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Racemization Catalyst for Amino Acids. I.*[1]
Racemization with Derivatives of *o*-Nitrosophenol

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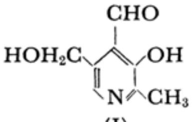
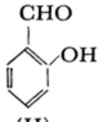
The racemization of amino acid catalyzed by several derivatives of *o*-nitrosophenol in the presence of metal ion was studied in a non-enzymatic system. The racemizations of L-alanine with 1-nitroso-2-naphthol, 2-nitroso-1-naphthol, 2, 4-dinitrosoresorcinol and disodium 1-nitroso-2-naphthol-3, 6-disulfate as catalysts were measured in the presence of metal ion and their activities were compared using various metal ions in these racemizations. Furthermore, racemizations with 1-nitroso-2-naphthol were carried out at various pH values in the presence of cupric or zinc ion and the optima pH values were measured. The racemization activities of 1-nitroso-2-naphthol and 4-nitroso-1-naphthol were compared to elucidate the relationship between the structural features and the activities of these catalysts. A possible mechanism for the catalytic racemization of amino acids is discussed briefly.

A number of studies[1-3] have revealed that pyridoxal (I) serves as a coenzyme in such biochemical reactions as transamination and racemization. Most of these reactions have been imitated non-enzymatically in model reactions using pyridoxal in the presence of metal ion. The catalytic activities of salicylaldehyde (II) and its derivatives, which possess the same functional group as pyridoxal, in the racemization[4-9] of amino acids were also measured by one of the present authors.

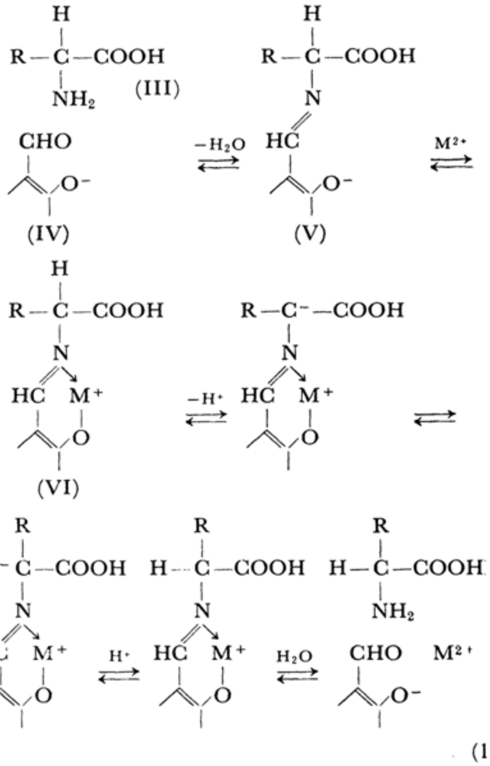


(I)



(II)

However, derivatives of salicylaldehyde are difficult to prepare, because their aldehyde groups are unstable becoming oxidized to carboxylic acids and moreover the introduction of an aldehyde group in the ortho position to the phenolic group is comparatively troublesome. On the other hand, several derivatives of *o*-nitrosophenol, which were examined as racemization catalysts in this work, can easily be synthesized from phenols, because the nitroso groups can be directly introduced in the ortho position to the phenolic groups by the



(1)

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*[1] Presented at the 19th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1966.

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2) E. E. Snell and W. T. Jenkins, *J. Cell. and Comp. Physiol.*, **54**, 161 (1959).

3) A. E. Braunstein, "The Enzymes," Vol. 2, Academic Press, New York (1960), p. 113.

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5) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York (1952), p. 397.

6) K. Toi, Y. Izumi and S. Akabori, This Bulletin, **35**, 1422 (1962).

7) K. Toi, *ibid.*, **36**, 739 (1963).

