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The purpose of this work was to reveal the identity of the deactivation reaction involved in the Fenton's oxidation of aromatic compounds. The effect of reaction conditions on the yield of phenol in the oxidation of benzene was systematically studied. The maximum yield of phenol (93% based on H_2O_2 added) was obtained at low concentrations of both phenol formed and Fe(II) ion added. The predominant deactivation reaction of hydroxyl radicals in the Fenton's oxidation was found to be the reaction of hydroxyl radical with phenol to give phenoxy radical, followed by the reduction of phenoxy radical with Fe(II) ion to regenerate the phenol molecule.

The hydroxylation of aromatic compounds were Fenton's reagent has been a subject of profound interest.¹⁻⁸ The active species generated from Fenton's reagent is hydroxyl radicals, and the first step of the hydroxylation of, for instance, benzene, is the addition of hydroxyl radicals to benzene nuclei to form hydroxycyclohexadienyl radicals (1).¹⁻³ Reaction mechanisms of enzymatic hy-

droxylations and their model systems such as Udenfriend's^{9,10} and Hamilton's reagents¹¹⁻¹³ have always been discussed in comparison with that of Fenton's oxidation, especially with respect to the isomer distributions of substituted phenols.14

In the Fenton's oxidation of benzene itself, the reaction mechanism for the formation of phenol and biphenyl is well established and is summarized in Scheme I.¹⁻⁵ The yield of phenol based on hydrogen peroxide, however, varies markedly depending on the reaction conditions, i.e., "less than 5%,"³ 57%,⁵ 40-50%,⁶ 12%.⁷ Since the byproduct is only a small amount of biphenyl, more than 43% of hydrogen peroxide has been wastefully consumed in some of the side and/or deactivation reactions shown in Scheme I. It is not clear, however, which of the reactions in Scheme I is responsible for the low yield of phenol observed. In the present work, the effect of reaction conditions on the yield of phenol in the Fenton's oxidation of benzene has been systematically investigated in order to reveal the identity of the side and/or deactivation re-

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Table I. Effect of Counteranion of Iron(II) Salts on Yield of Phenol^a

| | % yield of phenol ^b | | |
|-----------|---|--|--|
| Fe(II)/mM | $ \begin{array}{c} \operatorname{Fe}(\operatorname{ClO}_4)_2 \text{ in } 100 \\ \operatorname{mM} \operatorname{HClO}_4 \end{array} $ | FeSO ₄ in 50 mM H ₂ SO ₄ | |
| 5 | 79 ± 2 (4) | 83 ± 3 (3) | |
| 10 | $69 \pm 3 (4)$ | $71 \pm 3 (4)$ | |

^a In 25 mL of 50 mM H₂SO₄ or 100 mM HClO₄ containing Fe(II) ion, 30 mM CuSO₄, and 0.75 mL of benzene at 25 °C. The amounts of H_2O_2 added were 131-133 and 276-283 µmol at 5 and 10 mM of Fe(II) ions, respectively. ^bThe yield of phenol was based on H_2O_2 added. The values in parentheses are the number of repetitions.

action(s) operative in the Fenton's oxidation.

Results and Discussion

The wide range of phenol yields (5-57%) obtained under the comparable reaction conditions³⁻⁷ suggests that an unidentified reaction factor is operating in the Fenton's oxidation. Prior to examining the effect of the initial concentrations of benzene, H_2O_2 , and Fe(II) ions on the vield of phenol, some preliminary experiments were conducted in order to determine a standard for the reaction conditions.

