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Decarboxylation Reaction of Oxalacetic Acid by Metal Chelates[1)]

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Decarboxylation of oxalacetic acid (OAA) by metal chelates was examined, using copper (II) as a metal and seven kinds of ligand, which forms N,N-coordinated five membered ring chelate, used were 6,6′-dimethyl-α,α′-dipyridyl, 4,4′-dimethyl-α,α′-dipyridyl, α,α′-dipyridyl, 5-nitro-*o*-phenanthroline, *o*-phenanthroline, 4,7-dimethyl-*o*-phenanthroline, and ethylenediamine. When the concentration ratio of dipyridyl to copper (II) was varied, 1 : 1 ratio of copper to dipyridyl effected the fastest decarboxylation rate of OAA, and the rate was faster than that by copper (II) ion alone.

Effect of each ligand on the reaction rate was examined and the reaction rate fell in the order of α,α′-dipyridyl, *o*-phenanthroline, and ethylenediamine; 5-nitro-*o*-phenanthroline, *o*-phenanthroline, and 4,7-dimethyl-*o*-phenanthroline; α,α′-dipyridyl, 6,6′-dimethyl-α,α′-dipyridyl, and 4,4′-dimethyl-α,α′-dipyridyl.

When the acetate buffer concentration was 0.1M, the reaction rate was faster, the smaller the p*K*a, and greater the catalytic effect. When the buffer concentration was 1.0M, the above order did not hold. It was also revealed that steric effect of the ligand affected this reaction.

The intermediate formed in this decarboxylation reaction was examined by the measurement of ultraviolet and infrared spectra, and by isolation of this intermediate, and formation of a mixed chelate compound of OAA, copper, and ligand was confirmed.

It is known that enzymatic activity of pyruvate carboxylase and oxalacetate decarboxylase is accelerated by metals like manganese(II) and magnesium(II).[3–5)] In connection with studies on these metal activated enzymes, effect of several metal ions on enzymatic decarboxylation of oxalacetic acid (OAA) has been reported by Speck[6)] and Herbert.[7)] Non-enzymatic reaction of OAA has also been examined and it has been revealed that the addition of a number of polyvalent metal ions, such as copper(II), manganese(II), magnesium(II), *etc.*, to the aqueous solution of OAA accelerates its decarboxylation.[6,8)]

There has been little work on the decarboxylation of OAA by metal chelates, and report to date is confined to manganese(II) and zinc(II) chelates of *o*-phenanthroline derivatives.[9–11)] We reported in our preceding paper that β-diketones formed a mixed complex with copper(II)-α, α′-dipyridyl.[12)] Since OAA has a chelating ability same as β-diketone, there is a possibility that it would react with copper-dipyridyl to form a mixed complex. Therefore, examinations were made on the effect of the nature of ligands, which formed a chelate with copper(II) on the

1) A part of this work was presented before in the 90th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, July 1970.
2) Location: *Yayoi-cho, Chiba-shi.*
3) J.J.B. Cannata and A.O.M. Stoppani, *J. Biol. Chem.*, **238**, 1919 (1963).
4) A.S. Mildvan, M.C. Scrutton, and M.F. Utter, *J. Biol. Chem.*, **241**, 3488 (1966).
5) G.W. Kosicki and F.H. Westheimer, *Biochemistry*, **7**, 4303 (1968).
6) J.F. Speck, *J. Biol. Chem.*, **178**, 315 (1949).
7) D. Herbert, "Methods in Enzymology," Vol. 1, ed. by S.P. Colowick and N.O. Kaplan, Academic Press, Inc., New York, N.Y., 1955, p. 753.
8) G.W. Kosicki and S.N. Lipovac, *Nature*, **26**, 359 (1963).
9) J.U. Rund and R.A. Plane, *J. Am. Chem. Soc.*, **86**, 367 (1964).
10) K.G. Claus and J.U. Rund, *Inorg. Chem.*, **7**, 890 (1968).
11) K.G. Claus and J.U. Rund *Inorg. Chem.*, **8**, 59 (1969).
12) Y. Yamane, M. Miyazaki, S. Ikeda, and N. Yamaji, *Chem. Pharm. Bull.* (Tokyo), **18**, 1589 (1970).

