On Isotope Effects for the Cytochrome P-450 Oxidation of Substituted *N*,*N*-Dimethylanilines

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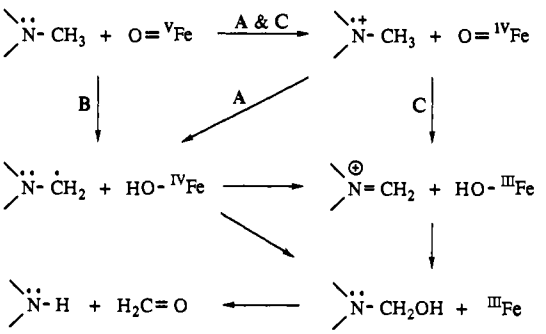
Abstract: Isotope effects were determined for the oxidative demethylation of the substituted *N*-methyl-*N*-(trideuteriomethyl)anilines **1a–d**, and the corresponding *N*,*N*-bis(dideuteriomethyl)anilines **2a–d**, by microsomal cytochrome P-450. The pairs of *p*-cyano- and *p*-nitro-*N*,*N*-dimethylanilines were found to have the same intramolecular isotope effects, while the unsubstituted and *p*-chloro derivatives had different isotope effects. It is concluded that, in general, intramolecular isotope effects measured for the enzymatic oxidations of *N*-methyl-*N*-(trideuteriomethyl)anilines are susceptible to masking. The isotope effect for the hydrogen (deuterium) atom abstractions from $PhN(CH_3)_2$ vs $PhN(CD_3)_2$ by the *tert*-butoxy radical was found to be 2.5. Interestingly, this is the same as the isotope effect measured for the cytochrome P-450 oxidation of *N*,*N*-bis(dideuteriomethyl)aniline (**2a**). These results are discussed with respect to the use of isotope effects for distinguishing the oxidative dealkylation mechanisms of amines by cytochrome P-450 and by related enzymes.

Introduction

Three major mechanisms have survived more than a decade of research on the oxidative dealkylation of amines by cytochrome P-450: (A) an electron/proton transfer mechanism, (B) a hydrogen atom transfer mechanism, and (C) an electron/hydrogen atom transfer mechanism.[2,3] Hydrogen/deuterium isotope effects for amines labeled at the carbon atom α to nitrogen are one of the criteria commonly used to distinguish between these mechanisms.[4] Most of these studies have relied on the mechanistic criterion first proposed by Miwa and co-workers in 1983.[5] It states that small kinetic isotope effects (e.g. $k_H/k_D \leq 4$) should be observed for the electron/proton transfer mechanism (A) and large isotope effects (e.g. $k_H/k_D \geq 7$) should be observed for the hydrogen atom transfer mechanism (B). The isotope effects for the electron/hydrogen atom transfer mechanism (C) remained uncertain. On the basis that the P-450 oxidation of *N*-methyl-*N*-(trideuteriomethyl)aniline showed an intramolecular isotope effect of 1.78, Miwa et al. proposed an electron/proton transfer mechanism for this reaction.[5] The same mechanistic conclusion was reached in most related P-450 studies.[4]



Recently, we prepared the isolable amine cation radical salt $(p\text{-}CH_3OPh)_2NCH_3^{\bullet+}$ AsF_6^- and directly studied its deprotonation by a series of substituted quinuclidine bases.[6] The H/D isotope effects for the deprotonations of $(p\text{-}CH_3OPh)_2NCH_3^{\bullet+}$ vs $(p\text{-}CH_3OPh)_2NCD_3^{\bullet+}$ varied between 6 and 9 depending on the base. These results demonstrated that tertiary amine cation radical deprotonations need not have small isotope effects, as was previously assumed. We also determined the pK_a of $(p\text{-}CH_3OPh)_2NCH_3^{\bullet+}$ ($\approx$10 in CH_3CN) and showed that it was nearly the same as that estimated for $PhNMe_2^{\bullet+}$ ($pK_a \approx 9$ in CH_3CN). On the basis of this comparison we proposed that our results with $(p\text{-}CH_3OPh)_2NCH_3^{\bullet+}$ might be relevant to other amine cation radical deprotonations, where the cation radical salts could not be isolated. This proposal has recently been confirmed by Parker and Tilset.[7] Using electrochemical methods, they measured isotope effects between 5 and 22 for the deprotonation of several substituted *N*,*N*-dimethylaniline cation radicals by pyridine. In addition, Mariano and co-workers have recently reported reasonably large isotope effects (k_H/k_D = 5–6) for the deprotonation of several photochemically generated trialkylamine cation radicals by enone anion radicals.[8] These combined studies unequivocally demonstrate that the deprotonation of amine cation radicals need not have small isotope effects, i.e. $k_H/k_D \leq 4$.

In this paper we show that the second part of the isotope effect magnitude criterion, namely, that hydrogen atom abstraction at the α-carbon of amines should have large kinetic isotope effects,

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(2) For reviews see: (a) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315. (b) Wislocki, P. G.; Miwa, G. T.; Lu, A. Y. H. In *Enzymatic Basis of Detoxification*; Jakoby, W. B., Ed.; Academic Press: New York, 1980; Vol. 1, p 135. (c) Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9. (d) *Cytochrome P-450*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; Chapter 1. (e) Dawson, J. H.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255. (f) Guengerich, F. P. *Crit. Rev. Biochem. Mol. Biol.* **1990**, *25*, 97. (g) Guengerich, F. P.; Macdonald, T. L. *FASEB J.* **1990**, *4*, 2453. (h) Porter, T. D.; Coon, M. J. *J. Biol. Chem.* **1991**, *266*, 13469. (i) Hollenberg, P. F. *FASEB J.* **1992**, *6*, 686.

(3) For simplicity, the P-450 reactive species in the mechanistic scheme is shown as a formal iron(V)–oxo species. Current experimental evidence suggests that the species is more likely a porphyrin cation radical/iron(IV)–oxo species. (a) Dawson, J. H. *Science* **1988**, *240*, 433. (b) Reference 2e and references therein.

(4) (a) Shono, T.; Toda, T.; Oshino, N. *J. Am. Chem. Soc.* **1982**, *104*, 2639. (b) Hollenberg, P. F.; Miwa, G. T.; Walsh, J. S.; Dwyer, L. A.; Kedderis, G. L. *Drug Metab. Dispos.* **1985**, *13*, 272. (c) Guengerich, F. P.; Böcker, R. H. *J. Biol. Chem.* **1988**, *263*, 8168. (d) Kurebayashi, H. *Arch. Biochem. Biophys.* **1989**, *270*, 320. (e) Born, J. L.; Hadley, W. M. *Chem. Res. Toxicol.* **1989**, *2*, 57. (f) Guengerich, F. P. *Chem. Res. Toxicol.* **1990**, *3*, 21. (g) Okazaki, O.; Guengerich, F. P. *J. Biol. Chem.* **1993**, *268*, 1546.

