Synthetic Resins Catalyzing the Racemization of Amino Acids. I. The Preparation of the Resins

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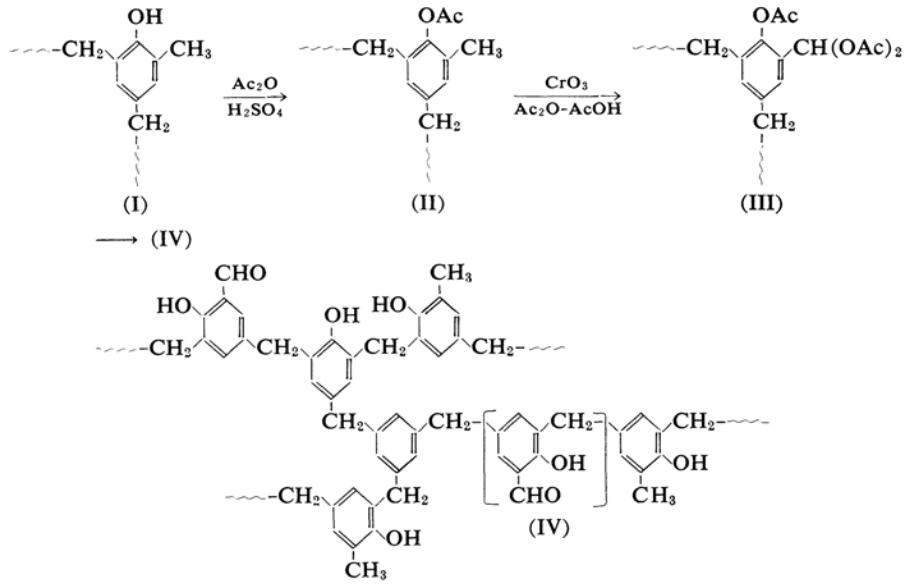
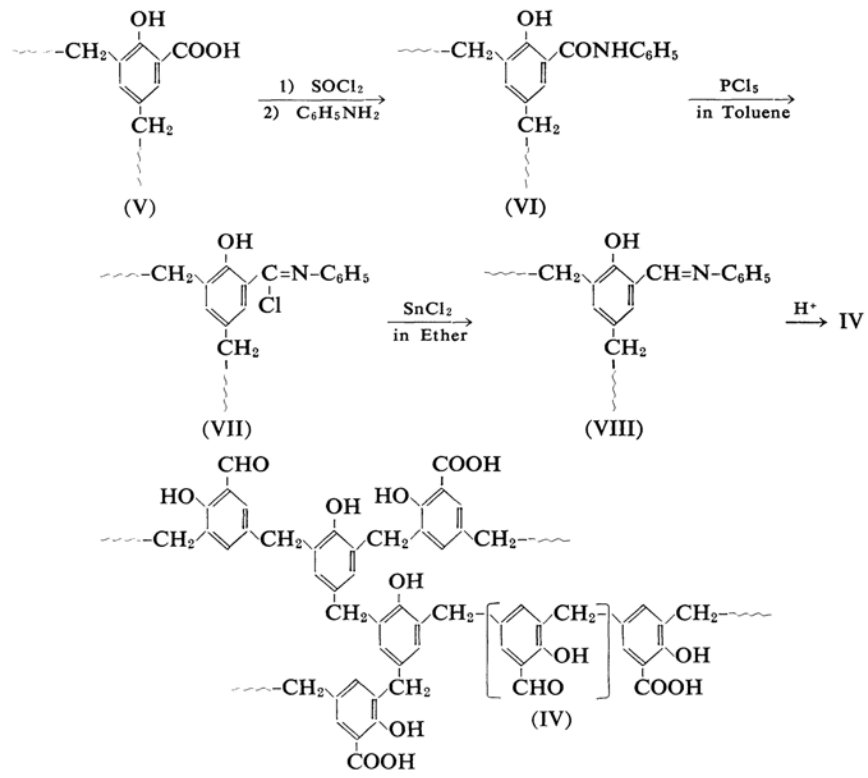
In general, optically-active free amino acids racemize only slowly even in a strongly alkaline or acidic solution at a boiling temperature. Heating the solution at a much higher temperature under high pressure accelerates the reaction, but this acceleration is accompanied by an appreciable decomposition of the amino acids. The racemization processes most commonly used involve heating suitable derivatives of optically-active amino acid with alkali or treating free amino acid with acetic anhydride or ketene in the presence of alkali and hydrolyzing the resulting acetyl-DL-amino acid. In the latter method, however, the recovery of the racemized amino acid has been achieved only with some difficult. Thus, it would be useful to prepare a catalyst which would accelerate the racemization of optically-active free amino acids under milder conditions.