1. Standard Reaction Method. In the Fenton's oxidation procedure, there is ambiguity with respect to the order of mixing of the reagents that make up Fenton's reagent. Norman³ and Walling⁵ adopted a procedure to add aqueous H_2O_2 dropwise into an Fe(II) solution, although the yield of phenol was not reproducible, i.e., less than 5%³ and 57%.⁵ Since Norman³ and Walling⁵ used two different sources of Fe(II) ions for the Fenton's

Table II. Effect of Mixing Method on Yield of Phenol^a

| mixing method | % yield of phenol | |
|---|----------------------|--|
| H_2O_2 (100 mM) was added into 10 mM FeSO ₄ solution containing 30 mM CuSO ₄ | 71 ^b | |
| H_2O_2 (100 mM) was added into 20 mM FeSO ₄ solution containing 30 mM CuSO ₄ | 65 | |
| $FeSO_4$ (10 mM) was added into 10 mM H_2O_2 solution containing 30 mM $CuSO_4$ | 71 | |
| $FeSO_4~(50~mM)$ was added into 10 mM H_2O_2 solution containing 20 mM $CuSO_4$ | 67 | |
| H ₂ O ₂ (20 mM) was mixed with 20 mM FeSO ₄ containing 60 mM CuSO ₄ by use of T-shaped capillary tube | 53° | |
| "HO and FaSO ware concretely dissolved in 50 n | M H.SO. | |

⁶H₂O₂ and FeSO₄ were separately dissolved in 50 mM H₂SO₄. ^bUnder the standard reaction conditions. ^cAn appreciable amount of biphenyl was formed.

Table III. Effect of Dioxygen on Yield of Phenol at 25 °C^a

| FeSO₄/mM | atm | % yield of phenol ^b | |
|----------|------------------|--------------------------------|--|
| 10 | N ₂ | 71° | |
| 10 | $\overline{O_2}$ | 50 | |
| 20 | N_2 | 58 | |
| 20 | O_2 | 38 | |

 $^{\rm a}\,In$ 25 mL of 50 mM H_2SO_4 containing FeSO₄, 30 mM CuSO₄, and 0.75 mL of benzene. $^{\rm b}Based$ on H_2O_2 added. $^{\rm c}Under$ the standard reaction conditions.

reagent, i.e., iron(II) sulfate in 0.2 M H_2SO_4 and iron(II) perchlorate in 0.05 M HClO₄, respectively, the difference in the counteranions might be responsible for the large discrepancy observed in the yields of phenol. The effect of the counter ions on the yield of phenol in the Fenton's oxidation of benzene was, however, not significant, as seen in Table I.

Accordingly, we tested three different methods of mixing the reagents, namely, (i) dropwise addition of H_2O_2 into an Fe(II) solution, (ii) dropwise addition of Fe(II) into an H_2O_2 solution, and (iii) rapid mixing of Fe(II) and H_2O_2 solutions by use of a T-shaped capillary tube. All the reactions were carried out in the presence of 30 mM copper(II) sulfate in order to oxidize the radical 1 effectively (see Figure 1). As seen in Table II, the effect of the order of addition of H_2O_2 and Fe(II) on the yield of phenol is not significant, while mixing with a T-shaped capillary tube gave a lower yield of phenol with concomitant formation of an appreciable amount of biphenyl 2. Since the yield of phenol is sensitive to the concentration of Fe(II) but not of H_2O_2 as described below (see Figures 2 and 3), the method of mixing was standardized as the dropwise addition of 110 mM H_2O_2 in 50 mM H_2SO_4 into 25 mL of a solution that was 50 mM H₂SO₄, 10 mM FeSO₄, 30 mM CuSO₄, and 0.75 mL of benzene. The yield of phenol obtained under the standard reaction conditions was 71 \pm 3% based on the amount of H₂O₂ added (273-288 μ mol).