(5) Miwa, G. T.; Walsh, J. S.; Kedderis, G. L.; Hollenberg, P. F. *J. Biol. Chem.* **1983**, *258*, 14445.

(6) Dinnocenzo, J. P.; Banach, T. E. *J. Am. Chem. Soc.* **1989**, *111*, 8646.

(7) Parker, V. D.; Tilset, M. *J. Am. Chem. Soc.* **1991**, *113*, 8778.

(8) (a) Xu, W.; Mariano, P. S. *J. Am. Chem. Soc.* **1991**, *113*, 1431. (b) Xu, W.; Zhang, X.-M.; Mariano, P. S. *J. Am. Chem. Soc.* **1991**, *113*, 8863.

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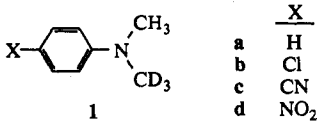
is similarly flawed. Originally, this proposal[5] was based on the relatively large isotope effect observed for the reaction of benzylamine with permanganate ion (k_H/k_D = 7),[9] which was assumed to proceed via a hydrogen atom abstraction mechanism. There is no compelling mechanistic evidence that this reaction proceeds by such a mechanism, however. More importantly, the criterion is at variance with the one seemingly clear-cut case of hydrogen atom abstraction from a tertiary amine. In 1981, Griller and Scaiano reported rate constants for hydrogen atom abstractions from trimethylamine and trimethylamine-d_9 by the *tert*-butoxy radical (•OBu^t) and found k_H/k_D to be only 1.4.[10] Herein we show that low isotope effects for hydrogen atom abstraction from tertiary amines is not unique to trimethylamine. We measured isotope effects for hydrogen atom abstraction from *N,N*-dimethylaniline by •OBu^t and found them to be similarly small (see below).

It is now clear that the mechanisms proposed for the oxidative dealkylation of amines by P-450 cannot be distinguished by the magnitude of the isotope effects. We have therefore begun an experimental investigation to test an alternate mechanistic strategy. Our approach is to determine the functional dependence of isotope effects for the P-450 oxidation of aryl-substituted *N,N*-dimethylanilines on the aromatic ring substituents and then to compare this dependence with those of model hydrogen atom abstractions and cation radical deprotonations. Of course, intrinsic kinetic isotope effects for the P-450 oxidations are necessary prerequisites for this study. It has previously been assumed that they are equal to the intramolecular isotope effects determined from the oxidations of *N*-methyl-*N*-(trideuteriomethyl)anilines.[5] These substrates suffer from a potential problem, however. In an asymmetric enzyme site, the methyl groups of *N,N*-dimethylaniline are necessarily diastereotopic, and therefore, unless positional exchange of the methyl groups is rapid with respect to the (presumably) irreversible C–H(D) cleavage step, the measured isotope effect will be "masked". Hawkins and Dawson have recently demonstrated isotope effect masking for the oxidation of dimethylamine by secondary amine monooxygenase.[11] They found different intramolecular isotope effects for the oxidation of methyl(trideuteriomethyl)amine and bis(dideuteriomethyl)amine. We found similar isotope effect differences for the P-450 oxidation of substituted *N*-methyl-*N*-(trideuteriomethyl)anilines and *N,N*-bis(dideuteriomethyl)anilines. These experiments are described herein.

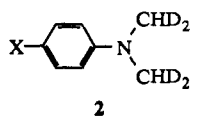
Results

Substituted *N*-methyl-*N*-(trideuteriomethyl)anilines (**1**) and *N,N*-bis(dideuteriomethyl)anilines (**2**) were synthesized by standard methods (see Experimental Section). The deuterium content of each amine was analyzed by mass spectrometry using low-energy, electron impact conditions so as to minimize ion fragmentation. In all cases, <0.18% of the M – 1 peak was observed. The percent deuterium content of the synthesized amines was ≥99% in all cases.



1

	X
a	H
b	Cl
c	CN
d	NO_2



2

The oxidative monodemethylations of anilines **1a–d** and **2a–d** were carried out by using phenobarbital-induced microsomal P-450 preparations.[12] Oxidative regeneration of the enzyme was performed using two different systems: (i) O_2/NADPH and (ii) O_2/NADP plus lactate/lactate dehydrogenase to reduce the NADP to NADPH. The isotope effects (k_H/k_D) for **1a–d** are defined as the ratios of the d_3/d_0 substituted *N*-methylanilines produced from the P-450 oxidations, which were determined by mass spectroscopic analysis. The isotope effects for the oxidations **2a–d** are defined as 2 times the d_2/d_1 formaldehyde product ratios, which were determined by the mass spectroscopic analysis of their dimedone adducts. The results are shown in Table I.

Table I. Isotope Effects for the P-450 Oxidations of **1a–d** and **2a–d**

	$(k_H/k_D)^{a,b}$ for **1**		$(k_H/k_D)^{a,b}$ for **2**	
substituent	NADPH	lactate	NADPH	lactate
H (**a**)	1.8(1)	1.9(1)	2.5(3)	2.5(1)
Cl (**b**)	2.0(1)	2.2(1)	2.7(1)	2.9(1)
CN (**c**)	3.1(1)	3.3(1)	3.2(1)	3.3(1)
NO_2 (**d**)	3.5(1)	3.5(3)	3.6(1)	3.4(2)

[a] See text for the definition of k_H/k_D. [b] Averages of at least three independent determinations. Standard deviation in the last significant figure is given in parentheses.

Rate constants for the reaction of •OBu^t with $PhN(CH_3)_2$ and $PhN(CD_3)_2$ were measured by using the diphenylmethanol-competition method of Scaiano.[13] The *tert*-butoxy radical was generated by nanosecond pulsed-laser photolysis of *tert*-butyl peroxide, and the rate constants for appearance of the diphenylketyl radical were determined as a function of amine concentration. Plots of k_{obs} vs amine concentration showed good linearity, and the slopes of these plots gave the second-order rate constants for the reaction of •OBu^t with the amines. The rate constants for reaction with $PhN(CH_3)_2$ and $PhN(CD_3)_2$ were $1.4(1) \times 10^8$ and $5.7(4) \times 10^7$ M^{-1} s^{-1}, respectively, in 1:2 benzene/$(t\text{-BuO})_2$ at 22 °C.[14] After correction for a small amount of incomplete deuterium incorporation into the $PhN(CD_3)_2$, the isotope effect is calculated to be 2.5(2). To determine if a change in the dielectric of the medium changed the isotope effect, the rate constants were also measured in a solvent system consisting of 1:2 CH_3CN/$(t\text{-BuO})_2$. The rate constants for reaction with $PhN(CH_3)_2$ and $PhN(CD_3)_2$ were only slightly altered in this solvent system: $k_H = 1.24(5) \times 10^8$ and $k_D = 5.2(3) \times 10^7$ M^{-1} s^{-1}. The corrected isotope effect, k_H/k_D = 2.5(2), is the same as in benzene/$(t\text{-BuO})_2$. The measured static dielectric constants of the benzene/$(t\text{-BuO})_2$ and CH_3CN/$(t\text{-BuO})_2$ solvent systems were 2.33 and 15.8, respectively.