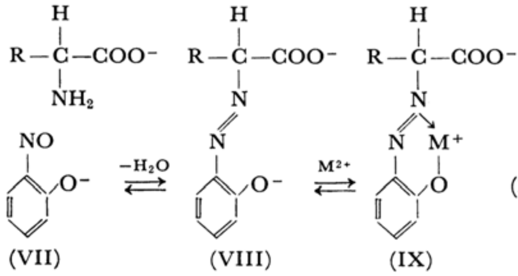
8) Y. Yoshikawa and Y. Ueda, Abstract of the 18th Annual Meeting of the Chemical Society of Japan, April, 1965, p. 229.

9) Y. Yoshikawa, K. Kuga, Y. Ueda and M. Goto, Abstract of the 18th Annual Meeting of the Chemical Society of Japan, April, 1965, p. 431.

action of nitrous acid or its esters. For example, 1-nitroso-2-naphthol, which is standard chelating reagent for metal ions, can be prepared in good yield by the nitrosation of 2-naphthol.

Catalytic racemization⁽¹⁰⁾ with pyridoxal or salicylaldehyde involves a chelation reaction between metal ion and Schiff base (V) formed with amino acid (III) and pyridoxal (IV) or salicylaldehyde (IV) as shown in reaction 1. A decrease in electron density at the α-nitrogen atom of the amino acid by the formation of a chelate ring (VI) makes the α-hydrogen atom more easy to ionize and promotes the racemization of the amino acid.

A nitroso group partially simulates an aldehyde group in its reactions with amino groups.⁽¹¹⁾ Thus, the former condenses with amines and forms azo linkages while the latter forms Schiff bases. Therefore, *o*-nitrosophenol (VII) reacts with the amino group of the amino acid and produces an azo compound (VIII), capable of forming a chelation compound (IX) with metal ion, as shown in reaction 2.



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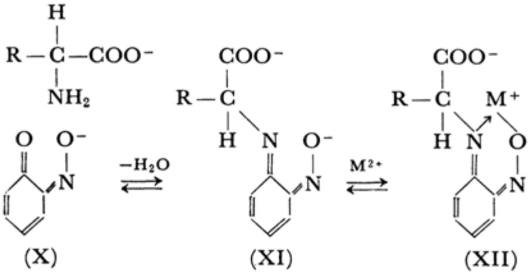

(3)

On the other hand, *o*-nitrosophenol has another tautomer,⁽¹²⁾ namely the quinone type X besides the phenol type VII. *o*-Quinone oxime (X) reacts with amino acid to form an *o*-quinone oxime imine compound (XI), which chelates with metal ion to produce the chelation compound (XII) as shown in reaction 3. These chelate ring formations (IX and XII), like VI in pyridoxal- or salicylaldehyde-catalyzed racemization, should be able to promote the racemization reaction of amino acids.

Experimental

Preparation of Catalysts. 1-Nitroso-2-naphthol, 2, 4-dinitrosoresorcinol monohydrate and disodium 1-nitroso-2-naphthol-3, 6-disulfonate were obtained commercially. 2-Nitroso-1-naphthol and 4-nitroso-1-naphthol were prepared by nitrosation of 1-naphthol.⁽¹³⁾

Racemization Procedure. Five milliliters of a solution containing 5 mmol of L-alanine and 0.2 mmol of metal salt, the pH of which had been adjusted to the required value with 40 per cent sodium hydroxide, was vigorously shaken with 0.2 mmol of catalyst in a sealed tube at 100°C. The reaction mixture was diluted to 10 m*l* with 12N hydrochloric acid and then the catalyst was removed by filtration. After the filtrate had been decolorized with charcoal, the optical rotation $[\alpha]_D$ of the solution was measured.

