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# Iron(II)/Reductant ($DH_2$)-Induced Activation of Dioxygen for the Hydroxylation of Aromatic Hydrocarbons and Phenols: Reaction Mimic for Tyrosine Hydroxylase

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**Abstract:** Several iron(II) complexes [($Fe^{II}L_x$); $Fe^{II}(DPAH)_2$ ($DPAH_2$ = 2,6-dicarboxylpyridine), $Fe^{II}(PA)_2$ (PAH = picolinic acid), and $Fe^{II}(bpy)_2^{2+}$ (bpy = 2,2′-bipyridine)] in combination with a reductant [$DH_2$; PhNHNHPh (mimic of dihydroflavin and tetrahydropterins)] catalytically activate $O_2$ (1 atm) for the hydroxylation of phenol and substituted phenols [e.g., PhOH → *o,p*-$C_6H_4(OH)_2$; 4-$XC_6H_4OH$ → 4-$XC_6H_3(OH)_2$ (X = Cl, H, *t*-Bu, MeO)]. This chemistry, which appears to involve a Fenton-like reactive intermediate, [$L_xFe^{IV}OOH(DH)$] (**1c**), mimics that of the tyrosine hydroxylase systems. With phenol as the reactant, the dominant product is catechol with the *o/p* ratio dependent on the $DH_2/Fe^{II}L_x$ ratio. As the latter approaches unity, catechol is favored. With $Fe^{II}(DPAH)_2$ in 3:1 (mole ratio) acetonitrile/pyridine [$(MeCN)_3py$], the relative reactivity for a series of substituted phenols (4-$XC_6H_4OH$; X = Cl, H, *t*-Bu, MeO) is substituent dependent: Cl > H > *t*-Bu > MeO.

Phenol is produced on an industrial scale via the autoxidation of cumene.[1] The direct conversion of benzene to phenol, which would decrease the number of synthetic steps and avoid the production of acetone, is a more desirable route. Pulse radiolysis results have shown that the addition of the hydroxyl radical to the aromatic ring is extremely rapid ($k = (3-8) \times 10^9$ $M^{-1}$ $s^{-1}$)[2] and that Fenton reagents (1:1 $Fe^{II}L_x$/HOOH) produce phenol from benzene.[3-6] Recent work[7,8] has demonstrated that the reactive intermediate with Fenton reagents is a nucleophilic adduct (rather than free HO•):

$$Fe^{II}L_x + HOOH \overset{B}{\rightleftharpoons} [L_x^{-}Fe^{II}OOH(BH^{+})] \quad (1)$$
$$\mathbf{1}$$

This same intermediate is the effective reactant for the hydroxylation of aromatic substrates.[9] Thus, the product profiles and substrate reactivity parameters for Fenton reagents are different from those for free hydroxyl radicals and depend upon the transition metal, the ligand, and the solvent matrix.

The predominant product observed for the reaction of Fenton reagents with a substituted aromatic is the *para*-hydroxylated isomer. The Fenton reactive intermediate (**1**) appears to react with substituted aromatic substrates ($XC_6H_4H$; X = *t*-Bu, Cl, Me, MeO, H) via (HO•) transfer from **1** and stabilization of the

[⊗] Abstract published in *Advance ACS Abstracts*, May 15, 1995.

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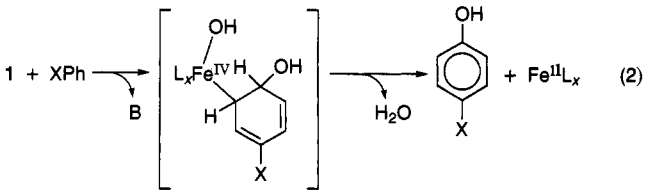
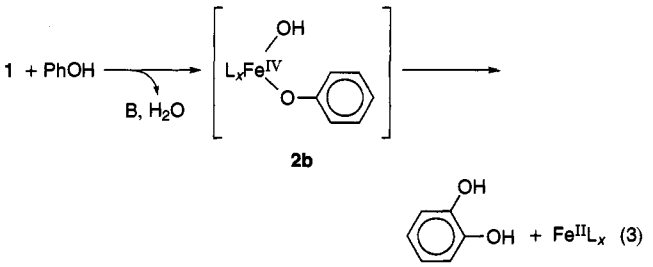
resultant carbon radical via an iron–carbon bond (**2a**):[9]




