# Kinetics of the Oxidation of Ascorbic Acid by the Copper(II) Ion in an Acetate Buffer Solution

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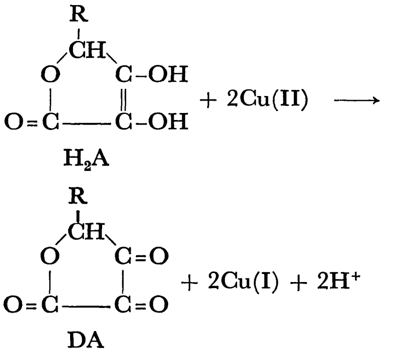
The kinetics of the oxidation of ascorbic acid by the copper(II) ion has been studied spectrophotometrically by a stopped-flow technique. At pH 4—6, the initial rate of the decrease in the monohydroascorbate anion is of the second-order with respect to the total concentration of the copper(II) ion and of the first-order with respect to the concentrations of the monovalent and divalent ions of ascorbic acid:

$$-(\mathrm{d}[\mathrm{HA}^-]/\mathrm{d}t)_{t=0} = k_1[\mathrm{Cu(II)}]_\mathrm{T}^2[\mathrm{HA}^-] + k_2[\mathrm{Cu(II)}]_\mathrm{T}^2[\mathrm{A}^{2-}]$$

where $\mathrm{HA}^-$ and $\mathrm{A}^{2-}$ are the monovalent and divalent ions of ascorbic acid respectively. $k_1$, $k_2$, and the activation parameters are obtained as follows: $k_1 = 3.1\times10^4$, $k_2 = 6.1\times10^{10}\ \mathrm{M}^{-2}\,\mathrm{s}^{-1}$, $\Delta H^{\ddagger} = 17$ kcal/mol, $\Delta S^{\ddagger} = 8.3$ cal/deg mol, and $\Delta G^{\ddagger} = 15$ kcal/mol. The EPR signal from the ascorbate radical is not observed in the kinetic run by either a flow technique or a quenching technique. Some discussions of the mechanism of the reaction are given.

It is known that the oxidation of ascorbic acid, $\mathrm{H_2A}$, to dehydroascorbic acid, DA, by molecular oxygen is catalyzed with a remarkable activity by the copper(II) ion. Many kinetic studies of the reaction have been made.[1–7] However, the rate of the reaction depends complicatedly on the pH, the catalyst, the oxygen pressure, the buffer, *etc.* and so the rate equation has not been determined.

The present study was undertaken to obtain some information on the kinetics of the reaction between ascorbic acid and the copper(II) ion in the absence of oxygen as a fundamental study of the copper(II) ion-catalyzed autoxidation of ascorbic acid. The electron paramagnetic resonance (EPR) spectra were measured in order to obtain some information on the mechanism of the reaction. The rates were measured in an acetate buffer solution. The stoichiometry of the reaction is as follows:



(1)

## Experimental

*Reagents.* The ascorbic acid, copper(II) sulfate, copper(II) acetate, acetic acid, and sodium acetate were obtained commercially (all GR). The copper(II) sulfate and copper(II) acetate were purified by recrystallization. The other materials were used without further purification. The water used was distilled and bubbled by nitrogen gas for 30 min before the preparation of the reactant solutions. The acetate buffer solution, which had been prepared from 0.1 M acetic acid and 0.1 M sodium acetate, was used between pH 4—6. The ascorbic acid solution was freshly prepared for every kinetic run.

*Kinetic Measurements.* The absorbance maximum of ascorbic acid is 265 nm in a neutral solution and 245 nm in an acidic solution for the monohydroascorbate anion, $\mathrm{HA}^-$, and ascorbic acid, $\mathrm{H_2A}$, respectively. At pH 4—6, the monohydroascorbate anion is the predominant species present in solution. In the rate study, the decrease in the absorbance was followed at 265 nm by a stopped-flow apparatus constructed in our laboratory, using a Shimadzu SV 50 spectrophotometer and a 5 mm quartz cell. The acetate buffer and the 1 mM $\mathrm{CuSO_4}$ solution do not interfere with the absorbance at 265 nm. The initial rate method was used for the analysis of the rate in order to neglect the complexity resulting from the decrease in the reactants and the increase of products during a kinetic run. Nitrogen gas was bubbled through the reactant solutions for 30 min before each kinetic run and during each kinetic run in order to exclude the oxygen. The pH was measured using a Hitachi-Horiba F-5 pH meter. The formation of precipitates by the hydrolysis of the copper(II) ion and copper(I) oxide produced interfered with the kinetic measurements above pH 6.

