Oxidative N-Dealkylation of *p*-Cyclopropyl-*N,N*-dimethylaniline. A Substituent Effect on a Radical-Clock Reaction Rationalized by Ab Initio Calculations on Radical Cation Intermediates

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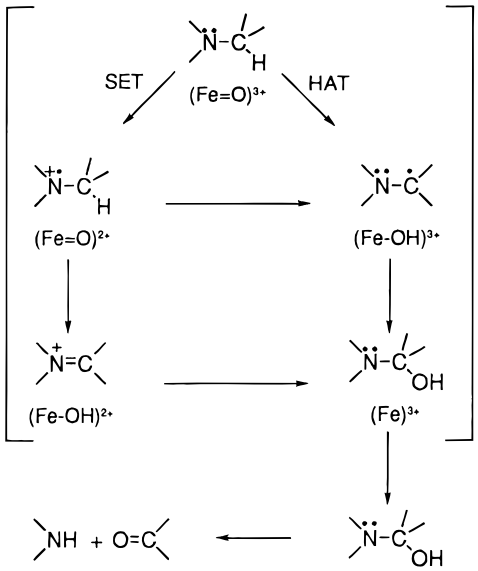
Introduction

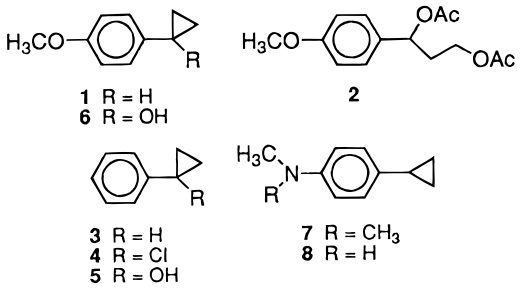
Cytochrome P450 (P450) catalyzed oxidative N-dealkylation is a major biotransformation pathway for many nitrogen-containing xenobiotics, yet after more than 40 years of study important uncertainties over the mechanism(s) of this reaction persist.[1] One view holds that N-dealkylation involves a classical hydrogen atom transfer/hydroxyl recombination sequence analogous to aliphatic hydroxylation and O-dealkylation (hydrogen atom transfer, HAT in Scheme 1). This is supported by observations that in some cases carbinolamines are isolable intermediates[2−5] and that their oxygen atom originates from molecular oxygen.[5] However, in contrast to the large deuterium isotope effects (DIEs (ca. 5−10)) observed for aliphatic hydroxylation[6−8] and O-dealkylation,[9,10] the uniformly low (≤2) DIEs for P450-catalyzed N-dealkylation[11,12] seem inconsistent with this view.

The idea that cytochrome P450 might initiate N-dealkylation by abstracting an *electron* from the amine (single electron transfer, SET in Scheme 1) was first suggested by studies with cyclopropylamines[13−16] and 4-alkyl-1,4-dihydropyridines[17] as suicide substrates for P450 enzymes. Later, SET mechanisms were suggested to account for the low DIEs observed for N-dealkylation of easily oxidizable aniline derivatives[18] and the aromatization of 1,4-dihydropyridine derivatives,[19] while the high (ca. 3.5−6.5) intramolecular DIEs observed for N-dealkylation of amides was explained by postulating a change of mechanism from SET with low-$E_{1/2}$ amines to HAT for high-$E_{1/2}$ amides.[2,20,21] A mechanism in which SET is the initial step in the oxidation of compounds with low-$E_{1/2}$ values, but not for compounds with $E_{1/2}$ > ca. 1.5 V, is consistent with experimental results for P450 oxidation of amines, sulfur compounds, and strained alkanes.[16,22,23] However, interpretation of isotope effects on P450-mediated N-dealkylation remains controversial[1,24−26] and DIEs alone do not distinguish unambiguously between HAT and SET mechanisms.

Scheme 1. Mechanisms of Cytochrome P450 Catalyzed *N*-Dealkylation: Single Electron Transfer (SET) and Hydrogen Atom Transfer (HAT)



Cyclopropyl groups[27] have shown promise as probes for differentiating SET from HAT mechanisms in P450-catalyzed[22,28] and other reactions.[29,30] For example, SET oxidation of **1** ($E_{1/2}$ = 1.40 V vs Ag/AgCl) with $Mn(OAc)_3$



produces exclusively the ring-opened compound **2** in high yield (Scheme 2).[30] Similarly, the cation radicals of **1**, **3** and several other *p*-substituted cyclopropylbenzenes,

