Synthetic Resins Catalyzing the Racemization of Amino Acids. II. The Activity of the Resins

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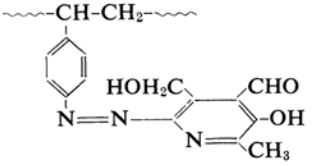
(Received March 5, 1963)

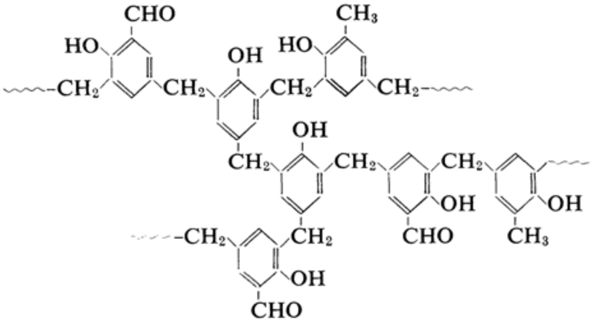
In the previous paper it was reported that some synthetic resins, to which pyridoxal is partially attached or which have a structure partially similar to salicylaldehyde, catalyzed the racemization of amino acids in the presence of cupric ions[1,2]. It was suggested that the conditions under which these catalytic resins are maximally active probably depend on many factors, such as the pH of the reaction mixture, the reaction temperature, the concentration of substrate and the presence of metal ions. Thus, in the present work, a study will be made of the influence of these conditions on the resin-catalyzed racemization.

Studies were made using two typical resins in order to elucidate the general properties of the resins. One (Resin I) is a styrene-divinylbenzene resin, to part of which pyridoxal is attached through azo bondings.



Partial structure of Resin I

The other (Resin II) is chemically-modified *o*-cresol-phenol-formalin resin, part of which is structurally similar to salicylaldehyde, —a benzene ring with formyl and hydroxy groups ortho to each other—



Partial structure of Resin II

Experimental

Amino Acid Solutions.—These solutions were prepared by dissolving 0.1 mol. of an amino acid and a given amount of a metal salt in a 0.1 M buffer solution and then adjusting it to the desired pH in a final volume of 100 ml. The buffers used were as follows: pH 4 to 5, acetate; pH 6 to 8, phosphate; pH 9 to 12, borate.

Catalytic Resins.—The preparation of Resins I and II was described in the previous paper[2]. Resin I was prepared from a styrene-divinylbenzene resin by nitration, reduction, diazotization, and subsequent coupling with pyridoxal in a mixture of water and pyridine. The formyl content was 0.10 mmol. per g. of dry resin. Resin II was prepared from *o*-cresol-formalin resin by converting the methyl groups in the resin to formyl groups. The crushed resin was oxidized with cromium trioxide in a mixture of acetic acid and acetic anhydride, and the acetal product was hydrolyzed with hydrochloric acid. The formyl content of Resin II was 0.24 mmol. per g. of dry resin.

Racemization Procedure.—A mixture of 5 ml. of an amino acid solution, prepared as described above, and 1 g. of Resin I or II was heated in a sealed test tube while being vigorous shaken in a water bath of the desired temperature. After the reaction, the catalyst was removed by filtration. The filtrate was diluted with a definite volume of 12 N hydrochloric acid, and its optical rotation was measured. For glutamic acid, the measurements were made after this acidic solution had been heated for 2 hr. at 100°C to reconvert the pyroglutamic acid formed during the racemization reaction to glutamic acid[3].

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

[2] K. Toi, Y. Izumi and S. Akabori, ibid., **36**, 734 (1963).

[3] H. Wilson and R. K. Cannan, *J. Biol. Chem.*, **119**, 309 (1937).

Determination of the Amino Acid Content of the Reaction Mixture.—An aliquot of the solution used for optical rotation measurements was evaporated to dryness. The residue was dissolved in a small amount of water, and the solution was made alkaline. The alkaline solution was evaporated to dryness. The residue was dissolved in water, and the amino acid content of the solution was determined by the ninhydrin colorimetric method[4].