*EPR Measurements.* To obtain the EPR signal from the kinetic run, the continuous-flow technique was used with various flow rates.[8] Also, to obtain the EPR signals from the solid phase solutions containing 0.05 M $\mathrm{CuSO_4}$ and 0.13 M ascorbic acid in 0.1 M NaOH aqueous solutions (pH *ca.* 5) were rapidly injected into liquid nitrogen and the mixing solutions were quickly quenched after the mixing.[9] The EPR spectra were measured for the solid sample at the temperature of liquid nitrogen. The EPR spectra were recorded using an X-band spectrometer with a 455 kHz field modulation constructed by the Professor Kuwata's Laboratory in Osaka University.

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1) Y. Ogata, Y. Kosugi and T. Morimoto, *Tetrahedron*, **24**, 4054 (1968).

2) A. Weissberger and J. E. LuVall, *J. Amer. Chem. Soc.*, **66**, 700 (1944).

3) E. Silverblatt, A. L. Robinson and C. G. King, *ibid*, **65**, 137 (1943).

4) L. W. Mapson, *Biochem. J.*, **39**, 228 (1945).

5) I. Ohnishi and T. Hara, This Bulletin, **37**, 1317 (1960).

6) V. S. Butt and M. Hallaway, *Arch. Biochem. Biophys.*, **92**, 24 (1961).

7) A. O. Dekker and R. G. Dickinson, *J. Amer. Chem. Soc.*, **62**, 2165 (1940).

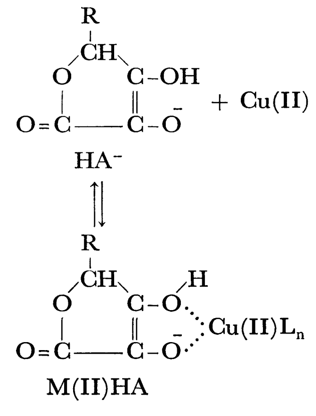
8) I. Yamazaki, H. S. Mason, and L. Piette, *J. Biol. Chem.*, **235**, 2444 (1960).

9) Y. Shimizu, T. Shiga and K. Kuwata, *J. Phys. Chem.*, **74**, 2929 (1970).

## Results and Discussion

The plot of the absorbance at 265 nm, $D_{265}$, *versus* the concentration of $HA^-$, which was calculated using the equilibrium constant ($pK_1=4.12$) for $H_2A \rightleftarrows HA^- + H^+$, is given in Fig. 1 in the absence of copper(II). As a straight line passing through the original point is obtained, it may be concluded that the absorbance at 265 nm is based on $HA^-$ under the present experimental conditions; this is consistent with the report by Ogata *et al.*[10] The molar extinction coefficient of $HA^-$ obtained at 265 nm, $\varepsilon_{HA}$, is $(1.62 \pm 0.02) \times 10^4$ $M^{-1}\,cm^{-1}$ at 20 °C. The spectra of 0.2 mM $CuSO_4$ and the buffer solution and a kinetic run at pH *ca.* 5 are given in Fig. 2. It is found that the absorbance of $CuSO_4$ and the buffer solution do not interfere with the absorbances of $HA^-$ at 265 nm. The absorption bands of the copper(I) compound and dehydroascorbic acid produced by the reaction are absent between 240 to 350 nm. At pH 6.5, the formation of the precipitate of copper (I) oxide[11] interfere with the measurement of the spectra. The copper(II) ion is present as the acetatocopper(II) complex ion in this buffer solution.

