

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

Sung Soo Kim* and Hwan Kyu Jung

Department of Chemistry and Center for Chemical Dynamics, Inha University, Incheon 402-751, South Korea

Received 10 September 2003; revised 15 January 2003; accepted 13 March 2003

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

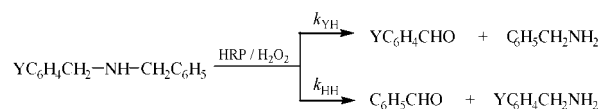
INTRODUCTION

The mechanism of the *N*-dealkylation of *N*-dimethylanilines catalyzed by heme enzymes and their analogs has attracted considerable attention.^{1–17} Iodosylbenzene (C₆H₅IO) 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 PhCH₂NMe₂ and PhCD₂NMe₂. The KIE and Hammett correlations in the oxidative *N*-demethylation of *N,N*-dimethylanilines catalyzed by tetrakis(pentafluorophenyl)porphyrinatoiron(III) chloride¹⁶ 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*-ditrideriomethylanilines. 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–H₂O₂ and 5,10,15,20-tetraphenyl-21H,23H-porphine-*p*, *p'*, *p''*, *p'''*-tetrasulfonic acid iron(III) chloride–H₂O₂ for a number of ring-substituted *N,N*-bis(dideuteriomethyl)anilines¹⁷ show bell-shaped curves. The intermolecular KIE of 4-*Y*-*N,N*-dimethylanilines and 4-*Y*-*N,N*-trideuteriomethylanilines by horseradish peroxidase (HRP) compound I¹⁵

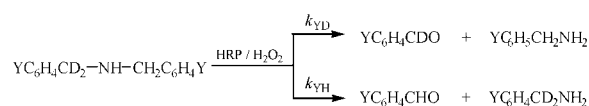
were observed to be $k_H/k_D = 1$, the values being constant with variation of *Y* from *p*-OMe to *p*-NO₂.

RESULTS AND DISCUSSION

The reactions are shown in Schemes 1 and 2. The competitive intramolecular *N*-debenzylation of *N*-benzyl-*N*-substitutedbenzylamines with HRP–H₂O₂ 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 [YC₆H₄CHO]/[C₆H₅CHO] 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

*Correspondence to: S. S. Kim, Department of Chemistry and Center for Chemical Dynamics, Inha University, Incheon 402-751, South Korea.

E-mail: sungsoo@inha.ac.kr

[†]This paper is dedicated to Professor Shinjiro Kobayashi on the occasion of his retirement.

Contract/grant sponsor: Korea Science and Engineering Foundation; Contract/grant number: F 01-2000-6-123-01-2.

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

	<i>p</i> -OCH ₃	<i>p</i> -CH ₃	<i>p</i> -H	<i>p</i> -Cl	<i>m</i> -Cl	<i>p</i> -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*-NO₂ to *p*-OCH₃. 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-C₆H₄N(CH₃)CD₃¹⁶ are 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 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*-OCH₃, $k_H/k_D = 3.43$ 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-C₆H₄N(CH₃)₂ and Y-C₆H₄N(CD₃)₂ utilizing horseradish peroxidase compound I¹⁵ 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*-OCH₃ ($k_H/k_D = 3.34$).