Most of the Fenton's oxidations hitherto reported were performed under a N₂ atmosphere.^{3,5} Since the key intermediate, hydroxycyclohexadienyl radicals (1), can be oxidized with O₂ as well as Cu(II) and Fe(III) ions to give phenol, Fenton's oxidation under O₂ atmosphere may increase the yield of phenol. The result in Table III shows that, on the contrary, lower yields are obtained with the experiments under O₂ atmosphere, probably because of the by-production of polyoxygenated products. In fact, the high-performance liquid chromatogram of the reaction mixture obtained under O₂ atmosphere gave an additional peak before that of phenol.^{6,7} The extra peak was not seen in the reaction run under N₂ atmosphere and was depleted

Table IV. Effect of Stream of N2 Gas on Yield of Phenola

| | · · · · · · · · · · · · · · · · · · · | | | |
|-------------|---------------------------------------|--------------------------------|--|--|
| H_2O_2/mM | N ₂ | % yield of phenol ^b | | |
| 110 | closed | 71° | | |
| 110 | flow | 49, 19 | | |
| 290 | closed | 71 | | |
| 290 | flow | 63, 46 | | |
| 580 | closed | 72 | | |
| 580 | flow | 42, 46 | | |
| | | | | |

^c In 25 mL of 50 mM H_2SO_4 containing 10 mM FeSO₄, 30 mM CuSO₄, and 0.75 mL of benzene. ^bBased on H_2O_2 added. ^cUnder the standard reaction conditions.

by addition of carbonyl reagents such as semicarbazide and hydroxylamine, indicating that it can be assigned to mucondialdehyde(s).⁸

There is another variable with respect to the N_2 atmosphere, i.e., whether N_2 gas is closed in the reactor or allowed to flow through the reactor during the oxidation of benzene. As Table IV clearly demonstrates the yield of phenol in the closed system was higher and more reproducible than that obtained in the flow system. Taking into account the high volatility of benzene, the low yield in the flow system must be due to the decrease in the effective concentration of benzene in the solution. especially at the surface of the solution where aqueous H_2O_2 is introducted. Accordingly, the standard protocol for the reaction was fixed as "the dropwise addition of aqueous H_2O_2 into aqueous Fe(II) solution containing benzene under N_2 atmosphere in a closed system at 25 °C" in the following experiments. The potential side reaction through peroxy radical 3, formed by the reaction of radical 1 with dioxygen (See Scheme I), therefore, can be excluded in the following discussion.

2. Stoichiometry for Reaction of H_2O_2 with Fe(II). Hydrogen peroxide is decomposed to give either H_2O or O_2 as a final product. The relative contribution of these decomposition reactions can be estimated from the stoichiometric relationship as follows. The decomposition of H_2O_2 promoted by Fe(II) ions can be expressed as:

$$H_2O_2 + Fe(II) + H^+ = H_2O + OH + Fe(III)$$
 (2)

$$OH + Fe(II) + H^{+} = H_2O + Fe(III)$$
 (3)

By adding eqs 2 to 3, eq 4 is obtained.

$$H_2O_2 + 2Fe(II) + 2H^+ = 2H_2O + 2Fe(III)$$
 (4)

According to (4), the molar ratio of Fe(II) oxidized to H_2O_2 consumed ($n = \Delta Fe(II)/\Delta H_2O_2$), which is originally defined as "consumption ratio" by Barb and co-workers,¹⁵ should be 2.

Competing with (3), hydroxyl radicals also react with H_2O_2 to give hydroperoxy radicals $(HO_2^{\bullet}).^{16}$

$$OH + H_2O_2 = H_2O + HO_2$$
 (5)

Hydroperoxy radicals can be oxidized with Fe(III) or Cu-(II) ions, if present, to evolve molecular oxygen.

$$HO_2^{\bullet} + Fe(III) = O_2 + Fe(II) + H^+$$
(6)

$$HO_2^{\bullet} + Cu(II) = O_2 + Cu(I) + H^+$$
 (6')

Summing up eqs 2, 5, and 6, one obtains eq 7. $2H O_{-} = 2H O_{+} O_{-}$

$$2H_2O_2 = 2H_2O + O_2 \tag{7}$$

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Provided (7) catalyzed by Fe(II)/Fe(III) redox couple occurs appreciably during the Fenton's oxidation of benzene, the consumption ratio n should be less than 2 and the yield of phenol should decrease with decreasing n

