The first calibration of an aminiumyl radical ion clock: why *N*-cyclopropylanilines may be poor mechanistic probes for single electron transfer†

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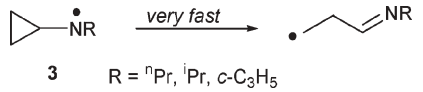
Using direct and indirect electrochemical methods, the rate constant for ring opening of the radical cation generated from *N*-cyclopropyl-*N*-methylaniline was found to be 4.1 × 10^4 s^{-1}.

N-Cyclopropylamines have been used in attempts to detect single electron transfer (SET) mechanisms for amine oxidations in both chemical and biochemical systems. The supposition is that if electron transfer occurs, the resulting aminiumyl radical ions (**1**$^{\bullet+}$) will undergo rapid ring opening (Scheme 1, path a) to generate distonic radical cations **2**$^{\bullet+}$, in direct analogy to the cyclopropylcarbinyl → homoallyl neutral free radical rearrangement.[1] The isolation or detection of cyclopropane ring-opened products, or a loss of catalytic activity for an enzyme-catalyzed process, provides evidence that a radical cation was formed, and thus an SET event had occurred. The most compelling evidence for radical intermediates in oxidations catalyzed by cyctochrome P450s,[2] horseradish peroxidase,[3] and monoamine oxidase A and B[4] is derived from the behavior of *N*-cyclopropylamines (**1**).

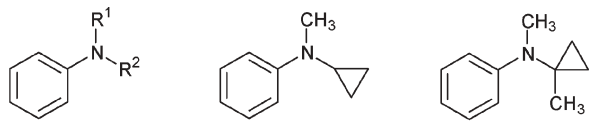
Despite their widespread use, there remain several unresolved issues with *N*-cyclopropyl compounds as probes for SET in amine oxidations, the most important of which is that no rate constants for ring opening of the corresponding radical cations (k_o) have ever been directly measured. It is often assumed that the rate constant for ring opening of these radical cations will be of comparable magnitude to that of the neutral radical (*e.g.*, **3**), whose rate constant for ring opening is estimated to be $>10^7$ s^{-1} (eqn (1)).[5]



3 R = ^{n}Pr, ^{i}Pr, *c*-C_3H_5 (1)

Indirect evidence and MO calculations imply that *N*-cyclopropyl radical cations generated from *aliphatic* amines undergo rapid ring opening.[6–9] Less is known about the behavior of aromatic aminiumyl radical ions such as those derived from *N*-cyclopropyl-*N*-methylaniline, and derivatives, which have been used as SET probes in both enzymatic[10–13] and chemical systems.[14,15] In this paper we report results pertaining to the mechanism and kinetics of radical cations generated from *N*-cyclopropylanilines which directly address these issues.

Direct photoionization (266 nm, 5% CH_3OH in CH_3CN) of *N*,*N*-dimethylaniline (**4**), *N*-ethyl-*N*-isopropylaniline (**5**), *N*-methyl-*N*-cyclopropylaniline (**6**) and *N*-methyl-*N*-(1-methylcyclopropyl)aniline (**7**) generated transients with absorption maxima in the region 460–490 nm, consistent with the reported spectroscopic features of **4**$^{\bullet+}$.[16] The lifetimes of each of these radical cations were nearly identical (*ca.* 0.2–0.7 μs) and their decay is attributable to their consumption in bimolecular reactions with other paramagnetic species produced by photoionization. The introduction of an *N*-cyclopropyl group (*e.g.*, **6**$^{\bullet+}$, **7**$^{\bullet+}$) did not affect the lifetime, suggesting the rate of cyclopropane ring opening is not competitive with these other processes, and that, k_o must be $\leqslant 10^6$ s^{-1} (*i.e.*, ring opening occurs slowly on the LFP time scale).

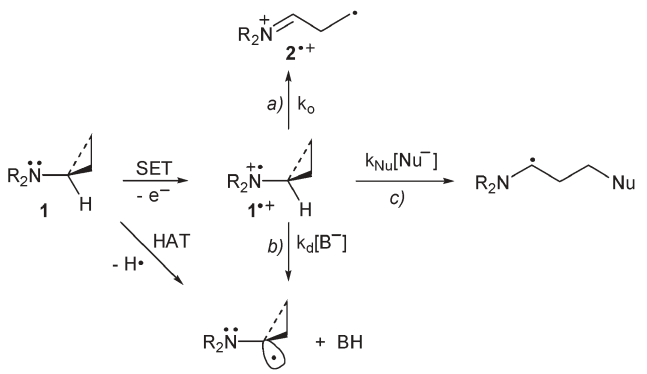


4 (R^1 = R^2 = Me)
5 (R^1 = Et, R^2 = ^{i}Pr)

