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Oxidative *N*-debenzylation of *N*-benzyl-*N*-substituted benzylamines catalyzed by horseradish peroxidase†

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ABSTRACT: *N*-Benzyl-*N*-substituted benzylamines and compound I of horseradish peroxidase engender electron transfer yielding the corresponding nitrogen radical cation **1**, which is simultaneously converted into **2** and **3**. Subsequently, expulsion of proton and hydroxylation yielding α-hydroxylamines are followed by the formation of benzaldehydes and benzylamines. Copyright © 2003 John Wiley & Sons, Ltd.

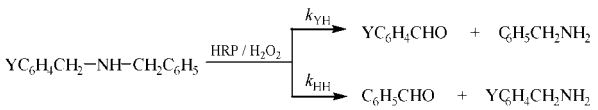
KEYWORDS: oxidation; *N*-debenzylation; horseradish peroxidase; hydrogen peroxide; amines

INTRODUCTION

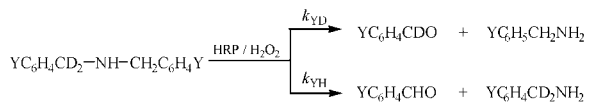
The mechanism of the *N*-dealkylation of *N*-dimethylanilines catalyzed by heme enzymes and their analogs has attracted considerable attention.[1–17] Iodosylbenzene (C_6H_5IO) catalyzed by tetraphenylporphyrinatoiron(III) chloride [Fe(III)TPPCl][12] oxidizes *N,N*-dimethylbenzylamines by an initial electron transfer (ET) process. The reactions indicate a small negative ρ value ($\rho = -0.41$) 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)porphyrinatoiron (III) chloride[16] were investigated. The intramolecular KIE of 4-Y-*N*-methyl-*N*-trideuteriomethylanilines are much larger than intermolecular KIE with 4-Y-*N,N*-dimethylanilines and 4-Y-*N,N*-ditrideuteriomethylanilines. The Hammett correlations 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 curves. The intermolecular KIE of 4-Y-*N,N*-dimethylanilines and 4-Y-*N,N*-trideuteriomethylanilines by horseradish peroxidase (HRP) compound I[15] were observed to be $k_H/k_D = 1$, the values being constant with variation of Y from *p*-OMe to *p*-NO_2.

RESULTS AND DISCUSSION

The reactions are shown in Schemes 1 and 2. The competitive intramolecular *N*-debenzylation of *N*-benzyl-*N*-substitutedbenzylamines with HRP–H_2O_2 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 $[YC_6H_4CHO]/[C_6H_5CHO]$ ratios. The $\log(k_Y/k_H)$ values were plotted against σ and σ^+ to yield better correlation with σ ($\rho = -0.76$; $r = 0.997$) than with σ^+ ($\rho = -0.51$, $r = 0.965$).



Scheme 1



Scheme 2

The Hammett correlations for the oxidation of *N,N*-dimethylanilines ($\rho^+ = -0.88$)[16] may suggest that the electron transfer step for the formation of radical cation is involved with rate-determining step. The negative sign of $\rho^+ = -0.88$ is also parallel with their oxidation potentials

