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

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Cytochrome P450 (P450)/O_2/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 a-hydroxylamines are followed by formation of benzaldehydes and benzylamines.

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

Introduction

Numerous enzymes and their man-made mimics catalyze[1-17] *N*-demethylation of *N*,*N*-dimethylanilines. The axial coordination site of P450 is thiolate ligation while histidine ligand is employed with horseradish peroxidase (HRP). Iodosylbenzene (C_6H_5IO) catalyzed by tetraphenylporphyrinatoiron (III) chloride ($Fe^{III}TPPCl$)[12] oxidizes *N*,*N*-dimethylbenzylamines by an initial electron transfer (ET) process. The reactions indicate small negative ρ value (ρ = -0.41 for $Fe^{III}TPPCl$) and marginal intermolecular kinetic isotope effect (KIE), k_H/k_D = 1.3 with $PhCH_2NMe_2$ and $PhCD_2NMe_2$. The KIE and Hammett correlations in the oxidative N-demethylation of *N*,*N*-Dimethylanilines catalyzed by tetrakis(pentafluorophenyl)porphyrin iron(III) chloride[16] were investigated. The intramolecular KIE (4-Y-*N*-methyl-*N*-trideuteriomethylanilines) are much larger than intermolecular ones (4-Y-*N*,*N*-dimethylanilines and 4-Y-*N*,*N*-di-trideuteriomethylanilines). 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 k_H/k_D *vs* the pK_a of the aniline radical cations) by lignin peroxidase/H_2O_2 and 5,10,15,20-tetraphenyl-21H,23H-porphine-*p*,*p'*,*p''*,*p'''*-tetrasulfonic acid iron(III) chloride/H_2O_2 for a number of ring-substituted *N*,*N*-bis(dideuteriomethyl) anilines[17] show bell-shaped curve.

Experimental Section

Materials and Methods. Benzylamine, substituted benzaldehydes (Y = *p*-OCH_3, *p*-CH_3, H, *p*-Cl, *m*-Cl, *p*-CN, and *p*-NO_2), substituted benzonitriles (Y = *p*-OCH_3 and *m*-Cl), $LiAlD_4$ 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-1column and FID.

Preparation of *N*-Benzyl-*N*-4-methoxybenzylamines. Benzylamine (0.02 mmol) in benzene solution was added to benzene solution (15 mL) of *p*-anisaldehyde (0.02 mmol) in 100 mL flask for 10 min. This solution was stirred for 3 h and Na_2SO_4 was added to eliminate H_2O. The formation of imine was confirmed when evaporating benzene by rotary evaporator. To the imine portion dissolved in methanol in ice bath, 1.5 equivalents of $NaBH_4$ was added very slowly. The reaction mixture was stirred at RT for 1 h. The solvent was evaporated and the remain was extracted with CH_2Cl_2/H_2O. The CH_2Cl_2 layer was dried with Na_2SO_4 to give the pure product (4.34 g, 95% yield). **^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (d, 7H, CH_3O, $2CH_2$), 6.9 (d, 2H, C_6H_4), 7.2 (d, 2H, C_6H_4), 7.3 (m, 5H, C_6H_5).

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

***N*-Benzyl-*N*-4-methylbenzylamines: ^{1}H NMR ($CDCl_3$, 200 MHz)** δ 2.4 (s, 3H, CH_3), 3.8 (d, 4H, $2CH_2$), 7.2-7.4 (m, 9H, C_6H_5, C_6H_4).

***N*-Benzyl-*N*-4-chlorobenzylamines: ^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (d, 4H, $2CH_2$), 7.2-7.4 (m, 9H, C_6H_5, C_6H_4).

***N*-Benzyl-*N*-3-chlorobenzylamines: ^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (d, 4H, $2CH_2$), 7.2-7.4 (m, 9H, C_6H_5, C_6H_4).

***N*-Benzyl-*N*-4-cyanobenzylamines: ^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (d, 4H, $2CH_2$), 7.2-7.6 (m, 9H, C_6H_5, C_6H_4).

***N*-Benzyl-*N*-4-nitrobenzylamines: ^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (d, 4H, $2CH_2$), 7.2-7.4 (m, 5H, C_6H_5) 7.5 (d, 2H, C_6H_4), 8.2 (d, 2H, C_6H_4).