Discussion

The data in Table I show that, using either enzyme regeneration system, the isotope effects for the P-450 oxidative demethylation of the pairs **1c/2c** and **1d/2d** are the same within experimental error, while those of **1a/2a** and **1b/2b** are not. It is possible that the isotope effect differences for the pairs **1a/2a** and **1b/2b** could be due to different α secondary isotope contributions. This explanation seems unlikely, however, because the isotope effects for **1c/2c** and **1d/2d** are the same, which suggests that the secondary isotope effect contributions are small and cannot account for the isotope effect differences observed for **1a/2a** and **1b/2b**. It is more likely that these isotope effect differences are due to masking, i.e. the rate constants for exchange of the CH_3/CD_3 groups of **1a** and **1b** in the enzyme active site are comparable

(9) Wei, M.-M.; Stewart, R. *J. Am. Chem. Soc.* **1966**, *88*, 1974.
(10) Griller, D.; Howard, J. A.; Marriott, P. R.; Scaiano, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 619.
(11) (a) Hawkins, B. K.; Dawson, J. H. *J. Am. Chem. Soc.* **1992**, *114*, 3547. For related studies see: (b) Hanzlik, R. P.; Hogberg, K.; Moon, J. B.; Judson, C. M. *J. Am. Chem. Soc.* **1985**, *107*, 7164. (c) Hanzlik, R. P.; Schaefer, A. R.; Moon, J. B.; Judson, C. M. *J. Am. Chem. Soc.* **1987**, *109*, 4926.
(12) Porter, W. R.; Branchflower, R. V.; Trager, W. F. *Biochem. Pharmacol.* **1977**, *26*, 549.
(13) (a) Reference 10. (b) Small, R. D., Jr.; Scaiano, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 296. (c) Paul, H.; Small, R. D., Jr.; Scaiano, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 4520. (d) Small, R. D., Jr.; Scaiano, J. C.; Patterson, L. K. *Photochem. Photobiol.* **1979**, *29*, 49. (e) Scaiano, J. C. In *Oxygen Radicals in Biology and Medicine*; Simic, M. G., Taylor, K. A., Ward, J. F., von Sonntag, C., Eds.; Plenum Press: New York, 1988; p 59.
(14) The standard deviation in the last significant figure is given in parentheses.

Table II. Comparison of $\Delta G_{et}°$ and $\Delta G^{\ddagger}$ for $ArNMe_2$ + $^{\bullet}OBu^t$

substituted *N,N*-dimethylaniline	E_{ox},[a] V (SCE)	$\Delta G_{et}°$, kcal/mol	$\Delta G^{\ddagger}$, kcal/mol
p-H	0.767	>6.2	6.3
p-Cl	0.789	>6.7	6.4
p-CN	1.049	>12.7	6.7

[a] In acetonitrile (ref 7).

to the rate constants for the isotopically sensitive step that breaks the C–H(D) bond.

The reason that isotope effect differences are observed for **1a/2a** and **1b/2b** but not for **1c/2c** and **1d/2d** is not yet clear. It is interesting to note, however, that the differences decrease as the aromatic ring substituents become more electron-withdrawing. One explanation consistent with this observation is that the amine nitrogen is hydrogen-bonded to a group in the active site of the enzyme. In this case, the rate of CH_3/CD_3 exchange should depend on the strength of the hydrogen-bonding interaction. On the basis of the basicities of the four amines,[15] the interaction is expected to be strongest for **1a** and weakest for **1d**, consistent with the observed results.

Next we turn to the isotope effects for the reaction of $PhNMe_2$ with $^{\bullet}OBu^t$. These isotope effects were measured to test the hypothesis that hydrogen atom abstraction from amines should show large isotope effects (e.g. $k_H/k_D \geq 7$).[5] Griller and Scaiano have previously shown that tertiary amines react with $^{\bullet}OBu^t$ to give α-amino radicals.[10] The rate constant they measured for the reaction of $^{\bullet}OBu^t$ with $PhNEt_2$ (1.3×10^8 M^{-1} s^{-1}) is nearly the same as the value we determined for $PhNMe_2$ (1.4×10^8 M^{-1} s^{-1}). The mechanism for the reaction of amines with $^{\bullet}OBu^t$ was previously assumed to involve a direct hydrogen atom transfer. It is possible, however, that the mechanism could instead involve a two-step, electron-transfer/proton-transfer process. We now show that this latter mechanism is unlikely on the basis of several lines of evidence. The first involves a comparison of the free energies of activation ($\Delta G^{\ddagger}$) for the reaction of the substituted *N,N*-dimethylanilines with $^{\bullet}OBu^t$ to the free energies of electron transfer from the *N,N*-dimethylanilines to $^{\bullet}OBu^t$ ($\Delta G_{et}°$). In general, if $\Delta G^{\ddagger} < \Delta G_{et}°$, then an electron-transfer mechanism is thermodynamically excluded, while if $\Delta G^{\ddagger} > \Delta G_{et}°$, then an electron transfer is thermodynamically permitted, although not necessarily kinetically permitted.[16] $\Delta G_{et}°$ can be determined from the oxidation potentials of the amines (Table II) and the reduction potential of $^{\bullet}OBu^t$ (ca. 0.5 V vs SCE).[17] The $\Delta G_{et}°$ values in Table II should be considered *minimum* estimates since the redox potentials apply to CH_3CN as solvent, while the actual reactions were done in far less polar media. The actual $\Delta G_{et}°$ values may be higher by ca. 0.2–0.3 V.[18] The values for $\Delta G^{\ddagger}$ (Table II) were determined by using our rate constant for $PhNMe_2$, the relative rate constants for reaction of $^{\bullet}OBu^t$ with various substituted *N,N*-dimethylanilines,[19] and the Eyring equation.

The data for the *p*-chloro- and *p*-cyano-*N,N*-dimethylanilines in Table II exclude an electron-transfer/proton-transfer mechanism for the reactions with $^{\bullet}OBu^t$. Although the data for the unsubstituted amine do not permit a definitive mechanistic conclusion, an electron-transfer mechanism seems unlikely for four reasons. First, a Hammett plot of the rate constants for reaction of substituted *N,N*-dimethylanilines with $^{\bullet}OBu^t$ shows good linearity for substituents ranging from *p*-$N(CH_3)_2$ to *p*-CN.[19] If the reaction mechanism changed with ring substitution, the rate constants for *N,N*-dimethylanilines with electron-donating substituents should have shown positive deviations in the Hammett plot. They do not. Second, as explained above, $\Delta G_{et}°$ for $PhMe_2$ + $^{\bullet}OBu^t$ in benzene/$(t\text{-BuO})_2$ is expected to be significantly higher than the value in Table II. Third, the activation free energy for electron transfer must be greater than $\Delta G_{et}°$. Fourth, the insensitivity of the rate constant for reaction of $PhMe_2$ with $^{\bullet}OBu^t$ to the solvent polarity argues against an electron-transfer mechanism. Taken together, these facts provide strong evidence against an electron-transfer/proton-transfer mechanism for the reaction of $^{\bullet}OBu^t$ with all of the substituted *N,N*-dimethylanilines. Finally, the relatively small isotope effects observed for the reaction of amines with $^{\bullet}OBu^t$ are consistent with a hydrogen atom abstraction mechanism. On the basis of the weak α C–H bonds in amines (84 and ca. 80 kcal/mol for NMe_3 and $PhNMe_2$, respectively)[20] and the strong O–H bond in $HOBu^t$ (105 kcal/mol),[21] the hydrogen atom abstractions are predicted to be strongly exothermic. On the basis of the Hammond–Leffler postulate,[22] the abstractions are therefore expected to have relatively early transition-state structures which, according to Westheimer's kinetic isotope effect model,[23] should lead to isotope effects that are well below the maximum theoretical value.

