

Stability of Sulpyrine. III.¹⁾ Copper(II) Ion Catalyzed Oxidation by Molecular Oxygen²⁾

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The kinetics of the copper(II) ion catalyzed oxidation of sulpyrine by molecular oxygen was investigated.

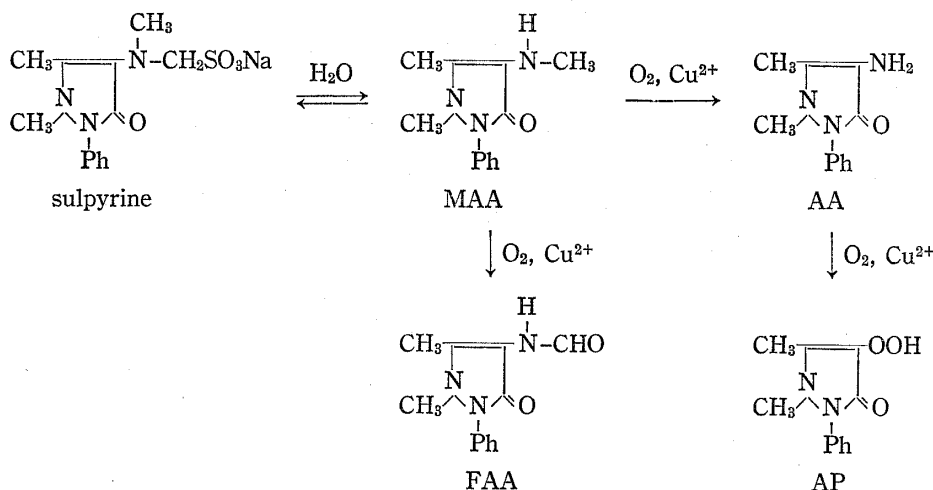
Sulpyrine was hydrolyzed to 4-methylaminoantipyrene (MAA) and then MAA was oxidized to antipyrinyl-4-peroxide (AP) *via* 4-aminoantipyrene (AA), and hydroxy-methanesulfonate formed in the hydrolysis is not effective on the rate of the oxidation of MAA. Below pH 4 the oxidation of MAA to AP was the rate-determining step. On the other hand above pH 5 the hydrolysis of sulpyrine to MAA was the rate-determining step.

The rate constants of the oxidative reactions of MAA to AA, and of AA to AP could not be determined because of the difficulties of determining the reaction orders. The complexation of MAA and AA with copper(II) ion and molecular oxygen was suggested from the observation that the both reactions were of first order with respect to copper(II) ion and of 0.5 order with respect to molecular oxygen.

Keywords—stability; sulpyrine; kinetics; 4-aminoantipyrene; molecular oxygen; copper(II) ion catalyzed oxidation; antipyrinyl-4-peroxide

Because of its side action such as allergy sulpyrine has been considered to have many problems to solve. In the previous study,^{1,4)} the hydrolysis of sulpyrine in aqueous solution was investigated and the relationship was clarified between sulpyrine and the hydrolyzed products, 4-methylaminoantipyrene (MAA), 4,4'-[methylenebis(methylimino)] diantipyrene and 4-(N-hydroxymethyl-N-methyl)aminoantipyrene.

In addition to hydrolysis sulpyrine has been known to undergo oxidative degradation. In the previous paper,⁵⁾ the copper(II) ion catalyzed oxidation of sulpyrine by molecular



- 1) Part II: S. Yoshioka, H. Ogata, T. Shibazaki, and T. Inoue, *Chem. Pharm. Bull.* (Tokyo), **25**, 484 (1977).
- 2) A part of this work was presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1977.
- 3) Location: 18-1, *Kamiyoga 1-chome, Setagaya, Tokyo 158, Japan.*
- 4) S. Yoshioka, H. Ogata, T. Shibazaki, and T. Inoue, *Chem. Pharm. Bull.* (Tokyo), **25**, 475 (1977).
- 5) S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Arch. Pharm.* "accepted".

oxygen was studied, and 4-aminoantipyrine (AA), antipyrinyl-4-peroxide (AP) and 4-formyl-aminoantipyrine (FAA) were characterized as the oxidized products. The reaction pathways shown in Chart 1 were proposed. In these reactions, sulpyrine was oxidized mainly to AP not to FAA when the initial concentration is low below 6×10^{-3} M. The purpose of this study is to determine the kinetics of the oxidation of sulpyrine to AP on solution below 6×10^{-5} M.