Results and Discussion

Effect of the pH Value of the Reaction Mixture.—The resin-catalyzed racemization of amino acids was tested as a function of pH in the presence of cupric ions. The test was conducted with L-alanine at 98°C. The results are shown in Figs. 1 and 2.

The reaction is influenced by the number of cupric ions, as is shown in Fig. 5. An increase in the concentration of cupric ions accelerates the reaction, but it also causes the racemization of amino acids in the absence of resin on heating in an alkaline medium, especially above pH 11. Therefore, it was undesirable to use much cupric salt in this test.

An aliquot of the L-alanine solution used for the test was simultaneously heated at the same temperature in the absence of resin, as a control for the resin-catalyzed racemization. The degree of racemization of the control was substracted from that of the sample heated with resin.

The results show that the catalysts have optima at about pH 10.5. In their work on the pyridoxal-catalyzed racemization of L-alanine in a non-enzymatic system, Snell et al. found that the optimum pH of pyridoxal was between 9 and 10[5]. Thus the two results agree roughly with each other.

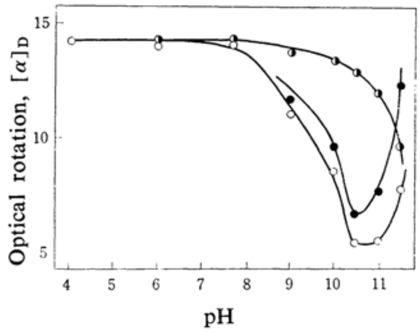

Fig. 1. Effect of pH on racemization of L-alanine by Resin I.

The reaction mixture contained 1 g. of Resin I and 5 ml. of amino acid solution containing 1 M L-alanine, 0.025 M $Cu(CH_3COO)_2\cdot H_2O$, and 0.1 M concentrations of the following buffers: pH 4 to 5, acetate; pH 6 to 8, phosphate; pH 9 to 12, borate. Reaction time, 1 hr. at 98°C.

○ $[\alpha]_D$ resin-catalyzed racemization
◑ $[\alpha]_D$ recemization in absence of resin
● Difference between ○ and ◑

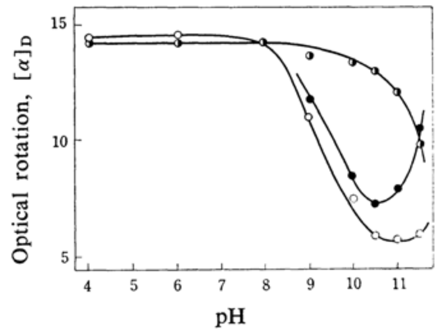

Fig. 2. Effect of pH on racemization of L-alanine by Resin II.

The reaction mixture contained 1 g. of Resin II and 5 ml. of amino acid solution containing 1 M L-alanine, 0.025 M $Cu(CH_3COO)_2\cdot H_2O$, and 0.1 M concentrations of the following buffers: pH 4 to 5, acetate; pH 6 to 8, phosphate; pH 9 to 12, borate. Reaction time, 1 hr. at 98°C.

○ $[\alpha]_D$ resin-catalyzed racemization
◑ $[\alpha]_D$ racemization in absence of resin
● Difference between ○ and ◑

Effect of the Reaction Temperature. — The racemization of L-alanine with Resins I and II was carried out at 60, 80 and 97°C. The results are shown in Figs. 3 and 4.

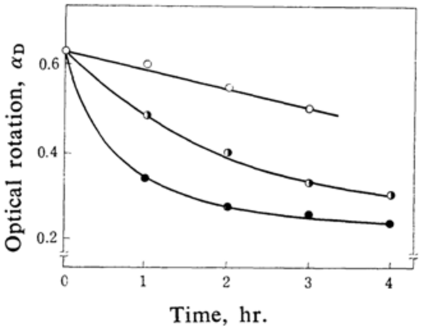

Fig. 3. Apparent racemization of L-alanine by Resin I at 60, 80 and 97°C (pH 10).

The reaction mixture contained 1 g. of Resin I and 5 ml. of amino acid solution containing 1 M L-alanine, 0.025 M $Cu(CH_3\cdot COO)_2\cdot H_2O$ and 0.1 M borate buffer.