Lastly, regarding the mechanism of the oxidative dealkylation of amines by P-450, it is interesting to note that the iron–oxo species presumed to be responsible for the reactivity of P-450 is, like $^{\bullet}OBu^t$, thought to be a good hydrogen atom abstractor.[24] On the basis of the primary and secondary isotope effects observed for the C-1 hydroxylation of *n*-octane by P-450,[25] Trager and co-workers have argued that the reaction involves a symmetric transition-state structure for hydrogen atom transfer, which implies that the bonds being broken and being made have similar energies (ca. 101 kcal/mol for *n*-octane).[26] Therefore, the quantitatively similar hydrogen atom abstracting abilities of $^{\bullet}OBu^t$ and the iron–oxo species suggest that the identical isotope effects observed for the hydrogen atom abstraction from *N,N*-dimethylaniline by $^{\bullet}OBu^t$ and the oxidative demethylation of **2a** by P-450 may not be fortuitous.[27] Doubtless, more compelling evidence for a hydrogen atom abstraction mechanism for P-450 would be provided if the isotope effect profile for the reaction of $^{\bullet}OBu^t$ with the three substituted *N,N*-dimethylanilines was the same as that observed for the P-450 oxidations of **2b–d**. Unfortunately, this comparison is not yet possible due to the fact that the substituted *N,N*-dimethylanilines strongly absorb in the spectral region where $(t\text{-BuO})_2$ can be photochemically excited to produce *tert*-butoxy radicals. We are currently testing other photochemical precursors of the *tert*-butoxy radical that will hopefully circumvent this problem.

(15) The pK_a's of the protonated *p*-H, *p*-Cl, *p*-CN, and *p*-NO_2 *N,N*-dimethylanilines are 5.1, 4.4, 1.8, and 0.6, respectively. Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965.

(16) For a discussion on the estimation of electron-transfer activation energies to assess reaction mechanism, see: Eberson, L. *Electron Transfer Reactions in Organic Chemistry*; Springer-Verlag: New York, 1987; Chapter 4.

(17) (a) Eberson, L. *Acta Chem. Scand., Ser. B* **1984**, *38*, 439. (b) Wayner, D. D. M. In *CRC Handbook of Organic Photochemistry*; Scaiano, J. C., Ed.; CRC Press: Boca Raton, 1989; Vol. II, Chapter 18.

(18) Weller, A. *Z. Phys. Chem. Neue Folge* **1982**, *133*, 93.

(19) Uneyama, K.; Namba, H.; Oae, S. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 1928.

(20) (a) Burkey, T. J.; Castelhano, A. L.; Griller, D.; Lossing, F. P. *J. Am. Chem. Soc.* **1983**, *105*, 4701. (b) Reference 6.

(21) McMillen, D. F.; Golden, D. M. *Annu. Rev. Phys. Chem.* **1982**, *33*, 493.

(22) (a) Hammond, G. S. *J. Am. Chem. Soc.* **1955**, *77*, 334. (b) Leffler, J. E. *Science* **1953**, *117*, 340.

(23) Westheimer, F. H. *Chem. Rev.* **1961**, *61*, 265.

(24) See: McMurray, T. J.; Groves, J. T. In *Cytochrome P-450*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; Chapter 1.

(25) Jones, J. P.; Rettie, A. E.; Trager, W. F. *J. Med. Chem.* **1990**, *33*, 1242.

(26) The bond dissociation energy for the Cl–H bond of *n*-octane is assumed to be equal to the Cl–H bond energy in *n*-heptane, which can be obtained from the heats of formation of the *n*-heptyl radical, the hydrogen atom, and *n*-heptane: 4, 52.1, and –44.8 kcal/mol, respectively. Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. No. 1.

(27) In this regard, it is interesting to note that the Hammett ρ value obtained from a plot of $\log(V_{max})$ vs σ for the P-450 oxidation of substituted *N,N*-dimethylanilines ($\rho \approx -0.6$: Burka, L. T.; Guengerich, F. P.; Willard, R. J.; Macdonald, T. L. *J. Am. Chem. Soc.* **1985**, *107*, 2549) is remarkably similar to that found for the reactions of substituted *N,N*-dimethylanilines with the *tert*-butoxy radical ($\rho \approx -0.4$).[19]

Summary

Direct measurements of isotope effects for the deprotonation of amine cation radicals and for hydrogen atom abstraction from amines demonstrate that isotope effect magnitudes do not provide decisive probes of the oxidative dealkylation mechanism of amines by cytochrome P-450 and by related enzymes. Although the identical isotope effects observed for the reaction of *N,N*-dimethylaniline with $^{\bullet}OBu^{t}$ and the oxidative demethylation of **2a** by P-450 provide support for a hydrogen atom transfer mechanism for the P-450 reaction, we do *not* think a compelling mechanistic assessment is possible on the basis of the present data. A more convincing test of the hydrogen atom abstraction mechanism awaits a comparison of the isotope effects for hydrogen atom abstraction for a series of substituted *N,N*-dimethylanilines by $^{\bullet}OBu^{t}$ with the isotope effects determined from the P-450 oxidation of substituted *N,N*-bis(dideuteriomethyl)anilines.[28] Critical tests of the other two oxidative dealkylation mechanisms could be provided by appropriate model isotope effect comparisons for these systems.

Experimental Section

General. Unless otherwise stated, all reactions were done under an atmosphere of dry nitrogen. Diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. Dimethyl sulfoxide and benzene were distilled from calcium hydride. Iodomethane-d_2 (99.5% atom D), paraformaldehyde-d_1 (99.4% atom D), paraformaldehyde-d_2 (99.3% atom D), and sodium borodeuteride (98.3% atom D) were obtained from MSD Isotopes. Lactate dehydrogenase was obtained from Boehringer Mannheim Biochemicals, and all other biochemicals were obtained from Sigma. All other chemicals and reagents were obtained from Aldrich and used as received except *N*-methylaniline and 4-chloro-*N*-methylaniline, which were purified by recrystallization of their acetanilides,[29] followed by hydrolysis. 4-Cyano-*N*-methylaniline was prepared by a literature procedure.[30] Di-*tert*-butyl peroxide was purified as previously reported,[31] and diphenylmethanol was sublimed before use. Melting points were obtained on a Thomas-Hoover Uni-Melt apparatus in open capillary tubes. Dielectric constants were measured using a cylindrical liquid cell with a General Radio 1689 Precision Digibridge operating at 10 kHz. The capacitance was corrected for stray capacitances of the electrical leads and other instrumental effects. The temperature of the cell was maintained at 25 °C by flowing water through a jacket in the cell using a VWR Scientific Model 1440 constant temperature bath.

