Oxidative N-Debenzylation of N-Benzyl-N-substituted Benzylamines Catalyzed by Cytochrome P450

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Cytochrome P450 (P450)/O2/NADPH engender electron transfer reaction of N-benzyl-N-substituted benzylamines to yield corresponding radical cation 1 that is simultaneously converted into 2 and 3. Subsequently, expulsion of proton and hydroxylation yielding α-hydroxylamines are followed by formation of benzaldehydes and benzylamines.

Key Words: Oxidations, N-Debenzylation, Horseradish peroxidase, Hydrogen peroxide, Amines

Introduction

Numerous enzymes and their man-made mimics catalyze1-17 N-demethylation of N,N-dimethylanilines. The axial coordination site of P450 is thiolate ligation while histidine ligand is employed with horseradish peroxidase (HRP). Iodosyl-benzene (C6H5IO) catalyzed by tetraphenylporphyrinatoiron (III) chloride (FeIIITPPCl)12 oxidizes N,N-dimethylanilines by an initial electron transfer (ET) process. The reactions indicate small negative ρ value (ρ = -0.41 for FeIIITPPCl) and marginal intermolecular kinetic isotope effect (KIE), kD/kH = 1.3 with PhCH2NMe2 and PhCD2NMe2. The KIE and Hammett correlations in the oxidative N-demethylation of N,N-Dimethylanilines catalyzed by tetra-kis(pentafluorophenyl)porphyrin iron(III) chloride16 were investigated. The intramolecular KIE (4-Y-N-methyl-N-trideuteriobenzylamines) are much larger than intermolecular ones (4-Y-N,N-dimethylanilines and 4-Y-N,N-di-trideuteriobenzylamines). The Hammett correlations also give rise to better correlations with σ’ (r = 0.995) than with σ (r = 0.993). The KIE profiles (plot of kD/kH vs the pKa of the aniline radical cations) by lignin peroxidase/H2O2 and 5,10,15,20-tetraphenyl-21H,23H-porphine-pp’pp”p”’-tetrasulfonic acid iron(III) chloride/H2O2 for a number of ring-substituted N,N-bis(dideuteriethyl) anilines17 show bell-shaped curve.

Experimental Section

Materials and Methods. Benzylamine, substituted benzaldehydes (Y = p-OCH3, p-CH3, H, p-Cl, m-Cl, p-CN, and p-NO2), substituted benzonitriles (Y = p-OCH3 and m-Cl), LiAlD4 and other reagents were purchased from the major suppliers. VARIAN GEMINI 2000 NMR spectrometer was used for the identification of the compounds. Relative quantities of the aldehydes were obtained with VARIAN 3300 GC with DB-1 column and FID.

Preparation of N-Benzyl-N-4-methoxybenzylamines.


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reduced with NaBH₄ to give \( p\)-CH₃OC₆H₄CD₂NHCH₂C₆H₄ (4.98 g, 72%). \(^{1}H\) NMR (CDCl₃, 200 MHz) \( \delta \) 3.8 (s, 5H, OCH₃, CH₂), 6.9 (d, 2H, C₆H₄), 7.2-7.4 (m, 7H, C₆H₅, C₆H₄).

Other deuterated benzylamines were similarly prepared and their NMR spectra are listed below.

\[ m\text{-Cl C}_{6}H_{4}CH_{2}NHCD_{2}C_{6}H_{5}; \quad ^{1}H\text{ NMR (CDCl}_{3}, \quad 200 \text{ MHz}) \quad \delta \quad 3.8 \quad (s, \quad 2H, \quad CH_{2}), \quad 7.2-7.4 \quad (m, \quad 9H, \quad C_{6}H_{5}, \quad C_{6}H_{4}) \]

\[ m\text{-Cl C}_{6}H_{4}CD_{2}NHCH_{2}C_{6}H_{5}; \quad ^{1}H\text{ NMR (CDCl}_{3}, \quad 200 \text{ MHz}) \quad \delta \quad 3.8 \quad (s, \quad 2H, \quad CH_{2}), \quad 7.2-7.4 \quad (m, \quad 9H, \quad C_{6}H_{5}, \quad C_{6}H_{4}) \]

Oxidations by P₄₅₀/O₂/NADPH. To 650 \( \mu L \) of distilled water were added in the order of 200 \( \mu L \) of potassium phosphate buffer (pH=7.4), 40 \( \mu L \) of microsomal P₄₅₀ (final concentration: 0.5 mg/1 mL), 10 \( \mu L \) of a substrate dissolved in CH₃OH (final concentration: 1 mg/1 mL) and 100 \( \mu L \) of NADPH so that the total volume becomes 1000 \( \mu L \). The reaction mixture was incubated at 37 °C for 30 minutes with vigorous stirring. The reaction mixture was then cooled with ice bath and 1 mL of 5% HCl solution was added to make the salt of substituted benzylamines. 10 \( \mu L \) of 0.014 mM of bibenzyl was added to reaction mixture as an internal standard. CH₂Cl₂ (3 mL × 3) was added to extract the organic layer. This was dried with anhydrous Na₂SO₄ and concentrated to 20 \( \mu L \) for GLC analysis.

Kinetic Isotope Effects. were determined indirectly as follows. \( k_{YH}/k_{YD} = k_{YH}/k_{HH} \times k_{HH}/k_{YD} \) when \( Y \) is \( p\)-OCH₃ and \( p\)-Cl. \( k_{HH}/k_{HD} = k_{HH}/k_{m-ClH} \times k_{m-ClH}/k_{HD} \) can be obtained when \( Y \) is H using \( m\text{-Cl C}_{6}H_{4}CH_{2} \) as an internal basis.