CONCLUSIONS

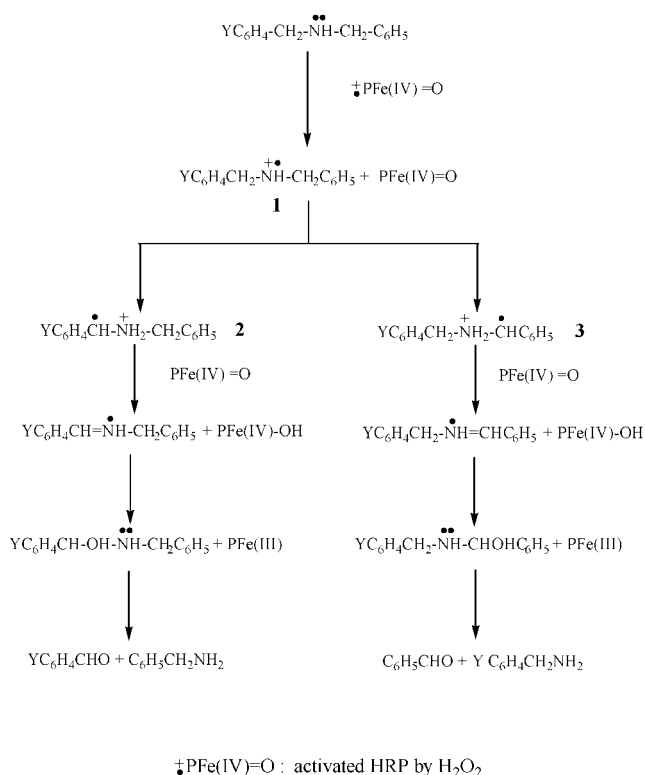
N-Demethylation of DMA proceed through the formation of the radical cation Y-C₆H₄N^{•+}(CH₃)₂. The reversible formation of YC₆H₄N^{•+}(CH₃)₂ should be influenced by the substituent(Y) and the kind of oxidant. The reversibility of formation of our radical cation, YC₆H₄CH^{•+}—NH₂—CH₂C₆H₅, by HRP compound **1** increases as the substituent (Y) becomes more electron donating.

EXPERIMENTAL

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 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 Na₂SO₄ was added to eliminate H₂O. 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 NaBH₄ 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 CH₂Cl₂–H₂O. The CH₂Cl₂ layer was dried with Na₂SO₄ to give the pure product (4.34 g, 95% yield). NMR (CDCl₃): δ 3.8 (d, 7H, CH₃O, 2CH₂), 6.9 (d, 2H, C₆H₄), 7.2 (d, 2H, C₆H₄), 7.3 (m, 5H, C₆H₅).

Other benzylamines were similarly synthesized and their NMR data are given below.

**Scheme 3**

N-Benzyl-*N*-4-methylbenzylamine. NMR (CDCl₃): δ 2.4 (s, 3H, CH₃), 3.8 (d, 4H, 2CH₂), 7.2–7.4 (m, 9H, C₆H₅, C₆H₄).

N-Benzyl-*N*-4-chlorobenzylamine. NMR (CDCl₃): δ 3.8 (d, 4H, 2CH₂), 7.2–7.4 (m, 9H, C₆H₅, C₆H₄).

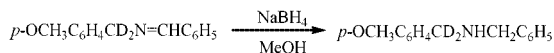
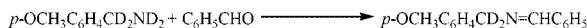
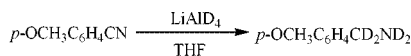
N-Benzyl-*N*-3-chlorobenzylamine. NMR (CDCl₃): δ 3.8 (d, 4H, 2CH₂), 7.2–7.4 (m, 9H, C₆H₅, C₆H₄).

N-Benzyl-*N*-4-cyanobenzylamine. NMR (CDCl₃): δ 3.8 (d, 4H, 2CH₂), 7.2–7.6 (m, 9H, C₆H₅, C₆H₄).

N-Benzyl-*N*-4-nitrobenzylamine. NMR (CDCl₃): δ 3.8 (d, 4H, 2CH₂), 7.2–7.4 (m, 5H, C₆H₅), 7.5 (d, 2H, C₆H₄), 8.2 (d, 2H, C₆H₄).

Preparation of *p*-CH₃OC₆H₄CD₂NHCH₂C₆H₅. 4-Methoxybenzonitrile (0.03 mol) in 20 ml of THF was added very slowly to an LiAlD₄ (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 NaBH₄ to give *p*-CH₃OC₆H₄CD₂NHCH₂C₆H₄ (4.98 g, 72% yield). NMR (CDCl₃): δ 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.