In the presence of the copper(II) ion, the formation of the monohydroascorbatocopper(II) complex ion, M(II)HA, has been reported by studies of the copper(II) ion-catalyzed autoxidation of ascorbic acid[1,12] and by spectroscopic studies.[10]



(2)

where L is the other ligand. The $\lambda_{max}$ of the complex is reported to shift to a shorter wavelength than that of $HA^-$,[10] but the molar extinction coefficient of the complex at 265 nm is not given. The fact that the absorbance, $D_{265}$, after a mixing of the reactants is equal to a half of the absorbance before mixing suggests that the concentration of the complex, $C_{MHA}$, is small compared with that of $HA^-$ and/or that the molar extinction coeffieient of M(II)HA is nearly equal to that of $HA^-$. From Equilibrium (2), $C_{MHA}=K_{MHA}C_MC_{HA}$, where $K_{MHA}$ is the formation constant of the complex. As $C_M$ is *ca.* 0.1 mM and $K_{MHA}$ is *ca.* 40 $M^{-1}$,[12] it is found that $C_{MHA}$ is very small compared with $C_{HA}$ under the present experimental conditions. Therefore, the decrease in $D_{265}$ corresponds to that of $HA^-$.

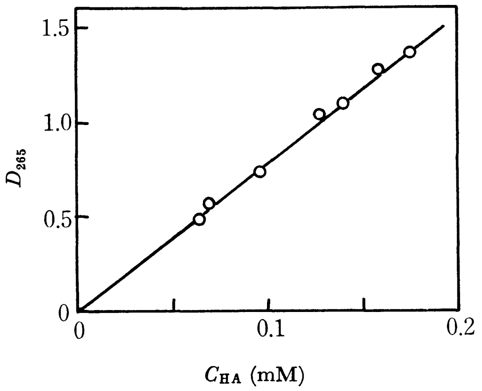


Fig. 1. The plot of the absorbance at 265 nm *versus* the concentration of monohydroascorbate ion; the total concentration of ascorbic acid: 0.08—0.2 mM.

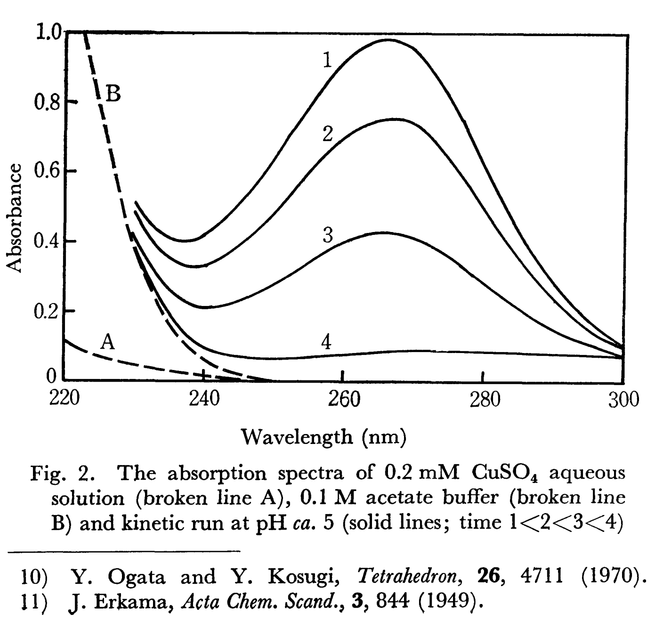


Fig. 2. The absorption spectra of 0.2 mM $CuSO_4$ aqueous solution (broken line A), 0.1 M acetate buffer (broken line B) and kinetic run at pH *ca.* 5 (solid lines; time 1<2<3<4)

TABLE 1. APPARENT FIRST-ORDER RATE CONSTANT $k'$
Temperature 30 °C, $[H_2A]_0$ $(0.73-2.53)\times 10^{-4}$ M

| $CuSO_4$ ($\times 10^4$M) | $k'$ ($sec^{-1}$) pH=4.80 | pH=4.96 | pH=5.25 | pH=5.50 |
|---|---|---|---|---|
| 4.81 | 170±2 | 204±2 | 345±4 | 457±5 |
| 4.01 | 128±2 | 161±5 | 262±3 | 335±5 |
| 3.21 | 95±1 | 102±1 | 185±3 | 241±2 |
| 2.40 | 59±3 | 65±4 | 147±1 | 168±3 |
| 1.60 | 47±2 | 46±2 | 96±7 | 113±4 |

The initial rate, $V_0(=-(dD/dt)_{t=0})$, is directly proportional to the initial concentration of ascorbic acid, $C_A{}^0$. It is found that this reaction is a first-order reaction with respect to $C_A{}^0$. The apparent first-order rate constants, $k'$, are given in Table 1. The apparent first-order rate constant is directly proportional to the square of the initial concentration of the copper(II) ion, $C_M{}^0$. It is found that the reaction is a second-order reaction with respect to $C_M{}^0$. Therefore, the rate equation is as follows:

$$V_0 = C_A{}^0(k''C_M{}^{02}+a) \tag{3}$$

where it is considered that the second term is a term resulting from the effect of a very small quantity of oxygen dissolved in the solution. $k''$ and $a$ are given in Table 2.