decarboxylation of OAA by using the ligands of α,α′-dipyridyl and *o*-phenanthroline which form N,N-coordinated five-membered ring chelates. It was thereby found that the reaction rate was faster when the copper(II) to ligand ratio 1: 1 than with copper(II) ion alone. Further, relationship between the pK_a[13] and steric effect of the ligand and its reaction rate were clarified. Examination was also made on the effect of buffer concentration at the time of the reaction. The ligands used here were 6,6′-dimethyl-α,α′-dipyridyl, 4,4′-dimethyl-α,α′-dipyridyl, α,α′-dipyridyl, 5-nitro-*o*-phenanthroline, *o*-phenanthroline, ethylenediamine, and 4,7-dimethyl-*o*-phenanthroline.

With respect to the mechanism of the catalytic reaction of copper(II) ion, it is assumed that the reaction progresses through five-membered ring chelation at the α-keto acid position of OAA[14-17] by the use of OAA labeled with ^{13}C, dimethyl OAA, and its ester of OAA. However, intermediate of this reaction, the chelate of OAA, has not been isolated as yet. We found that 5-nitro-*o*-phenanthroline reacted with OAA at below pH 6.5 to produce a greenish white precipitate which was found to be a mixed complex, and it was proved that the reaction progressed by the formation of a metal chelate by OAA.

Result and Discussion

Catalytic Acceleration of Copper(II)-α,α′-dipyridyl

Reaction rate of the decarboxylation of OAA was examined with a Warburg manometer by changing the ratio of α,α′-dipyridyl to copper(II) in 0.1M acetate buffer of pH 5.5. The course of this reaction rate is shown in Fig. 1.

The reaction rate of this decarboxylation was the fastest when the copper to dipyridyl ratio was 1: 1, and became slower as the ratio changed to 1: 2, 1: 3, and 1: 4. Decarboxylation of OAA by dipyridyl alone was the same as the rate of decarboxylation by autodecomposition of OAA itself, indicating that dipyridyl had no catalytic effect in itself. The fact that the catalytic activity was the highest in 1: 1 mixture of copper: dipyridyl indicates that this reaction requires the coordination sites of copper(II), and also suggests the formation of a mixed complex as the intermediate of this reaction. The 1: 1 mixture of copper

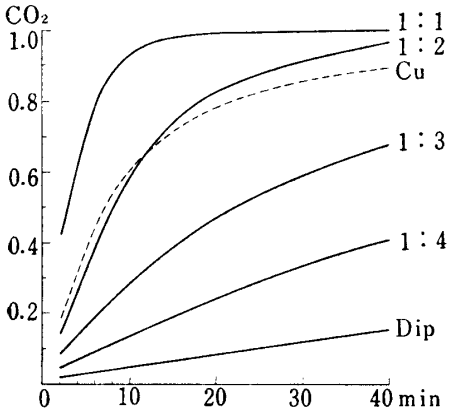

Fig. 1. 0.1M Acetate Buffer pH 5.5
Cu:Dip=1:0, 1:1, 1:2, 1:3, 1.4, 0:1

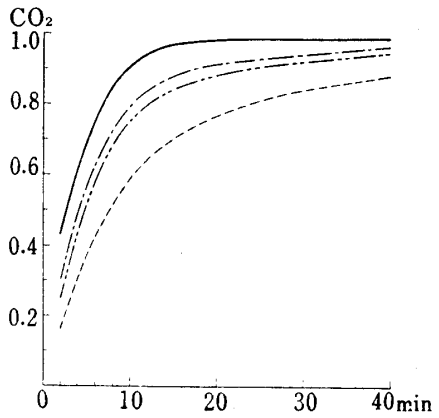

Fig. 2. 0.1M Acetate Buffer pH 5.5
Dip. Phen. En. $CuSO_4$

——— : Cu-Dip ----- : Cu-Phen
-·-·-·- : Cu-En ------ : $CuSO_4$

13) B.R. James and R.J.P. Williams, *J. Chem. Soc.*, **1961**, 2007.
14) S. Settzer, *J. Am. Chem. Soc.*, **81**, 4018 (1959).
15) R. Steinberger and F.H. Westheimer, *J. Am. Chem. Soc.*, **71**, 4158 (1949).
16) R. Steinberger and F.H. Westheimer *J. Am. Chem. Soc.*, **73**, 429 (1951).
17) E. Gelles and R.W. Hay, *J. Chem. Soc.*, **1958**, 3673.

and dipyridyl effected faster reaction than by copper (II) ion alone and the effect of chelate formation between copper and dipyridyl is apparent.

