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Catalytic Activities of Salicylaldehyde Derivatives. I. Catalytic Effects of 4-Formyl-3-hydroxyphenyltrimethylammonium Bromide on the Racemization of L-Amino Acid

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The pyridoxal-like catalytic effects of salicylaldehyde derivatives on the racemization of amino acid in the presence of a metal ion is known to be promoted by the substitution of an electron-attracting group in the benzene ring. Of the salicylaldehyde derivatives tested thus far, 4-formyl-3-hydroxyphenyltrimethylammonium bromide shows the most effective catalytic activity of racemization at pH 10 and at 80°C; the rate constant was 11.0×10^{-3} min^{-1}. The catalytic activity of this compound was studied under various conditions; the activation energy of the racemization of L-glutamic acid at pH 10 was 14.3 kcal/mol.

In the presence of a metal ion, pyridoxal has such a catalytic effects on amino acid as racemization and transamination. The benzene analogues were studied by Snell *et al.*,[1,2] who found that 4- and 6-nitrosalicylaldehydes had catalytic effects similar to those of pyridoxal on L-amino acid.[2] Thereafter, in the studies of the racemization of amino acid, it was recognized that even salicylaldehyde showed catalytic activity in the presence of a metal ion.[3,4] Recently, Yoshikawa *et al.* found that nitro-, sulfo-, and sulfonamide-derivatives of salicylaldehyde had also catalytic effects on the racemization of L-glutamic acid in the presence of a metal ion, and observed that an electron-attracting substituent on the salicylaldehyde ring promoted the catalytic activities, among which *p*-substitution was the most effective.[5] Since, as is well known, the tetrasubstituted ammonium group is a strong electron-attracting group, the *p*-trimethylammonium derivating of salicylaldehyde may be expected to be a stronger catalyst in racemization than all others known. This paper will deal with the synthesis of 4-formyl-3-hydroxyphenyltrimethylammonium bromide and with the measurement of its catalytic effect on the racemization of L-glutamic acid in the presence of a metal ion.

Experimental

Synthesis of 4-Formyl-3-hydroxyphenyltrimethylammonium Bromide (I). To a solution of sodium hydroxide (5 g) in methyl aclohol (200 m*l*), *p*-*N*,*N*-dimethylaminosalicylaldehyde[6,7] (15 g) and methyl iodide (50 g) were added successively, and then the solution was boiled for 24 hr. After the solution had been evaporated to syrup *in vacuo*, the syrup was extracted with an equivolume mixture of water and ethyl acetate (the aqueous layer was acidic); the aqueous solution was then evaporated under diminished pressure and treated with acetone. The precipitate, a mixture of *N*-methylated and *N*,*O*-dimethylated compound, was collected on a filter paper. It was very difficult to control the reaction to give the *N*-methylated compound alone, or to separate the *N*-methylated compound from the *N*,*O*-dimethylated product. Therefore, the mixture was boiled for 1 hr with 48% hydrobromic acid (50 m*l*), and then, after the solution had been cooled and diluted with water (100 m*l*), the resinous material was filtered off with charcoal. The filtrate was concentrated to a syrup under reduced pressure and digested with methanol to crystallize it. After recrystallization from methyl alcohol, the crystalline product was shown by a study of the analytical data to be a mixture of I and a small amount of the corresponding iodide. In order to convert the iodide into I, the mixture was dissolved in water (20 m*l*) and stirred with freshly-precipitated silver bromide (prepared from silver nitrate (5 g)) at room temperature for 3 hr. The silver halide was then filtered off and the filtrate was concentrated at diminished pressure. After recrystallization from methanol, 5.3 g of I were obtained. mp 197°C dec. (uncorrected). Found: C, 46.12; H, 5.11; N, 5.61; Br, 30.79%. Calcd

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[1] D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).

[2] M. Ikawa and E. E. Snell, *ibid.*, **76**, 653 (1954).

[3] K. Ohno, I. Sasaji and M. Hara, Japanese Pat. 295110 (1961).

[4] K. Toi, Y. Izumi and S. Akabori, This Bulletin, **35**, 1422 (1962); K. Toi, *ibid.*, **36**, 739 (1963).

[5] S. Yoshikawa, K. Kuga, Y. Ueda, M. Goto and H. Sugiyama, *Kogyo Kagaku Zasshi (J. Chem. Soc. Japan, Ind. Chem. Sect.)*, **70**, 331 (1967).