Experimental

Materials—Sulpyrine was of J.P.IX grade. MAA was prepared by the method described previously.⁴ Other chemicals used were of reagent grade.

Kinetic Studies—The kinetic study was done at 30° for the aqueous solutions of sulpyrine, MAA and AA respectively, which concentrations were 6×10^{-5} M unless otherwise stated.

The solutions were prepared in metal-free distilled water. The concentration of copper(II) ion was adjusted with CuSO_4 , and standardized by titration in ammoniacal solution with standard disodium salt of ethylenediamine tetracetic acid (EDTA). The other metal ions were added as $\text{Fe}_2(\text{SO}_4)_3$, MgSO_4 , ZnSO_4 , CoSO_4 , and MnCl_2 .

During each run the pH was maintained constant by automatic titration of standard HCl or NaOH. The ionic strength was maintained at 0.1 with KCl.

Pure oxygen or mixture of oxygen and nitrogen was passed through the experimental solution at 60 ml/min. Since the dissolution rate of oxygen is considered much faster than that of reaction in this condition, the amount of oxygen present at a given time may be constant and dependent only on its partial pressure. The gas mixture which has the desired partial pressure of oxygen was made by means of the method reported by Inoue.⁶

During each run the light was shielded.

Determination of MAA and AA by Means of Liquid Chromatography—MAA was determined separately from AA which was formed as an intermediate in the oxidation of MAA.

A 10 μl of reaction solution was withdrawn by microsyringe and injected accurately at appropriate intervals. The height of each peak at 22.4 and 27.0 min, which was due to AA and MAA respectively, was determined.

The operating conditions were as follows; column: Hitachi Gel 3010, 5 mm.i.d. \times 50 cm, column temperature: room temp., mobile phase: 90% (v/v) MeOH, flow rate: 0.75 ml/min, detector: UV 254 nm.

Determination of Sum Amount of Sulpyrine, MAA and AA by Means of Colorimetry—Sulpyrine, the hydrolyzed product MAA and the oxidized product AA were colored with the same absorbance per mole by means of the method reported in the previous paper.⁴ The value determined by this method represented the sum amount of sulpyrine, MAA and AA, and the decrease in the value meant the formation of AP which was not colored by this method.

A 2 ml of reaction solution was withdrawn at appropriate intervals, and shaken quickly with 0.1 ml of 0.02 M EDTA solution in order to stop the reaction. The sum amount of 4-aminoantipyrine derivatives was determined by colorimetry.

Determination of Absorbed Oxygen by Means of Warburg Apparatus—MAA was dissolved in 0.14 M acetic acid buffer (pH 5) to be 6×10^{-4} and 9×10^{-4} M, and oxidized at 40° in the presence of 2×10^{-3} M copper(II) ion in a reaction vessel of Warburg apparatus. The volume of absorbed oxygen was determined from the difference of levels of manometer at appropriate interval. At the same time MAA decomposed to AP was determined by colorimetry.

Results and Discussion

Oxidation of MAA

Since sulpyrine has been found to be oxidized *via* MAA as described later, the kinetics of the oxidation of MAA was first studied.

The trace heavy metal ions have been known to be serving as catalyst in oxidation.⁷ Therefore effects of various metal ions and chelating agents such as EDTA and citric acid on the oxidation of MAA were studied and represented in Table I. Though ferric ion was as effective at pH 2 as copper(II) ion at pH 6, magnesium, zinc, cobalt and manganese ions

6) S. Inoue, *Yakugaku Zasshi*, **91**, 81 (1971).

7) A.H. Beckett, A.R. Purkaystha, and P.H. Morgan, *J. Pharm. Pharmacol.*, **29**, 15 (1977).

were respectively less effective than copper(II) ion. The removal of metal ions by adding chelating agents to the reaction solutions delayed the oxidation of MAA.

Figure 1 shows the time course of the oxidation of MAA at pH 5. As MAA was decomposed AA and AP were formed, and AP was gradually accumulated. Figure 2 illustrates the

TABLE I. Effect of Metal Ion and Chelating Agent on Oxidation of MAA

Metal ion ^{a)}	Chelating agent ^{b)}	$T_{1/2}$ (min) ^{c)}
—	—	$\gg 480$ ^{d)}
Cu^{2+} (pH 5)	—	9.5
Cu^{2+} (pH 5)	EDTA	$\gg 480$ ^{e)}
Cu^{2+} (pH 5)	Citric acid	$\gg 300$ ^{f)}
Cu^{2+} (pH 6) ^{g)}	—	2.0
Fe^{3+} (pH 2) ^{g)}	—	1.6
Mg^{2+} (pH 7) ^{g)}	—	360
Zn^{2+} (pH 7) ^{g)}	—	290
Co^{2+} (pH 7) ^{g)}	—	390
Mn^{2+} (pH 7) ^{g)}	—	340