Catalytic effect of copper-α, α′-dipyridyl in oxidation and hydrolysis reactions has been reported already[18-21] and now we have found that it also has a catalytic action on the decarboxylation of OAA.

Relationship between pK_a of the Ligand and Reaction Rate

In order to examine what nature of the ligand was responsible for the catalytic effect in this reaction, examination was made on *o*-phenanthroline and ethylenediamine, which also undergoes N, N-coordinated five-membered ring chelation, same as α,α′-dipyridyl. Since the catalytic effect was the greatest when the copper to dipyridyl ratio was 1:1, the ratio of copper to ligand was made 1:1 for all subsequent experiments.

The decarboxylation rate of OAA in 0.1M acetate buffer (pH 5.5) using 1:1 mixtures of copper (II) with α,α′-dipyridyl, *o*-phenanthroline, or ethylenediamine, or with copper (II) ion alone, is compared in Fig. 2.

The reaction rate fell in the order of dipyridyl (pK_a 4.50±0.02), phenanthroline (pK_a 4.97 ±0.02), ethylenediamine (pK_a 7.30±0.05), and copper (II). The values in parentheses indicate the pK_a in aqueous solution.[13] This order indicates that the reaction rate is faster, the smaller the pK_a of the ligand. This order was the same even in 0.1M acetate buffer of pH 6.3 or 5.9.

The same examinations were made with phenanthroline derivatives of different pK_a and, in this case also, the reaction rate was found to become faster with smaller pK_a, the reaction rate falling in the order of 5-nitro-*o*-phenanthroline (pK_a 2.80±0.05), *o*-phenanthroline (pK_a 4.53±0.03), 4,7-dimethyl-*o*-phenanthroline (pK_a 5.40±0.02), and copper (II). Here, the values in parentheses show pK_a in 50% (v/v) dioxane–water.[13] Since copper-5-nitro-*o*-phenanthroline reacted with OAA to form a greenish white precipitate at below pH 6.5, these comparative examinations were made in 0.1M tris-acetate buffer of pH 6.7.

Effect of the buffer was then examined. The reaction was the same in 0.1M tris-acetate buffer of pH 5.5 as with 0.1M acetate buffer of pH 5.5, and the kind of buffer used did not affect the reaction rate. When the concentration of acetate buffer of pH 5.5 was changed from 0.1M to 1.0M, the difference in the reaction rates among dipyridyl, phenanthroline, and ethylenediamine was hardly perceptible. This is probably due to the effect of componental concentration of the buffer on the chelate formation reaction.

Steric Effect

Comparison of the reaction rate among dipyridyl, 6,6′-dimethyl dipyridyl, and 4,4′-dimethyl dipyridyl is given in Fig. 3. In this case also, the same relationshipb etween pK_a and reaction rate was recognized as in the case of dipyridyl, ethylenediamine, and phenanthroline, and the reaction rate fell in the order of dipyridyl (pK_a 3.62±0.02), 6,6′-dimethyl dipyridyl (pK_a 4.23±0.02), 4,4′-dimethyl dipyridyl (pK_a 4.40±0.03), and copper (II), where the values in parentheses indicate pK_a in 50% (v/v) dioxane–water.

However, in spite of the small difference in pK_a between the 6,6′-dimethyl derivative and 4,4′-dimethyl derivative, the reaction rate was considerably faster in the 6,6′-dimethyl derivative, especially at the initial stage, and this fact indicates the presence of a factor other than the difference in pK_a between the two derivatives.

Comparison of reaction rates among the dipyridyl derivatives in 1.0M acetate buffer of pH 5.5 is shown in Fig. 4. In this case, difference between dipyridyl and 4,4′-dimethyl

18) S. Sakai, N. Kasuya, and Y. Ishii, *Kogyo Kagaku Zasshi*, **72**, 1687 (1969).
19) S. Isaka, *Nature*, **16**, 578 (1957).
20) K. Kobashi, *Biochem. Biophys. Acta*, **158**, 239 (1968).
21) T. Wagner-Jauregg, B.E. Hackley, Jr., T.A. Lies, O.O. Owens, and R. Proper, *J. Am. Chem. Soc.*, **77**, 922 (1955).