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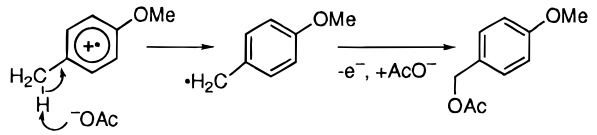
Scheme 2



generated photochemically[31,32] or anodically,[33] undergo cyclopropane ring opening essentially exclusively to yield propane derivatives. This process has been studied in detail and shown to occur via nucleophilic attack with inversion of configuration at the reacting cyclopropane carbon.[31,32] On the other hand **3** ($E_{1/2}$ = 1.70 V vs Ag/AgCl), which is not oxidized by $Mn(OAc)_3$, easily undergoes free-radical (i.e., HAT) chlorination to yield substantial amounts (up to 41%) of **4**, in which the cyclopropane ring remains intact. Although other chlorinated products are also formed in this reaction (e.g. Ar−Cl isomers and 1-phenyl-1,3-dichloropropane), none of these is derived from the 1-phenylcyclopropyl radical, the key intermediate in the benzylic chlorination of **3**, which remains intact long enough to capture a chlorine atom and generate **4**.[30]

Thus, if an arylcyclopropyl radical derived by HAT from **1** or **3** were formed as an intermediate in a P450-catalyzed hydroxylation process, it might reasonably be expected to survive long enough to capture a hydroxyl group and yield a cyclopropanol product. On the other hand, if enzymatic oxidation of **1** and **3** involved an SET process, one might expect predominantly if not exclusively ring-opened products. A possible caveat in the case of P450 oxidation is that if there were no water or hydroxide present in the active site cavity to participate in a nucleophilic ring-opening process, an SET mechanism might still lead to a cyclopropanol with the latter being mistaken to indicate a HAT mechanism (viz. Scheme 1). On the other hand, the fact that cyclopropylamines covalently inactivate P450 enzymes is not inconsistent with the alkylation of a *protein* nucleophile by a cation−radical intermediate. Additionally, given the large size of some P450 substrates (e.g. steroids) compared to **1**, **3**, or **7**, entry of one of the latter into the P450 active site might be insufficient to displace all water. Indeed, isotope effect experiments indicate that substrates as large as *p*-xylene tumble more rapidly within the P450 active site than they exchange with free *p*-xylene in solution.[7] Horseradish peroxidase is a heme protein thought to oxidize most substrates by SET processes.[34] HRP substrates are excluded by the apoprotein from access to the heme ferryl group, so their oxidation tends to occur at the solution−hemoprotein interface, leaving cation−radical products accessible to nucleophiles and thus obviating this caveat.

Unfortunately, we find that neither **1** nor **3** is oxidized by horseradish peroxidase, probably because their oxidation potentials are too high.[35] On the other hand, with cytochrome P450, **3** undergoes mainly benzylic hydroxylation to ring-intact **5**, along with some *p*-cyclopropylphenol, while **1** undergoes benzylic hydroxylation to ring-intact **6**, along with extensive O-demethylation.[28] The formation of these products is in agreement with ab initio calculations[36] of the relative energies and spin distributions of putative radical intermediates derived enzymatically from **1** via HAT processes. However, their high oxidation potential may also have contributed to this outcome by precluding SET oxidation even by P450, whose oxidation potential is much higher than that of HRP.[37] To circumvent this potential limitation on the utility of arylcyclopropanes as probes of enzymatic reaction mechanisms, we turned to *p*-cyclopropyl-*N*,*N*-dimethylaniline (**7**), which we anticipated would have a much lower oxidation potential than **1** or **3** and thus be susceptible to oxidation by HRP as well as P450. Indeed **7** is much more easily oxidized than **1** or **3**, but the outcome of its oxidation was quite unexpected.

Experimental and Computational Methods

Synthesis. Compound **7** was prepared by reductive methylation[38] of 4-cyclopropylaniline.[39] Kugelrohr distillation afforded a colorless oil which was characterized for identity and purity (>98%) by NMR and GC/MS: ^{1}H NMR δ 0.62 (m, 2H), 0.89 (m, 2H), 1.83 (m, 1H), 2.89 (s, 6H), 6.64 (m, 2H), 6.96 (m, 2H); ^{13}C NMR δ 8.74, 15.01, 41.54, 113.72, 127.16, 132.52, 149.47; EI-MS (electron impact mass spectrometry) *m*/*z* (%) 161 (M^+, 88), 160 (100), 146 (17), 131 (28), 117 (38).