6

7

Next, the oxidation of **4** and **6** was studied by cyclic and linear sweep voltammetry (Pt electrode, CH_3CN solvent). In these experiments, the rate law for radical cation decay can be determined by the characteristics of the voltammogram.[17] Consistent with previous electrochemical studies,[18,19] the radical cation derived from *N*,*N*-dimethylaniline (**4**$^{\bullet+}$) undergoes bimolecular decay.[19] The product of the electrochemical oxidation is the expected dimeric product *N*,*N*,*N*′,*N*′-tetramethylbenzidine (**8**).[18] In contrast, the radical cation generated from *N*-methyl-*N*-cyclopropylaniline (**6**$^{\bullet+}$) undergoes clean, first-order decay, consistent with a unimolecular rearrangement attributable to



Scheme 1 Possible SET and HAT pathways for the reaction of an *N*-cyclopropylamine.

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cyclopropane ring opening (Scheme 1, path a). The fact that the decay is zero order in methanol effectively rules out the nucleophile-assisted pathway for ring opening, analogous to that documented for cyclopropylarene radical cations[20,21] (Scheme 1, path c), at least for methanol as a nucleophile.

Bulk electrolysis of **6** proved problematic. Reasonable mass balances could only be obtained when the product mixture was quenched with $NaBH_4$. For example, electrolysis of **6** (2 equiv. electrons) resulted, after $NaBH_4$ treatment, in 21% *N*-methylaniline, 10% *N*-methyl-1,2,3,4-tetrahydroquinoline (**9**), and 70% unreacted starting material. Prolonged electrolysis produced these same products in approximately the same ratio, but with lower total mass balance. Formation of **9** is consistent with ring opening of $\mathbf{6}^{\bullet+}$ to the distonic radical cation followed by radical cyclization as was observed by Hanzlik *et al.* in horseradish peroxidase incubation mixtures of **6**.[12,22] No dimeric products of any type were detected, consistent with the results described above which revealed overall first order decay of $\mathbf{6}^{\bullet+}$.

Because of these low mass balances, products arising from the oxidation of **4** and **6** were studied further by electrochemical-electrospray ionization/mass spectrometry (EC-ESI/MS).[2] This technique involves pumping a solution containing the electroactive species through an electrochemical flow cell that is coupled directly to an electrospray ionization mass spectrometer. The mass voltammogram (plot of ion intensity *vs.* electrochemical cell potential) is presented in Fig. 1. The observed ion intensities of the product(s) relative to the starting material suggest good mass balance under these conditions for the oxidation of **4** and **6**.

Structural assignments for products arising from **4** and **6** are discussed in detail in the ESI.† To summarize: only dimeric products were observed for the EC-ESI/MS oxidation of **4**, consistent with the dimerization as the only available pathway for decay of $\mathbf{4}^{\bullet+}$. In contrast, only cyclopropane ring-opened products were observed for **6**; product assignments appear in Fig. 1. No products arising from radical cation deprotonation were detected in these products studies. This is not surprising since there is no base present in sufficiently high concentration for this pathway to become competitive with dimerization of **4**, or ring opening of **6**.

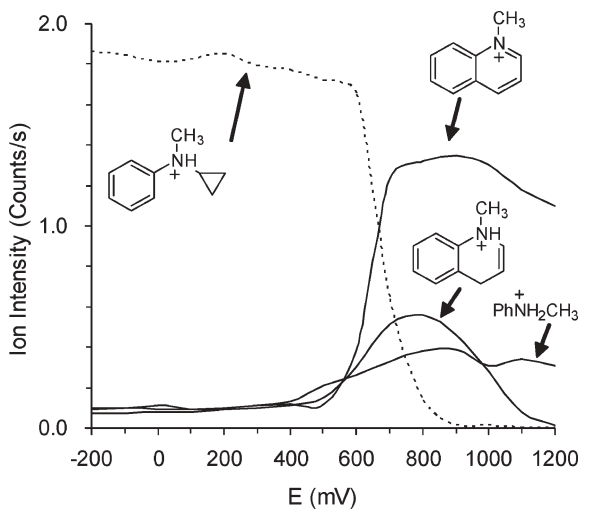


Fig. 1 The mass voltammogram of *N*-cyclopropyl-*N*-methylaniline (**6**) in 70% methanol–30% water–0.7% formic acid at a flow rate of 50 μL min^{-1}.