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[10] Y. Ogata and Y. Kosugi, *Tetrahedron*, **26**, 4711 (1970).

[11] J. Erkama, *Acta Chem. Scand.*, **3**, 844 (1949).

[12] M. M. T. Khan and A. E. Martell, *J. Amer. Chem. Soc.*, **89**, 4176 (1967).

TABLE 2. RATE CONSTANT $k''$ AND $a$ IN EQUATION (3) AT 30 °C

| pH | $k''$ ($\times 10^{-8}$ sec$^{-1}$M$^{-2}$) | $a$ (sec$^{-1}$) |
|---|---|---|
| 4.80 | $61\pm1$ | $29\pm4$ |
| 4.96 | $81\pm3$ | $23\pm4$ |
| 5.25 | $119\pm3$ | $69\pm4$ |
| 5.50 | $166\pm1$ | $71\pm1$ |

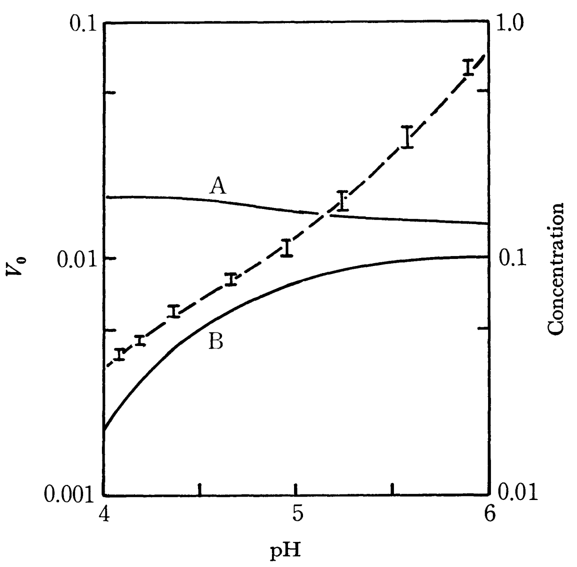

Fig. 3. Dependence of the initial rate at 0.37 mM Cu(II) ion and 0.11 mM ascorbic acid on pH (I) and the dependences of the concentrations of mono- and bis-acetatocopper(II) complex ion on pH (solid line A and B respectively). The broken line is calculated from the next equation:

$V_0=7.5\times10^{-3}/(1+1.3\times10^4C_H)+6.8\times10^{-8}/C_H(1+1.3\times10^4C_H)$.

The logarithm plot of $V_0$ *versus* the concentration of the hydrogen ion, $C_H$, is given in Fig. 3 at 0.11 mM $H_2A$ and 0.25 mM Cu(II). It is considered that the dependence of the rate on pH is primarily due to the difference in reactivity between the undissociated form and the dissociated forms of ascorbic acid and, secondary, to the difference in reactivity between the copper(II) ion and the acetatocopper(II) complex ion. According to Weissberger *et al.*, the apparent first-order rate constants of the copper(II)-catalyzed autoxidation of ascorbic acid obtained in phosphate, acetate, and carbonate buffer solutions depend on the pH with a smooth curve.2) This suggests that the second effect is small. Figure 3 shows that the dependence of the rate on the pH is not consistent with the dependence of the concentrations of the acetatocopper(II) complex ions (solid line), which are calculated using the formation constants of the mono- and bis-acetatocopper(II) complex ions ($K_1=251$ M$^{-1}$ and $K_2=7.94$ M$^{-1}$ respectively13)). From the plot of $1/V_0$ *versus* $C_H$ in Fig. 4, the next relations are obtained:

