A Stereospecific Racemization Catalyst for Amino Acid1)

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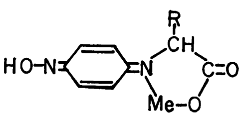
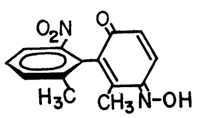
(Receied May 7, 1971)

It is known that pyridoxal, salicylaldehyde, *o*- or *p*-nitrosophenol, and aurintricarboxylic acid catalyze2–6) non-enzymatically, in alkaline media, the racemization of optically-active amino acid in the presence of a metal ion. These reactions involve the formation of the chelate compounds of a Schiff base, which compounds promote the release of the proton at the α-carbon of the amino acid and the racemization of the amino acid. A possible chelate structure in *p*-nitrosophenol (*p*-quinoneoxime), as an example, is shown in I.



(I)



(II) (oxime type)

Therefore, when the optically-active asymmetric structure is introduced to the catalyst, it could bring about differences in the stabilities or in the rates of the formation of these chelates with a metal ion between L- and D-amino acids. Those differences could appear as differences in the racemization rates between L- and D-amino acids. Therefore, we wish to report here the first observation of the stereospecific racemization reaction for amino acid with the hindered biphenyl, (S)-2′-nitro-2-hydroxy-5-nitroso-6,6′-dimethylbiphenyl(II).7) The racemization activities of the catalyst for L- and D-alanine respectively were studied in the presence of the cupric ion.

The procedure for the racemization reaction was as ollows; the reaction mixtures containing L- or D-alanine (0.5 mmol), cupric sulfate pentahydrate (0.05 mmol), and the catalyst (II) (0.0125 mmol) were adjusted to pH 10 with sodium hydroxide to make a total volume of 0.5 m*l* with water, and then the mixture was shaken in a sealed tube at 31° or 40°C. After the reaction, 6 N hydrochloric acid (0.5 m*l*) was added to stop the racemization reaction. The determination of the ratio of L- and D-alanines was performed as follows; the reaction mixtures were centrifuged to precipitate the used catalyst, and the supernatants were dried up. After the residues had been converted into l-menthyl D- and L-trifluoroacetylalaninates, the ratio of L- and D-alanines was determined by means of gas chromatography. Careful measurements, compared with the calibrated curve, led us to an estimated error of ±0.5%.

The catalytic activities for L- and D-alanines are compared in Table 1 in terms of the contents of the racemized D- and L-alanines and the difference between the two. Though the racemic catalyst, of course, showed no difference in the catalytic activities between L- and D-alanines, II possessed some stronger activities for L-alanine than for D-alanine. The difference in the catalytic activity lay in the range from 3.0 to 5.0% in the contents of L- and D-alanines racemized from the D- and L-alanines substrates respectively. Such a catalytic property may serve as a model for the high stereospecificities often observed in the interactions of metallo-enzymes and their substrates. A stereochemical relationship between the catalyst and substrate will be discussed in a presentation of a molecular model in This Bulletin in the near future.

TABLE 1. RACEMIZATION ACTIVITY OF THE CATALYST

Configuration of catalyst	Substrate	Exp. 1.		Exp. 2		Exp. 3	
		D- or L-ala. content (%)b)	Differencec)	D- or L-ala. content (%)	Difference	D- or L-ala. content (%)	Difference
Racemic	L-alanine D-alanine	D : 20.4 L : 19.8	0.6	D : 28.5 L : 29.0	0.5	D : 34.0 L : 34.5	0.5
S	L-alanine D-alanine	D : 20.1 L : 16.9	3.2	D : 26.2 L : 21.2	5.0	D : 29.5 L : 26.5	3.0

Reaction mixture (0.5 m*l*) containing 0.5 mmol alanine, 0.05 mmol $CuSO_4 \cdot 5H_2O$ and 0.0125 mmol catalyst (II) was shaken at pH 10.0.

a) Racemization conditions were as follows:
Exp. 1: at 31°C for 162 hr. Exp. 2: at 31°C for 168 hr. Exp. 3: at 40°C for 72 hr.

b) Content of D- or L-alanine after the racemization reaction.

c) Difference between contents of L- and D-alanines racemized from D- and L-alanines, respectively.

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1) Presented at the 24th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1971.

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7) II was derived from (S)-2′-nitro-2-amino-6,6′-dimethylbiphenyl; its diazonium salt was hydrolyzed to the phenol derivative, and then treated with sodium nitrite. The method will be reported in detail in the near future.