^{1}H NMR spectra were recorded with a General Electric/Nicolet QE-300 spectrometer. Proton chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane or in parts per million relative to the singlet at 7.24 ppm for the residual $CHCl_3$ in the chloroform-*d* or the multiplet at 1.93 ppm for the residual CHD_2CN in the acetonitrile-d_3. Proton–proton coupling constants are reported in hertz (Hz) and reflect assumed first-order behavior. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; and br, broad. Gas chromatography was performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a HP 7673A automatic sampler on a 30-m DX3 capillary column from J&W Scientific. The gas chromatograph was interfaced to an Everex 286/16 computer with a HP 7673A controller. Mass spectral analyses were performed on a GC–MS system made up of a Hewlett-Packard 5890 Series II gas chromatograph (with a 30-m DX3 capillary column) which was interfaced to a Hewlett-Packard 5970 Series mass selective detector equipped with a Hewlett-Packard 6212C variable power supply. UV–vis spectra were recorded with a Beckman DU-65 spectrophotometer. A Beckman L8-55 ultracentrifuge equipped with a type 60TI rotor was used for high-speed centrifugation.

Preparation of *N*-(Trideuteriomethyl)aniline. This material was prepared by a literature procedure,[32] except iodomethane-d_3 (99.5+ atom % D) was used. In the glovebox, a 50-mL round-bottomed flask was charged with sodium hydride (0.107 g, 4.458 mmol). After removal from the glovebox, the flask was further charged with tetrahydrofuran (10 mL) and *N*-(*tert*-butoxycarbonyl)aniline[32] (0.702 g, 3.63 mmol). After the mixture was stirred for 10 min, iodomethane-d_3 (99.5+ atom % D, 0.27 mL) was added dropwise over 2 min, and the mixture was refluxed for 5 h. After cooling, water (2 mL) was added to the milky white reaction mixture, which then became clear. Hydrochloric acid (6 M, 18 mL) was added to the reaction mixture, which turned pale yellow. After refluxing for 10 h, the reaction mixture was made basic by adding sodium hydroxide solution, was washed successively with 20-mL portions of water and brine, and was dried over anhydrous sodium sulfate. Filtration and solvent removal *in vacuo* gave a tan oil (0.389 g). Chromatography on silica gel using 50:50 hexane/diethyl ether as an eluant followed by solvent removal gave an oil (0.312 g), which was distilled under vacuum (0.25 mmHg, 35 °C) to give a colorless oil (0.280 g, 71%). ^{1}H NMR (CD_3CN): δ 7.13 (t, J = 7.5, 1.98 H), 6.56–6.63 (m, 3.02 H), 4.29 (br s, 0.99 H).

Preparation of 4-Cyano-*N*-(trideuteriomethyl)aniline. This material was prepared in a similar manner as above except 4-[*N*-(*tert*-butoxycarbonyl)amino]benzonitrile[32] (0.50 g, 2.29 mmol) was used. The resulting product was chromatographed on silica gel using 50:50 diethyl ether/chloroform as an eluant, which upon solvent removal gave a white solid (0.262 g, 85%). ^{1}H NMR ($CDCl_3$): δ 7.42 (d, J = 8.72 Hz, 1.99 H), 6.53 (d, J = 8.71 Hz, 2.01 H), 4.22 (br s, 0.99 H).

Preparation of 4-Nitro-*N*-(trideuteriomethyl)aniline. This material was prepared in 89% yield using the same procedure described above except that 4-nitro-*N*-(*tert*-butoxycarbonyl)aniline[32] was used. ^{1}H NMR ($CDCl_3$): δ 8.09 (d, J = 9.14 Hz, 2.02 H), 6.51 (d, J = 9.14 Hz, 1.97 H), 4.52 (br s, 1.0 H).

Preparation of 4-Chloro-*N*-(trideuteriomethyl)aniline. A 250-mL three-necked flask equipped with a reflux condenser was charged with lithium aluminum deuteride (98 atom % D, 0.205 g, 4.88 mmol) and tetrahydrofuran (10 mL). A solution of ethyl *N*-(4-chlorophenyl)carbamate[33] (0.487 g, 2.44 mmol) in 8 mL of tetrahydrofuran was added via syringe over 45 min. The reaction mixture was refluxed for 6 h and then cooled to room temperature. A saturated aqueous potassium sodium tartrate solution (40 mL) was added dropwise, and the mixture was stirred for 2 h. The organic layer was separated and washed successively with 10-mL portions of water and brine and was dried over anhydrous sodium sulfate. Filtration and solvent removal *in vacuo* gave a tan oil (0.298 g). Chromatography on silica gel using diethyl ether as an eluant gave, after solvent removal, a pale yellow oil (0.250 g), which was further distilled under vacuum (80 °C, 0.3 mmHg) to give a colorless oil (0.174 g, 50%). ^{1}H NMR ($CDCl_3$): δ 7.12 (d, J = 8.75 Hz, 1.99 H), 6.51 (d, J = 8.78 Hz, 1.99 H), 3.67 (br s, 1.02 H).

Preparation of *N*-Methyl-*N*-(trideuteriomethyl)aniline. A 25-mL round-bottomed flask equipped with a reflux condenser was charged with *N*-methylaniline (0.657 g, 6.13 mmol), tetra-*n*-butylammonium iodide (0.158 g, 0.428 mmol), potassium hydroxide (0.822 g, 14.6 mmol), benzene (7 mL), and water (1 mL). After 10 min, iodomethane-d_3 (99.5+ atom % D, 0.402 mL, 6.29 mmol) was added dropwise over 2 min and stirred at room temperature for 7 h. Additional tetra-*n*-butylammonium iodide (0.078 g, 0.21 mmol), potassium hydroxide (0.363 g, 6.46 mmol), and iodomethane-d_3 (0.070 mL) were added and stirred at room temperature for an additional 11 h. The organic layer was separated and washed successively with 10-mL portions of water, saturated sodium carbonate, and brine and dried over anhydrous sodium sulfate. Filtration and solvent removal *in vacuo* gave a tan oil (0.733 g). Chromatography on silica gel using 90:10 hexane/diethyl ether as an eluant gave, after solvent removal, a light yellow oil (0.569 g), which was distilled under vacuum (40 °C, 0.85 mmHg) to give a colorless liquid (0.502 g, 66%). ^{1}H NMR (CD_3CN): δ 7.19 (t, J = 7.3 Hz, 1.97 H), 6.74 (d, J = 8.4, 1.97 H), 6.65 (t, J = 7.3, 1.07 H), 2.89 (s, 2.99 H).