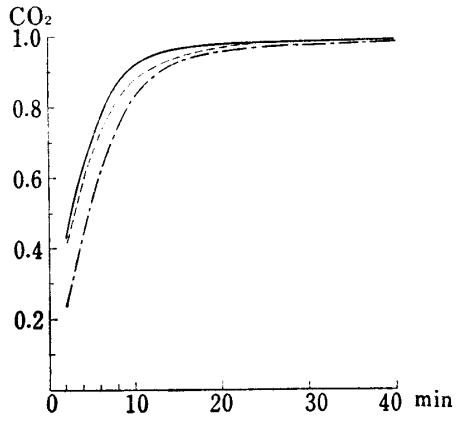

Fig. 3. 0.1M Acetate Buffer pH 5.5
Dip. Derivatives
———: Cu-Dip ·····: Cu-6.6 Me_2Dip
– – – –: Cu-4.4′ Me_2Dip

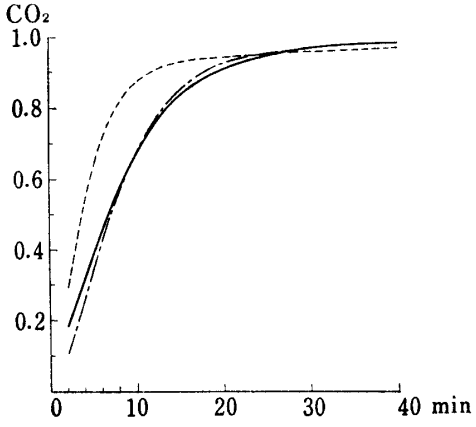

Fig. 4. 1.0M Acetate Buffer pH 5.5
Dip. Derivatives
———: Cu-Dip ·····: Cu-6.6′ Me_2Dip
– – – –: Cu-4.4′ Me_2Dip

derivative has become very small, and this may be interpreted in the same way as the lessening of difference in reaction rates among dipyridyl, phenanthroline, and ethylenediamine in 1.0M buffer concentration. The outstanding feature of the 1.0M buffer is that the accelerative effect of the 6,6′-dimethyl derivative was the greatest and the difference between the 6,6′-dimethyl derivative and 4,4′-dimethyl derivative became much greater. This was considered to be due to the steric effect because the apparent difference between these two derivatives is only the difference in the position of their substituents. This steric effect is considered to affect the stability of the 1:1 metal chelate, another factor that accelerates this reaction other than pK_a. As will be clear from the experiment in which the copper (II) to dipyridyl ratio was varied (Fig. 1), 1:1 metal chelate has the highest catalytic activity. The fact that 1:1 metal chelate of 6,6′-dimethyl dipyridyl is stable is probably due to the repulsion of the methyl groups against each other which destabilizes disproportionation of 1:1 metal chelate to 2:1 chelate. Similar discussion was made by Martell and others on the hydrolytic action of copper-tetramethylethylenediamine on diisopropylphosphorofluoridate.[22]

The effect of variation in buffer concentration mentioned above is produced by the relative proportion of metal chelate concentration and buffer concentration. Consequently, in order to examine the pure effect of ligand in catalytic relations with this kind of metal chelates, buffer concentration should be made as low as possible and the chelate concentration made high but without giving other effects.

Ultraviolet Spectra

Decarboxylation of OAA by copper-dipyridyl was examined through ultraviolet spectra.

(i) Formation of Mixed Complex——All the ultraviolet (UV) spectra were measured in abs. ethanol. Addition of abs. ethanol solution of $CuSO_4 \cdot 5H_2O$ to the abs. ethanol solution of OAA results in the shift of absorption maximum at 260 mμ to 286 mμ. Measurement of the difference spectrum of a sample of OAA solution added with copper-dipyridyl, the latter being used as reference standard, showed the same shift as in the case of copper sulfate and OAA, and indicated the maximum absorption at 289 mμ. The absorption maximum at 289—290 mμ is considered to be the absorption due to OAA and metal chelate,[17,23] the presence of absorption maximum at 289 mμ in the difference spectrum of copper-dipyridyl suggests the formation of a mixed complex between copper-dipyridyl and OAA (α,α′-dipyridyl, λ_{max} 237 and 283 mμ; copper-dipyridyl, λ_{max} 301 and 311 mμ).