(2)

**2a**

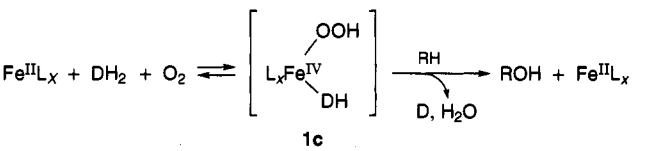
In the case of phenol (PhOH), however, the predominant product is the *ortho*-hydroxylated product, catechol. In this case **1** reacts via a stabilized HO• to abstract the phenolic H atom and stabilize the resultant phenolic radical through an iron oxygen bond (**2b**):




(3)

**2b**

Several iron(II) complexes ($Fe^{II}L_x$) in combination with various reductants ($DH_2$) catalytically activate $O_2$ for the hydroxylation of saturated hydrocarbons (RH).[10,11] This hydroxylation chemistry, which parallels that of the cytochrome P-450 proteins, appears to involve a reactive intermediate (**1c**) that has similar reactivity to that for Fenton reagents (**1**, eq 1):




(4)

**1c**

The successful hydroxylation of hydrocarbons by this system has prompted a systematic study of $O_2$ activation by several iron(II) complexes [$Fe^{II}(DPAH)_2$ ($DPAH_2$ = 2,6-dicarboxylpyridine), $Fe^{II}(PA)_2$ (PAH = picolinic acid), and $Fe^{II}(bpy)_2^{2+}$ (byp = 2,2′-bipyridine)] in combination with a reductant [$DH_2$; PhNHNHPh (reaction mimic for dihydroflavin and tetrahydropterin)] for the hydroxylation of aromatics, phenol, and substituted phenols. The goals have been (a) to characterize the reactive intermediates for the hydroxylation of aromatic/phenolic substrates and (b) to ascertain the relevance of these systems as mechanistic models for tyrosine hydroxylase.

Tyrosine hydroxylase is a non-heme iron(II) monoxygenase (mixed-function oxidase) that catalyzes the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa), which is the initial and rate-limiting step in catecholamine neurotransmitter biosynthesis.[10] The reductase for this system is tetrahydrobiopterin. Although reduced iron is an essential component of the active enzyme,[13] its role in the catalytic cycle of $O_2$ activation and tyrosine hydroxylation has not been established.[14]

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## Experimental Section

**Equipment.** The reaction products were separated and identified with a Hewlett-Packard 5880A Series gas chromatograph equipped with a HP-1 capillary column (cross-linked methyl silicone gum phase, 12 m × 0.2 mm i.d.) and by gas chromatography–mass spectrometry (Hewlett-Packard 5790A Series gas chromatograph with a mass-selective detector).

**Chemicals and Reagents.** The reagents for the investigations and syntheses were the highest purity commercially available and were used without further purification. Burdick and Jackson "distilled in glass" grade acetonitrile (MeCN, 0.004% $H_2O$) and pyridine (py, 0.014% $H_2O$) were used as solvents. All compounds were dried in vacuo over $CaSO_4$ for 24 h prior to use. Picolinic acid (PAH, 99%), 2,6-pyridinedicarboxylic acid ($DPAH_2$, 99%), 2,2′-bipyridine (bpy, 99+%), and 1,2-diphenylhydrazine (95%) were obtained from Aldrich. The organic substrates obtained from Aldrich included: benzene, *tert*-butylbenzene (99%), phenol (99%), 4-chlorophenol (99%), 4-*tert*-butylphenol (99%), and 4-methoxyphenol (99%).

**Syntheses of ($Me_4N$)PA and ($Me_4N$)DPAH.** Tetramethylammonium picolinate [($Me_4N$)PA] and tetramethylammonium dipicolinate [($Me_4N$)DPAH] salts were prepared by the neutralization of picolinic acid (PAH) and 2,6-pyridinedicarboxylic acid ($DPAH_2$) with tetramethylammonium hydroxide pentahydrate in aqueous solution. ($Me_4N$)PA was recrystallized from acetonitrile and ($Me_4N$)DPAH from 95% MeCN/5% MeOH. The hydroscopic products were stored under vacuum.