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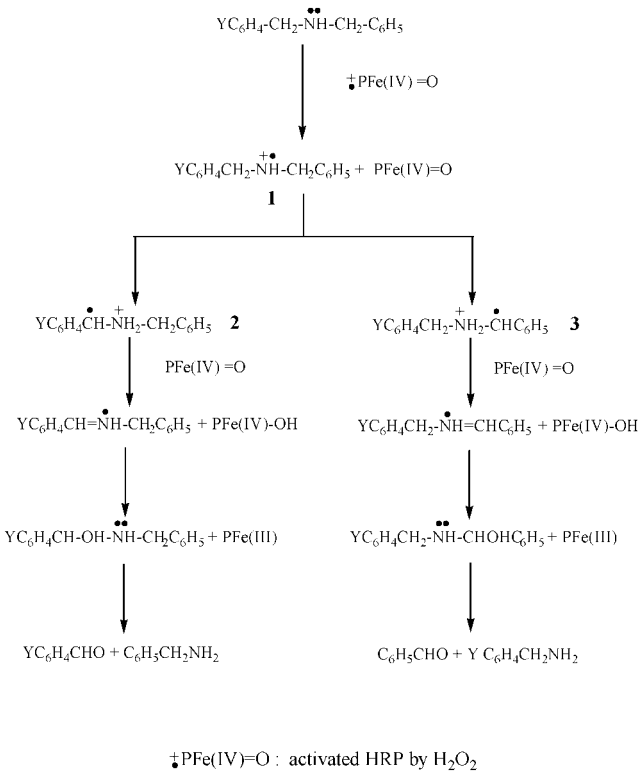
Table 1. Kinetic data for oxidative *N*-debenzylations of *N*-benzyl-*N*-substituted benzylamines by HRP

	p-OCH_3	*p*-CH_3	*p*-H	*p*-Cl	*m*-Cl	*p*-CN
k_{YH}/k_{HH}	1.64	1.30	1	0.609	0.491	0.310
			$\rho\ (r) = -0.76\ (0.997);\ \rho^{+}(r) = -0.51\ (0.965)$			
k_{YH}/k_{YD}	3.43 ± 0.3		1.34 ± 0.06		1.17 ± 0.02	

decreasing from *p*-NO_2 to *p*-OCH_3. The better correlation with σ in Table 1 indicates that positive charge is localized on the nitrogen atom. Compound **1** can be simultaneously transformed into either **2** or **3** (Scheme 3) since both of them are more stable than **1**. The intramolecular KIE values for 4-Y-$C_6H_4N(CH_3)CD_3$[16] are 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 the magnitude of pK_a of the corresponding radical cation, and suggests that there is a significant reverse electron transfer which competes with the α-deprotonation. The KIE for *p*-OCH_3, $k_H/k_D = 3.43$ in Table 1, shows a similar situation for the reversibility. In contrast, when electron transfer is the rate-determining step, no such KIE would be observed, that is, $k_H/k_D = 1$. The intermolecular KIE of Y-$C_6H_4N(CH_3)_2$ and Y-$C_6H_4N(CD_3)_2$ utilizing horseradish peroxidase compound I[15] shows an absence of KIE. Our KIE for *m*-Cl, $k_H/k_D = 1.17$, may indicate that reverse electron transfer occurs to a small extent. Therefore, the degree of the reversibility increases as the substituent gradually approaches electron donating, *m*-Cl ($k_H/k_D = 1.17$), H ($k_H/k_D = 1.34$), *p*-OCH_3 ($k_H/k_D = 3.34$).



Scheme 3

CONCLUSIONS

N-Demethylation of DMA proceed through the formation of the radical cation Y-$C_6H_4\dot{N}(CH_3)_2$ The reversible formation of $YC_6H_4\dot{N}(CH_3)_2$ should be influenced by the substituent(Y) and the kind of oxidant. The reversibility of formation of our radical cation, $YC_6H_4\dot{C}H—\overset{+}{N}H_2—CH_2C_6H_5$, by HRP compound **1** increases as the substituent (Y) becomes more electron donating.

EXPERIMENTAL

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 commercial products. Horseradish peroxidase was of type VI and was obtained from Sigma. A Varian Gemini 2000 NMR spectrometer was used for the identification of the compounds. Relative quantities of the aldehydes were obtained using a Varian 3300 gas chromatograph with a DB-1 column and a flame ionization detector.