22) R.C. Coutney, R.L. Gustafson, S.J. Westerback, H. Hyytiaminen, S.C. Chaberek, Jr. and A.E. Martell, *J. Am. Chem. Soc.*, **79**, 3030 (1957).
23) G.W. Kosicki, *Canadian J. Chem.*, **42**, 403 (1963).

(ii) Periodical Variation (Spectral Changes by Decarboxylation)——Periodical changes in the ultraviolet spectra of OAA alone and a mixture of copper-dipyridyl and OAA are shown in Fig. 5. Decrease of absorption in the spectra of OAA alone was extremely slight after 1 hr, but a great reduction in the absorption was seen after 10 min in the spectrum of copper-dipyridyl and OAA. Shift of the absorption was no longer apparent after 45 min, indicating completion of the reaction, and only the absorption of pyruvic acid remained. This indicates the catalytic action of copper-dipyridyl.

Properties of the Intermediate, 5-Nitro-*o*-phenanthroline-Copper-OAA (1: 1: 1)

Warburg manometric examination proved the evolution of carbon dioxide from the greenish white precipitate obtained by the reaction of OAA with copper-5-nitro-*o*-phenanthroline, suggesting that the precipitate is the reaction intermediate, a 1: 1: 1 complex of 5-nitro-*o*-phenanthroline, copper, and OAA. Properties of this intermediate complex were examined by ultraviolet and infrared spectral measurements.

Ultraviolet Spectra

Ultraviolet spectra of a mixture of copper-5-nitro-*o*-phenanthroline and OAA, copper-5-nitro-*o*-phenanthroline, and the synthesized mixed complex are shown in Fig. 6. All the

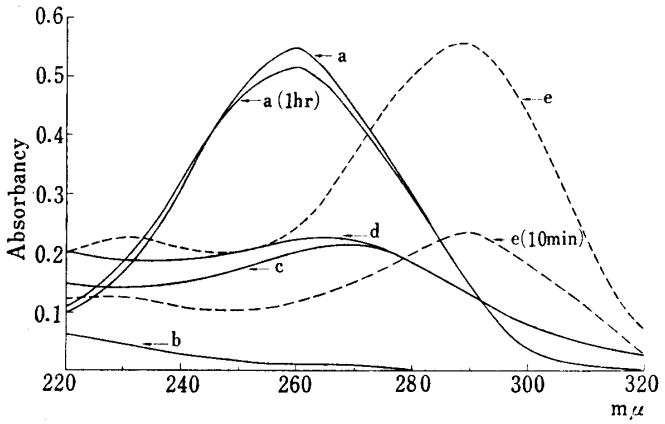

Fig. 5. Absorption Spectra (in EtOH)

a: OAA (10^{-4}M) b: PyA (10^{-4}M) c: $CuSO_4$ (10^{-4}M)
d: $CuSO_4$+PyA (10^{-4}M) e: CuDip+OAA (10^{-4}M)
difference spectrum

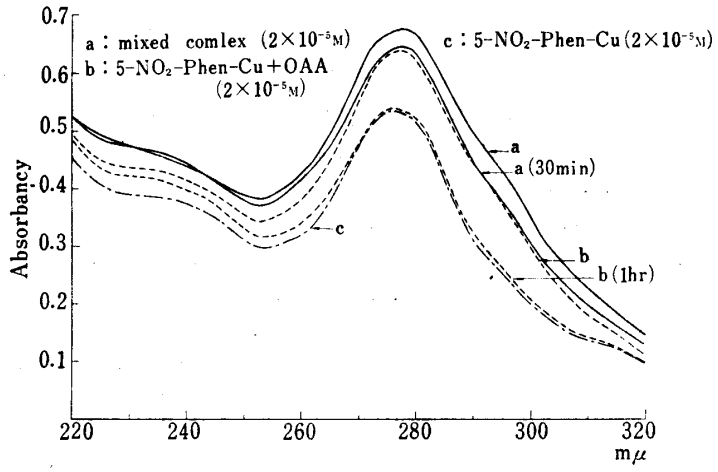

Fig. 6. Absorption Spectra (in EtOH)