**$[Fe^{II}(MeCN)_4](ClO_4)_2$.** The $[Fe^{II}(MeCN)_4](ClO_4)_2$ complex was prepared by multiple recrystallizations of $[Fe^{II}(H_2O)_6](ClO_4)_2$ from MeCN.

**Iron(II) Bis(picolinate) and Iron(II) Bis(dipicolinate) Solutions.** Solutions of $Fe^{II}(PA)_2$ and $Fe^{II}(DPAH)_2$ were prepared in situ by mixing $[Fe^{II}(MeCN)_4](ClO_4)_2$ with stoichiometric ratios of ligand anion.

**Iron(II) Bis(2,2′-bipyridine) Solutions.** Solutions of $Fe^{II}(bpy)_2^{2+}$ were prepared in situ by mixing $[Fe^{II}(MeCN)_4](ClO_4)_2$ in MeCN with stoichiometric ratios of bpy.

**Methods.** The investigations of $O_2$/reductant ($DH_2$) activation by the iron complexes ($Fe^{II}L_x$) used solutions that contained 1.0 M substrate (RH), 1–10 mM $Fe^{II}L_x$, and 5–100 mM $DH_2$ in 3–4 mL of the appropriate solvent. The total solution volumes were 5.0 mL. The process was initiated by the addition of 1 atm of $O_2$ (7 mM) into the septum-covered glass reaction cell (volume, 21 mL; 16 mL of headspace). After 3 h with constant stirring at room temperature (24 ± 2 °C), samples of the reaction solutions were injected into a capillary-column gas chromatograph for analysis. All of the reactions reached completion within 3 h (consumption of the reductant). In some cases, the reaction was quenched with water, and the product solution was extracted with diethyl ether. Product species were characterized by GC–MS. Reference samples were used to confirm product identifications and to produce standard curves for quantitative assays of the product species.

The observed initial rates of reaction were determined via extrapolation of product assays at early times during the course of a reaction. The results were used to determine the order for each reactant in the hydroxylation of substituted phenols ($XC_6H_4OH$). For example, with the $Fe^{II}(DPAH)_2$ (1–2 mM)/PhOH (0.5–1.0 M)/$O_2$ (1.6–8.1 mM)/PhNHNHPh (10–50 mM) system, the product yields were determined at reaction times of 1, 2.5, 5, 7.5, and 10 min for various combinations of initial concentrations. The experiments were designed to be limited by reductant ($DH_2$) in order to (a) evaluate the primary reaction efficiency with respect to $DH_2$, (b) minimize secondary reactions with the primary products, and (c) limit the extent that $DH_2$ acted as a competitive substrate.

## Results

Table 1 summarizes the product yields and reaction efficiencies for six substrates [benzene (PhH), *tert*-butylbenzene (*t*-BuPh), phenol (PhOH), 4-*tert*-butylphenol (4-*t*-$BuC_6H_4OH$), 4-chlorophenol (4-$ClC_6H_4OH$), and 4-methoxyphenol (4-$MeOC_6H_4OH$)] that result from their combination with 5 mM $Fe^{II}L_x$ [$Fe^{II}(DPAH)_2$, $Fe^{II}(PA)_2$, and $Fe^{II}(bpy)_2^{2+}$] and 50 mM