Oxidation by Classical SET Oxidants. In a modification of literature procedures[40,41] $Mn(OAc)_3 \cdot 2H_2O$ (0.42 mmol) was weighed into a 16 × 100 mm screwcap culture tube. A small stirbar and 2.0 mL of solvent (MeCN/HOAc/Ac_2O, 90:5:5 v/v) was added, followed by **7** (0.20 mmol, added neat from a microliter syringe). The mixture was degassed for 2 min with a stream of nitrogen and the tube was capped and heated at 70 °C for 3 h with stirring. After cooling, K_2HPO_4 solution (2 mL, 3.0 M) was added and the mixture extracted with EtOAc (4 × 2 mL). The extract was dried and the residue reconstituted in EtOAc containing an internal standard (2,6-di-*tert*-butyl-4-methylphenol) for analysis by GC/MS. A similar procedure was used for the $Ce(HSO_4)_4$ reaction except that the reaction solvent was HOAc/Ac_2O (90:10, v/v), NaOAc (5 mol/mol Ce^{4+}) was added as a buffer, the workup used concentrated NH_4OH (2.5 mL) instead of K_2HPO_4 solution, and products were extracted with ether. In no case was unreacted substrate detected; the only significant product (yield ⩾ 70% in most cases) was the corresponding *N*-methyl-4-cyclopropylacetanilide.

Oxidation by Horseradish Peroxidase. Horseradish peroxidase (HRP) was used as received from Sigma (A_{403}/A_{275} = 0.5). Incubations were conducted in 12 × 50 mm screwcap vials and contained, in order of addition, 895 ± 25 μL of buffer (0.4 M potassium phosphate, pH 5.5), 10 μL of HRP stock solution (ca. 80 μg/mL buffer), and 95 ± 25 μL of **7** (10.4 mM in MeCN). Reactions (total volume 1.0 mL) were initiated by addition of H_2O_2 (5 μL, 97.9 mM), incubated for 20 min at room temperature, and stopped by adding 60% trichloroacetic acid (450 μL) and an aliquot of *N*-ethylaniline as an internal standard (10 μL, 10 mM in MeCN). Aliquots of stopped reactions were analyzed directly on reverse phase HPLC (10 μm, C_{18}, 4.6 × 250 mm;

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elution with 50 mM NH_4OAc/MeCN, 55:45 v/v) with UV detection (254 nm) and electronic peak integration. Typical conversions were 5−15% over 20 min.

Oxidation by Induced Microsomes and Cytochrome P450 2B1. Liver microsomes from phenobarbital-induced rats were prepared as described.[42,43] Typical incubations (1.5 mL) contained 1.5−4.0 mg of microsomal protein, 1 μmol of NADPH, and 2−6 μmol of **7** (added in 20 μL MeCN). For the reconstituted system purified cytochrome P450 2B1 and NADPH cytochrome P450 reductase (0.1 nmol of each) were incubated for 3 min at 37 °C with sonicated dilauroylphosphatidylcholine (30 μg) in 0.1 M phosphate buffer (pH 7.4, 900 μL). Reactions were initiated by adding substrate (0.1−1 μmol in 10 μL MeCN) and NADPH (1 μmol in 90 μL buffer). After shaking at 37 °C for 20 min, reactions were terminated by adding 15% $ZnSO_4$ (200 μL) and internal standard (0.3 μmol *N*-ethylaniline). After centrifugation, aliquots of the supernatant were analyzed by HPLC as described for the HRP incubations.

Ab Initio Calculations. The initial conformations of the compounds were generated using the modeling package ChemX.[44] The quantum chemical package GAMESS−UK[45,46] (implemented on IBM RS6000 workstations and a CRAY−YMP) was used for the ab initio calculations. All radical cations were optimized at the UHF (unrestricted Hartree−Fock) level with the STO-3G (Slater type orbitals comprised of three Gaussians)[47] minimal basis set. On the resulting geometry a single point (open shell) RHF (restricted Hartree−Fock) energy and DMA (distributed multipole analysis) calculation[48] in a SV (split valence) 6-31G[49,50] basis set was performed to derive unpaired spin distributions and charge distributions per atom.

Results and Discussion

In parallel with the amide vs amine comparison discussed above, the high $E_{1/2}$ values of **1** and **3** may have precluded their SET oxidation by P450, leaving the HAT mechanism as the most viable alternative. Aniline derivative **7** was therefore synthesized and, as expected, its $E_{1/2}$ value (0.75 V) was found to be much lower than those of **1** or **3**. Subsequently **7** was submitted to oxidation by the classical SET oxidants $Mn(OAc)_3$ and $Ce(HSO_4)_4$, by horseradish peroxidase, and by liver microsomes from phenobarbital-induced rats as well as purified reconstituted cytochrome P450 2B1. Consistent with its low $E_{1/2}$, **7** was an excellent substrate in all oxidation systems, and in all cases the only reaction detected was N-demethylation to **8** (or its *N*-acetylated derivative in the case of the inorganic systems); no ring-opened products could be detected, although at longer reaction times in the HRP system, further N-demethylation of **8** to *p*-cyclopropylaniline occurred to a small extent.