○ Optical rotation in reaction at 60°C
◑ Optical rotation in reaction at 80°C
● Optical rotation in reaction at 97°C

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4) E. W. Yemm and E. C. Cocking, *Analyst*, **80**, 209 (1955).

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

TABLE I. COMPAPISON OF ACTIVITIES OF METAL IONS IN THE RESIN-CATALYZED RACEMIZATION OF L-ALANINE

Metal ion	Solution of L-alanine		Racemization							
			with Resin I				with Resin II			
	α_D	$[\alpha]_D$	α_D	Recovery of L-alanine	$[\alpha]_D$	Racemization yield	α_D	Recovery of L-alanine	$[\alpha]_D$	Racemization yield
Cu^{2+}	0.43	15	0.20	94	7.2	52	0.06	86	2.3	85
Co^{2+}	0.43	15	0.35	90	11	27	0.24	78	10	33
Ni^{2+}	0.44	15	0.40	87	15	0	0.29	73	13	13
Mg^{2+}	0.44	15	0.39	89	15	0	0.28	72	13	13
Zn^{2+}	0.44	15	0.40	94	14	7	0.32	79	14	7

The reaction mixture contained 1 g. of Resin I or II and 5 ml. of amino acid solution (pH 10) containing 1.0 M L-alanine, 0.06 M a metal salt, and 0.1 M borate buffer. The metal salts used were $Cu(CH_3COO)_2 \cdot H_2O$, $CoCl_2 \cdot 6H_2O$, $Ni(CH_3COO)_2 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$ and $Zn(CH_3COO)_2 \cdot 2H_2O$. Reaction time, 1 hr. at 98°C. The optical rotation α_D was measured in acidic solution prepared by diluting the reaction mixture with two volumes of 6 N HCl.

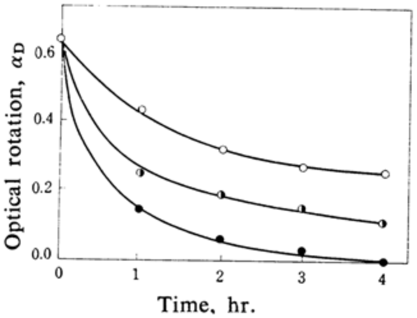

Fig. 4. Apparent racemization of L-alanine by Resin II at 60, 80 and 97°C (pH 10).

The reaction mixture contained 1 g. of Resin II and 5 ml. of amino acid solution containing 1 M L-alanine, 0.06 M $Cu(CH_3 \cdot COO)_2 \cdot H_2O$ and 0.1 M borate buffer.

○ Optical rotation in reaction at 60°C
◑ Optical rotation in reaction at 80°C
● Optical rotation in reaction at 97°C

In the presence of a given amount of cupric ion, the racemization reaction proceeded like an ordinary chemical reaction in that, as the temperature was increased, the rate of the reaction increased.

Effect of the Type of Metal Ion Present.—The racemization of amino acids catalyzed by the resin proceeds well in the presence, but not in the absence, of metal ions. A comparison was made of the effect of fixed concentrations of metal ions on the catalysis of the racemization by the resin. Table I shows the results of this test. Of the metal ions tested, cupric ions were the most effective, while cobaltous ions were quite active. Experiments with ferric and aluminum ions were impossible because these metals precipitated as hydroxides under the conditions used.

The concentration of metal ions in the reaction mixture has a considerable effect on the rate of racemization (Fig. 5). An increase in concentration of metal ions raises the rate of the reaction.

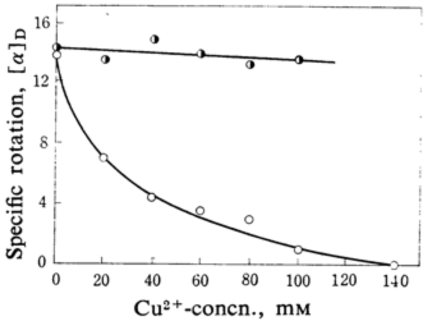

Fig. 5. Effect of the Cu^{2+}-concentration on racemization of L-alanine by Resin II.

The reaction mixture contained 1 g. of Resin II and 5 ml. of amino acid solution (pH 10) containing 1.0 M L-alanine and 0.1 M borate buffer. Reaction time, 1 hr. at 98°C.