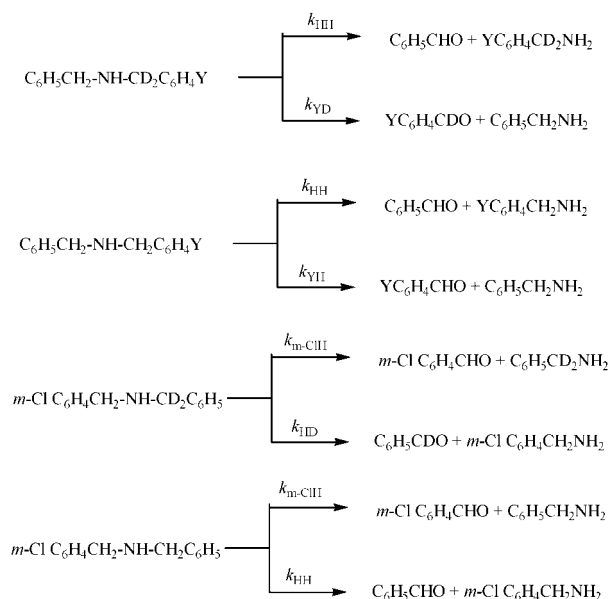
m-Cl C₆H₄CH₂NHCD₂C₆H₅. NMR (CDCl₃): δ 3.8 (s, 2H, CH₂), 7.2–7.4 (m, 9H, C₆H₅, C₆H₄).

m-ClC₆H₄CD₂NHCH₂C₆H₅. NMR (CDCl₃): δ 3.8 (s, 2H, CH₂), 7.2–7.4 (m, 9H, C₆H₅, C₆H₄).

Oxidations by HRP/H₂O₂. To 650 μ l of distilled water were added in the following order 200 μ l of sodium phosphate buffer (pH 7.4), 40 μ l of HRP (2.5 nmol), 10 μ l of substrate (2.5 μ mol) dissolved in CH₃OH and 100 μ l of H₂O₂ (25 μ mol). The reaction mixture (total volume

1000 μ l) 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. CH₂Cl₂ (3 ml $\times$ 3) was added to extract the organic layer. This was dried with anhydrous Na₂SO₄ and concentrated to 20 μ l for GC analysis.

Kinetic isotope effects. These were determined indirectly as follows. $k_{\text{YH}}/k_{\text{YD}} = k_{\text{YH}}/k_{\text{HH}} \cdot k_{\text{HH}}/k_{\text{YD}}$ was used when Y = *p*-OCH₃ and *p*-Cl, and $k_{\text{HH}}/k_{\text{HD}} = k_{\text{HH}}/k_{\text{m-Cl}}$. $k_{\text{m-Cl H}}/k_{\text{HD}}$ can be obtained when Y = H using *m*-ClC₆H₄CH₂ as an internal standard.



Acknowledgements

We warmly thank the Korean Science and Engineering Foundation for financial support through F 01-2000-6-123-01-2.

REFERENCES

- Ortiz de Montellano PR. *Cytochrome P450. Structure, Mechanism, and Biochemistry* (2nd edn). Plenum Press: New York, 1995.
- Guengerich FP, Macdonald TL. *Acc. Chem. Res.* 1984; **17**: 9–16.
- Miwa GT, Walsh JS, Kedderis GL, Hollenberg PF. *J. Biol. Chem.* 1983; **258**: 14445–14449.
- Miwa GT, Garland WA, Hodshon BJ, Lu AYW, Norhrop DB. *J. Biol. Chem.* 1980; **255**: 6049–6054.
- Guengerich FP, Yun CH, Macdonald TL. *J. Biol. Chem.* 1996; **271**: 27321–27329.
- Okazaki O, Guengerich FP. *J. Biol. Chem.* 1993; **268**: 1546–1552.
- Macdonald TL, Gutheim WG, Martin RB, Guengerich FP. *Biochemistry* 1989; **28**: 2071.
- Griffin BW, Ting PL. *Biochemistry* 1978; **17**: 2206–2211.
- 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 JJ, Dinnocenzo JP, Higgins L, Jones JP. *J. Am. Chem. Soc.* 1997; **119**: 5069–5070.
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.