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Chemical Model Studies on the Monoamine Oxidase-B Catalyzed Oxidation of 4-Substituted 1-Cyclopropyl-1,2,3,6-tetrahydropyridines

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Abstract—Two catalytic pathways have been proposed for the flavoenzyme monoamine oxidase B (MAO-B)—one based on an initial single electron transfer (SET) step from the nitrogen lone pair and the second based on an initial α-carbon hydrogen atom transfer (HAT) step. The SET pathway is consistent with the mechanism based inactivation properties of various cyclopropylamines. The observation that MAO-B catalyzes the efficient oxidation of certain 1-cyclopropyl-4-substituted-1,2,3,6-tetrahydropyridines to the corresponding dihydropyridinium metabolites suggests that the catalytic pathway for these cyclic tertiary allylamines may not proceed via the putative SET generated aminyl radical cations. The present paper describes the chemical fate of a series of *N*-cyclopropyltetrahydropyridines examined under reaction conditions that model the SET and the HAT pathways. All of the test compounds were rapidly converted under HAT reaction conditions to their dihydropyridinium products. Although the test compounds also were oxidized rapidly under SET conditions, no evidence for dihydropyridinium product formation was observed. The products that were identified most likely were formed after cyclopropyl ring opening of the initially formed cyclopropylaminyl radical cation. The results are discussed in terms of the mechanism of MAO-B catalysis. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Introduction

The flavoproteins monoamine oxidase (MAO) A and B catalyze the α-carbon oxidation of a variety of endogenous and exogenous amines.[1,2] Two distinct pathways have been proposed to account for MAO's catalytic activity (see Scheme 1). One is based on a single electron transfer (SET) step to form an aminyl radical cation.[3–6] The second pathway involves α-hydrogen atom transfer (HAT) as the initial step.[7–9] We have focused our MAO-B mechanistic studies on 1-methyl and 1-cyclopropyl-1,2,3,6-tetrahydropyridinyl derivatives bearing a variety of C-4 substituents.[10–21] These studies have been driven in part by interest in the MAO-B dependent toxicological properties of the parkinsonian inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) and the observation that these are the only cyclic amines which show MAO-B substrate properties.

MPTP is converted in an MAO-B catalyzed reaction to the unstable dihydropyridinium species **4** (Scheme 1)[22] which subsequently is oxidized further to the ultimate neurotoxin, the 1-methyl-4-phenylpyridinium species **5**.[23] According to the SET pathway, product formation proceeds via the aminyl radical cation **2** and α-carbon radical **3** intermediates. According to the HAT pathway the substrate molecule is converted directly to the α-carbon radical **3**. In an attempt to distinguish between these pathways, we examined the behavior of various 4-substituted 1-methyltetrahydropyridinyl derivatives under reaction conditions that model the SET and HAT pathways. The reactants were oxidized to the corresponding dihydropyridinium and pyridinium species under both reaction conditions. However, we were unable to make useful correlations between how the compounds behaved in these chemical models with their MAO-B substrate behavior.[24]

Key words: Monoamine oxidase; chemical model; SET (single electron transfer); cyclopropylamine; tetrahydropyridine.

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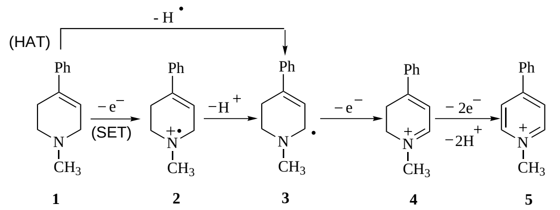
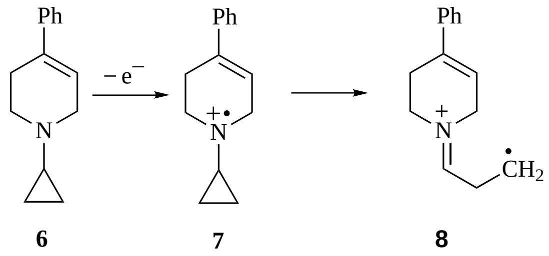


Scheme 1. Proposed pathways for the MAO catalyzed oxidation of MPTP.



Scheme 2. Proposed SET pathway mediating the inactivation properties of **6**.

