May, 1982] © *1982 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn.*, ***55***, *1657—1658 (1982)* 1657

The Racemization of Glutamic Acid Induced by Aliphatic Aldehydes

Mihoko Yamamoto, Hiroshi Ozaki, Akira Tai,* and Yoshiharu Izumi
Division of Organic Chemistry, Institute for Protein Research, Osaka University,
3-2 Yamada-oka, Suita, Osaka 565

(Received July 20, 1981)

Synopsis. The real catalytic species in the racemization of glutamic acid induced by acetaldehyde was investigated and found to be 2-butenal produced *in situ* from acetaldehyde.

It has been reported that the catalytic species of the racemization of alanine induced by Cu_2O was acetaldehyde produced in alanine and Cu_2O–O_2 system.[1)] In the course of a study of the racemization of glutamic acid with acetaldehyde, it has also been made clear that acetaldehyde functions not only as a moderately active racemization catalyst, but also as a precursor of the highly active racemization catalyst produced in the course of the reaction. In a patent, aliphatic aldehydes have been reported to be a good catalyst for the racemization of amino acid.[2)] However, no systematic study has been carried out. In this paper, the function of aliphatic aldehydes in the racemization of glutamic acid will be discussed.

The racemizations of glutamic acid with various aliphatic aldehydes were carried out in the presence of Cu^{2+} ions. The time courses of the racemization yield and the consumption of aldehyde are shown in Fig. 1. The time courses of racemization with different aldehydes showed different features. The activity induced by acetaldehyde was much higher than that induced by the other aldehydes. The order of activity was: acetaldehyde>propionaldehyde>formaldehyde>isobutyraldehyde.

As may be found in Fig. 1, the racemization induced by acetaldehyde proceeds with the consumption of acetaldehyde. The high rate of racemization was unchanged even after 90% of the acetaldehyde has disappeared in the reaction mixture. This result strongly suggests that, although acetaldehyde itself may have some catalytic activity, another compound having a much higher racemization activity than acetaldehyde is produced *in situ* at an early stage of the reaction.

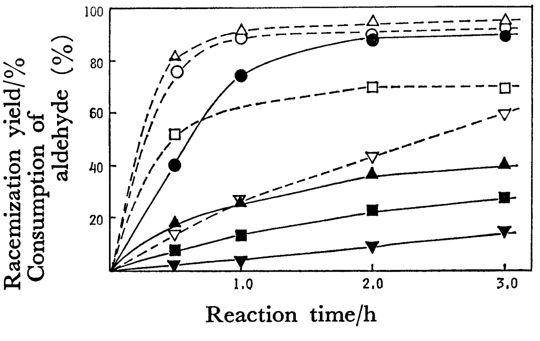

Fig. 1. Time course of racemization with acetaldehyde (—●—), propionaldehyde (—▲—), formaldehyde (—■—), and isobutyraldehyde (—▼—), and time course of consumption of acetaldehyde (—○—), propionaldehyde (—△—), formaldehyde (—□—), and isobutyraldehyde (—▽—).

Propionaldehyde shows the same consumption curve as acetaldehyde. However, the rate of racemization with propionaldehyde was found to be slow. That is, the racemization activity of the compound produced from propionaldehyde *in situ* was not expected to be so strong as that produced from acetaldehyde. When isobutyraldehyde was used, the consumption of the aldehyde was very slow, and the rate of racemization was almost negligible compared to that with acetaldehyde.

In the case of formaldehyde, the rate of racemization was unexpectedly slow, and the consumption of aldehyde was also slow.

The analyses of carbonyl compounds in the reaction mixture indicated that 2-butenal (I), 3-hydroxybutanal (II), and 2-oxoglutaric acid (III) were formed even at an early stage of the reaction. I and II were the products of the aldol condensation of acetaldehyde, while III was the product of the oxidative deamination of glutamic acid. As may be found in Fig. 2, the products of the aldol condensation showed a high racemization activity, while III showed no appreciable activity. Thus, the aldol condensation of acetaldehyde in the reaction system was expected to play a major role in giving a highly active catalyst for the racemization *in situ*. The order of the activity

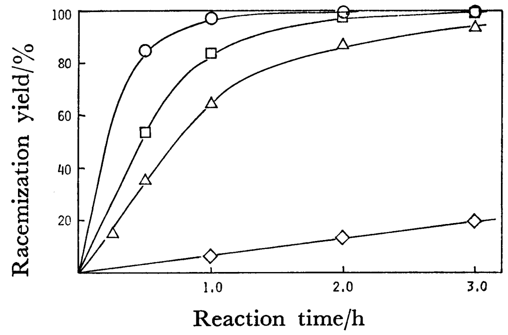

Fig. 2. Time course of racemization (at 56 °C) with acetaldehyde (—△—), II (—□—), I (—○—), and III (—◇—).

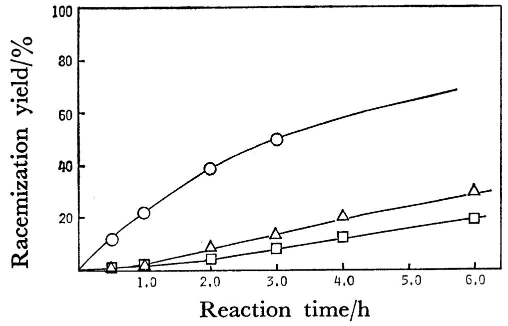

Fig. 3. Time course of racemization (at 25 °C) with acetaldehyde (—△—), II (—□—), and I (—○—).