- a) The concentration of each metal ion was 5×10^{-4} M.
- b) The concentration of each chelating agent was 0.01 M.
- c) The half-life period was determined by colorimetry.
- d) The time of 0.8% decrease of MAA.
- e) The time of 0.8% decrease of MAA.
- f) The time of 11% decrease of MAA.
- g) The half-life period at the pH where each metal ion was most effective was shown.

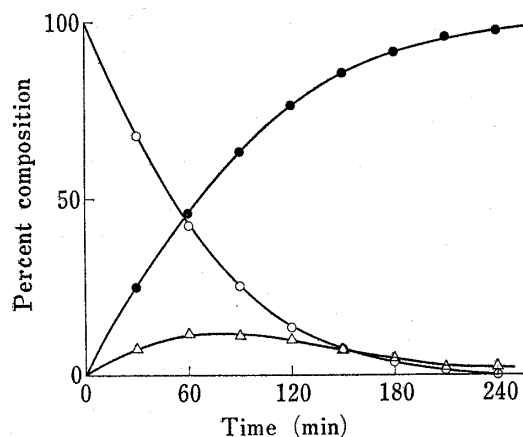


Fig. 1. Oxidation of MAA at pH 5

$[\text{Cu}^{2+}]$: 7.5×10^{-5} M.
 ○: MAA, △: AA, ●: AP.^{a)}
 a) The amount of AP was calculated from the amounts of MAA and AA.

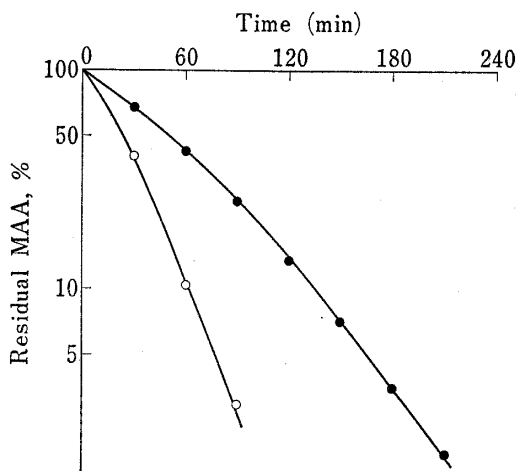


Fig. 2. Oxidation of MAA to AA at pH 5

$[\text{Cu}^{2+}]$ ●: 7.5×10^{-5} M, ○: 1.5×10^{-4} M.

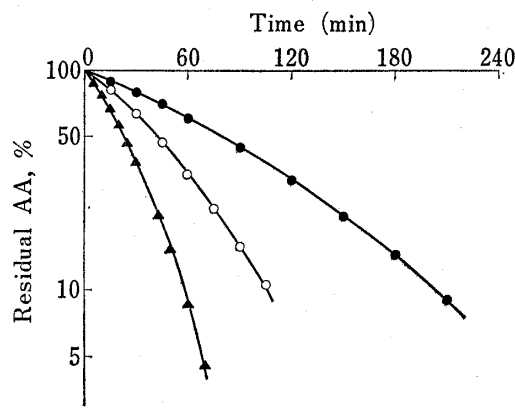


Fig. 3. Oxidation of AA to AP at pH 5

$[\text{Cu}^{2+}]$ ●: 7.5×10^{-5} , ○: 1.5×10^{-4} , ▲: 3.0×10^{-4} M.

TABLE II. Rate of Oxidation

Oxidation	Reciprocal of half-life period ($\times 10^{-2} \text{min}^{-1}$) $[\text{Cu}^{2+}]$ (M)		
	7.5×10^{-5}	1.5×10^{-4}	5.0×10^{-4}
MAA→AA	1.96	4.17	13.3
MAA, AA→AP ^{a)}	1.52	3.57	10.5
AA→AP	1.25	2.50	8.20

a) The half-life period of the sum amount of MAA and AA.

plot for the decrease of MAA in semilogarithmic scale, and indicates that the reaction of MAA to AA was not of first order. The time course of the oxidation of AA is represented in Fig. 3, and indicates that the reaction of AA to AP was not of first order similarly to the reaction of MAA to AA. Though the reaction of MAA to AP was a consecutive reaction, the rate constants were not calculated because of the difficulties of determining the reaction order. Therefore the rate was represented in terms of the reciprocal of the half-life period. The reciprocal values of the half-life period for the reaction of MAA to AA and that of AA to AP were represented in Table II.