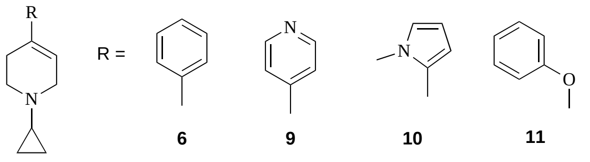
It is well known that cyclopropylamines, presumably via an SET generated cyclopropylaminyl radical cation (Scheme 2), can be efficient time and concentration dependent (mechanism based) inactivators of MAO-B.[25] For example, although MPTP is an excellent MAO-B substrate,[13] the corresponding *N*-cyclopropyl analog **6** is an efficient MAO-B mechanism based inactivator with such poor substrate properties that metabolite formation could not be detected spectrophotometrically.[16] The inactivation pathway presumably proceeds via the cyclopropylaminyl radical cation **7** followed by ring opening to form the distonic radical cation **8** that alkylates the enzyme active site (Scheme 2). The recent characterization of a three carbon adduct, derived from the cyclopropyl group of an *N*-benzylcyclopropylaminyl inactivator, to a cysteinyl residue of beef liver MAO-B provides strong evidence in support of the SET pathway.[26]

The 4-(4-pyridinyl) analogue **9** and several structurally related heteroaryl analogues also display MAO-B mechanism based inactivation properties.[14] Other experimental evidence, however, suggests that aminyl radical cations may not be obligatory intermediates in the MAO-B catalyzed oxidation of tetrahydropyridines.[17,18] For example, the 4-(1-methyl)pyrrol-2-yl analogue **10** is a good substrate and poor inactivator of MAO-B[14] while the 4-phenoxy analogue **11** is an excellent substrate that displays no inactivator properties (Table 1)[19]. In an effort to characterize more completely the pathway(s) by which MAO-B processes these types of cyclopropyltetrahydropyridinyl derivatives, we have examined the chemical fate of the *N*-cyclopropyltetrahydropyridinyl derivatives **6** and **9–11** when treated under SET and HAT oxidizing conditions. The results are discussed in terms of the behavior of these compounds in the presence of MAO-B.

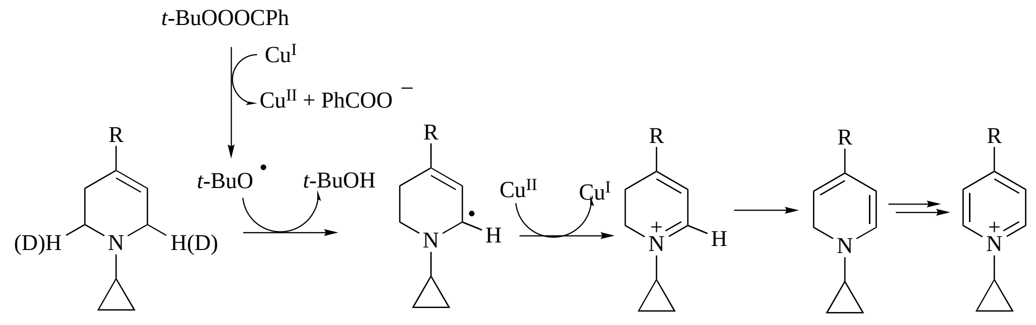
Results and Discussion

Treatment of **6** and **9–11** under HAT conditions (*t*-butyl peroxybenzoate in the presence of catalytic amounts of Cu^I) followed by reduction of the reaction mixtures with $NaBD_4$ resulted in the corresponding tetrahydropyridine-2,6-d_2 species **6-d_2** and **9-d_2-11-d_2**, respectively (Scheme 3).[27,28] The reactions were rapid in all cases since gas chromatography-electron ionization mass spectral (GC-EIMS) analyses of reaction mixtures treated with $NaBD_4$ showed that none of the starting d_0 materials were present after a 10 min reaction time. This behavior is similar to that observed when a series of 1-methyltetrahydropyridinyl analogues was examined under HAT reaction conditions.[24] We conclude,

Table 1. MAO-B substrate and inactivator properties of various *N*-cyclopropyl MPTP analogs



Compound	kcat (min^{-1})	Km (mM)	k_{cat}/K_M ($min\,mM^{-1}$)	k_{inact} (min^{-1})	K_I (mM)	k_{inact}/K_I ($min\,mM^{-1}$)
6	Turnover too slow to detect			0.7	0.18	0.1
9	Turnover too slow to detect			0.1	1.4	0.1
10	58	0.2	290	Rate of inactivation too slow to measure		
11	215	0.13	1650	Rate of inactivation too slow to detect		