Preparation of *N,N*-Bis(dideuteriomethyl)aniline. This material was prepared in 81% yield using the same procedure described above except that iodomethane-d_2 (99.4 atom % D) and aniline were used. ^{1}H NMR (CD_3CN): δ 7.19 (t, J = 7.3 Hz, 1.99 H), 6.74 (d, J = 8.3, 1.97 H), 6.66 (t, J = 7.3, 1.05 H), 2.85 (m, 1.99 H).

(28) At this point, we cannot exclude the possibility that some isotope effect masking occurs even for the substituted *N,N*-bis(dideuteriomethyl)-anilines **2a–d**. Masking could occur if the methyl group rotational barriers are comparable to the barriers for removal of H or D by P-450. Methyl rotational barriers for tertiary amines have been estimated from microwave studies to be ca. 3–4 kcal/mol. (a) Lide, D. R.; Mann, D. E. *J. Chem. Phys.* **1958**, *28*, 572. (b) Wollrab, J. E.; Laurie, V. W. *J. Chem. Phys.* **1971**, *54*, 532. (c) Cervellati, R.; Corbelli, G.; Dal Borgo, A.; Lister, D. G. *J. Mol. Struct.* **1981**, *73*, 31.

(29) Fanta, P. E.; Tarbell, D. S. *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. III, p 661.

(30) Kadin, S. B. *J. Org. Chem.* **1973**, *38*, 1348.

(31) Nazran, A. S.; Griller, D. *J. Am. Chem. Soc.* **1983**, *105*, 1970.

(32) Croce, P. D.; Rosa, C. L.; Ritieni, A. *J. Chem. Research, Synop.* **1988**, 346.

(33) Michman, M.; Patai, S.; Wiesel, Y. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1705.

Preparation of 4-Chloro-*N*-methyl-*N*-(trideuteriomethyl)aniline. A 25-mL round-bottomed flask equipped with a reflux condenser was charged with 4-chloro-*N*-methylaniline (0.897 g, 6.33 mmol), tetra-*n*-butylammonium iodide (0.165 g, 0.45 mmol), potassium hydroxide (0.904 g, 16 mmol), benzene (7 mL), and water (1 mL). After 10 min, iodomethane-d_3 (99.5+ atom % D, 0.473 mL, 7.44 mmol) was added dropwise over 2 min and heated to reflux for 6 h. After cooling, the organic layer was washed successively with 10-mL portions of water, saturated sodium carbonate, and brine and was dried over anhydrous sodium sulfate. Filtration and solvent removal *in vacuo* gave a pale yellow liquid (1.00 g). Chromatography on silica gel using 40:60 diethyl ether/pentane as an eluant gave, after solvent removal, a white solid (0.835 g, 83%). ^{1}H NMR ($CDCl_3$): δ 7.18 (d, J = 9.15 Hz, 1.99 H), 6.64 (d, J = 9.11 Hz, 2.00 H), 2.94 (s, 3.00 H).

Preparation of 4-Chloro-*N,N*-bis(dideuteriomethyl)aniline. This material was prepared in 87% yield using the same procedure described above except that iodomethane-d_2 (99.4 atom % D) and 4-chloroaniline were used. ^{1}H NMR ($CDCl_3$): δ 7.15 (d, J = 8.94 Hz, 1.98 H), 6.62 (d, J = 8.94 Hz, 2.01 H), 2.87 (m, 2.01 H).

Preparation of 4-Nitro-*N*-methyl-*N*-(trideuteriomethyl)aniline. A 25-mL round-bottomed flask equipped with a reflux condenser was charged with 4-nitro-*N*-methylaniline (0.910 g, 5.98 mmol), potassium hydroxide (0.908 g, 16 mmol), and dimethyl sulfoxide (6 mL). Iodomethane-d_3 (99.5+ atom % D, 0.42 mL) was added dropwise over 2 min and heated to 85 °C for 2 h. After cooling, the organic layer was washed successively with 10-mL portions of water, saturated sodium carbonate, and brine and was dried over anhydrous sodium sulfate. Filtration and solvent removal *in vacuo* gave yellow crystals (0.996 g). Chromatography on silica gel using 50:50 diethyl ether/chloroform as an eluant gave, after solvent removal, yellow needles (0.673 g, 67%). ^{1}H NMR ($CDCl_3$): δ 8.14 (d, J = 9.26 Hz, 1.91 H), 6.61 (d, J = 9.27 Hz, 2.00 H), 3.13 (s, 3.07 H).

Preparation of 4-Cyano-*N*-methyl-*N*-(trideuteriomethyl)aniline. A 25-mL round-bottomed flask was charged with 37% aqueous formaldehyde (0.057 g, 0.70 mmol) and sulfuric acid (3.0 mL of 3 M) and was placed in an ice/water bath. To this solution was added a slurry of 4-cyano-*N*-(trideuteriomethyl)aniline (0.0483 g, 0.357 mmol), sodium borohydride (0.070 g, 1.85 mmol), and tetrahydrofuran (7 mL) dropwise over 10 min. The ice/water bath was removed and the reaction mixture stirred for 1 h at room temperature. After recooling in an ice/water bath, 40% aqueous sodium hydroxide solution was added dropwise until the reaction mixture was basic. The organic layer was successively washed with 10-mL portions of water and brine and was dried over sodium sulfate. Filtration and solvent removal *in vacuo* gave a white solid (0.0525 g). Chromatography on silica gel using 50:50 diethyl ether/chloroform as an eluant gave, after solvent removal, a white solid (0.0482 g, 91%). ^{1}H NMR ($CDCl_3$): δ 7.45 (d, J = 8.9 Hz, 1.96 H), 6.62 (d, J = 8.9 Hz, 1.97 H), 3.02 (s, 3.04 H).

Preparation of 4-Cyano-*N,N*-bis(dideuteriomethyl)aniline.[34] A 25-mL round-bottomed flask was charged with paraformaldehyde-d_2 (0.1063 g, 3.31 mmol) and aqueous sodium hydroxide (40%, 2 mL). After stirring for 10 min, the reaction mixture was cooled with an ice/water bath, and sulfuric acid (3 M, 7 mL) was added. Then a slurry of 4-aminobenzonitrile (0.1306 g, 1.1 mmol), sodium borohydride (0.334 g, 8.8 mmol), and tetrahydrofuran (10 mL) was added dropwise over 10 min. The reaction mixture was allowed to warm to room temperature and was stirred for 1 h. After recooling in an ice/water bath, a 40% aqueous sodium hydroxide solution was added dropwise until the reaction mixture was basic. The organic layer was separated and successively washed with 10-mL portions of water and brine and was dried over anhydrous sodium sulfate. Filtration and solvent removal *in vacuo* gave a white solid (0.148 g). Chromatography on silica gel using 50:50 diethyl ether/hexane as an eluant gave, after solvent removal, a white solid (0.106 g, 65%). ^{1}H NMR ($CDCl_3$): δ 7.45 (d, J = 8.89 Hz, 1.94 H), 6.61 (d, J = 8.87 Hz, 2.07 H), 2.99 (m, 1.98 H).