As shown in Table II the reaction of MAA to AA was faster than that of AA to AP. This does not agree with the observation that AA was not so much accumulated in the oxidation of MAA to AP as shown in Fig. 1. Furthermore the half-life period of the sum amount of MAA and AA in the oxidation of MAA to AP was smaller than that of AA to AP as shown in Table II. This does not agree with that the reaction of MAA to AP is a simple consecutive one *via* AA. These are interpreted by assuming that a catalyst which facilitates the reaction of AA to AP is formed when MAA was decomposed to AA. Since the formation of hydrogen peroxide has been known in copper(II) ion catalyzed oxidation by molecular oxygen,^{8,9)} the effect of hydrogen peroxide on the oxidation rate of AA to AP was studied. As shown in Fig. 4, the addition of hydrogen peroxide to the reaction system containing copper(II) ion and oxygen resulted in the increase of the rate remarkably. The addition of hydrogen peroxide to the reaction system without oxygen resulted in the increase of the initial rate. Furthermore it was found that hydrogen peroxide was not effective without copper(II) ion and molecular oxygen. On the basis of these facts it may be considered that in the copper(II) ion catalyzed oxidation of MAA by molecular oxygen hydrogen peroxide is formed when MAA is decomposed to AA and then it facilitates the reaction of AA to AP.

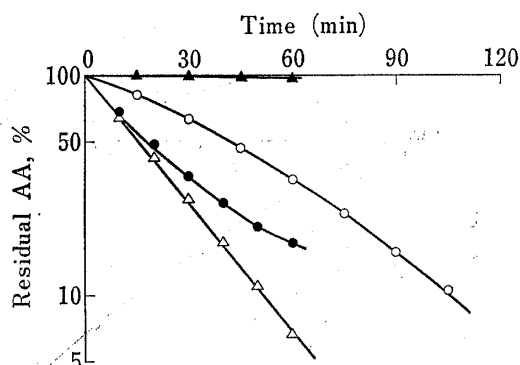


Fig. 4. Effect of Hydrogen Peroxide on Oxidation of AA

- : $[\text{Cu}^{2+}] 1.5 \times 10^{-4} \text{ M}, \text{O}_2 1 \text{ atm.}$
- △: $[\text{Cu}^{2+}] 1.5 \times 10^{-4} \text{ M}, \text{O}_2 1 \text{ atm.},$
 $[\text{H}_2\text{O}_2] 1.5 \times 10^{-4} \text{ M.}$
- : $[\text{Cu}^{2+}] 1.5 \times 10^{-4} \text{ M}, \text{N}_2 1 \text{ atm.},$
 $[\text{H}_2\text{O}_2] 1.5 \times 10^{-4} \text{ M.}$
- ▲: $[\text{H}_2\text{O}_2] 1.5 \times 10^{-4} \text{ M}, \text{N}_2 1 \text{ atm.}$

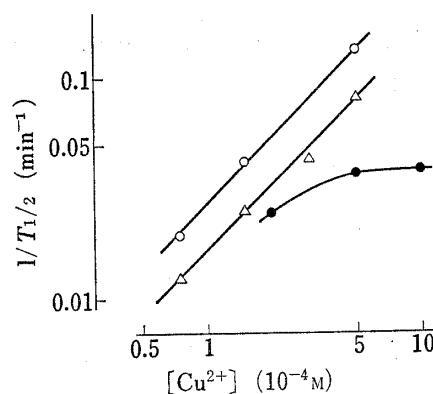


Fig. 5. Effect of Concentration of Copper (II) Ion on Oxidation

- : MAA→AA,
- △: AA→AP,
- : sulpyrine→AP.

The relationship between the reaction rate and the concentration of copper(II) ion is represented in Fig. 5. Figure 6 shows the effect of partial pressure of oxygen on the rate. In the cases of the reaction of MAA to AA and that of AA to AP the rate varied proportionally to the concentration of copper(II) ion. Furthermore the rate became greater with an increase in the partial pressure of oxygen and the order was 0.5. The dependence of the rate on the concentrations of copper(II) ion and oxygen may be similar to that reported in the copper(II)

8) M.M.T. Khan and A.E. Martell, *J. Am. Chem. Soc.*, **89**, 4176 (1967).