$$V_0 = k_1''/(1+bC_H) \qquad (\mathrm{pH}\leq5) \qquad (4a)$$

$$V_0 = k_2''/C_H \qquad (\mathrm{pH}>5) \qquad (4b)$$

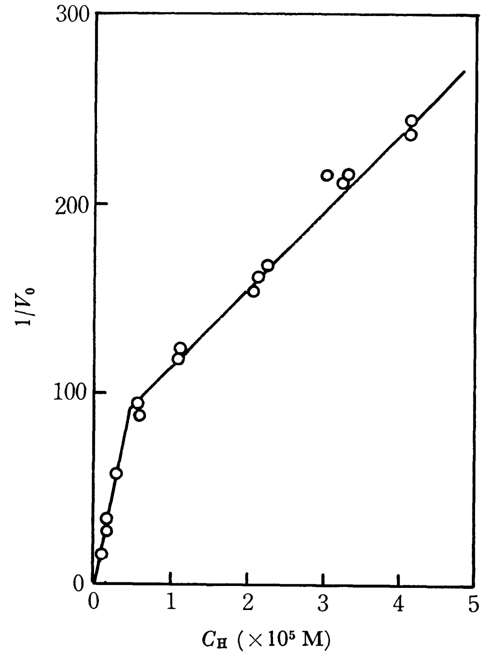

Fig. 4. Dependence of the reciprocal of the initial rate on the concentration of hydrogen ion.

In analogy with the autoxidation of ascorbic acid,14) it is considered that both the monovalent and divalent ions of ascorbic acid are able to participate in the present reaction. From the dissociation constants of ascorbic acid (p$K_1=4.04$ for $H_2A\rightleftarrows H^++HA^-$ and p$K_2=11.34$ for $HA^-\rightleftarrows H^++A^{2-}$ at 25 °C), $C_{HA}=C_A{}^0/(1+K_{-1}C_H)$ and $C_A=C_A{}^0/K_{-2}C_H(1+K_{-1}C_H)$ at pH 4—6, where $K_{-1}=1/K_1$, $K_{-2}=1/K_2$ and $C_{HA}$ and $C_A$ are the concentrations of $HA^-$ and $A^{2-}$ respectively. Therefore,

$$V_0 = k_1'C_A{}^0/(1+K_{-1}C_H) + k_2'C_A{}^0/K_{-2}C_H(1+K_{-1}C_H) \qquad (5)$$

Substituting the values of $k_1'C_A{}^0$, $k_2'C_A{}^0/K_{-2}$, and $K_{-1}$ ($7.5\times10^{-3}$, $6.8\times10^{-8}$, and $1.3\times10^4$ respectively, which are obtained using a curve-fitting method15)) in Eq. (5), the broken line in Fig. 3 is obtained. The curve is very consistent with the experimental data. Therefore, the experimental rate equation is as follows:

$$V_0/\varepsilon_{HA} = k_1C_M{}^{0\,2}C_A{}^0/(1+bC_H) + k_2C_M{}^{0\,2}C_A{}^0/C_H(1+bC_H) \qquad (6)$$

where $k_1=3.1\times10^4$ M$^{-2}$ s$^{-1}$, $k_2=0.28$ M$^{-1}$ s$^{-1}$, and $b=1.3\times10^4$ M$^{-1}$.

In the oxidation of $H_2A$ by the peroxidase and in the autoxidation of $H_2A$ at pH 6.6—9.6, the free radical from $H_2A$ was detected by EPR measurements.8,16,17) The EPR spectra of kinetic runs containing 0.05 M $CuSO_4$ and 0.13 M $H_2A$ in a 0.1 M NaOH aqueous solution (pH *ca.* 5) were measured using a continuous-flow technique and the quenching technique. The blue solution became brown quickly after mixing. The EPR signal from the copper(II) ion is observed, but that from the ascorbate radical is not observed under

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13) L. G. Sillén and A. E. Martell, "Stability Constants of Metal-ion Complexes," Chemical Society, London (1964), p. 365,

14) A. Weissberger, J. E. LuValle and D. S. Thomas, Jr., *J. Amer. Chem. Soc.*, **65**, 1934 (1943).

15) L. G. Sillén, *Acta Chem. Scand.*, **10**, 186 (1956); D. Dyrssen and L. G. Sillén, *ibid.*, **7**, 663 (1953).

16) I. Yamazaki and L. Piette, *Biochim. Biophys. Acta*, **50**, 62 (1961).

17) Ç. Lagercrantz, *Acta Chem. Scand.*, **18**, 562 (1964).

the condition of a varying flow rate. It is found by the stopped-flow technique that the EPR signal of the copper(II) ion from the brown solution decreases with the time because of the formation of the copper(I) compound, but the EPR signal from the ascorbate radical is not observed.