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Table V. Stoichiometry for Reaction of H₂O₂ with Fe(II) Ion^a

| | Cu(II)/ mM | $H_2O_2/\mu mol$ | | Fe(II)/µmol | | ΔFe(II)/ | |
|--|---------------|------------------|-------|-------------|-------|-----------------|--|
| | | initial | final | initial | final | ΔH_2O_2 | |
| | | 277 | 121 | 250 | 5 | 1.57 | |
| | 0 | 277 | 20 | 500 | 3 | 1.93 | |
| | 0 | 277 | 9 | 750 | 206 | 2.02 | |
| | 0 | 277 | 10 | 1000 | 465 | 2.00 | |
| | 30 | 280 | 145 | 250 | 1 | 1.84 | |
| | 30 | 277 | 37 | 500 | 3 | 2.07 | |
| | 30 | 280 | 9 | 750 | 205 | 2.01 | |
| | 30 | 280 | 8 | 1000 | 450 | 2.02 | |

^a In 25 mL of 50 mM H₂SO₄ at 25 °C.



Figure 1. Effect of copper(II) sulfate on the yield of phenol and biphenyl: O, phenol; \bullet , biphenyl. Solvent: 25 mL of 50 mM H₂SO₄; FeSO₄, 30 mM; CuSO₄, 30 mM; H₂O₂, 280 ± 8 μ mol; benzene, 0.75 mL (8.4 mmol). Temperature: 25 °C.

value. The experimental values of the consumption ratio n are, as summarized in Table V, equal to 2 within experimental error at the higher concentrations of Fe(II) ions (20-40 mM), though the yield of phenol was as low as 68-44% (Figure 3). The results in Table V and Figure 3 indicate that the low yield of phenol in the Fenton's oxidation cannot be attributed to the deactivation of hydroxyl radicals according to (5) followed by (6).

3. Effect of Cu(II) Ions on Yield of Phenol. In 1969, Norman found that the addition of copper(II) ions to the Fenton's system markedly increases the yield of oxidation products.⁴ The acceleration effect of copper(II) ions has been explained by the effective oxidation of the radical 1 to give phenol according to (8). Reaction 8 competes with

$$\bigcirc H + Cu(II) = \bigcirc -OH + Cu(I) + H^{+}$$
 (8)

the dimerization of the radical 1 to give biphenyl 2 (reaction 9), and the addition of copper(II) ions favors the

$$2 \left(\bigcirc H \right)^{OH} = \left(\bigcirc H \right)^{OH} + 2H_2O$$
 (9)

main reaction (8).

Walling proposed that, since the yield of phenol is only 57% even when the formation of biphenyl is negligible in the Fenton's oxidation of benzene, the low yield of phenol must be caused by the deactivation reaction of radical 1 to give cation radical 4, followed by reduction of 4 with iron(II) ions to regenerate benzene.⁵

$$\textcircled{OH}_{H} + H^{+} = \textcircled{O} + H_{2}O \qquad (10)$$

$$\textcircled{+} Fe(II) = \textcircled{+} Fe(III) \tag{11}$$

Provided that both the side reaction (9) and the deactivation reactions (10) and (11) are responsible exclusively for the low yield of phenol, the yield of phenol should



Figure 2. Effect of concentration of H_2O_2 on the yield of phenol: •, H_2O_2 in 50 mM H_2SO_4 saturated with benzene was added; 0, H_2O_2 in 50 mM H_2SO_4 was added. Solvent: 25 mL of 50 mM H_2SO_4 ; FeSO₄, 10 mM; CuSO₄, 30 mM; H_2O_2 , 277 \pm 25 μ mol; benzene, 0.75 mL (8.4 mmol). Temperature: 25 °C.