Preparation of N-benzyl-N-4-methoxybenzylamine. A solution of benzylamine (0.02 mmol) in benzene was added to a benzene solution (15 ml) of *p*-anisaldehyde (0.02 mmol) in 100 ml flask over 10 min. The mixture was stirred for 3 h and Na_2SO_4 was added to eliminate H_2O. The formation of imine was confirmed when evaporating benzene in a rotary evaporator. To the imine dissolved in methanol in an ice-bath, 1.5 equiv of $NaBH_4$ were added very slowly. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the remainder 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). NMR ($CDCl_3$): 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 data are given below.

N-Benzyl-N-4-methylbenzylamine. NMR ($CDCl_3$): 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-chlorobenzylamine. NMR ($CDCl_3$): δ 3.8 (d, 4H, $2CH_2$), 7.2–7.4 (m, 9H, C_6H_5, C_6H_4).

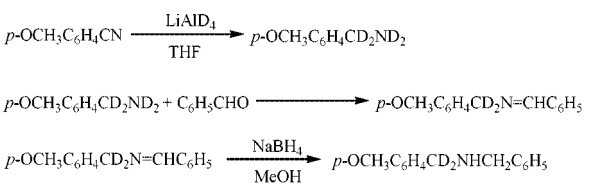
N-Benzyl-N-3-chlorobenzylamine. NMR ($CDCl_3$): δ 3.8 (d, 4H, $2CH_2$), 7.2–7.4 (m, 9H, C_6H_5, C_6H_4).

N-Benzyl-N-4-cyanobenzylamine. NMR ($CDCl_3$): δ 3.8 (d, 4H, $2CH_2$), 7.2–7.6 (m, 9H, C_6H_5, C_6H_4).

N-Benzyl-N-4-nitrobenzylamine. NMR ($CDCl_3$): 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 mol) in 20 ml of THF was added very slowly to an $LiAlD_4$ (0.045 mol) solution of THF in an ice-bath. The reaction mixture was then stirred for 1 day under nitrogen at room temperature. After reaction, 5% HCl solution was added slowly until the reaction mixture became acidic. The aqueous layer was separated by addition of benzene. To the aqueous layer, 3 M NaOH solution was added to make a basic solution and the amine layer was separated with benzene. 4-Methoxy(α,α-dideuterio)benzylamine (0.023 mol, 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 mol) and benzaldehyde (0.023 mol). This was reduced with $NaBH_4$ to give *p*-$CH_3OC_6H_4CD_2NHCH_2C_6H_4$ (4.98 g, 72% yield). NMR ($CDCl_3$): 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.

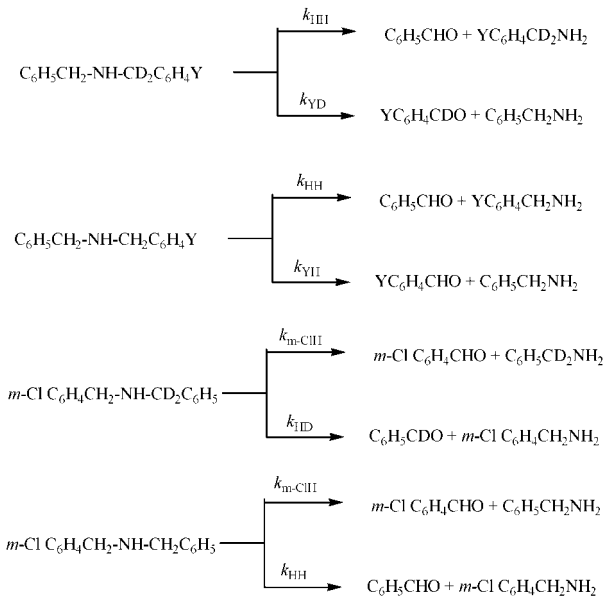


m-Cl $C_6H_4CH_2NHCD_2C_6H_5$. NMR ($CDCl_3$): δ 3.8 (s, 2H, CH_2), 7.2–7.4 (m, 9H, C_6H_5, C_6H_4).

m-Cl$C_6H_4CD_2NHCH_2C_6H_5$. NMR ($CDCl_3$): δ 3.8 (s, 2H, CH_2), 7.2–7.4 (m, 9H, C_6H_5, C_6H_4).