Preparation of *p*-$CH_3OC_6H_4CD_2NHCH_2C_6H_5$. 4-Methoxybenzonitrile (0.03 mole) in 20 mL THF was added very slowly to $LiAlD_4$ (0.045 mole) solution of THF in ice bath. The reaction mixture was then stirred for one day under nitrogen at RT. After reaction, 5% HCl solution was added slowly until the reaction mixture becomes acidic. The aqueous layer was separated by addition of benzene. To the aqueous layer, 3 N NaOH solution was added to make basic solution and the amine layer separated with benzene. 4-Methoxy(α,α-dideuterio) benzylamine(0.023 mole, 77%) was then obtained by evaporation of benzene. *N*-Benzylidene-4-methoxy(α,α-dideuterio)benzylamine was prepared by reaction of 4-methoxy(α,α-dideuterio)benzylamine (0.023 mole) and benzaldehyde(0.023 mole). This was

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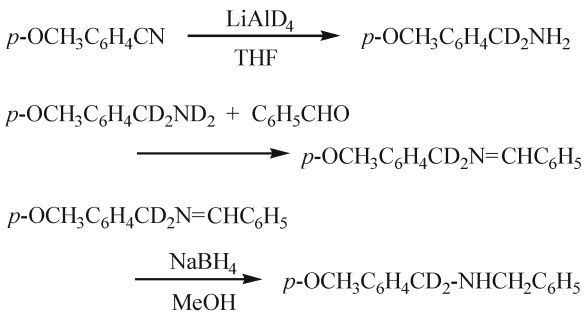
reduced with $NaBH_4$ to give *p*-$CH_3OC_6H_4CD_2NHCH_2C_6H_4$ (4.98 g, 72%). **^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (s, 5H, OCH_3, CH_2), 6.9 (d, 2H, C_6H_4), 7.2-7.4 (m, 7H, C_6H_5, C_6H_4).

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



***m*-Cl $C_6H_4CH_2NHCD_2C_6H_5$: ^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (s, 2H, CH_2), 7.2-7.4 (m, 9H, C_6H_5, C_6H_4).

***m*-Cl $C_6H_4CD_2NHCH_2C_6H_5$: ^{1}H NMR ($CDCl_3$, 200 MHz)** δ 3.8 (s, 2H, CH_2), 7.2-7.4 (m, 9H, C_6H_5, C_6H_4).

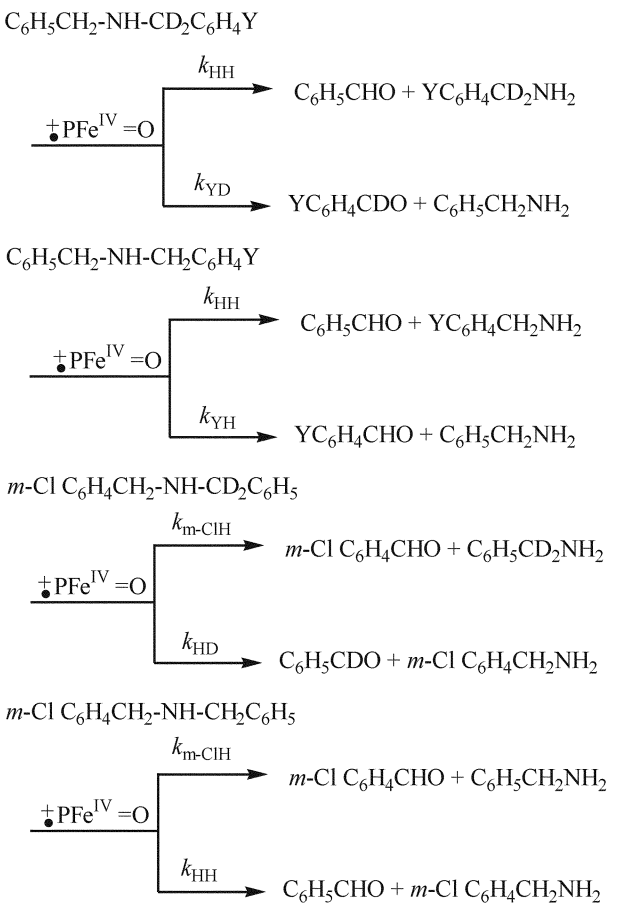
Oxidations by P450/O_2/NADPH. To 650 μL of distilled water were added in the order of 200 μL of potassium phosphate buffer (pH=7.4), 40 μL of microsomal P450 (final concentration: 0.5 mg/1 mL), 10 μL of a substrate dissolved in CH_3OH (final concentration: 1 mg/1 mL) and 25 mM of 100 μL of NADPH so that the total volume becomes 1000 μL. 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 μL of 0.014 mM of bibenzyl was added to reaction mixture as an internal standard. CH_2Cl_2 (3 mL × 3) was added to extract the organic layer. This was dried with anhydrous Na_2SO_4 and concentrated to 20 μL for GLC analysis