**Table 1.** $Fe^{II}L_x$(5 mM)/PhNHNHPh (50 mM)/$O_2$ (1 atm)-Induced Hydroxylation of Various Aromatic Substrates

| $Fe^{II}L_x$ | solvent (mol/mol) | substrate, RH (1 M) | products (mM, ±5%),[a] ROH | effcny[b] (%) |
|---|---|---|---|---|
| $Fe(DPAH)_2$ | $(MeCN)_3py$ | PhOH | 17 (*o*), 6.7 (*p*) | 48 |
| | | $4\text{-}ClC_6H_4OH$ | 20 | 40 |
| | | $4\text{-}t\text{-}BuC_6H_4OH$ | 12 | 24 |
| | | $4\text{-}MeOC_6H_4OH$ | 3.0 | 6 |
| | | PhH | 0.6, 0.5[c] | 1, 3 |
| | | *t*-BuPh | none | |
| | $(MeCN)_3(H_2O)_{1.5}py$ | PhOH | 14 (*o*), 5.5 (*p*) | 38 |
| | | PhH | 0.6, 2.2[d] | 1, 10 |
| | $(MeCN)_3H_2O$ | PhOH | 1.5 (*o*), 0.6 (*p*) | 4 |
| | | $4\text{-}t\text{-}BuC_6H_4OH$ | 1.8 | 4 |
| | | PhH | 0.5, 0.4[d] | 1, 3 |
| | | *t*-BuPh | none | |
| $Fe(PA)_2$ | $(MeCN)_3py$ | PhOH | 12 (*o*), 5.8 (*p*) | 35 |
| | | PhH | 0.5, 1.0[d] | 1, 5 |
| | $(MeCN)_3(H_2O)_{1.5}py$ | PhOH | 9.5 (*o*), 3.9 (*p*) | 26 |
| | | PhH | 0.4, 1.3[d] | 2, 6 |
| | $(py)_2HOAc$ | PhOH[e] | 1.8 (*o*), 1.3 (*p*) | 7 |
| | | PhH | 0.3, 0.8[d] | 1, 4 |
| $Fe(bpy)_2^{2+}$ | $(MeCN)_3py$ | PhOH | 11 (*o*), 5.4 (*p*) | 32 |
| | | PhH | none | |
| | $(MeCN)_3(H_2O)_{1.5}py$ | PhOH | 2.3 (*o*), 1.7 (*p*) | 8 |
| | | PhH | 0.3 | <1 |
| | $(MeCN)_3H_2O$ | PhOH | 1.9 (*o*), 1.3 (*p*) | 6 |
| | | PhH | 1.4 | 3 |
| $Fe(bpy)_2^{2+}$ | MeCN | PhOH | none | |
| | | PhH | 0.6 | 1 |

[a] Substrate (1 M), $Fe^{II}L_x$ (5 mM), and PhNHNHPh (50 mM) combined in designated solvent to give indicated initial concentrations in a total volume of 5.0 mL. Product solutions were analyzed by capillary-column gas chromatography and GC–MS after a reaction time of 3 h at 24 ± 2 °C. [b] Millimoles of hydroxylated product per millimole of reductant. [c] 0.2 mM $o\text{-}HOC_6H_4OH$, 0.3 mM $p\text{-}HOC_6H_4OH$. [d] $p\text{-}HOC_6H_4OH$ product. [e] After 24 h, 5.7 mM $o\text{-}HOC_6H_4OH$, 3.2 mM $p\text{-}HOC_6H_4OH$ (18% efficiency).

reductant [$DH_2$; PhNHNHPh] in the specified solvent matrix [mol/mol ratio; MeCN, $(MeCN)_3H_2O$, $(MeCN)_3py$, $(MeCN)_3(H_2O)_{1.5}py$, or $(py)_2HOAc$]. The major product from benzene is hydroquinone and *t*-BuPh is unreactive. Even the most effective system for the hydroxylation of benzene [$Fe^{II}(DPAH)_2$/PhNHNHPh in $(MeCN)_3(H_2O)_{1.5}py$] is only 10% efficient.

In sharp contrast to benzene, phenolic substrates are readily hydroxylated, with catechol the dominant product from phenol (Table 1). On the basis of the product yields from the combination [5 mM $Fe^{II}L_x$/50 mM $DH_2$/1 M PhOH] in $(MeCN)_3py$, the relative efficiency (product per PhNHNHPh) for the three catalysts is in the order of $Fe^{II}(DPAH)_2$ (48%) > $Fe^{II}(PA)_2$ (35%) > $Fe^{II}(bpy)_2^{2+}$ (32%) . When the solvent is changed to include $H_2O$ [$(MeCN)_3(H_2O)_{1.5}py$], the $Fe^{II}(DPAH)_2$ efficiency decreases to 38%, $Fe^{II}(PA)_2$ to 26%, and $Fe^{II}(bpy)_2^{2+}$ to 8%. When no pyridine is present in the solvent matrix [$(MeCN)_3H_2O$], the $Fe^{II}(DPAH)_2$ efficiency decreases to 4%.

With 5 mM $Fe^{II}(DPAH)_2$/50 mM PhNHNHPh in $(MeCN)_3py$, a series of substituted phenolic substrates ($4\text{-}XC_6H_4OH$, 1 M) give product profiles that indicate that their relative reactivities are in the order: $4\text{-}ClC_6H_3(OH)_2$ (20 mM) > $o\text{-}C_6H_4(OH)_2$ (17 mM) > $4\text{-}t\text{-}BuC_6H_3(OH)_2$ (12 mM) > $4\text{-}MeOC_6H_3(OH)_2$ (3.0 mM). Upon going from a chloro group (strongly electron withdrawing) to a methoxy group (strongly electron donating), the reaction efficiency decreases from 40% to 6%.