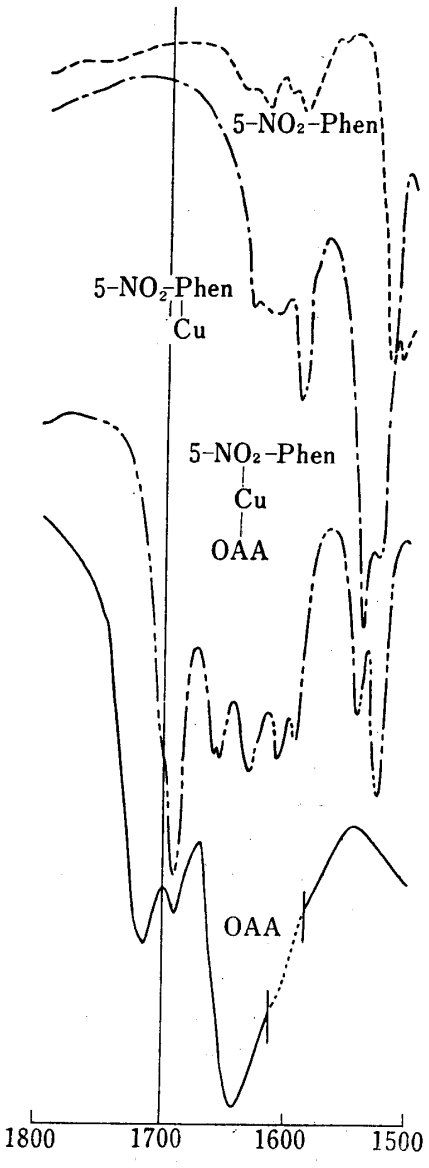

Fig. 7. IR Spectra

UV spectra were measured in abs. ethanol solution. The mixed complex also had absorption maximum at 278 mμ, same as the mixture of copper-5-nitro-*o*-phenanthroline and OAA, and both showed decrease of absorption with time, centering arround 290 mμ, the absorption of the chelate. After about 5 hr, the absorption maximum of the mixed complex at 278 mμ shifted to 276 mμ, almost the same as the absorption of copper-5-nitro-*o*-phenanthroline. The difference in the absorption below 260 mμ between a mixture of copper-5-nitro-*o*-phenanthroline and OAA, and copper-5-nitro-*o*-phenanthroline is probably due to the presence of pyruvic acid formed (Fig. 5, curve b). These data suggest the presence of copper-5-nitro-*o*-phenanthroline and OAA in the structure of synthesized mixed complex.

Infrared Spectra

In order to examine the chelate structure of the synthesized copper-5-nitro-*o*-phenanthroline-OAA complex, the infrared spectrum of this mixed complex was compared with those of 5-nitro-*o*-phenanthroline, copper-5-nitro-*o*-phenanthroline (1:1), and OAA. As will be seen from Fig. 7, the strong absorptions at 1503 and 1515 cm^{-1} in the spectrum of 5-nitro-*o*-phenanthroline are shifted to 1725 and 1537 cm^{-1}, respectively, in copper-5-nitro-*o*-phenanthroline, and the spectrum of the mixed complex also showed absorption of strong intensity in the same position as in the spectrum of 5-nitro-*o*-phenanthroline. The strong absorptions at 1587, 1605, and 1628 cm^{-1} in the spectrum of copper-5-nitro-*o*-phenanthroline are also seen in the spectrum of the mixed complex. These facts suggest the presence of a chelate bonding between copper and 5-nitro-*o*-phenanthroline in the structure of the mixed complex.

Further, the spectrum of the mixed complex exhibits strong absorptions at 1654 and 1690 cm^{-1} which do not appear in the spectrum of copper-5-nitro-*o*-phenanthroline. The spectrum of OAA has absorptions of strong intensity at 1690 and 1713 cm^{-1}, and the latter absorption is considered to have shifted to a lower wave number (1654 cm^{-1}) in the mixed complex by chelation between copper-5-nitro-*o*-phenanthroline and OAA. Further, the spectrum of copper-5-nitro-*o*-phenanthroline shows broad absorptions of strong intensity at 1100 and 620 cm^{-1} assigned to SO_4^{2-} but such absorptions are not found in the spectrum of the mixed complex. In addition, SO_4^{2-} was not detected in the mixed complex, and the presence of SO_4^{2-} in the structure of mixed complex can probably be denied.