The completely opposite behavior of **7** compared to **1** under SET conditions, i.e., heteroatom dealkylation compared to cyclopropyl ring opening, is striking. Understanding the origins of this difference is crucial to the informed use of these compounds as probes for reaction mechanism. In an attempt to rationalize this difference, we postulated that these differences in chemical behavior arose from differences in the distribution of spin and/or charge within the respective radical cations. To test this hypothesis, ab initio calculations were performed on the radical cations of **1** and **7**, as well as on their *p*-methyl- and para-unsubstituted analogues. As noted previously for radical cations of diaminobenzene derivatives,[51] conversion of all three anilines to their radical cations is accompanied by a change in hybridization of the nitrogen from sp^3 to sp^2 and a shortening of the $N-C_{aryl}$ bond, indicating a strong interaction of its half-filled p-orbital with the aromatic π-system. Significantly, the cyclopropane substituents in the radical cations of both **1** and **7** are in the bisected conformation, in which the electronic interaction of the cyclopropane ring with the π-system is maximized.[32,52] The distributions of unpaired spin and charge in the radical cations are summarized in Table 1.

Table 1. Calculated Spin and Charge Distributions in Radical Cations of Para-Substituted Anisoles and Para-Substituted *N,N*-Dimethylanilines Summarized over the Substituents and the Aromatic Ring[a]

	anisoles			*N,N*-dimethylanilines		
	R	C_6H_4	OCH_3	R	C_6H_4	$N(CH_3)_2$
R = cyclopropyl						
spin	0.064	0.840	0.097	0.015	0.354	0.631
charge	0.193	0.914	−0.108	0.135	0.398	0.468
R = methyl						
spin	0.019	0.874	0.105	0.005	0.311	0.686
charge	0.183	0.915	−0.099	0.146	0.339	0.514
R = hydrogen						
spin	0.000	0.886	0.113	0.000	0.262	0.740
charge	0.141	0.946	−0.086	0.120	0.317	0.562

[a] Net spins and charges were calculated over every atom of each radical cation and summed over the molecular fragments indicated. Original atom-by-atom data are given in Tables S1-S6 (Supporting Information), along with the conformations of the radical cations in Figures S1-S6 (Supporting Information).

The calculations suggest that in the anisole radical cations both the charge and the unpaired spin are centered primarily on the aromatic ring, a result consistent with published ESR spectra of the anisole radical cation[53] and in agreement with recent calculations on the cation radical of **3**.[32] As a consequence of this charge and spin distribution, *ring* substituents are activated. Thus, the radical cation of *p*-methylanisole undergoes deprotonation, giving a benzylic radical which is oxidized further to *p*-methoxybenzyl acetate (Scheme 2),[40,41] while the radical cation of **1** undergoes nucleophilic attack with cyclopropane ring opening giving benzylic radical which is further oxidized to **2** (Scheme 3, path a).[30] In contrast, calculations indicate that the majority of the charge and unpaired spin in the radical cations of the aniline derivatives is centered on the nitrogen atom rather than the aromatic ring. This conclusion is supported by ESR studies of the anodic oxidation[54] and the horseradish peroxidase oxidation[55] of aniline derivatives. Hence in

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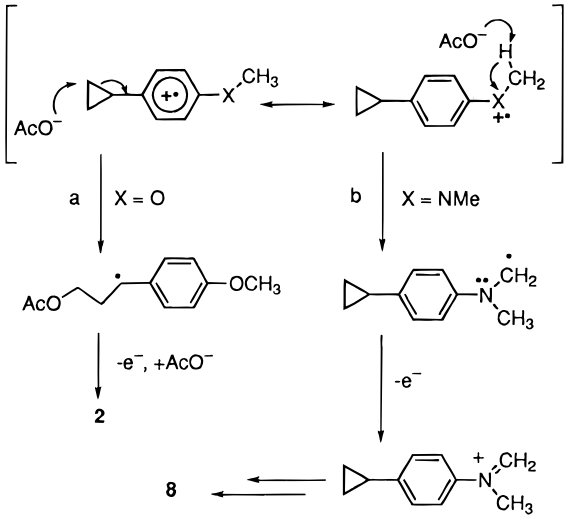
Scheme 3



the anilines the *nitrogen* substituents rather than the ring substituents are most activated, and *N*-dealkylation becomes the favored pathway.

Given the results of these calculations, the failure of **7** to yield ring-opened oxidation products analogous to **2** should not be taken as evidence against a radical cation as an obligatory intermediate in their oxidation. Rather, the invariant occurrence of N-dealkylation as the only oxidation route for this low-$E_{1/2}$ aniline derivative suggests that P450, like the inorganic oxidants and probably HRP, may well oxidize this and other *N*,*N*-dimethylanilines via an SET process. Further oxidation studies with *N*-cyclopropylalkylamines and *N*-cyclopropylarylamines, as well as chemical models for HAT processes, are underway in our laboratory.

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Supporting Information Available: A listing of the partial charges and spin distributions and conformations calculated for each atom of the radical cations of anisole, *N*,*N*-dimethylaniline, and their *p*-methyl and *p*-cyclopropyl derivatives (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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