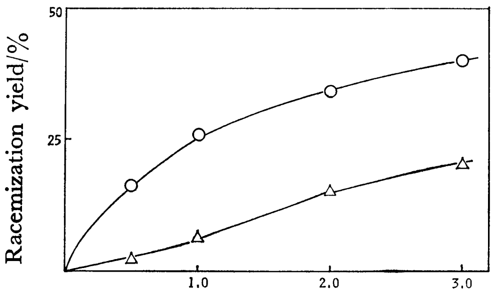

Fig. 4. Time course of racemization with propionaldehyde (—○—) and 2-methyl-2-pentenal (—△—).

at 50 °C was as follows: acetaldehyde<II<I. However, at 25 °C (Fig. 3), the order of II and acetaldehyde was reversed (II<acetaldehyde<I). Since the conversion of acetaldehyde and II to I must be slow at a lower reaction temperature, the order of the catalytic activities at 25 °C is considered to reflect the order of the intrinsic racemization activity of each compound. On the other hand, at a higher reaction temperature, 56 °C, II and acetaldehyde are converted to I. Since I is produced from II (one-step reaction) much more easily than from acetaldehyde (two-step reaction), a higher racemization rate was attained by the use of II than that of acetaldehyde. From the fact that I exhibited the highest activity both at a low and at a high reaction temperature, it is clear that the real active species in the racemization induced by acetaldehyde at a high temperature is I.

It is possible to convert propionaldehyde to 2-methyl-2-pentenal through aldol condensation and successive dehydration in the reaction system. However, the racemization activity of this compound was found to be lower than that of propionaldehyde itself (Fig. 4). Thus, the overall rate of the racemization induced by propionaldehyde was not enhanced by the accumulation of α,β-unsaturated aldehyde.

Both isobutyraldehyde, which does not afford α,β-unsaturated aldehyde, and formaldehyde, which does not produce an aldol-type compound, were found to induce a poor racemization activity.

These findings also support the idea that the high rate of racemization induced by acetaldehyde is attributable to the formation and to the accumulation of the I produced in a reaction system.

Experimental

The colorimetric determination was performed using a Hitachi 124 spectrometer. Thin-layer chromatography was carried out with a precoated plate of silica gel (E. Merck) with Solvent 1 (diethyl ether/cyclohexane=10/2.5) or Solvent 2 (1-butanol/1-propanol/benzene/conc. ammonia/water =3/10/3/0.5/3.5). The optical rotation was measured by the use of Perkin-Elmer 241 Polarimeter.

Materials. The (*S*)-glutamic acid and copper(II) sulfate pentahydrate were obtained from commercial sources. The acetaldehyde was generated by the decomposition of paraldehyde in the presence of sulfuric acid. The propionaldehyde, isobutyraldehyde, II, and I were distilled immediately before use.

Racemization. Into a slurry of (*S*)-glutamic acid (30 mmol) and $CuSO_4\cdot 5H_2O$ (3 mmol) in 10 ml of water, was added a 7 M (1 M=1 mol dm^{-3}) NaOH solution until the pH of the solution became 10.4. Then the mixture was made up to 30 ml with water.

In a shaker flask, a 25-ml portion of the solution and 2.5 mmol of aldehyde were placed, after which the mixture was shaken at the temperature stated in the text. At specific time intervals, a small portion of the reaction mixture was taken out for analysis.

Determination of the Racemization Yield. A 3-ml portion of the reaction mixture at each reaction time was acidified with 6 ml of 6 M HCl. After the removal of Cu^{2+} from the 3-ml portion of the acidified reaction mixture by treatment with H_2S, the resulting solution was made up to 5 ml with water and subjected to the determination of the optical rotation (α_D^{20}). The degree of racemization was calculated from the ratio of α_D^{20} at each reaction time to the α_D^{20} at the beginning of the reaction.

Determination of Aldehydes. A 3-ml portion of the acidified reaction mixture was mixed with 3 ml of a 1.5% aqueous solution of 4-nitrophenylhydrazine hydrochloride. The reaction product was extracted with 10 ml of ethyl butyrate.

A 100-μl portion of the extract was submitted to chromatography on a TLC plate with Solvent 1. The 4-nitrophenylhydrazone of aldehyde was recovered by scraping the corresponding part of the silica gel and eluting with 5 ml of ethanol. The amount of 4-nitrophenylhydrazone of aldehyde in the elute was determined by colorimetry with the absorbances at 380 nm (acetaldehyde), 390 nm (propionaldehyde and isobutyraldehyde), and 345 nm (formaldehyde).

Identification of Carbonyl Compounds. From the acidified reaction mixture induced by acetaldehyde, yellow products were obtained by a reaction with 4-nitrophenylhydrazine. The yellow crystals were found to contain 4-nitrophenylhydrazone derivatives of II, I, and III by a comparison of their Rf values and UV absorption spectra (λ_{max}) with those of authentic samples. The Rf values of the 4-nitrophenylhydrazone derivatives were found to be 0.095 for II, 0.47 for I, and 0.12 for III by the use of Solvent 1, while their λ_{max} values were 380 nm for II, 395 nm for I, and 383 nm for III.

The authors wish to express their thanks to Dr. Katsumoto Yamanouchi for his helpful suggestions. This work was supported in part by a Scientific Research Grant from the Ministry of Education, Science and Culture Japan (411108).

References

1) A. Tai, K. Okada, T. Masuda, and Y. Izumi, *Bull. Chem. Soc. Jpn.*, **49**, 310 (1976).

2) T. Ogasawara, H. Tachimichi, and S. Suzuki, Japan Patent 13445 (1967).