[6] Joh. Rud. Geigy & Co., German Pat. 105103 (1901).

[7] W. C. Baird, Jr., and R. L. Shriner, *J. Am. Chem. Soc.*, **86**, 3142 (1964).

for $C_{10}H_{14}O_2NBr$: C, 46.17; H, 5.43: N, 5.39: Br. 30.72 %

Preparation of Solution. *Solution of Amino Acid.* A solution of amino acid (0.1 mol) and cupric sulfate (2 mmol) in a small amount of water was adjusted to the required pH value with 2N sodium hydroxide, and then diluted with water to make a final volume of 100 m*l*.

Solution of Catalyst. The catalyst (2 mmol) was dissolved in 100 m*l* of the required Sörensen 0.05M borate buffer solution and the pH was corrected with a slight amount of 2N sodium hydroxide.

Procedure of Racemization. Unless otherwise stated, the reactions were carried out using the solutions described above. A mixed solution of amino acid (2 m*l*) and a catalyst (2 m*l*) was reacted in a tightly-stoppered test tube on a temperature-regulating water bath. When the required temperature was below room temperature, each solution was cooled for 1 hr at that temperature before mixing. After the required reaction time, the mixture was cooled under a trap and 6N hydrochloric acid (5 m*l*) was added immediately in order to stop the reaction. The optical rotation of the solution $[\alpha_t]$ was measured in a 10 cm tube by a Perkin-Elmer model 141 polarimeter (direct reading 0.001°) at 589 mμ. The racemization yield was calculated as follows:

$$100([\alpha_0]-[\alpha_t])/[\alpha_0]$$

Analysis of Amino Acid. After the reaction had been stopped with hydrochloric acid, the solution was diluted with water (250 fold). The amino acid content in the dilute solution (0.5 m*l*) was analyzed by a Mitamura Riken model II auto amino-acid analyzer.

Results and Discussion

Effect of Kind of Metal Ion Present. The effect of the kind of metal ion on the racemization was examined at 80°C at pH 10 for 3 hr. Some metal salts were not dissolved in the pH-adjusted 1M glutamic acid solution, so every metal salt was added in the solid state and reacted as it was. During the reaction, cuprous or ferrous ions might be partly turned to cupric and ferric ions respectively, but their effect was not detectable. The results are listed in Table 1. Since, of the metal ions tested, cupric, cuprous, ferric, ferrous, and aluminum ions were most effective, and since the cupric ion was the best of all, racemization was thereafter carried out in the presence of the cupric ion.

TABLE 1. COMPARISON OF METAL IONS IN RACEMIZATION OF L-GLUTAMIC ACID

Metal ion	Racemi. yield	Metal ion	Racemi. yield	Metal ion	Racemi. yield
Cu^{2+}	84.2%	Sn^{2+}	34.7%	Zn^{2+}	15.0%
Cu^{+}	81.7	Hg^{2+}	17.6	Pb^{2+}	14.3
Fe^{3+}	76.6	none	17.4	Co^{2+}	12.3
Fe^{2+}	75.0	Ba^{2+}	17.1	Ni^{2+}	10.4
Al^{3+}	71.2	Ca^{2+}	16.4	Mg^{2+}	9.8

Metal ion (0.04 mmol), catalyst (0.04 mmol), and L-glutamic acid (2 mmol) were used and reacted at 80°C for 3 hr at pH 10.

Effect of the Amounts of the Catalyst and the Cupric Ion. Racemization was carried out at pH 10 by altering the amounts of the catalyst and of the cupric ion (0—0.08 mmol) with 2 mmol of L-glutamic acid at 80°C for 1 hr. The results are shown in Fig. 1. As is shown in Fig. 1, the racemization was hastened when accompanied by an increase in the concentrations of the catalyst and of the cupric ion. However, its speed was gradually slowed when their concentrations became more than 0.04 mmol. Most reactions were undertaken in the presence of 0.04 mmol portions of the catalyst and the cupric ion. Of course, it is possible to enrich the catalyst more and more, because of its very high solubility in the reaction system.