In nature the so-called racemase catalyzes the racemization of amino acid under physiological conditions. These enzymes require pyridoxal phosphate as a coenzyme[1-5]. In

1) W. A. Wood and I. C. Gunsalus, *J. Biol. Chem.*, **190**, 403 (1951).
2) P. Ayengar and E. Roberts, ibid., **197**, 453 (1952).
3) W. A. Wood and S. Narrod, *Arch. Biochem. Biophys.*, **35**, 462 (1952).
4) H. T. Huarg, D. A. Kita and J. W. Davidson, *J. Am. Chem. Soc.*, **80**, 1006 (1958).
5) A. Ichihara, S. Furuya and M. Suda, *J. Biochem.*, **48**, 277 (1960).



Structure of Resin A

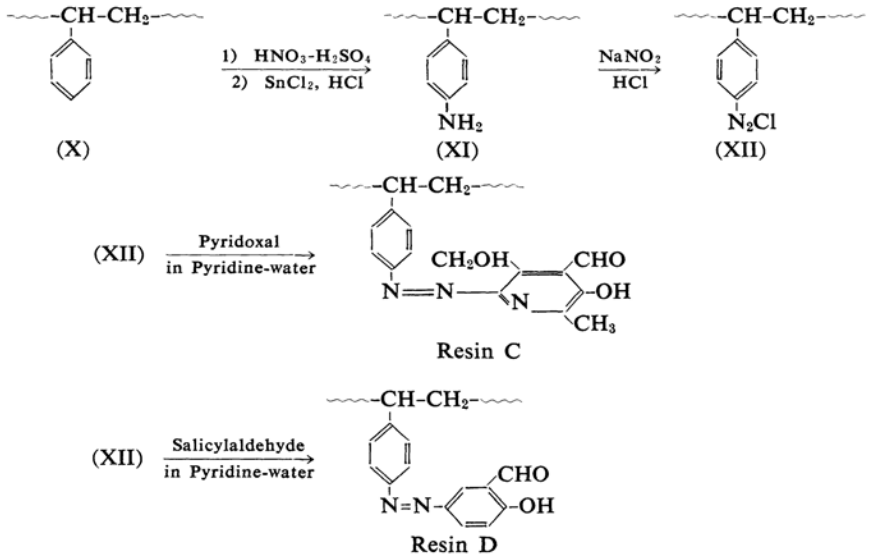


Structure of Resin B

studies of the non-enzymatic reaction between pyridoxal and amino acids, Snell et al. found that pyridoxal itself catalyzes the racemization of amino acids in the presence of some metal ions[6] and proposed a general mechanism for pyridoxal-catalyzed reactions[7].

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

7) M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 653 (1954).



Furthermore, it has been demonstrated that benzene analogues of pyridoxal, such as salicylaldehyde and its derivatives, namely 4- and 6-nitrosalicylaldehyde, also catalyze the racemization of amino acids in the presence of metal ions[8-10].

In a previous paper[11] it was reported that a resin, part of which was structurally similar to salicylaldehyde, namely, a benzene ring with formyl and hydroxy groups at positions ortho to each other (IV), accelerated the racemization of L-alanine, L-aspartic acid and L-glutamic acid in the presence of cupric ions.

In the present work, various methods of preparing such resin were investigated. Thus, four resins (Resins A—D) were prepared. All of them were found to be able to catalyze the racemization of amino acids in the presence of cupric ions.