Determination of Amino Acid. The alanine content of the reaction mixture was determined according to Toi's method.⁽⁷⁾

Results and Discussion

Effect of the pH Value of the Reaction Mixture. The effect of the pH value of the reaction mixture in the presence of cupric ion was tested in the pH range of 4—11 in a four hour reaction period at 100°C. The results are shown in Fig. 1. Catalytic activities for racemization were observed in the pH range of 4—10.5 and during the racemization alanine consumption was accompanied by its decomposition to the extent of 10—20% under alkaline conditions. Though with cupric ion, especially above pH 10.5, there was a considerable racemization in the absence of 1-nitroso-2-naphthol, the optimum pH for the racemization catalyzed by both catalysts was about 10. A similar optimum pH has also been observed in the non-enzymatic racemizations of L-alanine with pyridoxal and derivatives of salicylaldehyde by Snell *et al.*⁽¹⁴⁾ and Toi.⁽⁷⁾

Under the conditions described above, the racemization of L-alanine was tested in the presence of zinc ion, and the results are shown in Fig. 2. No definite optimum pH was observed in this case under the present conditions, but increase in the pH appeared to accelerate the racemization in contrast to the case with cupric ion.

Effect of the Kind of Metal Ion Present. The effect of the kind of metal ion on the racemization with 1-nitroso-2-naphthol was examined at a fixed metal ion concentration at pH 10 and results are summarized in Table 1. The racemization of amino acid by these catalysts proceeds in the presence, but not in the absence of metal ion.

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12) *Ibid.*, Vol. 9, Asakura, Tokyo (1959), p. 446.

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TABLE 1. COMPARISON OF ACTIVITIES OF METAL IONS IN CATALYSIS OF RACEMIZATION WITH *o*-NITROSOPHENOL DERIVATIVES

Catalyst	Metal ion								
	Cu^{2+}			Co^{2+}			Ni^{2+}		
	Obs.a) yield %	Decomp.b) %	Corr.c) yield %	Obs. yield %	Decomp. %	Corr. yield %	Obs. yield %	Decomp. %	Corr. yield %
2, 4-Dinitrosoresorcinol monohydrate	39	13	26	53	13	40	30	29	1
1-Nitroso-2-naphthol	65	9	56	38	4	34	9	9	0
2-Nitroso-1-naphthol	51	0	51	30	4	26	6	6	0
Disodium 1-nitroso-2-naphthol-3, 6-disulfonate	50	6	44	38	11	27	15	14	1

Catalyst	Metal ion								
	Hg^{2+}			Fe^{2+}			Zn^{2+}		
	Obs. yield %	Decomp. %	Corr. yield %	Obs. yield %	Decomp. %	Corr. yield %	Obs. yield %	Decomp. %	Corr. yield %
2, 4-Dinitrosoresorcinol monohydrate	19	16	3	69	23	46	35	11	24
1-Nitroso-2-naphthol	8	10	0	13	0	13	14	6	8
2-Nitroso-1-naphthol	19	10	9	9	6	3	16	4	12
Disodium 1-nitroso-2-naphthol-3, 6-disulfonate	22	8	14	25	9	16	16	6	10

Five milliliters of reaction mixture (pH 10.0) containing 5 mmol L-alanine, 0.2 mmol catalyst and 0.2 mmol metal salt was heated for 4 hr at 100°C. Then the reaction mixture was diluted to 10 m*l* with 12 N HCl and the optical rotation $[\alpha]_D$ was measured. The metal salts used were $CuSO_4\cdot 5H_2O$, $CoCl_2\cdot 6H_2O$, $NiCl_2\cdot 6H_2O$, $HgCl_2$, $FeSO_4\cdot 7H_2O$ and $Zn(CH_3COO)_2\cdot 2H_2O$.

a) Observed racemization yield calculated as follows; $[\alpha]_0-[\alpha]/[\alpha]_0\times 100$.

b) Decomposition per cent calculated from recovery of amino acid after the reaction.

c) Corrected value for racemization yield calculated by [Obs. yield] − [Decomp.].