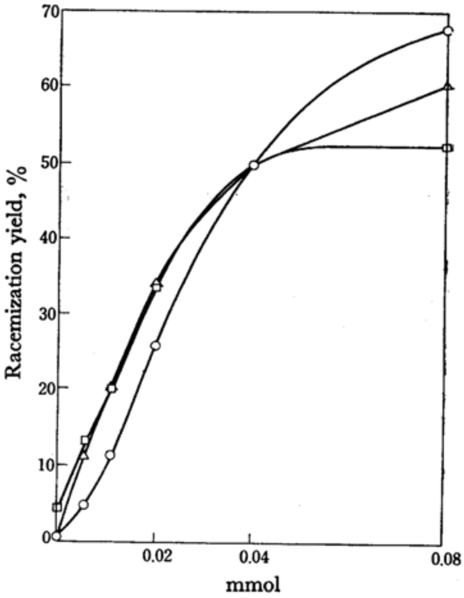

Fig. 1. Effect of the amounts of catalyst and cupric ion on racemization yield.
L-Glutamic acid (2 mmol) was used and reacted at pH 10 at 80°C for 1 hr.
○ Amounts of both catalyst and cupric ion were altered.
□ Amount of cupric ion was altered and that of catalyst was constant (0.04 mmol).
△ Amount of catalyst was altered and that of cupric ion was constant (0.04 mmol).

Effect of the Kind of Buffer Solution. Snell and Longenecker[8] have reported that in the catalytic α,β-elimination reaction of serine derivatives in the presence of pyridoxal and a metal ion, they observed the effect of the kind of buffer solution used. In order ourselves to study the effect of the kind of buffer solution, we dissolved 4-formyl-3-hydroxy-

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[8] J. B. Longenecker and E. E. Snell, *J. Biol. Chem.*, **225**, 409 (1957).

phenyltrimethylammonium bromide in borate, carbonate, and Britton-Robinson buffer solution (a mixture of phosphoric acid, acetic acid, boric acid, and sodium hydroxide solutions), all at pH 10. Because, at high temperatures, there was some uncertainty of this pH value being kept, the reaction was carried out at 25°C for 1 day and also for 7 days. The results are given in Table 2, which shows no serious effect of the kind of buffer solution on the racemization.

TABLE 2. COMPARISON OF KINDS OF BUFFER SOLUTIONS IN RACEMIZATION REACTION

Buffer Sol.	Racemi. yield	
	1 day	7 days
Borate	12.9%	60.5%
Carbonate	14.1	62.1
Mixed acid	14.7	64.8

Borate: 0.05M $Na_2B_4O_7$+0.1M NaOH.
Carbonate: 0.025M Na_2CO_3+0.025M $NaHCO_3$
Mixed Acid: 0.04M H_3PO_4+0.04M CH_3COOH +0.04M H_3BO_3+0.2M NaOH.

L-Glutamic acid (2 mmol), cupric sulfate (0.04 mmol), and catalyst (0.04 mmol) were used and reacted at 25°C at pH 10.

Effect of pH-Value. The effect of the pH-value on the catalytic racemization of glutamic acid was examined with or without cupric ions in a 0.05M borate buffer solution (pH 8—12) at 80°C for 1 hr; we also examined the effect on the racemization of L-alanine at pH 0—14 for 3 hr in a 0.04M Britton-Robinson buffer solution. The results are given in Fig. 2. As this figure shows, no actual racemization was observed below pH 8, and, in contrast with the case of pyridoxal,[9] no optimum pH value was observed, just as with 5-nitro- and 4-sulfo-salicylaldehydes;[5] namely, in the more alkaline solution, the faster racemization occurred in our experimental pH region. Because the racemization yields were reasonable compared with those of 5-nitro- and 4-sulfo-salicylaldehydes,[5] we can say that the zwitter ion is not formed between the ammonium group and the phenol group.

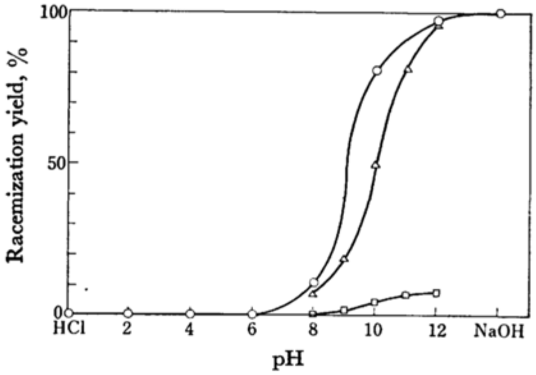

Fig. 2. Effect of pH value on racemization yield at 80°C.
△ Racemization of L-glutamic acid for 1 hr in the presence of cupric ion.
□ Racemization of L-glutamic acid for 1 hr without cupric ion.
○ Racemization of L-alanine for 3 hr in the presence of cupric ion.
HCl and NaOH mean that alanine salt is dissolved in 1N hydrochloric acid and 1N sodium hydroxide, respectively.