Results and Discussion

The competitive intramolecular \( N\)-debenzylation of \( N\)-benzyl-\( N\)-substituted benzylamines with P₄₅₀/O₂/NADPH has been studied through Hammett correlations and KIE. The relative rates caused by substituents (\( Y = p\)-OCH₃, \( p\)-CH₃, H, \( p\)-Cl, \( m\)-Cl, \( p\)-CN and \( p\)-NO₂) were obtained from the ratios of \([YC_{6}H_{4}CHO]/[C_{6}H_{5}CHO]\). The log \( k_{YH}/k_{H} \) values were plotted against \( \sigma \) and \( \sigma^{+} \) to yield better correlation with \( \sigma \) (r = -0.95; r = 0.993) than with \( \sigma^{+} \) (r = -0.71, r = 0.945). The intramolecular KIE was calculated in a indirect manner as follows \( k_{YH}/k_{YD} = k_{YH}/k_{HH} \times k_{HH}/k_{YD} \) when \( Y \) is \( p\)-OCH₃ and \( p\)-Cl. \( m\text{-Cl C}_{6}H_{4}CH_{2} \) substitution at phenyl ring has been employed for the KIE of \( k_{HH}/k_{HD} = k_{HH}/k_{m-ClH} \times k_{m-ClH}/k_{HD} \).

The Hammett correlations for the oxidation of \( N\)-dimethylanilines (\( \rho^{+} = -0.88 \)) may suggest that the electron transfer step for the formation of radical cation is involved with rate determining step. The negative sign of \( \rho^{+} = -0.88 \) is also parallel with their oxidation potentials decreasing from \( p\)-NO₂ to \( p\)-OCH₃. The better correlation with \( \sigma \) of Table 1 indicates that positive charge is localized on the nitrogen atom. \( \alpha\)-Deprotonation of \( 1 \) yields the two carbon centered \( \alpha\)-amino benzylic radicals, \( 2 \) and \( 3 \). Thus \( 1 \) can be simultaneously transformed into either \( 2 \) or \( 3 \) since both of them are
more stable than 1. The intramolecular KIE values for 4-Y-
C₆H₄N(CH₃)CD₃ are quite distinct and increase from
p-NO₂ (k_H/k_D = 2.0) to p-OCH₃ (k_H/k_D = 3.0). This increasing
trend parallels with magnitude of pKₐ of the corresponding
radical cation, 4-Y-C₆H₄(ıt(N(CH₃))₂) and suggests that there is a
significant reverse electron transfer which competes with the
α-deprotonation. The KIE for p-OCH₃ k_H/k_D = 4.56 in
Table 1 can be the similar situation for the reversibility. On
the contrary, when electron transfer is the rate determining
step, no such KIE would be observed that is
k_H/k_D = 1. Our KIE for m-Cl, k_H/k_D = 1.61 may indicate that reverse electron
transfer occurs to a small extent. Our KIE values range from
1.61(m-Cl), 1.91(H) to 4.56(p-OCH₃) which are larger than unity. The increasing trend with electron-donating sub-
stituents may indicate increase of the reversibility.

### Table 1. Kinetic Data of Oxidative N-Debenzylation of N-Benzyl-
N-substituted benzylamines by P450/O₂/NADPH

<table>
<thead>
<tr>
<th>Substituent</th>
<th>k_H/k_D</th>
<th>p-Cl</th>
<th>m-Cl</th>
<th>p-CN</th>
<th>p-NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-OCH₃</td>
<td>1.69</td>
<td>0.46</td>
<td>0.38</td>
<td>0.225</td>
<td>0.145</td>
</tr>
<tr>
<td>p-H</td>
<td>1.04</td>
<td>0.46</td>
<td>0.38</td>
<td>0.225</td>
<td>0.145</td>
</tr>
</tbody>
</table>

### Scheme 3

\[
\begin{align*}
&\text{YC}_6\text{H}_4\text{CH}_2\text{-NHCH}_2\text{C}_6\text{H}_5 \\
\rightarrow & \text{PFe}^{IV} = \text{O} \\
&\text{YC}_6\text{H}_4\text{CH}_2\text{-NHCH}_2\text{C}_6\text{H}_5 + \text{PFe}^{IV} = \text{O} \quad 1 \\
&\text{YC}_6\text{H}_4\text{CH}_2\text{-NHCH}_2\text{C}_6\text{H}_5 \quad 2 \\
&\text{PFe}^{IV} = \text{O} \\
&\text{YC}_6\text{H}_4\text{CH}_2\text{-NHCH}_2\text{C}_6\text{H}_5 + \text{PFe}^{IV} = \text{OH} \\
&\text{YC}_6\text{H}_4\text{CH}^-\text{OH}-\text{NHCH}_2\text{C}_6\text{H}_5 + \text{PFe}^{III} \\
&\text{YC}_6\text{H}_4\text{CHO} + \text{C}_6\text{H}_4\text{CH}_{2}\text{NH}_2 \\
\end{align*}
\]

### Conclusions

The KIE values are k_H/k_D > 1 and increase monotonically from m-Cl to p-OCH₃. N-Debenzylation of N-benzyl-N-
substituted benzylamines proceed through the reversible formation of
\( \text{YC}_6\text{H}_4\text{CH}^-\text{NH}_2\text{CH}_2\text{C}_6\text{H}_5 \) and \( \text{YC}_6\text{H}_4\text{CH}^-\text{NH}_2\text{CH}_2\text{C}_6\text{H}_5 \). The reversibility should be influenced by the substituent (Y) and kind of the oxidant. The variation of magnitude of k_H/k_D may tell increasing trend of reversibility when sub-
stituents becoming electron-donating.

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### References