R					
C_6H_5	**6** (**6-*d*₂**)	**12**	**16**	**20**	**24**
4-C_5H_4N	**9** (**9-*d*₂**)	**13**	**17**	**21**	**25**
2-$C_4H_3NCH_3$	**10** (**10-*d*₂**)	**14**	**18**	**22**	**26**
OC_6H_5	**11** (**11-*d*₂**)	**15**	**19**	**23**	**27**

Scheme 3. Proposed HAT pathway for the *t*-butoxy radical initiated oxidation of the 4-substituted 1-cyclopropyl-1,2,3,6-tetrahydropyridines **6** and **9–11**

therefore, that the *t*-butoxyl radical converts these tetrahydropyridinyl derivatives to the corresponding allylic radicals **12–15** which, in turn, are oxidized by the Cu^{II} species, formed in the reaction between *t*-butyl peroxybenzoate and Cu^I, to the dihydropyridinium intermediates **16–19** (Scheme 3). Deprotonation of **16–19** generates the corresponding free bases **20–23** that are converted to the pyridinium products **24–27** by an analogous oxidative sequence. Reduction of **24–27** with $NaBD_4$ provides the d_2 products **6-*d*₂** and **9-*d*₂-11-*d*₂** that were identified by GC-EIMS analysis.

The SET studies were performed using the Fe^{III} phenanthroline complex $Fe^{3+}(Phen)_3(PF_6^-)_3$ as the electron acceptor[29] in the presence of pyridine, the base used to deprotonate the intermediate cyclopropylaminyl radical cations. The reactions were run in CH_3CN at room temperature, conditions that worked well in the corresponding study with a series of 4-substituted 1-methyl-1,2,3,6-tetrahydropyridines.[24] GC-EIMS analysis of the reaction with **6** and the Fe^{+3} complex established that no starting material remained after 10 min. Furthermore, none of the starting material was observed in the GC-EIMS total ion current (TIC) chromatogram (Figure 1) of a 10 min reaction mixture that had been treated with $NaBH_4$. Consequently, unlike the behavior of the *N*-methyltetrahydropridines, **6** was not converted to its dihydropyridinium or pyridinium oxidation products under SET conditions.

The GC-EIMS total ion current (TIC) chromatogram (Figure 1) showed two major peaks [$t_R = 6.7$ min (73%) and $t_R = 7.3$ min (13%)] and three minor peaks. The appearance of the major peaks was dependent on $NaBH_4$ treatment. With time (16 h), neither of the major peaks was present and one of the minor peaks ($t_R = 6.0$ min) now dominated the tracing (>90% of the total ion current). The mass spectrum ($M^{\cdot+}$ 155) led to the assignment of 4-phenylpyridine (**28**) for the structure of the compound giving rise to the peak at 6.0 min. This assignment was confirmed by comparison of the corresponding spectrum obtained with an authentic sample of **28**. Treatment of reaction mixtures with $NaBD_4$ or with water alone gave the same product with no deuterium incorporation suggesting that **28** was formed during the course of the reaction, presumably from early arising intermediates that were converted with $NaBH_4$ to the two major products. The minor peak at 6.3 min has not been identified but may be derived from the tetrahydropyridinyl starting material since it is not present in the absence of this reactant. The minor peak at 7.2 min is derived from the phenanthroline reagent and was observed at all time points examined.

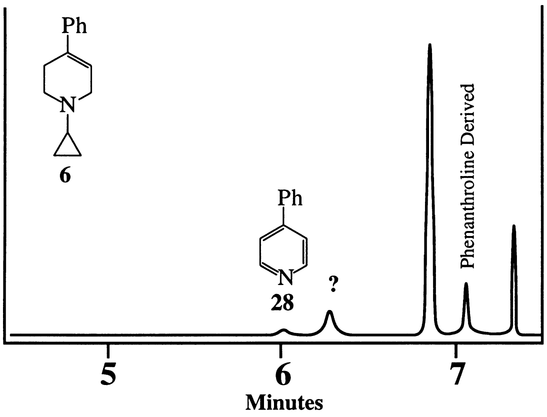


Figure 1. GC-EIMS total ion current chromatogram of a $NaBH_4$ treated 10 min SET reaction mixture containing **6**.