Resin A, the activity of which has been reported in a previous paper[11], was prepared from *o*-cresol-phenol-formalin resin (I) by converting the methyl groups in the resin to formyl groups. The crushed resin was oxidized with chromium trioxide in a mixture of acetic acid and acetic anhydride, and then the acetal product III was hydrolyzed with 4 N hydrochloric acid to give IV.

Resin B was prepared from salicylic acid-phenol-formalin resin (V) by reducing the carboxy groups in the resin to formyl groups according to the Sonn-Müller method[12]. The crushed resin was treated with thionyl chloride and then with aniline to give the anilides VI in the resin. The anilides were converted to chloroimides VII by treatment with phosphorous pentachloride in toluene, and the chloroimides were then reduced to aldimines VIII with stannous chloride in ether saturated with hydrogen chloride. The aldimines were finally converted to aldehyde groups IV by hydrolysis with N hydrochloric acid.

Resins C and D were obtained from diazotized poly-*p*-amino styrene (XII) by coupling with pyridoxal and salicylaldehyde respectively. A cross-linked polystyrene resin (X) was nitrated with a mixture of nitric acid and sulfuric acid, and then the nitro groupings in the resin were reduced to amino groupings with stannous chloride in hydrochloric acid according to the procedure of D'Alelio[13]. The amino-resin (XI) was then treated with sodium nitrite in hydrochloric acid, and the XII produced was subjected to coupling with pyridoxal or salicylaldehyde. The coupling was carried out in a mixture of pyridine and water. The resins so obtained had the dark red color characteristic of the azo groupings.

The amount of formyl group in the resin was measured by Schultes' procedure[14]. A given amount of the resin was treated with aqueous hydroxylamine sulfuric acid, and the amount of sulfuric acid liberated was determined quantitatively by alkaline titration (Table I).

The racemization experiment was conducted

8) K. Ohno, I. Sasaji and M. Hara, Abstract of the 12th Annual Meeting of the Chemical Society of Japan (1959), p. 27.
9) K. Ohno, I. Sasaji and M. Hara, Japanese Pat. 295110.
10) A. E. Martell and M. Carvin, "Chemistry of the Metal Chelate Compounds", Prentice-Hall, Inc., New York, N. Y. (1952), pp 397-401.
11) K. Toi, Y. Izumi and S. Akabori, This Bulletin, 35, 1422 (1962).
12) A. Sonn and E. Müller, *Ber.*, **52**, 1927 (1919).
13) G. F. D'Alelio, U. S. Pat. 2366008.
14) H. Schultes, *Z. Angew. Chem.*, **47**, 258 (1934).

TABLE I. ALDEHYDE CONTENTS OF THE RESINS

Resin	Aldehyde content mmol./g.
A	0.24
B	0.31
C	0.10
D	0.20

with L-alanine in the presence of cupric ions. The results are shown in Figs. 1 to 3. Racemization tests with Resin A for L-alanine, L-aspartic acid and L-glutamic acid have already been reported in a previous paper[11]. An aqueous L-alanine solution of pH 10 containing cupric ions and a borate buffer was heated with the resin at 100°C. Aliquots of the reaction mixture were removed at varying intervals and filtered. Each filtrate was diluted with an

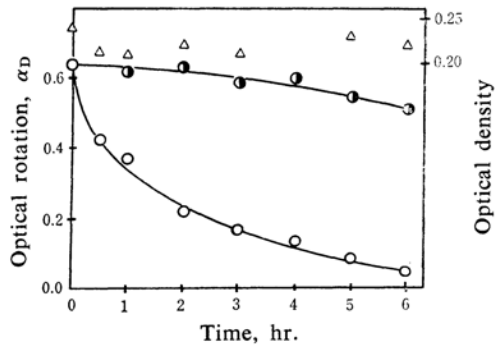


Fig. 1. Racemization of L-alanine by Resin B in the presence of cupric ion.
- ○ Optical rotation in the resin-catalyzed reaction
- ◑ Optical rotation of heated sample without resin
- △ Optical density measured by the ninhydrin method