From these ultraviolet and infrared spectral data, and from the elemental analytical values, the most appropriate structure of copper-5-nitro-*o*-phenanthroline-OAA complex would be as shown in Fig. 8.

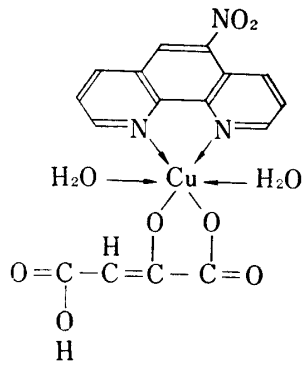

Fig. 8. Mixed Complex

The fact that copper-5-nitro-*o*-phenanthroline-OAA complex was obtained is considered to support the assumption that the mechanism of the catalytic reaction by metal chelate involves a mixed complex formation, and chelation of OAA with a metal was proved by the synthesis of this complex. The present evidence that the properties of the ligand affects the reaction rate and the formation of a mixed chelate suggests that, even in enzymatic reaction, OAA probably forms an enzyme-metal-substrate mixed complex.

Experimental

Apparatus——UV spectra were taken by Hitachi EPS-3T autorecording spectrophotometer. IR spectra were measured by Hitachi EPI-G31 autorecording spectrophotometer with nujol. Toa Dempa HM-5A pH meter was used for the measurement of pH of sample solutions.

Materials and Reagents——*cis*-Oxalacetic acid (OAA), $CuSO_4 \cdot 5H_2O$, the seven chelating agents, tris (hydroxymethyl)aminomethane, and sodium acetate were of analytical grade.

Procedure——The quantity of carbon dioxide evolved from the reaction mixture was determined by Warburg manometer by the use of flasks with one side arm, at $30 \pm 0.5°$. The reaction mixture was buf-

fered at the fixed pH by tris acetate or sodium acetate. 2 ml of 5×10^{-3}M copper-ligand solution (bufferized) was placed in the main chamber of the Warburg flask. In the side chamber, was placed 1 ml of OAA solution whose concentration was 1.5×10^{-2}M at pH 5.1, 5.5, 5.9, and 6.3, and 3.0×10^{-2}M at pH 6.7, respectively. Before mixing, the flasks were pre-incubated for 10 min, opening the choke of the manometer. Shutting the choke, the side arm containing the OAA solution was tipped in and mixed at zero time. The volume of carbon dioxide evolution was measured continuously. The relative rate of decarboxylation were determined by the volume of carbon dioxide at the respective time.

Synthesis of Copper-5-Nitro-*o*-phenanthroline (1 : 1)——121.7 mg of $CuSO_4\cdot5H_2O$ (4.87×10^{-4} mole) was dissolved in 200 ml of water, to which 89.7 mg of 5-nitro-*o*-phenanthroline (3.98×10^{-4} mole) was added with stirring at room temperature. The reaction mixture was filtered and the recrystalization from H_2O gave a green needle like crystal. *Anal.* Calcd. for $C_{12}H_{13}O_9N_3SCu$:C, 32.80; H, 2.61; N, 9.98. Found: C, 33.30; H, 3.03; N, 9.77.

Synthesis of Copper-5-Nitro-*o*-phenanthroline-Oxalacetic Acid (1 : 1 : 1)——5-Nitro-*o*-phenanthroline 112.5 mg (5×10^{-3} mole), $CuSO_4\cdot5H_2O$ 125.0 mg (5×10^{-3} mole), OAA 66.0 mg (5×10^{-3} mole) were mixed in 50 ml of water and stirred for 1 hr. The precipitate was filtered, washed with water, ethanol, and ether successively, and dried over P_2O_5. This greenish white solid showed dec. p. 132°. *Anal.* Calcd. for $C_{16}H_{13}O_9N_3Cu$:C, 42.19; H, 2.85; N, 9.23. Found: C, 41.76; H, 3.08; N, 9.30.