9) E.V. Shtamm and Y.I. Skurlatov, *Zhurnal Fizicheskoi Khimii*, **48**, 1454 (1974).

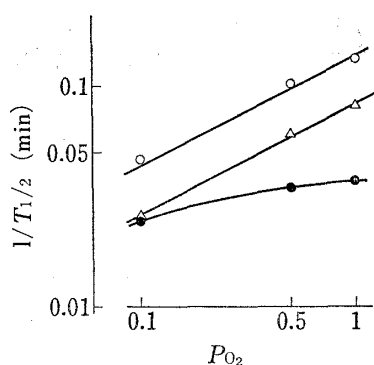


Fig. 6. Effect of Partial Pressure of Oxygen on Oxidation

○: MAA→AA,
 △: AA→AP,
 ●: sulpyrine→AP.

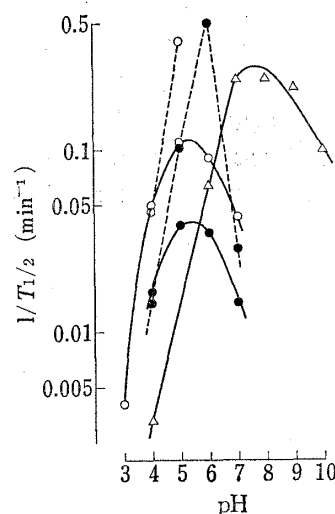


Fig. 7. Dependence of Rate^{a)} on pH

a) The half-life period was determined by colorimetry.
 —: sulpyrine, ----: MAA.
 ●: 30° [Cu²⁺] 5 × 10⁻⁴ M,
 ○: 40° [Cu²⁺] 5 × 10⁻⁴ M,
 △: 70° [Cu²⁺] 5 × 10⁻⁶ M.

ion catalyzed oxidation of ascorbic acid by molecular oxygen.^{8,9)} The oxidation of ascorbic acid has been reported to be of first order with respect to the concentration of copper(II) ion.^{8,9)} With respect to the partial pressure of oxygen it has been reported to be of first order⁸⁾ or of 0.5 order.⁹⁾ In order to study the action of oxygen the volume of oxygen absorbed during the oxidation of MAA was determined by means of a Warburg apparatus. The degradation of MAA was accompanied by the absorption of oxygen from the beginning of the reaction. These observations above suggest that the oxidation of MAA to AA and that of AA to AP may include the complexation of MAA and AA with copper(II) ion and molecular oxygen in the similar way as reported in the oxidation of ascorbic acid.

The effect of pH on the rate of the oxidation of MAA is represented in Fig. 7. When the concentration of copper(II) ion was 5 × 10⁻⁴ M, the maximum of the rate was observed nearly at pH 6. The decrease of the rate in acidic range suggests that the protonation of MAA may delay the oxidation. On the other hand the decrease of the rate above pH 7 may be ascribable to the decrease of the solubility of copper(II) ion, accompanied by the precipitation of copper hydrate. This is supported by the observation that the maximum was shifted to pH 8 when the concentration of copper(II) ion was reduced to 10⁻⁶ M and the temperature was risen to 70°.

Oxidation of Sulpyrine

In the case of the oxidation of sulpyrine to AP, the half-life period was represented as the time of 50% decrease of the sum amount of sulpyrine, MAA and AA.

The effect of pH on the rate of the oxidation of sulpyrine is represented in Fig. 7. Above pH 5 the rate of the formation of AP from sulpyrine was much smaller than that from MAA in the 5 × 10⁻⁴ M copper(II) ion. This suggests that the reaction of sulpyrine to MAA is the rate-determining step in this pH range since it is slower than the oxidation of MAA. The dependence of the oxidation rate of sulpyrine on the concentration of copper(II) ion and partial pressure of oxygen was not observed remarkably in comparison with those of AA and MAA at pH 5 as shown in Fig. 5 and 6. This suggests that the reaction of sulpyrine to MAA is a simple hydrolysis without the effects of oxygen and copper(II) ion.

At pH 4 the rate from sulpyrine was in agreement with that from MAA. This suggests that as the oxidation of MAA gets slower, this step becomes rate-determining and the rate from sulpyrine comes close to that from MAA.

These observations indicates that sulpyrine is hydrolyzed to MAA and then MAA is oxidized to AP *via* AA, and that hydroxymethanesulfonate formed in the hydrolysis is not effective on the rate of the oxidation of MAA.