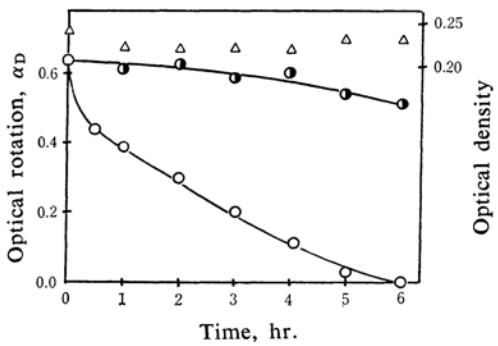


Fig. 2. Racemization of L-alanine by Resin C in the presence of cupric ion.
- ○ Optical rotation in the resin-catalyzed reaction
- ◑ Optical rotation of heated sample without resin
- △ Optical density measured by the ninhydrin method

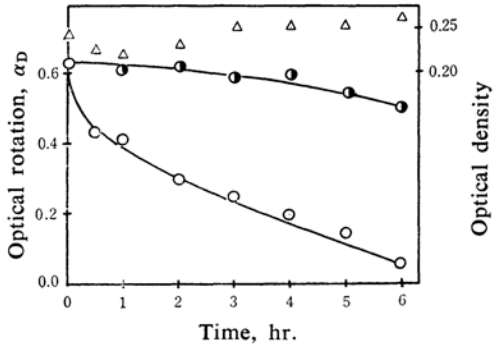


Fig. 3. Racemization of L-alanine by Resin D in the presence of cupric ion.
- ○ Optical rotation in the resin-catalyzed reaction
- ◑ Optical rotation of heated sample without resin
- △ Optical density measured by the ninhydrin method

equal volume of 12 N hydrochloric acid, and its optical rotation was measured. The amino acid contents of the reaction mixtures were checked in order to determine whether or not the simultaneous decomposition of amino acid had occurred. Appropriate aliquots of the acidic solutions, which had been prepared for rotation measurement, were evaporated to dryness. The residues were dissolved in water, and the solutions were made alkaline with sodium hydroxide. Each alkaline solution was evaporated to dryness. The residues was dissolved in water, and the amino acid content of each was determined by the ninhydrin colorimetric method[15]. The values of their optical densities are plotted in Figs. 1 to 3.

It is considered that the reaction conditions under which these catalysts will pass through their maximum activities depend on many factors, such as the pH value of the reaction mixture, the reaction temperature and the nature and concentration of metal ions used.

Experimental

Preparation of Resin A.—A mixture of 108 g. of *o*-cresol, 38 g. of phenol, 160 ml. of 37% formalin and 5 ml. of 50% aqueous sodium hydroxide was stirred at 80~90°C. When the mixture became viscous after 3 hr., stirring was stopped. This mixture was then heated at 100°C for 15 hr. The rigid mass which resulted was crushed to 50~200 mesh, thoroughly washed with water, and dried at 50°C under vacuum. Forty grams of this product were mixed with 240 ml. of acetic anhydride containing 5 ml. of concentrated sulfuric acid for 1 hr., and then 160 ml. of acetic acid were added. Into

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

this mixture were slowly stirred 16 ml. of concentrated sulfuric acid while it was being externally cooled. When the temperature of the mixture decreased to 0°C, 20 g. of chromium trioxide were added in small portions at such a rate that the temperature did not rise above 5°C. Stirring at 4~5°C was continued for 3 hr. after the addition of the chromium trioxide. The reaction mixture was vigorously stirred into 1.5 l. of water. The product was separated by decantation and washed with water. This materia! was then hydrolyzed with 300 ml. of 4 N hydrochloric acid under reflux for 3 hr. The final product was filtered with suction, washed with aqueous 5% sodium bicarbonate and then thoroughly with water, and dried at 50°C under vacuum.

Preparation of Resin B.—A mixture of 13.8 g. of salicylic acid, 4.7 g. of phenol, 36 g. of 37% formalin, and 45g. of 20% aqueous sodium hydroxide was stirred at 100°C. When the mixture became viscous, stirring was stopped and the mixture was allowed to stand at 100°C for 12 hr. The resulting rigid mass was crushed to 100~200 mesh, washed thoroughly with dilute hydrochloric acid and then with water, and dried.