Table VI. Effect of Initial Concentration of Fe(II) Ion on Yield of Phenol at 25 °C^a

| H _a O _a / | Fe(II)/ | % yield of phenol | | |
|---------------------------------|-----------------|-------------------|-------------------------------|--|
| µmol | mM | obsd ^b | calcd from eq 12 ^b | |
| 132 | | 82 ± 2 | 98.9 | |
| 132 | 20 ^d | 78 ± 1 | 95.8 | |
| 64 | 2.5° | 93 ± 1 | 99.5 | |
| 64 | 20^d | 89 ± 1 | 95.8 | |

 a Under the standard reaction conditions. $^{b}Based$ on $H_{2}O_{2}$ added. $^{c}125~\mu mol.$ $^{d}500~\mu mol.$ $^{c}62.5~\mu mol.$

increase up to 100% with increasing concentration of copper(II) ions added. Figure 1 shows that the yield of phenol increases with the addition of copper(II) sulfate but the plateau of the yield $(54 \pm 2\%)$ is obtained in the range of 25–60 mM of copper(II) sulfate.

The result in Figure 1 indicates that (i) the formation of biphenyl is negligible in the presence of more than 10 mM of copper(II) sulfate, (ii) the increment of the yield of phenol by the addition of copper(II) ions is, therefore, mainly due to suppression of the side reaction (9) and/or the deactivation reactions (10) and (11), but (iii) $46 \pm 2\%$ of hydrogen peroxide is still wastefully consumed according to some reactions even after complete suppression of (9), (10), and (11).

In light of the results obtained above, all the Fenton's oxidations described below were carried out in the presence of 30 mM copper(II) sulfate.

4. Effect of H_2O_2 Concentration. When H_2O_2 in initial concentrations from 58 to 590 mM was added dropwise into 10 mM of Fe(II) solution containing benzene, the yield of phenol was essentially constant (71 ± 4% based on H_2O_2 added) as seen in Figure 2.

5. Effect of Fe(II) Concentration. The effect of the initial concentration of iron(II) sulfate on the yield of phenol under the standard reaction conditions was, as shown in Table VI, very small (less than 4%).

The slight inhibition effect of Fe(II) ions at the higher concentrations can be explained by the competitive reactions (3) and (1) toward hydroxyl radicals, though Walling claimed that the rate constant for the reaction of hydroxyl radical with Fe(II) ions is too small $(3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1})^{16}$ to explain the observed low yield of phenol (57%). A simplified reaction model can help to estimate the contribution of (3). Assuming that the concentrations of both benzene and Fe(II) ions are approximately constant during the entire sequence of the oxidation, one can derive eq 12

yield of phenol =

$$\frac{[\text{PhOH}]}{[\text{PhOH}]_{o}} = \frac{k_{\text{B}}[\text{benzene}]}{k_{\text{B}}[\text{benzene}] + k_{\text{F}}[\text{Fe}(\text{II})]}$$
(12)

Table VII. Oxidation of Phenol with Fenton's Reagent^a

| H_2O_2/μ mol phenol/ μ mol | | µmol | catechol/ | hydroquinone/ | | |
|------------------------------------|-------|---------|-----------|---------------|-----------------------|-----------------------|
| initial | final | initial | final | consumed | μmol | μmol |
| 285 | trace | 125 | 105 | 20 | 2 (0.7%) ^b | 4 (1.4%) ^b |
| 662 | trace | 125 | 97 | 28 | 3 (0.5%) ^b | 5 (0.8%) ^b |

 o In 25 mL of 50 mM H₂SO₄ containing 20 mM FeSO₄ (500 μ mol) and 30 mM CuSO₄ at 25 °C. b The values in parentheses are % yield based on H₂O₂ added.