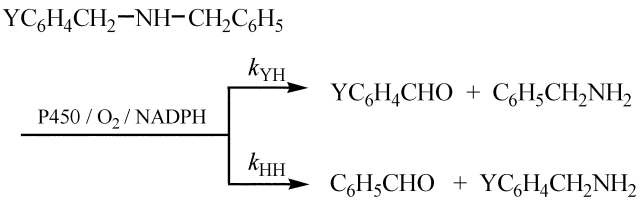
Kinetic Isotope Effects. were determined indirectly as follows. $k_{YH}/k_{YD} = k_{YH}/k_{HH}\cdot k_{HH}/k_{YD}$ was used when Y is *p*-OCH_3 and *p*-Cl. $k_{HH}/k_{HD} = k_{HH}/k_{m\text{-Cl H}}\cdot k_{m\text{-Cl H}}/k_{HD}$ can be obtained when Y is H using *m*-$ClC_6H_4CH_2$ as an internal basis.

Results and Discussion

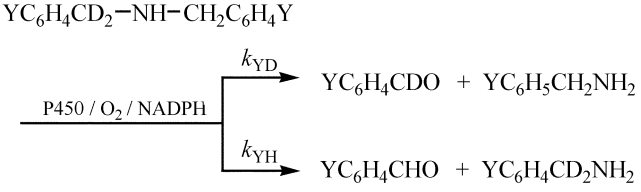
The competitive intramolecular *N*-debenzylation of *N*-benzyl-*N*-substituted benzylamines with P450/O_2/NADPH has been studied through Hammett correlations and KIE. The relative rates caused by substituents (Y = *p*-OCH_3, *p*-CH_3, H, *p*-Cl, *m*-Cl, *p*-CN and *p*-NO_2) were obtained from the ratios of [YC_6H_4CHO]/[C_6H_5CHO]. The log k_Y/k_H values were plotted against σ and σ^+ to yield better correlation with σ (r = -0.95; r = 0.993) than with σ^+ (ρ = -0.71, r = 0.945). The intramolecular KIE was calculated in a indirect manner as follows $k_{YH}/k_{YD} = k_{YH}/k_{HH}\cdot k_{HH}/k_{YD}$ when Y is *p*-OCH_3 and *p*-Cl. *m*-Cl substitution at phenyl ring has been employed for the KIE of $k_{HH}/k_{HD} = k_{HH}/k_{m\text{-Cl H}}\cdot k_{m\text{-Cl H}}/k_{HD}$.

The Hammett correlations for the oxidation of *N*,*N*-dimethylanilines (ρ^+= -0.88)[16] may suggest that the electron transfer step for the formation of radical cation is involved with rate determining step. The negative sign of ρ^+= -0.88 is also parallel with their oxidation potentials decreasing from *p*-NO_2 to *p*-OCH_3. The better correlation with σ of Table 1 indicates that positive charge is localized on the nitrogen atom. α-Deprotonation of **1** yields the two carbon centered α-amino benzyl radicals, **2** and **3**. Thus **1** can be simultaneously transformed into either **2** or **3** since both of them are





Scheme 1



Scheme 2

Table 1. Kinetic Data of Oxidative *N*-Debenzylations of *N*-Benzyl-*N*-substituted benzylamines by P450/O_2/NADPH

	p-OCH_3	*p*-H	*p*-Cl	*m*-Cl	*p*-CN	*p*-NO_2
k_{YH}/k_{HH}	1.69	1	0.46	0.38	0.225	0.145
	ρ (r) = -0.95 (0.993);			ρ^+ (r) = -0.71 (0.945)		
k_{YH}/k_{YD}	4.56 ± 0.2	1.91 ± 0.03		1.61 ± 0.03		

more stable than 1. The intramolecular KIE values for 4-Y-$C_6H_4N(CH_3)CD_3$[16] are quite distinct and increase from *p*-NO_2 (k_H/k_D = 2.0) to *p*-OCH_3 (k_H/k_D = 3.0). This increasing trend parallels with magnitude of p*K*a of the corresponding radical cation, 4-Y-$C_6H_4\overset{+\bullet}{N}(CH_3)_2$ and suggests that there is a significant reverse electron transfer which competes with the α-deprotonation. The KIE for *p*-OCH_3 k_{YH}/k_{YD} = 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 $k_H/k_D = 1$[15]. 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*-OCH_3) which are larger than unity. The increasing trend with electron-donating substituents may indicate increase of the reversibility.