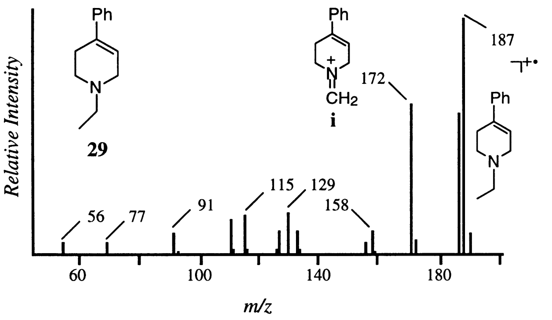


Figure 2. GC-EI mass spectrum of 1-ethyl-4-phenyl-1,2,3,6-tetrahydropyridine (**29**) isolated from a $NaBH_4$ treated 10 min SET reaction mixture containing **6**.

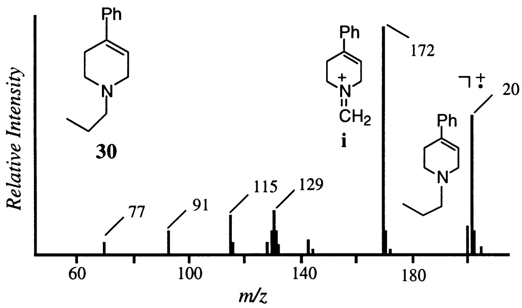


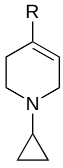
Figure 3. GC-EI mass spectrum of 1-propyl-4-phenyl-1,2,3,6-tetrahydropyridine (**30**) isolated from a $NaBH_4$ treated 10 min SET reaction mixture containing **6**.

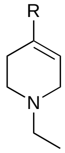
The mass spectrum of the major peak in the TIC tracing with $t_R = 6.7$ min is shown in Figure 2. The parent ion at *m/z* 187 is consistent with *N*-ethyltetrahydropyridinyl derivative **29**. As expected, a major fragment ion (**i**) at *m/z* 172 can be assigned to loss of a CH_3 radical. A synthetic standard of **29** gave an identical GC-EI mass spectrum. The structure of this compound was fully confirmed by ^1H NMR analysis of material isolated from a preparative scale SET reaction mixture.

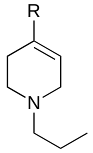
The mass spectrum (Figure 3) of the second major peak with $t_R = 7.3$ min is similar to the spectrum of compound **29** except that the parent ion (*m/z* 201) appears at 15 amu higher than the parent ion of **29**. This information plus the presence of the same fragment ion **i** at *m/z* 172 (loss of a CH_3CH_2 radical) led to the assignment of the *N*-propyl derivative **30** as the structure of this product. The identical spectrum was observed with a synthetic sample of **30**.

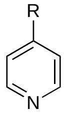
Similar experiments were carried out with the 4-(4-pyridinyl)-, 4-(1-methylpyrrol-2-yl)-, and 4-phenoxy-1-cyclopropyl-1,2,3,6-tetrahydropyridines (**9**, **10**, and **11**, respectively). In all cases, the starting materials were consumed rapidly and no evidence of ring α-carbon oxidation was obtained. Quantitatively, the fate of each molecule was dependent on the C4 substituent (see Table 2). Reaction of the pyridinyl analogue **9** led to the *N*-ethyl product **31** but the levels of the *N*-propyl product **34** were too low to detect. As with the phenyl case, however, the only product detected in the GC-EIMS TIC chromatogram after a 16 h reaction period was the pyridinyl product, in this case 4,4′-bipyridine (**37**). The intermediates leading to the *N*-ethyl (**32**) and *N*-propyl (**35**) products derived from the pyrrolyl analogue **10** appeared to be relatively stable since **32** and **35** represented the major species at all time points analyzed with only 5% of the pyridinyl species **38** being detected at 16 h. Finally, the behavior of the 4-phenoxy analogue **11** was intermediate in that about 50% of the starting

Table 2. GC-EI TIC yields[a] of various products from the cyclopropyltetrahydropyridine **6** and **9–11** observed following SET initiated oxidation and $NaBH_4$ treatment