where [PhOH]_o and [PhOH] are the theoretical and observed yields of phenol, [Fe(II)] and [benzene] are the concentrations of Fe(II) and benzene in the solution, and $k_{\rm Fe}$ and $k_{\rm B}$ are the rate constants for (3) and (1), respectively. Substituting the numerical values of $k_{\rm B}$ ((6.0 ± 1.7) × 10⁹ M⁻¹ s⁻¹),^{14,17,18} $k_{\rm Fe}$ (3 × 10⁸ M⁻¹ s⁻¹),¹⁶ and the saturated concentration of benzene in 50 mM of H₂SO₄ (0.023 M at 25 °C) into eq 12, one can estimate the yield of phenol at a given concentration of iron(II) ions. The observed yield of phenol in Table VI is slightly but definitely lower than those calculated from eq 12, although the yield of phenol decreases with increasing concentration of Fe(II), as predicted by eq 12, for a fixed amount of H₂O₂ added.

The results in Table VI suggest that the yield of phenol mainly depends on the amount of H_2O_2 added, not on the concentration of H_2O_2 as has already been shown in Figure 2.

6. Effect of Amount of H_2O_2 Added. When the amount of H_2O_2 was increased by increasing the duration of dropwise addition of 110 mM H_2O_2 into 50 mM H_2SO_4 containing benzene (0.75 mL), FeSO₄ (equivalent to H_2O_2), and CuSO₄ (30 mM), the % yield of phenol based on H_2O_2 significantly decreased, i.e., 93 ± 1 , 82 ± 3 , $71 \oplus 3$, $60 \pm$ 7 and $53 \pm 6\%$ for the addition of 64, 131, 277, 415, and 610 µmol of H_2O_2 , respectively. The maximum yield of phenol amounted to 93% (based on H_2O_2 added) at 2.5 mM of FeSO₄ and 64 µmol of H_2O_2 . The plot of % yield of phenol against the amount of phenol formed, rather than the amount of H_2O_2 added, is shown in Figure 3.

The next question to address is that of why the yield of phenol decreases with increasing amount of H_2O_2 added. One might expect that the consecutive oxidation of phenol to catechol, hydroquinone, and further oxidation products brings about the apparent decrease in the phenol yield. The yields of catechol and hydroquinone in the Fenton's oxidation of benzene were, however, less than 1% under the present reaction conditions, indicating that the consecutive oxidation of phenol is not responsible for the results in Figure 3.

7. Deactivation Mechanism. A separate experiment on the oxidation of *phenol* with the Fenton's reagent at 25 °C also showed that the yields of catechol and hydroquinone based on H_2O_2 added were very low (0.5–1.4%), as shown in Table VII.

It is noteworthy that the amount of phenol consumed is only 4-7% of H_2O_2 added. Taking into account that there was only a trace amount of H_2O_2 remaining in the oxidation mixture, a deactivation reaction (13) for hydroxyl radicals must be operating effectively in the Fenton's oxidation of phenol. The phenoxy radical formed is then

$$HO - O + OH = O - O + H_2O$$
(13)

reduced back to phenol with Fe(II) ion according to (14).

•O-
$$\langle O \rangle$$
 + Fe(II) + H^{*} = HO- $\langle O \rangle$ + Fe(III) (14)

Thus, (13) followed by (14) affords a mechanism for rapid consumption of hydroxyl radicals without oxidizing phenol,



Figure 3. Effect of concentration of phenol formed on the yield of phenol. Solvent: 25 mL of 50 mM H₂SO₄; FeSO₄, 20 mM (O), 2.5–20 mM (\odot); CuSO₄, 30 mM; H₂O₂, 64–613 μ mol; benzene, 0.75 mL (8.4 mmol). Temperature: 25 °C. The solid line is calculated from eq 18 using $k_{\rm B} = 4.3 \times 10^9$ M⁻¹ s⁻¹ and $k_{\rm p} = 1.4 \times 10^{10}$ M⁻¹ s⁻¹ (K = 70.7 M⁻¹).

as shown in Table VII. Summation of eqs 13 and 14 gives eq 3.