○ $[\alpha]_D$ with Resin II
◑ $[\alpha]_D$ in absence of resin

Retention of Resin Activity.—It is desirable that the catalytic resin should retain its activity during the reaction and on repeated use. The activity of Resins I and II on repeated use was, therefore, tested; the results are given in Table II. L-Alanine was tested with the resin for 1 hr. at 98°C in the presence of cupric ions, and the resin was recovered by filtration. After recovery, the catalyst was washed with borate buffer and used for the next run. The activity of the resin was retained for at least seven runs.

The Racemization of Various Amino Acids.—A study was made of the extent of racemization of a series of amino acids. As may be

Table II. Retention of activity

	Apparent racemization, α_D	
	Resin I	Resin II
Before reaction	0.42	0.42
Original resin	0.17	0.08
After 1st recovery	0.16	0.10
After 2nd recovery	0.18	0.09
After 3rd recovery	0.19	0.08
After 4th recovery	0.18	0.12
After 5th recovery	0.11	0.08
After 6th recovery	0.16	0.11

The reaction mixture contained 1 g. of Resin I or II and 5 ml. of amino acid solution (pH 10) containing 1 M L-alanine, 0.06 M $Cu(CH_3COO)_2 \cdot H_2O$ and 0.1 M borate buffer. Reaction time, 1 hr. at 98°C.

seen in Table III, each of the amino acids tested underwent racemization to some extent. In the case of L-phenylalanine, L-tyrosine and L-methionine, cobaltous ions were chosen instead of cupric ions because of the insolubility of the copper complexes of these amino acids under the conditions used. L-Alanine racemized most rapidly, and L-aspartic acid, at quite a high rate. In the course of the reaction, amino acids underwent some spontaneous decomposition. The recovery of amino acid was estimated by the optical density of the ninhydrin colorimetric reaction of the amino acid after its isolation from the reaction mixture on a paper chromatogram.

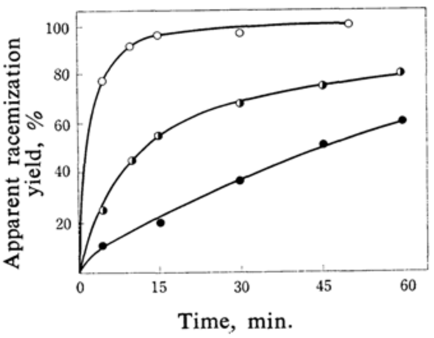

Fig. 6. Comparison of the activities of the resins and pyridoxal in catalysis of the racemization of L-alanine at 98°C.

The reaction system contained 1 g. of Resin I, 0.42 g. of Resin II, or 0.1 mmol. of pyridoxal hydrochloride and 6 ml. of L-alanine solution (pH 10.0) containing 0.83 M L-alanine, 0.083 M $Cu(CH_3COO)_2 \cdot H_2O$ and 0.1 M borate buffer. Reaction time, 1 hr. at 98°C.

○ Pyridoxal ◑ Resin II ● Resin I

A Comparison of the Activity of the Resin and of Pyridoxal.—In non-enzymatic systems, pyridoxal may be most effective as a catalyst for amino acid racemization. A comparison was made of the effects of the resin and of pyridoxal on the rate of racemization of L-alanine in the presence of cupric ions at 80 and 98°C, though pyridoxal was dissolved in the reaction systems. Conditions were used under which the catalytic resin passed through its maximum activity at a given temperature. The amount of each catalyst was adjusted to give equal contents of aldehyde.