Preparation of 4-Nitro-*N,N*-bis(dideuteriomethyl)aniline. This material was prepared in 85% yield using the same procedure described above except that 4-nitroaniline was used and the product was chromatographed on silica gel using 50:50 diethyl ether/chloroform as an eluant. ^{1}H NMR ($CDCl_3$): δ 8.11 (d, J = 9.36 Hz, 1.97 H), 6.58 (d, J = 9.33 Hz, 2.03 H), 3.07 (m, 2.00 H).

Preparation of *N,N*-Bis(trideuteriomethyl)aniline. This material was prepared in 85% yield using the same procedure described above except that sodium borodeuteride (98.3 atom % D) and aniline were used and the product was chromatographed on silica gel using 90:10 *n*-pentane/diethyl ether as an eluant. The product was then distilled under vacuum (45 °C, 1.1 mmHg) to give a clear liquid. ^{1}H NMR (CD_3CN): δ 7,21 (t, J = 8.0 Hz, 2.1 H), 6.65–6.76 (m, 2.9 H).

Preparation of the Dimedone Adduct of Formaldehyde-d_2 and -d_1.[35] A 200-mL round-bottomed flask was charged with paraformaldehyde-d_2 (99.3 atom % D, 0.0403 g, 1.26 mmol), 5,5-dimethyl-1,3-cyclohexanedione (0.884 g, 6.3 mmol), and aqueous sodium hydroxide (0.2 M, 40 mL). After stirring for 1 h, the reaction mixture was cooled in an ice/water bath, and hydrochloric acid (4 M, 5 mL) was added, which formed a white precipitate. The acidic reaction mixture was allowed to sit in an ice/water bath for 30 min, after which the precipitate was collected by filtration, washed with ice-cold water (2 × 10 mL) and ice-cold 50:50 ethanol/water (4 × 20 mL), and dried *in vacuo*. The material was recrystallized from hot 70:30 methanol/water (100 mL) to give white needles (0.281 g, 81%). Mp: 191–192 °C. ^{1}H NMR ($CDCl_3$): δ 11.56 (s, 2.0 H), 2.27 (s, 7.97 H), 1.04 (s, 12.03 H). The formaldehyde-d_1 adduct was similarly prepared in 82% yield except that paraformaldehyde-d_1 (99.4 atom % D) was used. Mp: 191–192 °C. ^{1}H NMR ($CDCl_3$): δ 11.56 (s, 2.0 H), 3,13 (s, 0.95 H), 2.27 (s, 8.05 H), 1.04 (s, 12.0 H).

Isotopic Composition of the Substituted *N*-(Trideuteriomethyl)anilines, 1a–d, and 2a–d. GC–MS data on the amines were collected in the selected ion monitoring mode and were analyzed at the following ionization voltages: *p*-H, 7.0 eV.; *p*-Cl, 7.0 eV.; *p*-CN, 7.5 eV.; *p*-NO_2, 7.5 eV. At these ionization voltages, the M – 1 contributions were less than 0.18% of the molecular ion, M^+. The isotopic composition percentages (standard deviation) summarized below are an average of at least three determinations.

isotopic compositions of 4-substituted *N*-(trideuteriomethyl)anilines

X	% d_3	% d_2
H	99.83(1)	0.17(1)
Cl	98.42(6)	1.58(2)
CN	99.72(1)	0.28(1)
NO_2	99.75(1)	0.25(1)

isotopic compositions of 4-substituted *N*-methyl-*N*-(trideuteriomethyl)anilines **1a–d**

X	% d_3	% d_2	% d_1
H	99.42(1)	0.45(1)	0.12(1)
Cl	99.49(6)	0.44(6)	0.06(1)
CN	99.52(4)	0.48(4)	
NO_2	98.9(1)	0.88(1)	0.06(1)

isotopic compositions of 4-substituted *N,N*-bis(dideuteriomethyl)anilines **2a–d**

X	% d_4	% d_3	% d_2
H	97.33(2)	2.39(1)	0.26(1)
Cl	97.36(8)	2.47(8)	0.15(1)
CN	99.21(4)	0.76(5)	0.02(1)
NO_2	99.25(2)	0.71(3)	0.03(1)

Microsomal Preparation.[12] Male Sprague-Dawley rats (200–250 g) were administered phenobarbital every 24 h (80 mg/kg body weight) and sacrificed on the fourth day. After decapitation, the livers were removed, weighed, and placed in an ice-cold isotonic phosphate buffer (pH 7.4). The livers were chopped and homogenized using Potter–Elvehjen tissue homogenizer, and the homogenate was centrifuged (9000*g*, 20 min, 10 °C). The supernatant was centrifuged again (100000*g*, 1 h, 5 °C). The supernatant was decanted and the microsomal pellet transferred to vials with a minimum amount of a 0.25 M sucrose solution and stored at –80 °C.

Enzymatic Incubations.[36] The microsomes were removed from the –80 °C freezer, thawed, homogenized in a Potter–Elvehjen tissue homogenizer using cold phosphate buffer (pH 7.4), and centrifuged (100000*g*, 1 h, 5 °C). A suitably diluted microsomal preparation was reduced by adding several milligrams of solid sodium dithionite and was then saturated with carbon monoxide by bubbling the gas into the solution for ca. 1 min. The microsomal P-450 content was determined by the method of Omura and Sato[37] from the difference in visible absorption spectra between 450 and 490 nm.

(34) Giumanini, A. G.; Verardo, G.; Gei, M. H.; Lassiani, L. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 255.

(35) Horning, E. C.; Horning, M. G. *J. Org. Chem.* **1946**, *11*, 95.

(36) Jones, J. P.; Korzekwa, K. R.; Rettie, A. E.; Trager, W. F. *J. Am. Chem. Soc.* **1986**, *108*, 7074.

(37) Omura, T.; Sato, K. *J. Biol. Chem.* **1964**, *239*, 2370.

Scintillation vials were charged with microsomal P-450 (12–15 nmol), NADPH (10 μmol), or a NADPH-regenerating system made up of NADP (10 μmol), D,L-lactic acid (sodium salt, 0.15 mmol), 250 units of lactate dehydrogenase,[38] and Tris buffer (0.1 M, pH 8.2) for a total volume of 2 mL. The mixture was preincubated under an air atmosphere at 37 °C for 5 min in a Dubnoff metabolic shaking incubator. After preincubation, substrates were added (10 μmol in 100 μL of methanol or acetone) and placed back in the incubator for 30 min. The incubations of **1a–d** were terminated by adding 5 mL of pentane and were stored at –80 °C before derivatization. The incubations of **2a–d** were terminated by adding a 40% sodium hydroxide solution (100 μL) and were immediately derivatized by adding 1 mL of a 0.12 M dimedone solution in 0.2 M sodium hydroxide. The mixture was shaken and allowed to sit at room temperature for 2 h. Then hydrochloric acid (4 M) was added dropwise until the mixture became acidic.