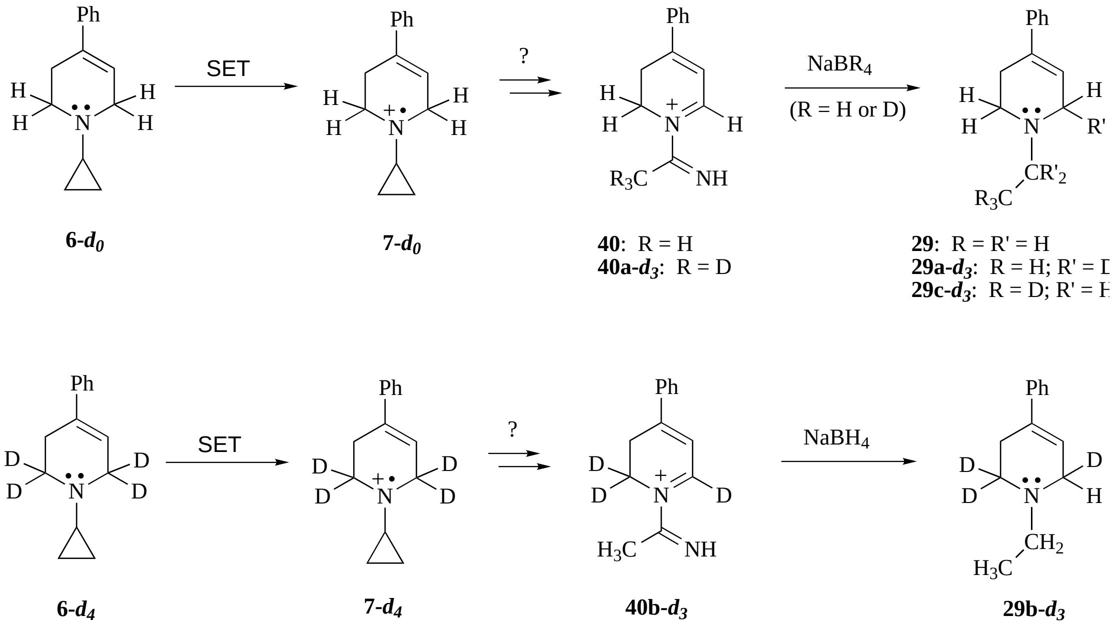






R			10 min	90 min	16 h		10 min	90 min	16 h		10 min	890 min	16 h
C_6H_5	**6**	**29**	70%	50%	0%	**30**	20%	6%	0%	**28**	10%	44%	100%
$4\text{-}C_5H_4N$	**9**	**31**	74	2	0	**34**	0	0	0	**37**	26	98	100
$2\text{-}C_4H_3NCH_3$	**10**	**32**	55	65	90	**35**	45	30	5	**38**	0	5	5
OC_6H_5	**11**	**33**	76	70	40	**36**	22	25	10	**39**	2	5	50

[a] The actual yields are somewhat less since these estimates do not take into account the contributions of the minor peaks (10% or less) to the integrated total ion current.

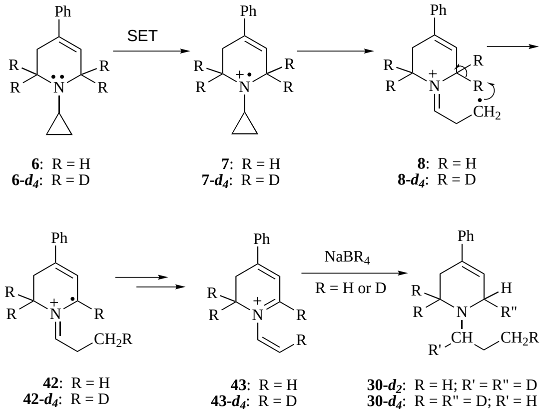


Scheme 4. Proposed pathway leading to various *N*-ethyl (**29**) products in the SET reaction.

material could be accounted for as the *N*-ethyl (**36**) and *N*-propyl (**39**) species after the 16 h reaction period with the remaining 50% recovered as the 4-pyridinyl species **39**. The differences in the life times of the intermediates leading to the *N*-ethyl and *N*-propyl products (see Schemes 4 and 5) presumably reflect stereoelectronic effects of the C4 substituents on the stability of these intermediates.

