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THE ACTION MODEL OF TYROSINASE. DIRECT OXIDATION OF 2-ANTHRANOL TO 1,2-ANTHRAQUINONE SPECIFICALLY CATALYZED BY CUPRIC ION

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L'oxydation directe par oxygène moléculaire des anthranols-2 aux anthraquinones-1,2 est étudiée. La réaction est spécifiquement catalysée par l'ion cuivrique. L'addition d'une molécule d'imidazole par atome de cuivre augmente énormément la vitesse initiale de la réaction.

In the course of our continuing study concerning the synthesis of "asymmetric inversion catalysts",$^{1)}$ 2-anthranols as synthetic intermediates were found to be autoxidized to 1,2-anthraquinones, specifically catalyzed by cupric ion, and further enormously enhanced by the addition of imidazole. These findings seemed to involve much more important significance in relation to an enzyme reaction. The oxidation of phenols to o-quinones is catalyzed by tyrosinase or laccase, both of which contain copper indispensable for the activities.$^{2)}$ Although the mode of action of the enzyme has not been well established, the state and the role of copper and the active site(s) of the enzyme have been gradually recognized through several investigations of chemical model systems.$^{3)}$ Unfortunately, however, in these model systems the chosen substrates seem to be unsuitable for the study of the mode of action of the enzyme since they form relatively unstable o-quinones and the reactions are so fast. On the other hand, 2-anthranols seem to provide more suitable substrates because of their slow oxidation and of the quantitative formation of relatively stable 1,2-anthraquinones. In this communication we wish to report the enzyme-like oxidation of 2-anthranol catalyzed by metal ions and of the effect of amines on the oxidation.

The preliminary reaction systems are shown in Table 1. The oxidation of 2-anthranol was followed by measuring an amount of oxygen uptake. The apparent rate constant, k_{obs}, was evaluated from the initial velocity of the curve obtained by plotting an amount of oxygen uptake vs. the reaction time. Metal ions were selected in consideration of the Irving-Williams order. For the investigation of the additional effect of amines on the metal-ion catalyzed oxidation, we chose imidazole (Im), an analogue of an active site of tyrosinase, and o-phenylenediamine (PDA).

Neither nickel (SN-2) nor zinc (SN-3) chlorides has catalytic activity because both reaction rates were the same as that of the system SN-1. By the addition of copper or iron, the reaction rate was strikingly accelerated. Surprisingly, however, the reaction products were completely different from each other. The oxidation of 2-anthranol catalyzed by cupric ion produced only 1,2-anthraquinone, while the reaction catalyzed by ferric ion afforded some coupling products of 2-anthranol whose structures will be reported elsewhere.

Some of the typical apparent rate constants, k_{obs}, are listed in Table 2. The catalytic activity of cupric ion is clearly shown by the comparison of SN-4 with SN-5. SN-9 showed nearly the same k_{obs} as that of SN-4. This observation together with the comparison of the apparent rate constant of SN-6 with that of SN-10 shows that PDA has little influence on the rate enhancement and only acted as the trapping reagent of 1,2-anthraquinone. On the other hand it is of interest in relation to the enzyme reaction that the addition of a molecule of imidazole per an atom of copper enhanced the reaction rate by ca. 70 times.

Present experimental results make us recognize that the ligation of imidazole to the coordination site(s) of cupric ion eases the incorporation and the transfer of oxygen molecule to 2-anthranols. In order to draw true image of the roles of cupric ion and amine, subsequent works are designed and in progress.

All the structures of the products were characterized by spectral measurements and elemental analyses.

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Table 1. Standard Reaction Systems. Added metal ion and amine for the oxidation of 5.16×10^{-4} mol of 2-anthranols. All of the reactions was carried out in 20 ml of ethanol at ambient temperature.

System No.	SN-1	SN-2	SN-3	SN-4	SN-5	SN-6	SN-7	SN-8	SN-9	SN-10
Metal Ion		Ni^{2+}	Zn^{2+}	Cu^{2+}	Cu^{2+}	Cu^{2+}	Fe^{3+}	Fe^{3+}	Cu^{2+}	Cu^{2+}
$(mol)\times 10^{4}$	—	3.47	3.47	3.47	6.94	3.47	3.47	3.47	3.47	3.47
Amine						Im		Im	PDA	Im, PDA
$(mol)\times 10^{4}$	—	—	—	—	—	3.47	—	3.47	5.16	3.47,5.16

Table 2. Summary of the typical apparent rate constant, k_{obs}, for the oxidation of 2-Anthranol (5.16×10^{-4} mol) catalyzed by cupric ion and imidazole.

System No.	SN-4	SN-5	SN-6	SN-10
k_{obs} $(ml/M \cdot min)\times 10^{-2}$	0.360	6.88	24.8	21.3
$k_{obs}/0.360$	1	19.1	68.8	59.1

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