The incubation mixtures were extracted with diethyl ether (3 × 10 mL) and dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo*. The extracts from the oxidations of **2a–d** were taken up in 100 μL of dry diethyl ether and stored at –20 °C in septum-capped vials until analysis. The extracts from the oxidations of **1a–d** were taken up in 250 μL of dry diethyl ether and derivatized with trifluoroacetic anhydride (60 μL, 0.42 mmol). This mixture was allowed to sit for 2 h, after which the solvent and excess trifluoroacetic anhydride were removed *in vacuo*. Then 5 mL of diethyl ether and ca. 0.15 g of poly(4-vinylpyridine) were added, and the mixture was allowed to sit for 12 h. After filtration, the solvent was removed *in vacuo*. The extracts were taken up in 100 μL of diethyl ether and stored at –20 °C until analysis.

Incubations of **1a–d** were separately analyzed by GC before derivatization to see if any secondary oxidation products could be detected, i.e. substituted anilines. In all cases, the substituted anilines detected were less than 1% of the substituted *N*-methylanilines.

GC–MS Analysis of Trifluoroacetylated Products from P-450 Oxidation of 1a–d. The trifluoroacetylated products were analyzed by GC–MS at 70 eV using the selected ion monitoring mode by a method similar to that used by Abdel-Monem[39] and Miwa.[5] A series of known mixtures, corrected for the deuterium contents in the starting dimethylanilines, were analyzed by the same method, and a standard curve was generated. The results (standard deviation) tabulated below are an average of at least three determinations. The ratios from the P-450 oxidations are given in Table I.

standard mixtures of trifluoroacetylated *N*-methylaniline-d_3 and -d_0

d_3/d_0, std	abund 206/abund 203, measd
0.50	0.55(2)
1.00	1.06(1)
2.02	2.04(1)
3.03	3.02(2)

standard mixtures of trifluoroacetylated 4-chloro-*N*-methylaniline-d_3 and -d_0

d_3/d_0, std	abund 240/abund 237, measd
0.52	0.58(2)
1.03	1.09(3)
2.06	2.15(5)
3.09	3.22(8)

standard mixtures of trifluoroacetylated 4-cyano-*N*-methylaniline-d_3 and -d_0

d_3/d_0, std	abund 231/abund 228, measd
0.57	0.59(1)
1.14	1.21(1)
2.29	2.38(5)
3.43	3.58(3)

standard mixtures of trifluoroacetylated 4-nitro-*N*-methylaniline-d_3 and -d_0

d_3/d_0, std	abund 251/abund 248, measd
0.60	0.64(1)
1.20	1.23(1)
2.41	2.43(2)
3.61	3.59(1)

GC–MS Analysis of Dimedone Adducts from P-450 Oxidation of 2a–d. The dimedone adducts were analyzed by GC–MS at 70 eV using the selected ion monitoring mode. At this ionization voltage the M – 1 ion for the dimedone adduct of formaldehyde-d_0 was 0.16% of the molecular ion, M^+. The abundances at m/z of 292, 293, 294, and 295 were analyzed for the mole fractions of the d_0, d_1, and d_2 dimedone adducts by the matrix method described by Brauman.[40] GC–MS analysis of oxidation mixtures that were not derivatized with dimedone gave no ions with m/z = 290–295. The isotope effects in Table I are equal to 2 times the d_2/d_1 dimedone adduct ratios from the P-450 oxidations.

A series of known d_2/d_1 dimedone adduct mixtures, corrected for the deuterium contents in the starting formaldehydes, were analyzed by the same method as above, and a standard curve was generated. The results (standard deviation) tabulated below are an average of at least three determinations.

d_2/d_1, std	d_2/d_1, obsd
0.50	0.49(1)
1.00	0.99(1)
1.49	1.47(2)
1.98	1.96(4)

Tests for Isotope Effects on the Derivatization of Formaldehyde with Dimedone. A 0.81:1 mixture of aqueous formaldehyde-d_2 and -d_0 was treated with dimedone using the same procedure used to derivatize the P-450 incubated samples. The concentration of formaldehyde used was in the same range as would be expected in the incubation mixture (ca. 0.1 mM). The ratio of d_2/d_0 dimedone adducts measured by GC–MS was 0.78(3).

A 2.4:1 mixture of formaldehyde-d_2 and -d_1 was added to a incubated set of microsomal P-450 oxidations of *N*,*N*-dimethylaniline-d_0. The incubation was terminated and derivatized in the same manner as for the deuteriated compounds described above. The d_2/d_1 ratio measured by GC–MS of the dimedone adducts was 2.5(2).

Nanosecond Laser Kinetics. The transient absorption apparatus used has been described previously.[41] Quartz cuvettes maintained at 22 °C and containing 3.0 mL of argon-purged solutions of diphenylmethanol (0.1 or 0.2 M) in 1:2 benzene/(t-BuO)$_2$ or 1:2 acetonitrile/(t-BuO)$_2$ were excited at 340 nm (ca. 15 ns, 1–3 mJ). Aliquots of argon-purged solutions of $PhN(CH_3)_2$ or $PhN(CD_3)_2$ were added by syringe, and the appearance of the diphenylketyl radical was monitored at 540 nm in a manner similar to that described by Scaiano.[10] For the range of laser energies used, there was no power dependence to the rate constants for appearance of the signal at 540 nm. Second-order rate constants for reaction with $PhN(CH_3)_2$ and $PhN(CD_3)_2$ were obtained from the slopes of plots of the first-order rate constants for the appearance of the diphenyl ketyl radical vs amine concentration (0 to ca. 0.1 M). The second-order rate constants are an average of at least three independent measurements. The measured ratio of second-order rate constants for the reactions of $PhN(CH_3)_2$ and $PhN(CD_3)_2$, $(k_H/k_D)_{obs}$, were corrected for incomplete deuterium incorporation in the $PhN(CD_3)_2$ using the equation below, which assumes a dominant, primary mechanistic isotope effect. The isotopic composition of $PhN(CD_3)_2$ was determined by GC–MS as described above and was found to be 89.3(2)% d_6, 10.3(2)% d_5, and 0.4(1)% d_4.

$$(k_H/k_D)_{corr} = \frac{(k_H/k_D)_{obs}[6(\%d_6) + 5(\%d_5) + 4(\%d_4)]/100}{6 - (k_H/k_D)_{obs}[\%d_5 + 2(\%d_4)]/100}$$

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(38) Fawcett, C. P.; Kaplan, N. O. *J. Biol. Chem.* **1962**, *237*, 1709.
(39) Abdel-Monem, M. M. *J. Med. Chem.* **1975**, *18*, 427.
(40) Brauman, J. I. *Anal. Chem.* **1966**, *38*, 607.
(41) Gould, I. R.; Ege, D.; Moser, J. E.; Farid, S. *J. Am. Chem. Soc.* **1990**, *112*, 4290.