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Racemization Catalyst for Amino Acids. II. Racemization with Aurintricarbonic Acid

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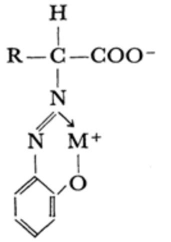
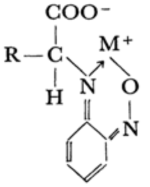
The catalytic racemization of amino acids with aurintricarboxylic acid was examined in the presence of cupric ion. The tests were conducted at various pH values to study the optimum pH for the racemization. Moreover the racemizations with aurintricarboxylic acid and with *o*-cresolphthalein were compared and the results are briefly discussed with regard to the relationship between the structural features and the activities of these catalysts.

In the previous paper[1] the authors reported that several derivatives of *o*-nitrosophenol catalyzed the racemization of amino acid in the presence of metal ion just as salicylaldehyde,[2-5] or pyridoxal[6] does in a non-enzymatic system. Derivatives of *o*-nitrosophenol were examined as catalysts because they were easy to prepare from phenols by the action of nitrous acid or its esters. However, they are unsuitable as catalysts because the nitroso groups are sensitive to oxidation or reduction. Thus it would be useful to prepare a catalyst which is stable during chemical treatments.

In a study[1] on the racemizations of amino acid with derivatives of *o*-nitrosophenol, the authors have postulated a mechanism in which the racemization of amino acids proceeds through formation of a reactive chelate intermediate such as I or II, which is formed by reaction of *o*-nitrosophenol with metal ion and amino acid.

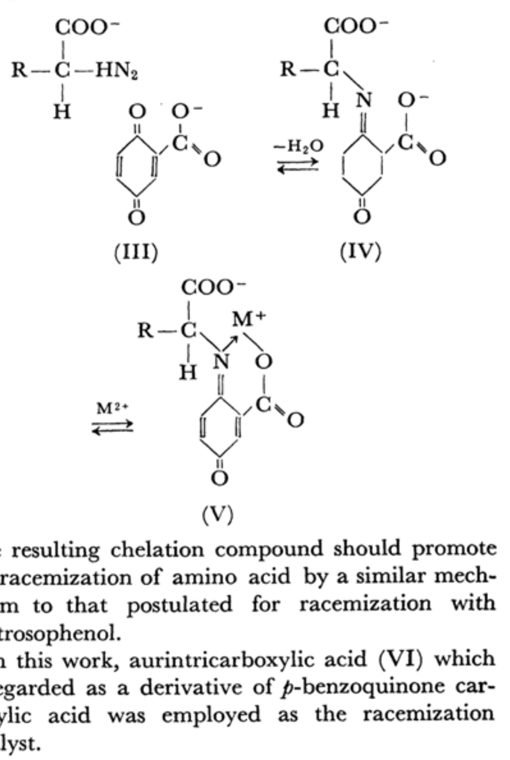


(I)



(II)

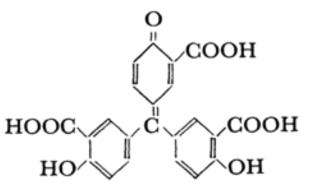
Similarly, *p*-benzoquinone carboxylic acid (III) reacts with amino acid to produce a *p*-benzoquinone imine compound (IV) and forms the chelation compound (V) as shown in the following scheme.



(III) (IV) (V)

The resulting chelation compound should promote the racemization of amino acid by a similar mechanism to that postulated for racemization with *o*-nitrosophenol.

In this work, aurintricarboxylic acid (VI) which is regarded as a derivative of *p*-benzoquinone carboxylic acid was employed as the racemization catalyst.



(VI)

This catalyst, the ammonium salt of which is well known as aluminon, can easily be prepared from salicylic acid, and its functional groups in the quinoid ring, that is the ketonic and carboxylic groups, are stable during chemical treatments, in

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[1] Part I of this series: K. Hirota and Y. Izumi, This Bulletin, **40**, 178 (1967).

[2] K. Ohno, I. Sasaji and M. Hara, Abstract of the 12th Annual Meeting of the Chemical Society of Japan, April, 1959, p. 27.

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

[4] K. Toi, *ibid.*, **36**, 739 (1963).