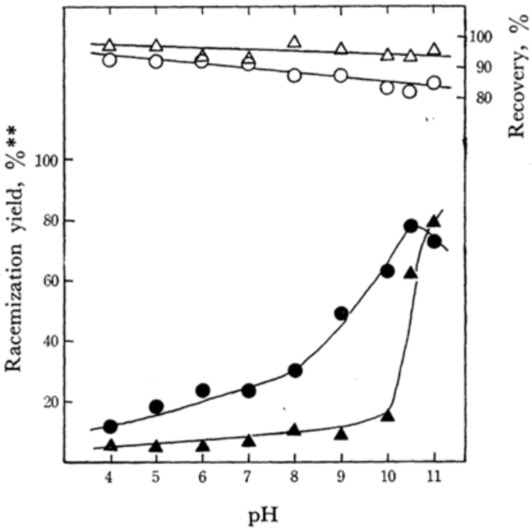

Fig. 1. Effect of pH on the racemization reaction in the presence of cupric ion.
Reaction mixtures of 5 m*l* contained 5 mmol L-alanine, 0.2 mmol 1-nitroso-2-naphthol, and 0.2 mmol $CuSO_4\cdot 5H_2O$. Reaction time 4 hr at 100°C.

Racemization with 1-nitroso-2-naphthol and $CuSO_4\cdot 5H_2O$.
- ● Racemization yield
- ○ Recovery of amino acid

Racemization with $CuSO_4\cdot 5H_2O$.
- ▲ Racemization yield
- △ Recovery of amino acid

* Observed racemization yield calculated as follows; $[\alpha]_0-[\alpha]/[\alpha]_0\times 100$.

Of the metal ions tested, cupric and cobaltous ions catalyze the racemization most effectively with every catalyst employed, while ferrous and zinc ions have some catalytic effect though zinc ion was less effective in the racemization than cupric ion at the various pH values tested, as may be seen in Fig. 2. On the other hand, nickelous and mercuric ions are not effective. 1, 3-Dinitrosoresorcinol, led to more extensive decomposition of the amino acid than other catalysts with every metal ion tested.

Effect of the Concentration of Catalyst. The concentrations of 1-nitroso-2-naphthol- and metal ion-catalysts which promote racemization of L-alanine were examined and the results are shown in Fig. 3. It can be seen that the rate of

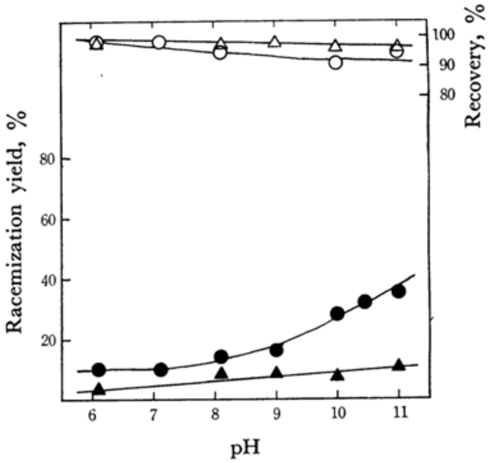

Fig. 2. Effect of pH on the racemization reaction in the presence of zinc ion.
Reaction mixtures contained 5 mmol L-alanine, 0.2 mmol 1-nitroso-2-naphthol, and 0.2 mmol $Zn(CH_3COO)_2 \cdot 2H_2O$. Reaction time, 4 hr at 100°C.
Racemization with 1-nitroso-2-naphthol and $Zn(CH_3COO)_2 \cdot 2H_2O$.
● Racemization yield
○ Recovery of amino acid
Racemization with $Zn(CH_3COO)_2 \cdot 2H_2O$.
▲ Racemization yield
△ Recovery of amino acid