The product yields for the hydroxylation of PhOH in $(MeCN)_3py$ with various ratios of $[DH_2]/[Fe^{II}L_x]$ (mM/mM) are summarized in Table 2. With $Fe^{II}(DPAH)_2$, as the ratio of $[DH_2]/[Fe^{II}L_x]$ increases from 5/1 to 100/1, the product concentration increases to reach a maximum at a ratio of 70/1 (17 mM catechol, 8.8 mM hydroquinone). The *o/p* ratio for hydroxylated products decreases from 3.0 to 1.9, while reaction efficiency (moles of hydroxylated product obtained per mole of reductant × 100) decreases from 72% to 23%. The reaction efficiency approaches 100% as the $DH_2/Fe^{II}L_x$ ratio approaches unity (the reductant is a competitive substrate).

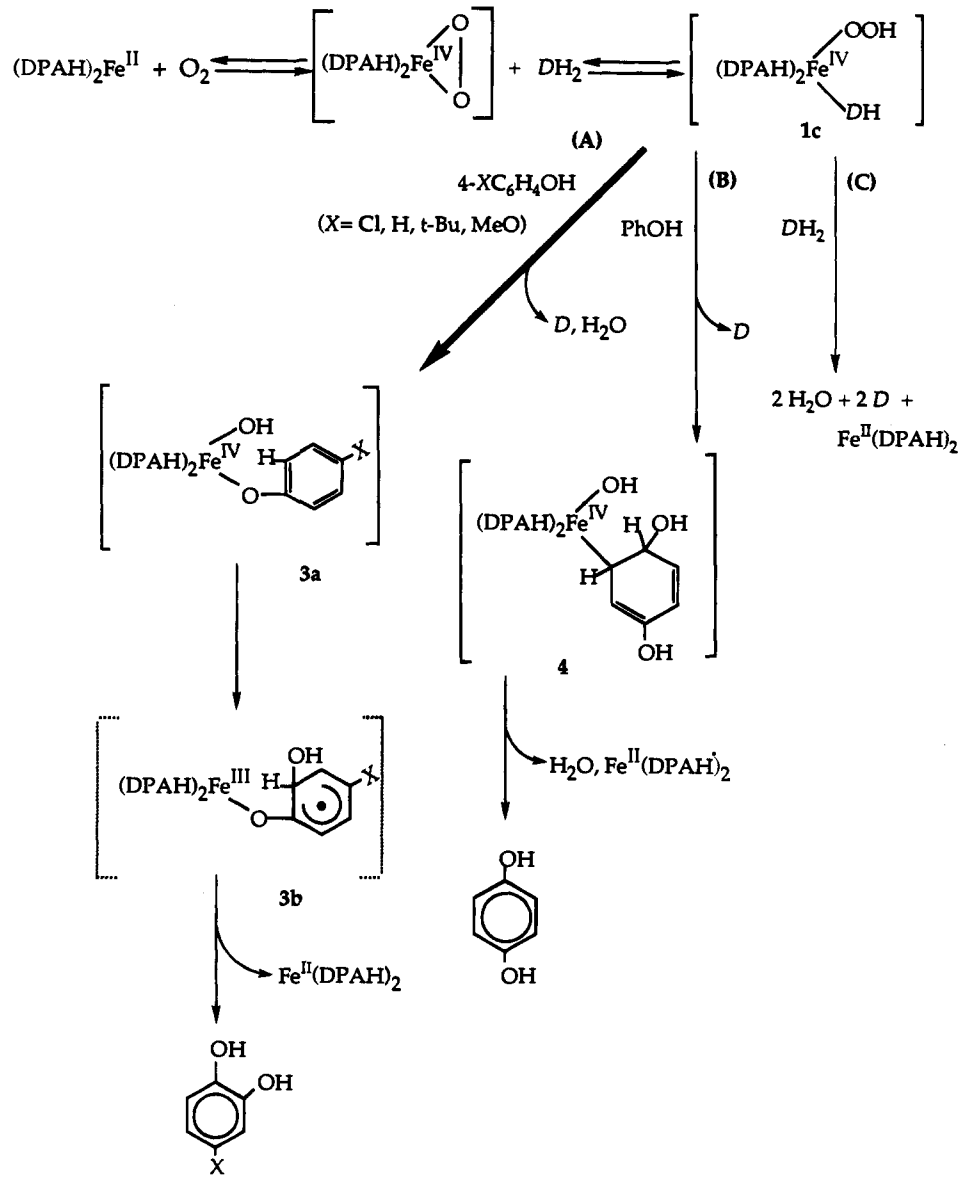
**Table 2.** $Fe^{II}L_x$/PhNHNHPh/$O_2$ (1 atm)-Induced Hydroxylation of Phenol: Effect of $[DH_2]/Fe^{II}L_x]$ Ratio on Reaction Efficiency and *o/p* Selectivity