The resin has no stereospecificity and can simultaneously catalyze the conversion of both the L- to the D-form and the D- to the L-form. Therefore, the racemization of amino acids is,

Table III. Racemization of various amino acids

Amino acid ([α]D)	Metal ion	pH	Racemization						
			without Resin	with Resin I			with Resin II		
			[α]D	[α]D	(Racemization yield, %)	Recovery of amino acid, %	[α]D	(Racemization yield, %)	Recovery of amino acid, %
Ala (+15)	Cu^{2+}	10.5	+14	+ 6.4	(57)	99	+ 2.2	(85)	91
Val (+25)	Cu^{2+}	10.5	+25	+25	(0)	99	+19	(24)	98
Asp (+26)	Cu^{2+}	10.0	+25	+23	(11)	95	+ 5.0	(81)	90
Glu (+32)	Cu^{2+}	10.0	+27	+26	(19)	95	+20	(37)	91
Arg (+29)	Cu^{2+}	10.0	+27	+27	(7)	87	+22	(24)	47
Lys (+26)	Cu^{2+}	10.0	+26	+22	(15)	74	+17	(35)	65
Phe (−9.1)	Co^{2+}	10.5	− 8.2	− 8.3	(9)	99	− 5.8	(36)	99
Tyr (−13)	Co^{2+}	10.5	−10	− 9.0	(31)	98	− 6.3	(52)	97
Met (+24)	Co^{2+}	10.5	+22	+20	(17)	100	+15	(38)	98

The reaction mixture contained 1 g. of Resin I or II and 5 ml. of amino acid solution, containing 1 M amino acid, 0.06 M metal salt, and 0.1 M borate. Because of the insolubility of their metal complexes in the case of L-valine, L-tyrosine and L-methionine, the concentration was reduced to 0.5 M amino acid and 0.03 M metal salt.

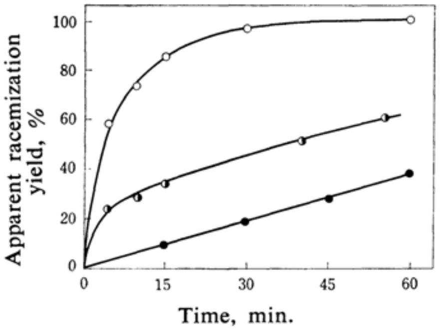

Fig. 7. Comparison of activities of the resins and pyridoxal in catalysis of racemization of L-alanine at 80°C.

The reaction system consisted of 1 g. of Resin I, 0.42 g. of Resin II, or 0.1 mmol. of pyridoxal hydrochloride and 6 ml. of L-alanine solution (pH 10.5) containing 0.83 M L-alanine, 0.033 M $Cu(CH_3COO)_2 \cdot H_2O$ and 0.1 M borate buffer. Reaction time, 1 hr. at 80°C.

○ Pyridoxal ◑ Resin II ● Resin I

in theory, a first-order reaction. The rate of the reaction should be proportional to the concentration of L-alanine at a fixed concentration of cupric ions.

The rate observed in the resin-catalyzed racemization of L-alanine described a curve like a first-order reaction, but it deviated from the theoretical course when the reaction period was long, as may be seen in Figs. 6 and 7. However, after a certain initial period, the rate may be approximately regarded as of the first order.

Thus, the rate constants, k, of the reaction with resin and pyridoxal were compared over the initial five-minute period. The values obtained are summarized in Table IV. At 98°C, the activities of Resins I and II were, respectively, about 8×10^{-2} and 2×10^{-1} of that of pyridoxal.

TABLE IV. RATE CONSTANT

Temp. °C	k, min^{-1}		
	Pyridoxal	Resin I	Resin II
98	3.0×10^{-1}	2.3×10^{-2}	6.4×10^{-2}
80	1.8×10^{-1}	9.2×10^{-3}	5.5×10^{-2}

The values of k were calculated with the formula,

$$k = \frac{2.3}{5} \log \frac{100}{100 - x}$$

where x is the apparent racemization yield.

Summary

1) A study has been made of the factors influencing the racemization of amino acids by resinous catalysts with structures partially similar to those of pyridoxal and salicylaldehyde.

2) The optimum pH of the reaction was about 10.5.

3) A rise in temperature resulted in an increase in the rate of the reaction.

4) The racemization reactions catalyzed by the resins required the presence of metal ions, and cupric ions were found to be most effective.

5) Of the amino acids tested, L-alanine was racemized most rapidly.

6) The resinous catalysts retained activity after repeated usage.

7) The racemization-activity of the resins was compared with that of pyridoxal.

The author wishes to express his thanks to Professor Shiro Akabori and Dr. Yoshiharu Izumi for their valuable advice and encouragement throughout this work.

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