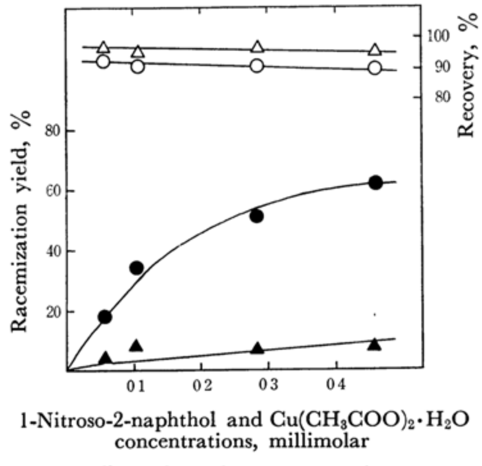

Fig. 3. Effect of catalyst concentration on racemization reaction (pH 9).
Reaction mixtures of 5 m*l* contained 5 mmol L-alanine, the indicated amount of 1-nitroso-2-naphthol and $Cu(CH_3COO)_2 \cdot H_2O$. Reaction time, 3 hr at 100°C.
Racemization with 1-nitroso-2-naphthol and $Cu(CH_3COO)_2 \cdot H_2O$.
● Racemization yield
○ Recovery of amino acid
Racemization with $Cu(CH_3COO)_2 \cdot H_2O$.
▲ Racemization yield
△ Recovery of amino acid

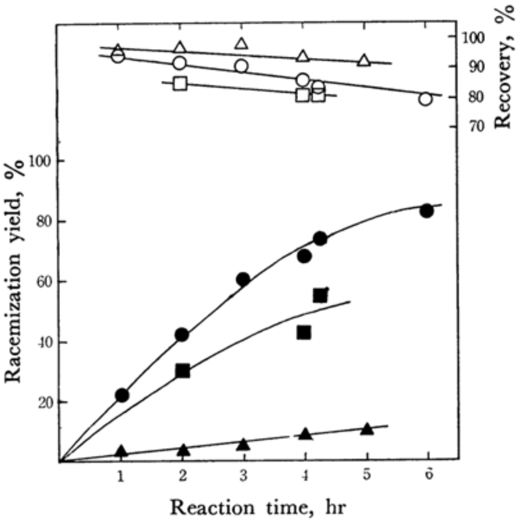

Fig. 4. Racemization activities of 1-nitroso-2-naphthol and 4-nitroso-1-naphthol in the presence of cupric ion.
Reaction mixtures of 5 m*l* (pH 10) contained 5 mmol L-alanine, 0.2 mmol 1-nitroso-2-naphthol or 4-nitroso-1-naphthol, and 0.2 mmol $CuSO_4 \cdot 5H_2O$. Reaction time, 4 hr at 100°C.
Racemization with 1-nitroso-2-naphthol and $CuSO_4 \cdot 5H_2O$.
● Racemization yield
○ Recovery of amino acid
Racemization with 4-nitroso-1-naphthol and $CuSO_4 \cdot 5H_2O$.
■ Racemization yield
□ Recovery of amino acid
Racemization with $CuSO_4 \cdot 5H_2O$.
▲ Racemization yield
△ Recovery of amino acid

the racemization reaction is highly dependent on the concentrations of both 1-nitroso-2-naphthol and metal ion, and that increase in the concentrations of the catalysts accelerates the reaction.

Relationship between the Activity and Structure of the Catalyst. The racemization activities of 1-nitroso-2-naphthol and 4-nitroso-1-naphthol were compared in the presence of cupric ion to elucidate the relationship between the structural features and the activities of these catalysts. Tests were carried out with L-alanine at pH 10 at 100°C and results are shown in Fig. 4. Racemization with 4-nitroso-1-naphthol is less than with 1-nitroso-2-naphthol. The results show that the presence of a hydroxy group at the ortho position to the nitroso group in the structure of the catalyst is desirable for racemization of amino acid. Moreover, results appear to support the formation of a reactive chelation intermediate such as IX or XII suggested to be formed during racemization with *o*-nitrosophenol catalyst, because a catalyst such as *p*-nitrosophenol, 4-nitroso-1-naphthol, cannot form the chelate ring which can promote the racemization of amino acid in reaction 2 or 3. The mechanism of this reaction will be discussed in detail in a forthcoming paper.