Oxidations by HRP/H_2O_2. To 650 µl of distilled water were added in the following order 200 µl of sodium phosphate buffer (pH 7.4), 40 µl of HRP (2.5 nmol), 10 µl of substrate (2.5 µmol) dissolved in CH_3OH and 100 µl of H_2O_2(25 µmol). The reaction mixture (total volume 1000 µl) was incubated at 37 °C for 30 min with vigorous stirring. The reaction mixture was then cooled with an ice-bath and 2 ml of 5% HCl solution were added to make the salt of substituted benzylamines. 1,4-Dibromobenzene (0.02 mmol) was added to the 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 GC analysis.

Kinetic isotope effects. These were determined indirectly as follows. $k_{YH}/k_{YD} = k_{YH}/k_{HH} \cdot k_{HH}/k_{YD}$ was used when Y = *p*-OCH_3 and *p*-Cl, and $k_{HH}/k_{HD} = k_{HH}/k_{m-Cl\ H} \cdot k_{m-Cl\ H}/k_{HD}$ can be obtained when Y = H using *m*-$ClC_6H_4CH_2$ as an internal standard.



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REFERENCES

1. Ortiz de Montellano PR. *Cytochrome P450. Structure, Mechanism, and Biochemistry* (2nd edn). Plenum Press: New York, 1995.
2. Guengerich FP, Macdonald TL. *Acc. Chem. Res.* 1984; **17**: 9–16.
3. Miwa GT, Walsh JS, Kedderis GL, Hollenberg PF. *J. Biol. Chem.* 1983; **258**: 14445–14449.
4. Miwa GT, Garland WA, Hodshon BJ, Lu AYH, Norhrop DB. *J. Biol. Chem.* 1980; **225**: 6049–6054.
5. Guengerich FP, Yun CH, Macdonald TL. *J. Biol. Chem.* 1996; **271**: 27321–27329.
6. Okazaki O, Guengerich FP. *J. Biol. Chem.* 1993; **268**: 1546–1552.
7. Macdonald TL, Gutheim WG, Martin RB, Guengerich FP. *Biochemistry* 1989; **28**: 2071.
8. Griffin BW, Ting PL. *Biochemistry* 1978; **17**: 2206–2211.
9. Van der Zee J, Duling DR, Mason RP, Eling TE. *J. Biol. Chem.* 1989; **264**: 19828–19836.

10. Kedderis GL, Hollenberg PF. *J. Biol. Chem.* 1983; **258**: 8129–8183.
11. Kedderis GL, Koop DR, Hollenberg PF. *J. Biol. Chem.* 1980; **255**: 10174–10182.
12. Lindsey Smith JR, Mortimer DN. *J. Chem. Soc., Chem. Commun.* 1985; 64–65.
13. Manchester JI, Dinnocenzo JP, Higgins L, Jones JP. *J. Am. Chem. Soc.* 1997; **119**: 5069–5670.
14. Karki SB, Dinnocenzo JP, Jones JP, Korzekwa KR. *J. Am. Chem. Soc.* 1995; **117**: 3657–3664.
15. Goto Y, Watanabe Y, Fukuzumi S, Jones JP, Dinnocenzo JP. *J. Am. Chem. Soc.* 1998; **120**: 10762–10763.
16. Baciocchi E, Lanzalunga O, Lapi A, Manduchi L. *J. Am. Chem. Soc.* 1998; **120**: 5783–5787.
17. (a) Baciocchi E, Gerini MF, Lanzalunga O, Lapi A, Mancinelli S, Mencarelli P. *Chem. Commun.* 2000; 393–394; (b) Baciocchi E, Gerini MF, Lanzalunga O, Lapi A, Piparo MGL, Mancinelli S. *Eur. J. Org. Chem.* 2001; 2305–2310.