Ten grams of this product were swollen with *N*, *N*-dimethylformamide and stirred at 0°C in 65 ml. of thionyl chloride for 3 hr. The resulting resin was filtered, washed with chloroform, and stirred at 0°C for 5 hr. in a mixture of 60 ml. of chloroform, 20 ml. of aniline and 10 ml. of triethylamine. After the mixture had been allowed to stand overnight at room temperature, the product was filtered washed with chloroform and dried. The anilides produced were added to a solution obtained by dissolving 12 g. of phosphorous pentachloride in 100 ml. of toluene and stirred at 90°C for 6 hr. The resulting product, the chloroimides, was filtered, washed with toluene, and then subjected to reduction by stirring it with a solution which was prepared by dissolving 40 g. of stannous chloride dihydrate in 200 ml. of ether saturated with hydrogen chloride. The product obtained was filtered, washed with ether, and then stirred in 200 ml. of N hydrochloric acid at 60°C for 6 hr. The final product was filtered, thoroughly washed with water, and dried under vacuum. Yield, 12 g.

Preparation of Resin C.—A cross-linked polystyrene resin (8% of divinylbenzene, 100~200 mesh) was converted to a poly-*p*-aminostyrene following the procedure of D' Alelio[13]. The polystyrene resin was nitrated with nitric acid and sulfuric acid, and then the nitro groups were reduced to amino groups by stannous chloride in hydrochloric acid. The total capacity of its anion exchange was 1.02 meq. per g.

Ten grams of poly-*p*-aminostyrene resin were stirred in 50 ml. of N hydrochloric acid at 0°C with external cooling. To this suspension 5 ml. of 20% aqueous sodium nitrite were added drop by drop, and stirring was continued for 2 hr. at 0°C. The resulting resin was filtered and washed with cooled aqueous urea and then with cooled water. The diazotized resin was suspended in a solution which was cooled to 0°C and which was prepared by dissolving 3.0 g. of pyridoxal hydrochloride in a mixture of 20 ml. of pyridine and 20 ml. of water. The suspension was stirred at 0°C for 15 hr. The resulting resin was filtered, washed successively with dilute hydrochloric acid, dilute sodium acetate and water, and then dried under vacuum. Yield, 10.3 g.

Preparation of Resin D.—Ten grams of poly-*p*-aminostyrene were diazotized as has been described in the previous paragraph on Resin C. A solution was made of 3.7 g. of salicylaldehyde and 1 g. of sodium carbonate in a mixture of 30 ml. of N sodium hydroxide and 20 ml. of pyridine. This salicylaldehyde solution was cooled to 0°C, and the diazotized resin was added. This mixture was stirred at 0°C for 15 hr. The resulting resin was filtered and washed thoroughly with water. The washed resin was dried under vacuum. Yield, 10.5 g.

Measurement of the Aldehyde Contents of the Resins.—One gram of a resin was shaken for 3 hr. at 60°C with 5 ml. of N hydroxylamine sulfuric acid in a sealed test tube. The mixture was filtered, and an aliquot of the filtrate was titrated with 0.1 N sodium hydroxide, using bromphenol blue as an indicator.

Racemization Test. — An aqueous solution of L-alanine was prepared; it was adjusted to pH 10 with sodium hydroxide and was found to contain 17.8 g. of L-alanine and 0.45 g. of $CuCl_2 \cdot 2H_2O$ in a total volume of 100 ml. Fifteen milliliters of the above solution, 15 ml. of 0.2 M borate buffer of pH 10, and 5 g of the resin were stirred at 100°C.

Summary

1) Four resinous catalysts (Resins A—D) for the racemization of amino acids were prepared. They had structures partially similar to that of salicylaldehyde or pyridoxal. Resins A and B were prepared by the chemical modification of *o*-cresol-phenol-formalin resin and salicylic acid-phenol-formalin resin respectively. Resins C and D were obtained by attaching chemically pyridoxal and salicylaldehyde residues respectively to a styrene-divinylbenzene lattice.

2) These resins catalyzed the racemization of amino acids in the presence of cupric ions at 100°C.

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