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

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

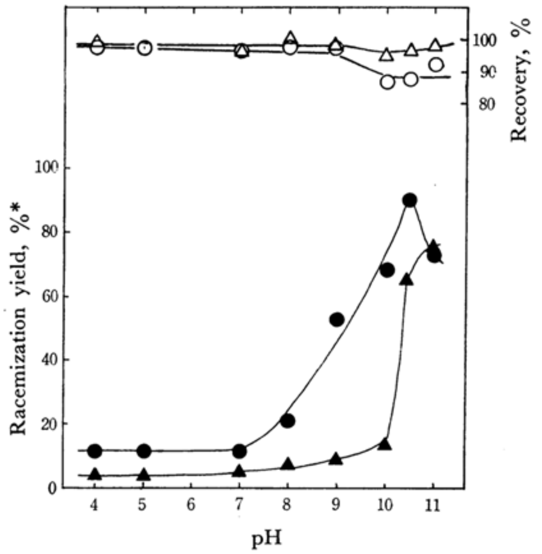


Fig. 1. Effect of pH on the racemization reaction in the presence of cupric ion.
Reaction mixtures of 5 m*l* contained 5 mmol L-alanine, 0.2 mmol aurintricarboxylic acid and 0.2 mmol $CuSO_4\cdot 5H_2O$. Reaction time, 4 hr at 100°C.
Racemization with aurintricarboxylic acid and $CuSO_4\cdot 5H_2O$.
● 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; $[\alpha]_0-[\alpha]/[\alpha]_0\times 100$.

contrast with the nitroso or aldehyde group in *o*-nitrosophenol or salicylaldehyde. From this point of view, aurintricarboxylic acid is suitable as a catalyst. Its racemization activity was measured using L-alanine in the presence of cupric ion.

Experimental

Catalysts. Aurintricarboxylic acid was prepared from salicylic acid and formaldehyde by the action of sodium nitrite according to the method reported by Heisig and Lauer.7) *o*-Cresolphthalein was obtained commercially.

Racemization Procedure. A solution containing 5 mmol of L-alanine, 0.2 mmol of cupric sulfate tetrahydrate and 0.2 mmol of catalyst was adjusted to the required pH value with 2N sodium hydroxide. And water was added to make final volume of five milliliters. Then it was vigorously shaken in a sealed tube at 100°C. The reaction mixture was diluted to 10 m*l* with 12N hydrochloric acid and the catalyst was removed by filtration. The filtrate was decolorized with charcoal and its optical rotation $[\alpha]_D$ was measured.

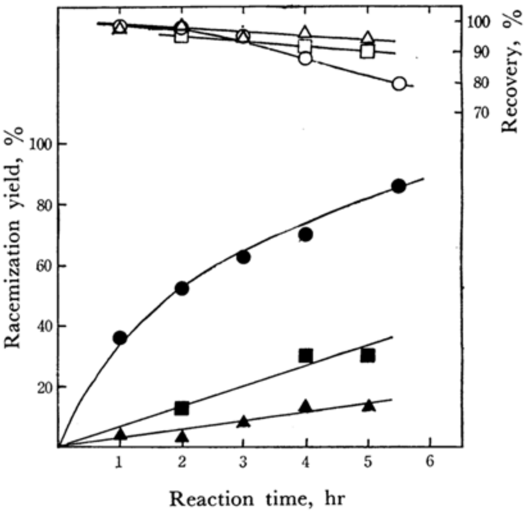
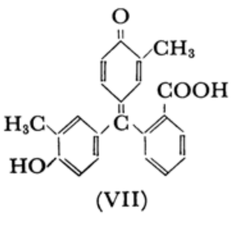


Fig. 2. Racemization activities of aurintricarboxylic acid and *o*-cresolphthalein in the presence of cupric ion.
Reaction mixtures of 5 m*l* (pH 10) contained 5 mmol L-alanine, 0.2 mmol aurintricarboxylic acid or *o*-cresolphthalein, and 0.2 mmol $CuSO_4\cdot 5H_2O$. Reaction time, 4 hr at 100°C.
Racemization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

Determination of Amino Acid. The alanine content of the reaction mixture was determined according to Toi's method.4)

Racemization at Various pH Values. Racemization of L-alanine with aurintricarboxylic acid was carried out at pH values of 4 to 11 for four hours at 100°C with cupric ion as the metal ion. Catalytic activity was observed between pH 4 and 10.5 with an optimum at about pH 10, as shown in Fig. 1. In the absence of aurintricarboxylic acid, especially above pH 10.5, cupric ion alone caused similar racemization. These results showed similar tendencies to those on the racemization of L-alanine with derivatives of *o*-nitrosophenol.1)

Relationship between the Activity and Structure of the Catalyst. The racemizations of L-alanine with aurintricarboxylic acid and with *o*-cresolphthalein (VII) in the presence of cupric ion were



(VII)

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7) G. B. Heisig and W. M. Lauer, "Organic Syntheses," Coll. Vol. I, p. 54 (1956).

compared and the results are shown in Fig. 2.

Aurintricarboxylic acid is more effective in racemization than *o*-cresolphthalein, which has a methyl group at the ortho position to the ketonic group in the quinoid ring. From the above results, the presence of a carboxylic group at the ortho position to the ketonic group seems essential for the racemization. Results also support the formation of chelation compound V during the racemization reaction, because catalysts such as *o*-cresolphthalein cannot form a chelation intermediate such as this which promotes the racemization.