| $Fe^{II}L_x$ | ratio (mM/mM), $DH_2/Fe^{II}L_x$ | products[a] (mM, ±5%) | effcny[b] (%) | *o/p* ratio |
|---|---|---|---|---|
| $Fe(DPAH)_2$ | 5/1 | 2.6 (*o*), 1.0 (*p*) | 72 | 2.6 |
| | 10/1 | 4.2 (*o*), 1.4 (*p*) | 56 | 3.0 |
| | 20/1 | 7.8 (*o*), 2.6 (*p*) | 52 | 3.0 |
| | 50/1 | 17 (*o*), 7.3 (*p*) | 49 | 2.3 |
| | 70/1 | 17 (*o*), 8.8 (*p*) | 37 | 1.9 |
| | 85/1 | 16 (*o*), 9.0 (*p*) | 29 | 1.8 |
| | 100/1 | 15 (*o*), 7.6 (*p*) | 23 | 1.9 |
| | 10/5 | 4.9 (*o*), 1.3 (*p*) | 62 | 3.8 |
| | 20/5 | 8.0 (*o*), 2.6 (*p*) | 53 | 3.1 |
| | 50/5 | 17 (*o*), 6.7 (*p*) | 48 | 2.6 |
| | 10/10 | 5.4 (*o*), 1.3 (*p*) | 67 | 4.1 |
| | 20/10 | 8.6 (*o*), 3.0 (*p*) | 58 | 2.9 |
| | 50/10 | 17 (*o*), 7.2 (*p*) | 49 | 2.4 |
| $Fe(PA)_2$ | 10/5 | 1.3 (*o*) | 13 | ∞ |
| | 20/5 | 2.2 (*o*), 1.0 (*p*) | 16 | 2.2 |
| | 50/5 | 12 (*o*), 5.8 (*p*) | 35 | 2.0 |
| | 10/10 | 1.0 (*o*) | 10 | ∞ |
| | 20/10 | 1.6 (*o*), 0.7 (*p*) | 12 | 2.3 |
| | 50/10 | 5.2 (*o*), 2.1 (*p*) | 15 | 2.5 |
| $Fe(bpy)_2^{2+}$ | 10/5 | 1.4 (*o*) | 14 | ∞ |
| | 20/5 | 3.9 (*o*), 1.0 (*p*) | 25 | 3.9 |
| | 50/5 | 11 (*o*), 5.4 (*p*) | 32 | 2.0 |
| | 10/10 | 0.6 (*o*) | 6 | ∞ |
| | 20/10 | 1.8 (*o*) | 9 | ∞ |
| | 50/10 | 8.9 (*o*), 3.1 (*p*) | 24 | 2.9 |

[a] Substrate (1 M), $Fe^{II}L_x$, and PhNHNHPh combined in $(MeCN)_3py$ solvent to give indicated initial concentrations in a total volume of 5.0 mL. Product solutions were analyzed by capillary-column gas chromatography and GC–MS after a reaction time of 3 h at 24 ± 2 °C. [b] Millimoles of hydroxylated product per millimole of reductant.