Conclusions

The KIE values are $k_{YH}/k_{YD} > 1$ and increase monotonically from *m*-Cl to *p*-OCH_3. *N*-Debenzylation of *N*-benyl-*N*-substitutedbenzylamines proceed through the reversible formation of $YC_6H_4\overset{\bullet}{C}H\text{-}\overset{+}{N}H_2\text{-}CH_2C_6H_5$ **2** and $YC_6H_4CH\text{-}\overset{+}{N}H_2\text{-}\overset{\bullet}{C}H_2C_6H_5$ **3**. The reversibility should be influenced by the substituent (Y) and kind of the oxidant. The variation of magnitude of k_{YH}/k_{YD} may tell increasing trend of reversibility when substituents becoming electron- donating.

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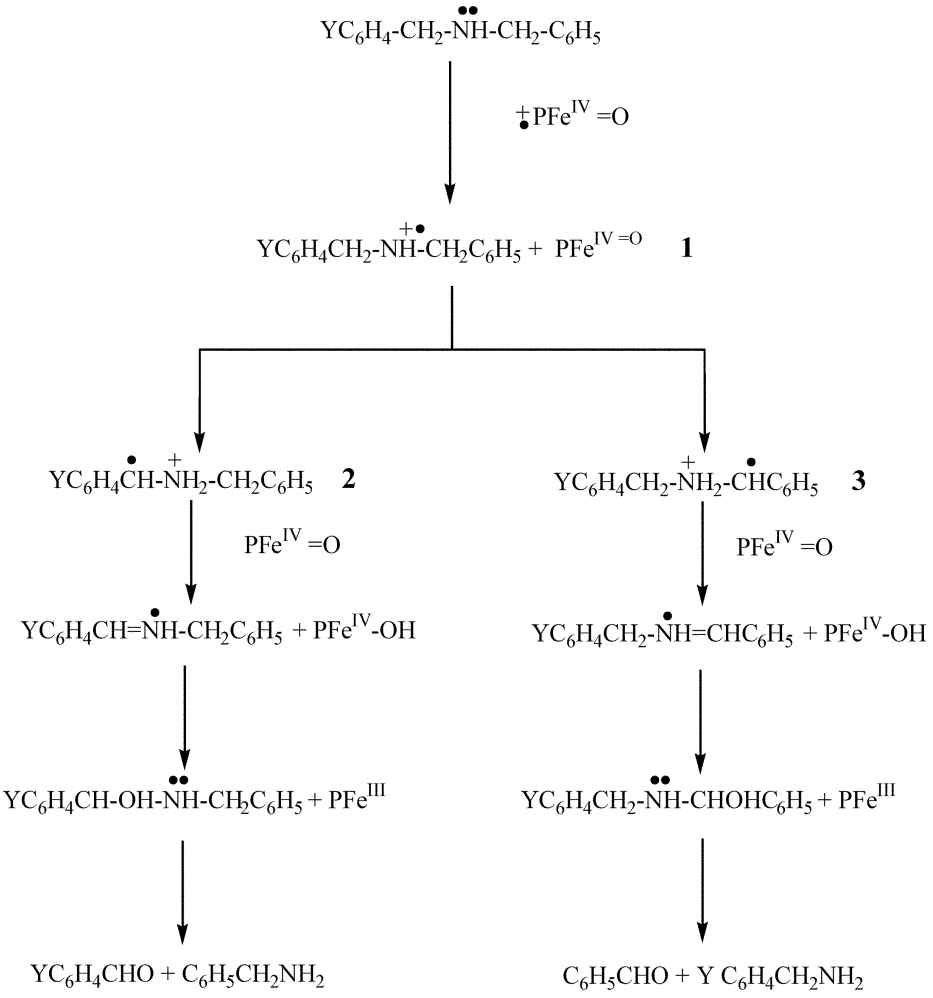

Scheme 3

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