According to this deactivation mechanism, the yield of phenol in the Fenton's oxidation of benzene can be expressed by eq 15, where $[H_2O_2]_0$ is the amount of hydrogen

yield of phenol =
$$\frac{[PhOH]}{[H_2O_2]_o} = \frac{[PhOH]}{[PhOH]_o} = \frac{1}{K[PhOH] + 1}$$
 (15)

peroxide added and $K = k_p/(k_B[\text{benzene}])$, in which k_p is the rate constant for the reaction of hydroxyl radical with phenol. Equation 15 is derived assuming that (i) benzene and phenol competitively react with hydroxyl radical according to (1) and (13) and (ii) the concentrations of benzene [benzene] and phenol [PhOH] are approximately constant during the entire sequence of the Fenton's oxidation of benzene. For small changes d[H₂O₂] and d-[PhOH] in the amounts of H₂O₂ and phenol, respectively, eq 16 holds. The minus sign on the left-hand side is

$$\frac{\mathrm{d[PhOH]}}{\mathrm{d[H_2O_2]}} \approx \frac{1}{K[\mathrm{PhOH}] + 1}$$
(16)

required since $d[H_2O_2]$ is always negative while d[PhOH] is positive. Equation 16 can be rewritten as

 $(K[PhOH] + 1)d[PhOH] = -d[H_2O_2]$ (17)

Integrating eq 17 from time = zero to t, one obtains

$$K/2[PhOH]^{2} + [PhOH] = [H_{2}O_{2}]_{o}$$

Therefore,

yield of phenol =
$$\frac{[PhOH]}{[H_2O_2]_o} = \frac{1}{(K/2)[PhOH] + 1}$$
 (18)

Substituting the numerical values of k_p (1.4 × 10¹⁰ M⁻¹ s⁻¹),¹⁹ k_B ((6.0 ± 1.7) × 10⁹ M⁻¹ s⁻¹),^{14,17,18} and the saturated concentration of benzene in 50 mM of H₂SO₄ (0.023 M at 25 °C), one can obtain the calculated yield of phenol. As shown in Figure 3, the plot of the yield of phenol observed in the Fenton's oxidation of benzene against the concentration of phenol formed is in good agreement with that calculated from eq 18 using $k_B = 4.3 \times 10^9$ M⁻¹ s⁻¹¹⁷ and $k_p = 1.4 \times 10^{10}$ M⁻¹ s⁻¹¹⁹ (K = 70.7 M⁻¹).

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⁽¹⁸⁾ Neta, P.; Dorfman, L. M. In Radiation Chemistry; Hart, E. J., Ed.; American Chemical Society: Washington D.C., 1968; Vol. 1, p 222.



In conclusion, the minimal reaction scheme for the Fenton's oxidation of benzene is outlined in Scheme II. The main deactivation reaction is the reaction of hydroxyl radical with phenol, followed by reaction of the resulting phenoxy radical with Fe(II) ion. The direct reaction of hydroxyl radical with Fe(II) ion causes the slight decrease (ca. 4% at 20 mM of Fe(II) ion) in the yield of phenol (Table 6).

Experimental Section

Materials. All chemicals used were commercial and reagent grade and were used without further purification. Hydrogen peroxide (35 wt %) was obtained from Katayama Kagaku Kogyou.

Analysis. Phenol, hydroquinone, and catechol were determined by reversed-phase HPLC using a JASCO Intelligent HPLC Pump (880-PU) and a JASCO Intelligent UV/vis detector (875-UV) coupled with a Shimadzu Chromatopac C-R6A. The separation column was a Cica-MERCK Hibar Lichrosorb RP-18 (5 μ m), and the eluent was a mixture of acetonitrile (14 vol %) and a 50 mM phosphate buffer (86 vol %, pH 3.5). Biphenyl was determined by GLC. Hydrogen proxide (titanium sulfate method)²⁰ and iron(II) ions (o-phenanthroline method) were de-

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termined spectroscopically by use of a JASCO UVIDEC 610 spectrophotometer.