As the $DH_2/Fe^{II}L_x$ ratio approaches unity, the favored product is catechol. With $Fe^{II}(DPAH)_2$, a $DH_2/Fe^{II}L_x$ ratio of 5/1 gives an *o/p* ratio of 2.6; 10/5 gives 3.8, and 10/10 gives 4.1. Similar trends are observed for $Fe^{II}(PA)_2$ and $Fe^{II}(bpy)_2^{2+}$. At $DH_2$/

**Scheme 1.** Proposed Reaction Pathways for the Hydroxylation of Phenol and Substituted Phenols with the $Fe^{II}(DPAH)_2/DH_2/O_2$ System




$Fe^{II}L_x$ ratios of 10/5 and 10/10 for $Fe^{II}(PA)_2$ and $Fe^{II}(bpy)_2^{2+}$, and 20/10 for $Fe^{II}(bpy)_2^{2+}$, the only detectable product is catechol.

The rate of formation of catechol and hydroquinone from phenol (0.5−1.0 M) by the $Fe^{II}(DPAH)_2$ (1−2 mM)/PhNHNHPh (10−50 mM)/$O_2$ (1.6−8.1 mM) system in $(MeCN)_3$-py conforms to a rate law that is first order each in the concentration of $Fe^{II}(DPAH)_2$, PhNHNHPh, and PhOH and zero order in the concentration of dioxygen. On the basis of initial rates of reaction, the apparent rate constant for the formation of catechol [$k_{ox}$(*ortho*)] is 0.07 $M^{-2}$ $s^{-1}$ and for the formation of hydroquinone [$k_{ox}$(*para*)] is 0.04 $M^{-2}$ $s^{-1}$ at 25 °C $\{d[C_6H_4(OH)_2]/dt = k_{ox}[Fe^{II}(DPAH)_2][PhNHNHPh][PhOH]\}$.

## Discussion and Conclusions

**Hydroxylation of Benzene and *tert*-Butylbenzene.** The various $Fe^{II}L_x/DH_2/O_2$/solvent systems are not reactive enough to hydroxylate *tert*-butylbenzene. Although benzene is hydroxylated, the yields are low and the predominant product is hydroquinone (Table 1). Clearly the reductant (PhNHNHPh) is a much more reactive substrate than aromatic hydrocarbons (at least 90% is consumed by the reactive intermediate before 1 M PhH becomes competitive).

**Hydroxylation of Phenol.** The several $Fe^{II}L_x$/PhNHNHNPh/$O_2$ systems catalytically hydroxylate phenol (with catalyst turnovers up to 25; Tables 1 and 2) and thereby mimic the catalytic chemistry of tyrosine hydroxylase (TH):

$$\text{Htyr} + DH_2 + O_2 \xrightarrow{\text{TH}} \text{HOtyr} + D + H_2O \qquad (5)$$

However, in the chemical system, the reductant ($DH_2$) is a competitive substrate, especially when present at the levels necessary to obtain adequate product for accurate assays. The $Fe^{II}(DPAH)_2$/PhNHNHPh system approaches 100% efficiency at low reductant concentrations (Table 2):

$$\text{PhOH} + \text{PhNHNHPh} + O_2 \xrightarrow{Fe^{II}(DPAH)_2} o,p\text{-}C_6H_4(OH)_2 + \text{PhN=NPh} + H_2O \qquad (6)$$

With 1 mM $Fe^{II}(DPAH)_2$, as the amount of PhNHNHPh is decreased from 100 to 5 mM, the reaction efficiency increases from 23% to 72%. If the amount of $Fe^{II}(DPAH)_2$ is increased while the concentration of reductant remains relatively low, the efficiency of the reaction remains high. Thus, a 5 mM $Fe^{II}$-$(DPAH)_2$/10 mM PhNHNHPh ratio produces 4.9 mM catechol

and 1.3 mM hydroquinone (an efficiency of 62%), while a 10 mM $Fe^{II}(DPAH)_2$/10 mM PhNHNHPh ratio produces 5.4 mM catechol and 1.3 mM hydroquinone (an efficiency of 67%). Even at a 5 mM $Fe^{II}(DPAH)_2$/50 mM PhNHNHPh ratio, the reaction produces 17 mM catechol and 7.2 mM hydroquinone (an efficiency of 49%).

