min corresponded to TMS-tyrosine. GCMS *m/e* 280, 218, 179. The carrier gas flow rates were 40 ml/min and 15—20 ml/min for nitrogen (GC) and helium (GCMS), respectively.

## Results and Discussion

*N*-Oxaloglycine was useful as a starting material in this experiment, since it could be obtained simply by mixing glyoxylic acid and ammonia in a neutral or weak acidic aqueous solution at room temperature.



$$\sim NH\text{-}\underset{\underset{H}{|}}{C}(CH_2\text{-}CN)\text{-}COOH \longrightarrow \sim NH\text{-}\underset{\underset{H}{|}}{C}(CH_2\text{-}\underset{\underset{NH_2}{|}}{C}{=}O)\text{-}COOH \longrightarrow \mathrm{NH_2}\text{-}\underset{\underset{H}{|}}{C}(CH_2\text{-}COOH)\text{-}COOH \quad (2)$$

The introduction of a $C_2$ unit to *N*-oxaloglycine was achieved with the use of acetonitrile. Since aspartic acid was found to be one of the final product following acid hydrolysis, following scheme has been proposed

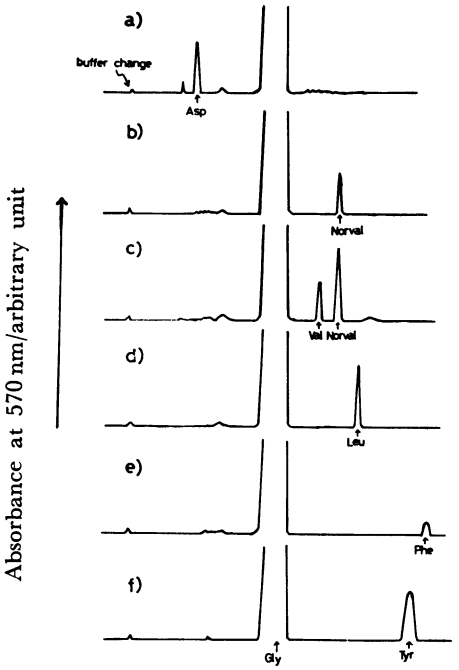



Fig. 1. Amino acid analyses of acid hydrolysates from reaction mixtures.
a): Reaction with acetonitrile, b): reaction with propylene, c): reaction with propylene in the presence of $10^{-4}$ M palladium(II) chloride, d): reaction with isobutylene, e): reaction with toluene, f): reaction with *p*-cresol.

as a plausible hydrolysis route. The cyanoalanine residue is finally hydrolysed to asparagine and aspartic acid. Acetate could not be introduced onto *N*-oxaloglycine in this experiment.

Norvaline was formed using propylene in the absence of metal ions (Fig. 1-(b)). The formation of valine (Fig. 1-(c)) may be inconsistent with the anti-Markownikov mode, if a radical mechanism is operative. However, added metal ions can react with propylene under the experimental conditions to result in a different product. This phenomenon was observed when either $PdCl_2$, $CoCl_2$, or $MnCl_2$ was added. Other metal ions such as $Fe^{2+}$, $Cu^{2+}$ gave no valine peak. When acrylonitrile was put into the reaction system, the resulting considerable precipitation under UV irradiation precluded an analysis of glutamic acid.

The $C_4$ unit was inserted onto *N*-oxaloglycine by using isobutylene as an alkylating agent. Except glycine only a leucine peak is evident in Fig. 1-(d). Only a trace amount of *cis* and *trans*-2-butene resulted in the formation of isoleucine as indicated by amino acid analysis sensitivity. Decisive identification was not possible under our experimental conditions.

In the system which included *p*-cresol as an alkylating agent, tyrosine was obtained from *N*-oxaloglycine. The hydroxyl group of *p*-cresol probably had no need of protection since there was no ninhydrin-positive by-product as evident from Fig. 1-(f). Since toluene is not very soluble in the aqueous-acetone mentioned above, *N*-oxaloglycine was suspended in a 1 : 1 aqueous acetone mixture under vigorous stirring, resulting in the formation of phenylalanine.

When using a low pressure Hg-lamp to excite *N*-oxaloglycine ($\varepsilon_{254}=450\ cm^{-1}\ M^{-1}$) and alkylating agents, no expected amino acids were produced. Acetone was then introduced into the system so as to initiate a photochemical process. Similar photochemical reactions were reported by Elad *et al.*[6] in organic solvents. The n-$\pi^*$ transition of a carbonyl compound brings about a biradical expressed as $>\dot{C}-\dot{O}$, which can abstract a active hydrogen on $\alpha$-carbon of glycine residue. The radical species can react with an alkylating agent to possibly form a branched $\alpha$-amino acid. On the other hand, it is necessary for the rather stable radicals to combine with the glycine residue. In fact, when this combination occurs, unsaturated bonds are situated close to the radical site. When glycine was used as a starting material, no converted amino acid was found. In this connection, *N*-oxaloglycine synthesized from glyoxylic acid and ammonia is suitable for conversion to other amino acids, in view of prebiotic synthesis and the organic chemistry involved. In strong acidic or basic media, the above reaction was found to be negligibly. In a non-deaerated system ninhydrin-positive by-products were formed to a considerable extent and the production of expected amino acids decreased by about one third. This may possibly confirm that a prebiotic neutral aqueous medium is suitable for forming amino acids photochemically, though the conversion yield was *ca.* 1% at this stage.

Also of interest is the relation between the synthetic route of amino acids and the origin of genetic code, for which a working hypothesis has been proposed by Egami.[7] The most important aspect of this matter is the interdependent genesis of protein amino acids and nucleic bases. According to Egami, synthetic route is reasonable in the case of aspartic acid from a glycine residue plus a $C_2$ unit, and valine from a glycine residue plus a $C_3$ unit.

In conclusion, several protein amino acids were formed from common materials such as glyoxylic acid, ammonia, nitrile, and hydrocarbons under UV light.

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