Oxidation of Benzene. The standard procedure for the Fenton's oxidation of benzene was as follows: Into a 100-mL Erlenmeyer flask with a rubber stopper equipped with four glass tubings was placed 25 mL of a solution that was 50 mM H₂SO₄, 10 mM FeSO₄, and 30 mM CuSO₄. After air was purged from the flask with N₂ through two glass tubings fitted to the rubber-stopper, 0.75 mL (8.4 mmol) of benzene was added through the third tubing using a microfeeder (Azuma Denki Kogyo, MF-2). With a magnetic bar stirring at 1000-1100 rpm, 100 mM H_2O_2 in 50 mM H_2SO_4 were added dropwise using another microfeeder (Azuma Denki Kogyo, MF-2) with a rate of 0.2759 mL min⁻¹. After addition of the precalculated amount of H₂O₂ at 25 °C, the reaction mixture was allowed to stand for 30 min with stirring. After evaporating the remaining benzene with a stream of N_2 , 5 mL of the reaction mixture was diluted to 50 mL with 25 mM EDTA. An aliquot of the diluted reaction mixture was subjected to HPLC analysis.

Oxidation of Phenol. Into a 100-mL Erlenmeyer flask with rubber-stopper was added 25 mL of a solution that was 50 mM H_2SO_4 , 5 mM phenol, 20 mM FeSO₄, and 30 mM CuSO₄. After air was purged from the flask with N₂, 100 mM H_2O_2 in 50 mM H_2SO_4 was added dropwise using the microfeeder with a magnetic stir bar. The reaction mixture was allowed to stand at 25 °C for 30 min and was subjected to HPLC analysis after dilution with 50 mM EDTA.

Registry No. C_6H_6 , 71-43-2; H_2O_2 , 7722-84-1; $CuSO_4$, 7758-98-7; $Fe(ClO_4)_2$, 13933-23-8; $FeSO_4$, 7720-78-7; C_6H_5 - C_6H_5 , 92-52-4; C_6H_5OH , 108-95-2; HOC_6H_4 -o-OH, 120-80-9; HOC_6H_4 -p-OH, 123-31-9; HO, 3352-57-6; H_2 , 1333-74-0.

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Bipyridyl Amino Acid-Metal Complexes and Their Characterization by Electrospray Mass Spectrometry

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The syntheses of bipyridylalanine and related bipyridyl-functionalized peptide derivatives are described. The copper complexes of several peptide-bipyridyls were prepared and characterized. Application of electrospray ionization mass spectrometry to the structure determination of cationic bipyridyl Cu⁺ complexes 2 and 3, as well as peptide-metal complexes 18-22, was explored.

As part of a program to develop new methods for peptide cross-linking, we are studying the use of a bipyridyl metal template, where the geometric requirements of coordination might be used to constrain a peptide to a well-defined conformation.¹ Metal binding sites in proteins are well-known to stabilize structure as well as to participate in catalytic enzyme function. Cross-linking of synthetic peptides with metals and specific derivatization of peptides with bipyridyl groups has only recently been reported in the literature.² Recent reports of bipyridylalanine complexes by Imperiali³ have prompted us to report our work in this area.⁴

Results and Discussion

Two different strategies have been explored to incorporate the bipyridyl ligand into a peptide. The first involves direct introduction of the bipyridyl (bpy) group onto a cysteine thiol or at the terminus of preformed peptides. The second route involves the preparation of a *new amino acid* containing the bipyridyl (bpy) side chain for use in peptide synthesis.

Electrospray ionization mass spectrometry (ESI-MS) has recently revolutionized the mass measurement of biological molecules.⁵ We have also shown that ESI-MS is quite

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