The ratio of reductant to iron complex not only affects the reaction efficiency, it also affects the *o/p* ratio of the products obtained. For each $Fe^{II}L_x$ studied, as the $DH_2/Fe^{II}L_x$ ratio approaches unity, the formation of catechol product is favored. With 10 mM PhNHNHPh and $Fe^{II}(DPAH)_2$, as the $DH_2/Fe^{II}L_x$ ratio goes from 10 down to 1, the *o/p* ratio goes from 3.0 to 4.1. Furthermore, at $DH_2/Fe^{II}L_x$ ratios greater than 50, the *o/p* ratio is less than 2 (1.8−1.9), which is effectively the statistical probability of hydroxylating the *ortho* position relative to the *para* position (2:1). With $Fe^{II}(PA)_2$ or $Fe^{II}(bpy)_2^{2+}$ and $DH_2/Fe^{II}L_x$ ratios of 1−2, only catechol is formed. At ratios of 2−5, the *o/p* ratio is greater than 2 (2.2−3.9), and at a ratio of 10, the *o/p* ratio is 2.0.

**Hydroxylation of Substituted Phenols.** The 4-position-substituted phenols ($4\text{-}XC_6H_4OH$; see Table 1) are transformed by the 5 mM $Fe^{II}(DPAH)_2$/50 mM PhNHNHPh/$O_2$ system to the 4-position-substituted catechols [$4\text{-}XC_6H_3(OH)_2$]. On the basis of the product profiles, their relative reactivity is in accord with the substituent group's electron-withdrawing ability: Cl > H > *t*-Bu > MeO.

Scheme 1 outlines a reasonable set of reaction pathways that are consistent with the product profiles and the substituent effects on the relative reactivities of substituted phenols. Reduced iron complexes [e.g., $Fe^{II}(DPAH_2)$] reversibly bind dioxygen[10] and in the presence of reductants ($DH_2$) undergoes H-atom addition to give reactive intermediate **1c**.[11] This hydroxylation intermediate has a reactivity and produces product profiles that are similar to that for the reactive intermediate of Fenton reagents (**1**, eq 1);[7,8] a stabilized HO• (via a weak MO−OH bond). Intermediate **1c** collapses to release a hydroxyl group that (a) abstracts an H atom from $4\text{-}XC_6H_4O\text{−}H$ via path A to form a water molecule, (b) releases D, and (c) binds the just-formed phenolic radical to the iron center to give intermediate **3a**. Hydroxyl radical transfer from the iron center to the *ortho* carbon of the bound phenol yields **3b**. The key step in the transformation from **3b** to product [$4\text{-}XC_6H_3(OH)_2$] is H-atom transfer from the aliphatic hydroxylated carbon to give catechol and $Fe^{II}(DPAH)_2$. The latter H-atom transfer is directly affected by a substituent group on the aromatic ring. A strong electron-withdrawing group would remove electron density from the aromatic ring and thereby decrease the C−H bond energy. In turn, this favors H-atom transfer to the O atom of the Fe−O bond (**3b**), which is further favored by the rearomatization of the ring upon release of catechol product.

When phenol is the substrate, a secondary reaction pathway (B) is possible. In this case, much as in the hydroxylation through the Fenton intermediate (**1**),[7] the stabilized HO• is transferred to the *para* position of the bound phenolic substrate with formation of an iron−carbon bond at the *meta* position to yield intermediate **4**. The stabilized HO• on the iron of **4** then abstracts an H atom to produce water while rearomatizing the ring to yield $Fe^{II}(DPAH)_2$ and hydroquinone. Alternatively, in the absence of a *para* substituent, intermediate **3a** may have HO addition at the *para* rather than the *ortho* position. The reductant ($DH_2$) is a competitive substrate that is dehydrogenated via path C (Scheme 1) when present in excess.

To date the highly reactive nature of the intermediates that are proposed in Scheme 1 [species **1c** (stabilized HO• group), **3a**, **3b**, and **4**] has precluded their detection. However, the product profiles (Tables 1 and 2) require a directing system that has the characteristics of the proposed intermediates.

The $4\text{-}t\text{-}BuC_6H_4OH$ substrate represents a reasonable model substrate for tyrosine. The results for this substrate with the $Fe^{II}(DPAH)_2$/PhNHNHPh/$O_2$ system confirm that it is a reaction mimic for tyrosine hydroxylase[12−14] and that Scheme 1 is a chemically reasonable reaction path for the enzyme system. This infers that the essential roles for the reduced iron of the enzyme are (a) binding of dioxygen, (b) stabilization of a phenoxy-radical intermediate, and (c) facilitation of H-atom transfer to form catechol.

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