The above facts and discussions suggest the following mechanism for the oxidation of ascorbic acid by the copper(II) ion at pH 4—6, $C_A{}^0$ *ca.* 0.1 mM, $C_M{}^0$ *ca.* 0.1 mM, and 30 °C:

$$\mathrm{H_2A} \overset{K_1}{\rightleftharpoons} \mathrm{H^+} + \mathrm{HA^-} \tag{7}$$

$$\mathrm{HA^-} \overset{K_2}{\rightleftharpoons} \mathrm{H^+} + \mathrm{A^{2-}} \tag{8}$$

$$\mathrm{HA^-} + \mathrm{Cu(II)} \overset{K_9}{\rightleftharpoons} \mathrm{Cu(II)HA} \tag{9}$$

$$\mathrm{A^{2-}} + \mathrm{Cu(II)} \overset{K_{10}}{\rightleftharpoons} \mathrm{Cu(II)A} \tag{10}$$

$$\mathrm{Cu(II)HA} + \mathrm{Cu(II)} \xrightarrow{k_{11}} \mathrm{DA} + 2\mathrm{Cu(I)} + \mathrm{H^+} \tag{11}$$

$$\mathrm{Cu(II)A} + \mathrm{Cu(II)} \xrightarrow{k_{15}} \mathrm{DA} + 2\mathrm{Cu(I)} \tag{12}$$

Assuming that Reactions (11) and (12) are the rate-determining steps and applying the preliminary equilibrium treatment to Reactions (7), (8), (9) and (10), the following expression is obtained:

$$\begin{aligned} V_0/\varepsilon_{\mathrm{HA}} &= k_{11}K_9C_M{}^{0^2}C_A{}^0/(1+K_{-1}C_H) \\ &\quad + k_{12}K_{10}C_M{}^{0^2}C_A{}^0/K_{-2}C_H(1+K_{-1}C_H) \end{aligned} \tag{13}$$

where the next approximations are used: $C_M \gg C_{MHA}$, $C_M \gg C_{MA}$, $C_A \ll C_{HA}$, and $C_A \ll C_A{}^0$. $k_{11}K_9 = 3.1 \times 10^4$ $\mathrm{M^{-2}\,s^{-1}}$, $k_{12}K_{10} = 6.1 \times 10^{10}$ $\mathrm{M^{-2}\,s^{-1}}$ (when $K_{-2} = 2.2 \times 10^{11}$ $\mathrm{M^{-1}}$ [12]) and $K_{-1} = 1.3 \times 10^4$ $\mathrm{M^{-1}}$ are also obtained. According to Khan *et al.*, $K_{-1} = 1.1 \times 10^4$ $\mathrm{M^{-1}}$ at 25 °C.[12] Before the rate-determining steps, the Cu(II)HA⇄Cu(I)HA· and Cu(II)A⇄Cu(I)A· equilibria are considered, but no evidence of the presence of a radical was obtained.

TABLE 3. EFFECT OF TEMPERATURE AND ACTIVATION PARAMETERS

pH 4.95, $[\mathrm{CuSO_4}]_0$ $3.01 \times 10^{-4}$ M, $[\mathrm{H_2A}]_0$ $(0.6—2.5) \times 10^{-4}$ M

| Temperature (°C) | $k'$ (sec$^{-1}$) |
|---|---|
| 24.5 | 70±3 |
| 32 | 137±3 |
| 38 | 281±4 |
| 42 | 360±6 |

$\Delta H^{\neq} = 17$ kcal, $\Delta S^{\neq} = 8.3$ cal/deg, $\Delta G^{\neq} = 15$ kcal

The dependence of $k'$ on the temperature is given in Table 3. From the Arrhenius plot based on the theory of the transition state, the activation parameters of the present reaction were obtained as follows: $\Delta H^{\neq} = 17$ kcal/mol, $\Delta S^{\neq} = 8.3$ cal deg$^{-1}$ mol$^{-1}$ and $\Delta G^{\neq} = 15$ kcal/mol. These values are comparable with those in the copper(II)-catalyzed autoxidation of $\mathrm{H_2A}$ ($\Delta H^{\neq} = 15.5$ kcal/mol, $\Delta S^{\neq} = 14$ cal deg$^{-1}$ mol$^{-1}$ and $\Delta G^{\neq} = 11.3$ kcal/mol[12]).

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