Racemization of Alanine Induced by Copper(I) Oxide

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Copper(I) oxide was found to be an excellent initiator for the racemization of alanine in alkaline aqueous solutions. The rate studies indicate that copper(I) oxide itself is not a catalyst for the racemization but is a pre-catalyst producing real racemization catalysts in the reaction system. The real catalytic system for racemization was found to consist of acetaldehyde and copper(II) ions. Acetaldehyde and copper(II) ions were produced by the oxidation of alanine and copper(I) oxide with oxygen in air, respectively. The function of copper(I) oxide is considered to produce oxidants by reaction with oxygen, which effects the oxidation of alanine.

In the course of studies on the catalytic racemization of alanine with nitrosophenol and copper(II) ions,[1] it was found that the addition of copper(I) oxide to the reaction system accelerates the rate of racemization. Further investigations revealed that even in the absence of nitrosophenol, racemization proceeded at a high rate so long as a catalytic amount of copper(I) oxide was present in the system.

The present paper describes the results of this finding and the function of the copper(I) oxide in this reaction.

Results and Discussion

The experimental systems are listed in Table 1 and the results of experiments 1, 2, and 3 are shown in Fig. 1.

Racemization proceeded at a high rate in the system of experiment 1. Since no detectable racemization was observed in experiment 2 carried out in a nitrogen atmosphere, copper(I) oxide itself was found to be inactive for racemization. From the results of experiment 3, it is also clear that copper(II) ions alone produce no racemization activity. Thus, the presence of copper(I) oxide and air appears to be an essential factor for racemization.

TABLE 1. COMPOSITION OF REACTION SYSTEM[a)]

Experiment	Copper compound	Additives	Atmosphere
1	Cu_2O	···	air
2	Cu_2O	···	nitrogen
3	$CuSO_4$	···	air
4	Cu_2O	pyruvic acid[b)]	air
5	$CuSO_4$	pyruvic acid	air
6	Cu_2O	pyruvic acid	nitrogen
7	Cu_2O	acetaldehyde[b)]	air
8	Cu_2O	acetaldehyde	nitrogen
9	$CuSO_4$	acetaldehyde	air

a) The amounts of alanine, the copper compounds and the buffer solution are given in the experimental section.
b) The amount is adjusted to the maximum amount found in the system of experiment 1; pyruvic acid: 59 mg, acetaldehyde: 20 mg.

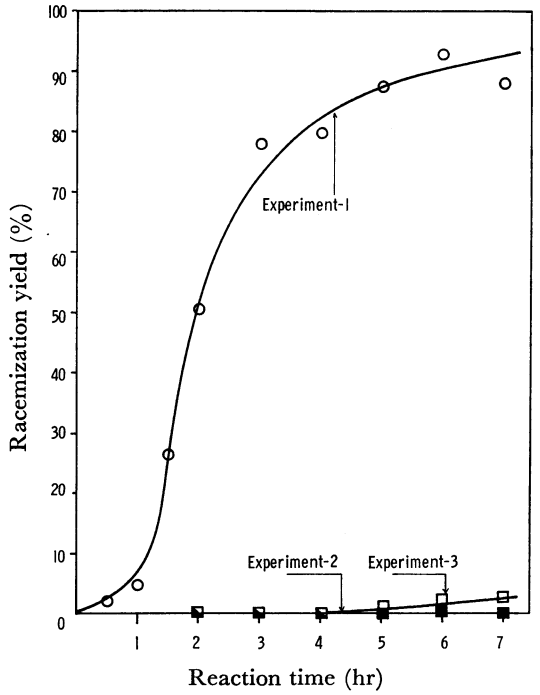

Fig. 1. Time course of racemization of D-alanine.
The reaction condition of each experiment is shown in Table 1.