Effect of pH on the Rate Constant of the Racemization of L-Glutamic Acid. The racemization yield of L-glutamic acid in the presence of cupric ions at 80°C and at pH 8—12 for various periods of time was determined. The racemization should theoretically be a first-order reaction, and the relation between the rate constant, k, the time, t, and the observed rotation, $[\alpha_t]$, at t can be written as follows:

$$kt = 2.30 \times (\log[\alpha_0] - \log[\alpha_t])$$

At pH 10, in contrast with the case of pyridoxal, the activity of which decreased during the reaction,[5] 4-formyl-3-hydroxyphenyltrimethylammonium bromide indicated a first-order reaction, except at pHs 11 and 12. The plots which obeyed the first-order rate are shown in Fig. 3; the rate constants at pH 8, 9, and 10 were $1.2\times10^{-3}\,\text{min}^{-1}$, $3.3\times10^{-3}\,\text{min}^{-1}$, and $11.0\times10^{-3}\,\text{min}^{-1}$ respectively. Of the salicylaldehyde derivatives tested to date, this compound was found to have the most effective activity.

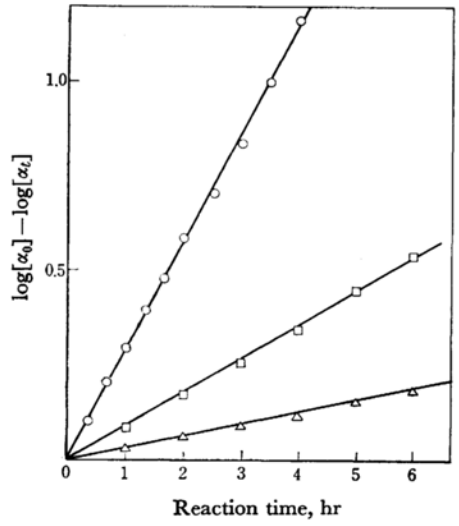

Fig. 3. Catalytic activities at pH 8, 9 and 10 in the presence of cupric ion at 80°C.
△ pH 8 □ pH 9 ○ pH 10

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[9] E. E. Snell, J. Olivard and D. E. Metzler, *J. Biol. Chem.*, **199**, 669 (1952)

Table 3. The rate constant k at various temperatures

Temp.	k
100°	28.9×10^{-3} min^{-1}
90	18.2
80	11.0
70	6.20
60	3.35
50	1.69
40	0.801
20	0.155

L-Glutamic acid (2 mmol), cupric sulfate (0.04 mmol), and catalyst (0.04 mmol) were used, and reacted at pH 10.

Activation Energy of the Racemization of L-Glutamic Acid. The effect of the temperature on the rate constant of the racemization of L-glutamic acid at pH 10 was also studied. The rate constants, k, at various temperatures are listed in Table 3. A linear relation between $\log k$ and $1/T$ was observed, as is shown in Fig. 4, and the activation energy was found to be 14.3 kcal/mol.

Determination of the Amount of Glutamic Acid. After the racemization reaction had been carried out at 80°C and at pH 10 for 3 hr or 6 hr, the amount of glutamic acid was changed within the limits of experimental error; although the amount of ammonia increased considerably, it was still an unmeasurable amount.

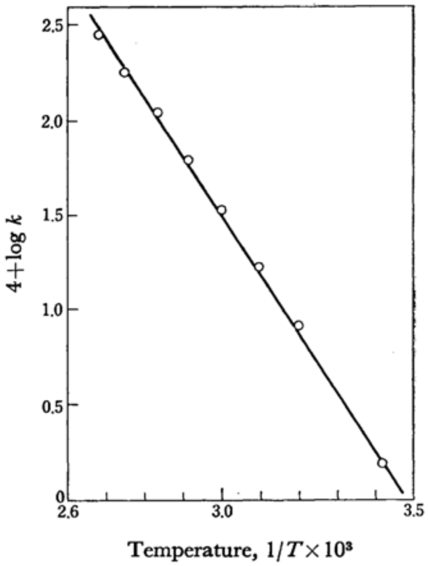

Fig. 4. Effect of temperature on rate constant at pH 10 in the presence of cupric ion.

It is easy to recover racemized free glutamic acid from the concentrated solution of a low pH, because the catalyst is very soluble in the solution.

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