Finally, homogeneous redox catalysis[17] was used to study further the electrochemical oxidation of **6**. Three ferrocene-based mediators were used, varying in oxidation potential. For each mediator, the chemical step was rate-limiting, with the electron transfer as a rapid pre-equilibrium step; the results are summarized in Fig. 2. Under these conditions, the composite rate constant $k_{obs} = k_o k_1/k_{-1} = k_o K_1$ can be determined by fitting of the i_p/i_{pd} *vs.* $\log(1/v)$ data to theoretical working curves.[17,23,24]

Reconciliation of the direct and indirect electrochemical results leads to the rate constant for ring opening ($k_o = 4.1 \times 10^4$ s^{-1}). The rate constant for ring opening of $\mathbf{6}^{\bullet+}$ is exceptionally low, likely too low for **6** to be a dependable probe for single electron transfer (*vide infra*). Two explanations are offered (Scheme 2): (1) the lowest energy conformation of $\mathbf{6}^{\bullet+}$ does not meet the stereoelectronic requirements for cyclopropane ring opening,[24,25] and (2) $\mathbf{6}^{\bullet+}$ is stabilized by resonance in the cyclopropane ring-closed form.

These results are of particular interest because *N*-cyclopropyl-*N*-methylaniline and several structurally related substrates have been used to probe for single electron transfer pathways in amine oxidations mediated by cytochrome P450. Based upon the results reported herein, if electron transfer were occurring in these systems, the sluggish rate of ring opening would be unable to compete radical cation deprotonation.

To amplify this statement: The probe approach requires that the rearrangement is rapid relative to other competing processes, such as deprotonation. If, in cases where a paramagnetic intermediate is almost certainly involved (*e.g.*, the cP450 system), the rate of ring opening is uncompetitive with deprotonation ($k_o < k_d[B^-]$, paths a and b, Scheme 1), then it is impossible to distinguish an SET pathway from other pathways such as direct hydrogen atom transfer (HAT). The rate constant for the deprotonation of *N*,*N*-dimethylaniline radical cation by acetate anion has been measured under very similar conditions ($k_d = 1.2 \times 10^8$ M^{-1} s^{-1}).[26,27] Assuming that the rate constant for deprotonation from the methyl group of $\mathbf{6}^{\bullet+}$ is the same ($k_d = 6 \times 10^7$ M^{-1} s^{-1}, correcting for the number of hydrogens) and 0.1 M acetate, the

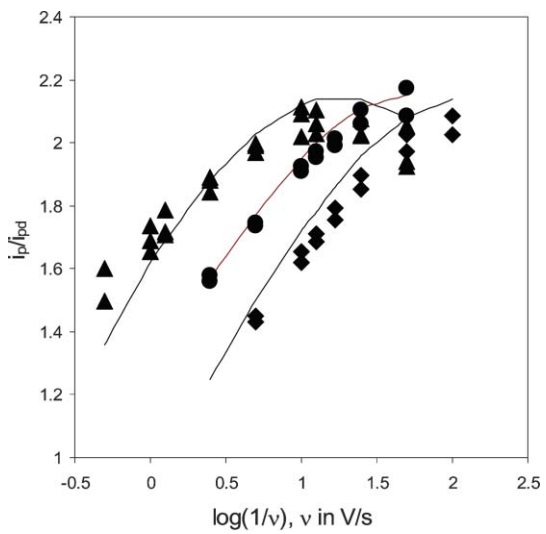
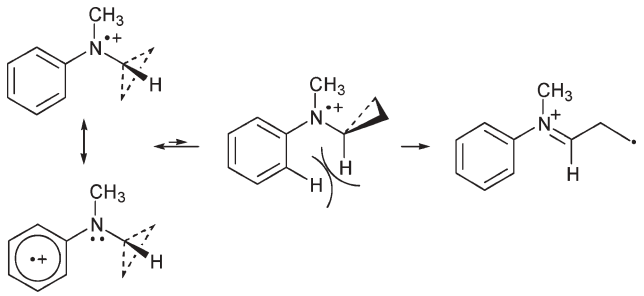


Fig. 2 Mediated oxidation of *N*-cyclopropyl-*N*-methylaniline by ferrocenecarbaldehyde (▲), benzoylferrocene (●), and ferrocene carboxylic acid (◆) at various concentrations of substrate; constant [M]/[substrate].



Scheme 2 Stereoelectronic and resonance effects which may hinder ring opening of the radical cation generated from *N*-cyclopropyl-*N*-methylaniline.

deprotonation pathway would overwhelm ring opening by a 150 : 1 ratio. In the context of an enzymatic study, a carboxylate base present in an effective molar concentration of 0.1 or greater would certainly overwhelm the ring opening in a similar manner, and a bona fide SET process would likely go undetected by this method.

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