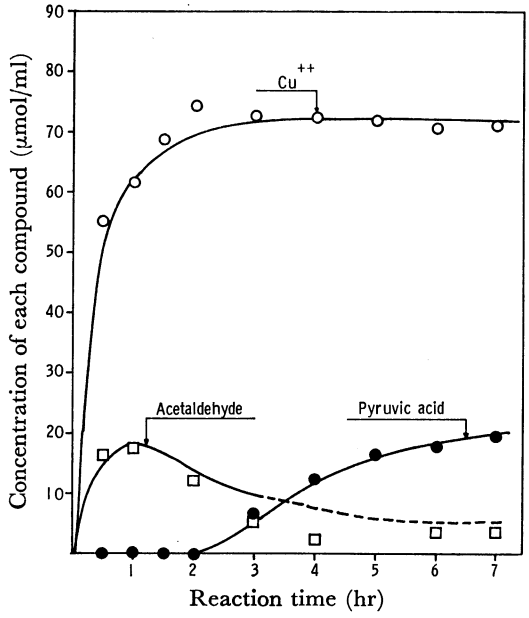

Fig. 2. Concentrations of cupric ion, acetaldehyde and pyruvic acid in the reaction mixture of experiment 1.
The concentrations of acetaldehyde shown in a part of broken line are estimated by the average of scatterd analytical resalts caused by the interference of pyruvic acid.

When the presence of the induction period in the course of experiment 1 is taken into account, real racemization catalysts appear to be produced from the reaction mixture in the course of the reaction.

Analysis of the products of experiment 1 during the reaction revealed the presence of small amounts of pyruvic acid and acetaldehyde. The formation of copper(II) ions was suggested by a deep blue coloration of the reaction mixture. Fig. 2 shows the amounts of copper(II) ions, pyruvic acid and acetaldehyde produced during the reaction of experiment 1.

The role of the pyruvic acid in racemization was investigated with systems containing pyruvic acid (experiments 4, 5, and 6 in Table 1). The time courses of racemization in experiments 4, 5, and 6 are shown in Fig. 3.

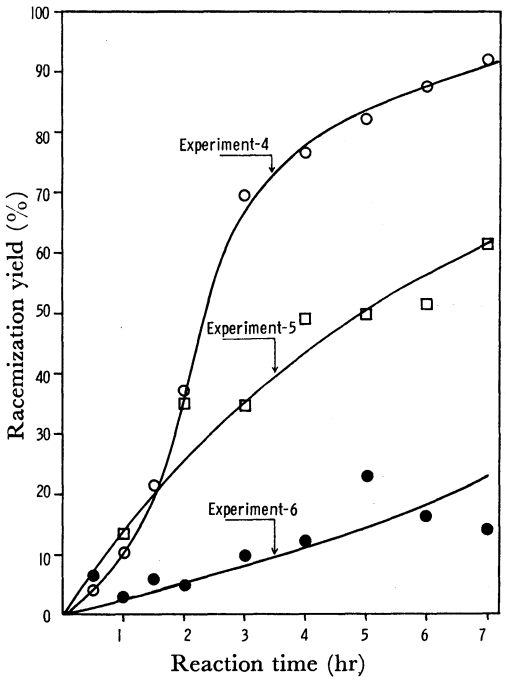

Fig. 3. Time course of racemization of D-alanine. The reaction condition of each experiment is shown in Table 1.

If the racemization of experiment 1 is catalyzed by pyruvic acid or by substances produced from pyruvic acid in the presence of copper(I) oxide or copper(II) ions, these experiments must result in an increase in the racemization rate and a reduction in the induction period. However, experiment 4 almost had the same rate and time course of racemization as did experiment 1 and experiments 5 and 6 resulted in racemization at very low or almost negligible rates.

Although the catalytic action of pyruvic acid is not completely neglected, it is not reasonable to consider pyruvic acid a primary factor governing the racemization in experiment 1.

A major part of the racemization in experiment 5 could be achieved by the transamination between alanine and pyruvic acid.[2)]

The action of acetaldehyde in the racemization was also examined with systems containing acetaldehyde

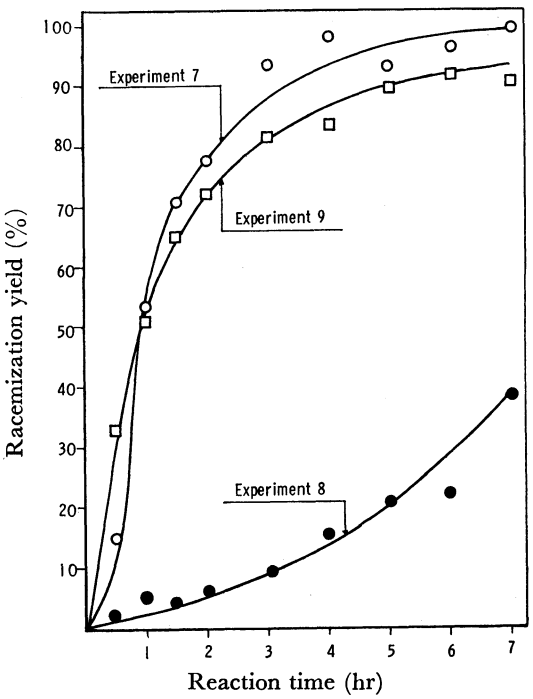

Fig. 4. Time course of racemization of D-alanine. The reaction condition of each experiment is shown in Table 1.

(experiments 7, 8, and 9 in Table 1). The time course of racemization of each experiment is shown in Fig. 4.

The results of experiment 7 reveal that acetaldehyde plays an important role in racemization. However, results of experiment 8 indicate that if there is no oxygen in the system, the rate of racemization becomes negligible even if sufficient amounts of acetaldehyde and copper(I) oxide are present. The fact that experiment 9 resulted in the absence of an induction period and high initial rate of racemization suggests that acetaldehyde is one of the essential components for the catalytic system and requires cupric ions to promote the racemization.

Since negligible effects of substances expected to be produced by the decomposition of alanine such as carbon dioxide, ammonia and ethylamine were confirmed experimentally, the real catalytic system participating in the racemization of experiment 1 was ascertained to consist of acetaldehyde and copper(II) ions.

The mechanism of racemization of alanine in experiment 1 is summarized in two steps: 1) oxidation of copper(I) oxide with oxygen produces copper(II) ions and oxidants, and the reaction of the oxidants with alanine results in the formation of acetaldehyde, and 2) catalytic racemization of alanine with acetaldehyde and copper(II) ions.

The detailed mechanism of catalytic racemization with acetaldehyde and copper(II) ions is not clear at the present time. Work is currently in progress to confirm this. Racemizations catalyzed by nitrosophenol, pyridoxal and their analogues are also being reinvestigated on the basis of this work.

Although the mechanism of the reaction between

copper(I) oxide and oxygen in the present experimental conditions has not been clarified, the formation of oxidants such as superoxide or hydroxy radicals can be expected from the reduction of oxygen with copper-(I) oxide.

The formation of acetaldehyde is considered to proceed by means of the oxidative decarboxylation of alanine because the results of experiments 4 and 5 exclude the possibility of its formation by means of the decarboxylation of pyruvic acid, as does the fact that the formation of acetaldehyde precedes that of pyruvic acid, as is shown in Fig. 2.

The direct formation of acetaldehyde and pyruvic acid from alanine and oxygen in the presence of copper-(I) oxide is interesting as a model reaction of amino acid oxidase. Studies along this line are also under way.

Experimental

All chemicals except those listed below were obtained from commercial sources and used without further purification. Copper(I) oxide was prepared by the reduction of copper(II) sulfate in an aqueous solution with glucose in the presence of potassium sodium tartrate and sodium hydroxide. Acetaldehyde was generated by the decomposition of paraldehyde in the presence of sulfuric acid. Diethyl ether was purified by the method described in Ref. 3).

The infrared spectra were recorded using a Shimadzu IR-27G spectrometer. Colorimetric determination was performed using a Hitachi 124 spectrometer. Gas chromatographic analysis was carried out with a Shimadzu 4APF gas chromatograph using a 300×0.5 cm glass column packed with neopentylglycol succinate (1.5%) on Chromosorb W. Thin-layer chromatography was performed on a precoated plate of Silica-gel HF (E. Merk) with Solvent 1 (hexane/ethyl formate=6/4) or Solvent 2 (butanol/ethanol/12% aqueous ammonia=7/1/2).

Racemization. In a shaker flask, D-alanine (25 m mol), a copper compound (2.5 m mol) and other additives as necessary were dissolved in 23 ml of an alkaline stock solution consisting of a 0.05 M solution of sodium borate and a 1M solution of sodium hydroxide in such a ratio that the pH of the resulting reaction mixture became 10.4 at 50 °C. The mixture was shaken at 50 °C in air or nitrogen. At specific time intervals, a small portion of the reaction mixture was taken out as a sample for analysis. The composition of each experiment is listed in Table 1.

Determination of Racemization Yield. The reaction mixture (0.2 ml) was acidified and dried *in vacuo*. The residue was heated with *l*-menthol (1.0 g). When the temperature of the mixture reached 120 °C, dry hydrogen chloride was passed into the mixture until the esterification of alanine was completed (requiring 1.5 hr). Excess *l*-menthol was removed from the mixture by sublimation at 110 °C at reduced pressure and the residue was allowed to react with trifluoroacetic anhydride (0.4 ml) at room temperature. The resulting *l*-menthyl L- and D-trifluoroacetylalaninate were subjected to gas chromatography at 170 °C. From the ratio of diastereomers, the racemization yield of D-alanine was calculated.

Determination of Copper(II) Ions. Copper(II) ions in the reaction mixture were determined by EDTA titration. The reaction mixture (2 ml) was acidified with 1 M sulfuric acid to pH 4—5, and was then diluted with 20 ml of an acetate buffer (pH 4.5). The solution was titrated with a 1/100 M EDTA solution in the presence of PAN as an indicator.

Characterization of Acetaldehyde and Pyruvic Acid. The reaction mixture of experiment 1 (20 ml) was acidified with hydrochloric acid and mixed with 20 ml of a 0.4% solution of 2,4-dinitrophenylhydrazine in 2 M hydrochloric acid. The reaction products were extracted three times with a 20 ml portion of ether. The combined extracts were extracted twice with a 30 ml portion of a 10% aqueous sodium carbonate solution.

The organic layer was concentrated to 0.5 ml and the reddish-orange crystals that precipitated were collected. The crystals (mp 162.0 °C) were identical with 2,4-dinitrophenylhydrazone of acetaldehyde (**1**) from a mixed melting-point determination, IR spectra and the R_f values (0.65) of tlc (developed with Solvent 1).

The aqueous layer was acidified with 2 M hydrochloric acid and the acid insoluble substance was extracted with a 30 ml portion of ethyl acetate. A yellow precipitate which appeared when the extract was concentrated to 0.3 ml was collected and recrystallized from ethyl acetate to give 2,4-dinitrophenylhydrazone of pyruvic acid (**2**) as yellow crystals (mp 162.5 °C), which was confirmed by a mixed melting-point determination with an authentic sample and by comparison of its IR spectrum and tlc R_f values of tlc (0.57) (developed with Solvent 2) with those of an authentic sample.

Determination of Acetaldehyde and Pyruvic Acid. The amounts of acetaldehyde and pyruvic acid in the reaction mixture were evaluated from those of their 2,4-dinitrophenylhydrazones, which were found in the reaction mixture treated with 2,4-dinitrophenylhydrazine.

The reaction mixture (0.3 ml) was treated with 1 ml of a 0.4% solution of 2,4-dinitrophenylhydrazine in 2 M hydrochloric acid. The products were extracted five times with a 4 ml portion of ether. The combined extracts were made up to 25 ml with ether in a volumetric flask.

a) Isolation and Determination of 1. A 2 ml portion of the extract was concentrated to 0.1 ml and applied on a tlc plate. The plate was developed with Solvent 1. **1** separated as a yellow band on the plate was recovered by scraping the corresponding part of the silica gel and eluting with 5 ml of methanol. The amount of **1** in the eluate was determined by colorimetry with the absorbance at 355 mμ.

b) Isolation and Determination of 2. A 0.6 ml portion of the extract was subjected to chromatography on a tlc plate with Solvent 2. **2** separated on the plate was recovered in the same way as in a) except for the use of a 0.1 M sodium bicarbonate solution instead of methanol. The amount of **2** in the eluate was determined by colorimetry with the absorbance at 355 mμ.

References

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