Additional information regarding the origins of the three products formed from **6** was sought with the aid of deuterium labeling (Table 3). GC-EIMS analysis of a 10 min reaction mixture treated with $NaBD_4$ established the following:

1. The *N*-ethyl product incorporated three deuterium atoms ($M\cdot^+$ at *m*/*z* 190). The presence of fragment ions at *m*/*z* 189 and 188, corresponding to $(M\cdot^+-1)^+$ and $(M\cdot^+-2)^+$, indicated the presence of one deuterium atom at the C6 position. A weak fragment ion at *m*/*z* 161 (observed at *m*/*z* 158 with **29** as the reactant), corresponding to $(M\cdot^+-29)^+$, is typical of C4-phenyl substituted tetrahydropyridines.[30] This fragment is formed by loss of the C2/C3 ethylene group plus one hydrogen atom from the phenyl ring. Consequently, no deuterium is present at the C2 or C3 positions. Finally, the loss of the terminal methyl group as $CH_3\cdot$ $(M\cdot^+-15)^+$ led to the conclusion that the remaining 2 deuterium atoms were located on the methylene carbon of the ethyl group. Based on these data, this d_3 product has been assigned the structure **29a–*d*₃** (Scheme 4).
2. The *N*-propyl product incorporated two deuterium atoms ($M\cdot^+$ *m*/*z* 203). Once again, the fragment ions observed at *m*/*z* 202 and 201 suggested the presence of both a proton and a deuteron at C6. The fragment ion resulting from loss of Et· (*m*/*z* 174, $M\cdot^+-29$) retained the two deuterium atoms establishing that no deuterium atom had been incorporated into the *N*-ethyl group. A structure consistent with these data is **30–*d*₂**.



Scheme 5. Proposed SET pathway for the formation of **30-d_2** from **6** (following $NaBD_4$ treatment) and **30-d_4** from **6-d_4** (following $NaBH_4$ treatment).

3. As mentioned earlier, the 4-phenylpyridine (**28**) recovered from this reaction contained no deuterium ($M^{\cdot +}$ *m*/*z* 155).

A third experiment was conducted with 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine-2,2,6,6-d_4 (**6-*d*$_4$**) as substrate. As expected, the 4-phenylpyridine formed from this reactant contained two deuterium atoms ($M^{\cdot +}$ 157), most likely at the 2- and 6-positions. The *N*-ethyl product contained three deuterium atoms ($M^{\cdot +}$ 190)$^+$. Since ions were observed at *m*/*z* 189, assigned to $(M^{\cdot +}-H\cdot)^+$, and *m*/*z* 188, assigned to $(M^{\cdot +}-D\cdot)^+$, we have concluded that one hydrogen and one deuterium atom are attached to C6. The loss of Me· from this product gives the base peak at *m*/*z* 175 and therefore all three deuterium atoms were retained in the fragment. These data are consistent with **29b-2,2,6-*d*$_3$** in which the proton at C6 is derived from $NaBH_4$ (Scheme 4).

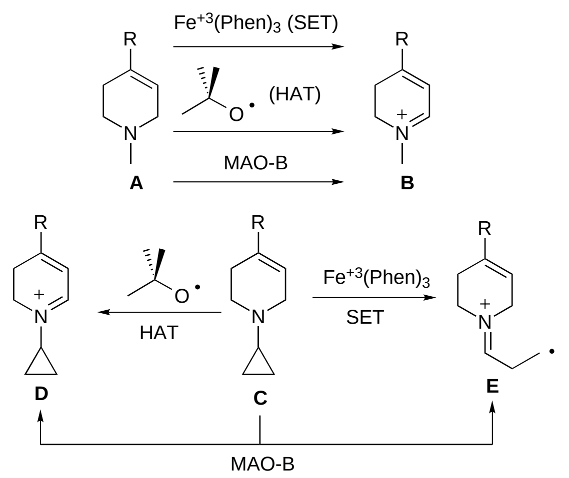
Since it was difficult to rationalize the conversion of an *N*-cyclopropyl group to an *N*-ethyl group, we considered CH_3CN as a potential source of the *N*-ethyl group. When the reaction was run with **6-*d*$_0$** in CD_3CN and worked-up with $NaBH_4$, the product again contained three deuterium atoms. Their location on the terminal methyl position of the *N*-ethyl group was readily demonstrated by mass spectral analysis since an intense ion was present at *m*/*z* 172 due to the loss of the terminal CD_3 radical. These data led to the assignment of **29c-*d*$_3$** for this product. We conclude, therefore, that acetonitrile is the source of the *N*-ethyl group of **29**. In addition, the corresponding reaction of **6** in proprionitrile does not lead to the formation of the *N*-ethyl derivative **29** but, as expected, only to the *N*-propyl derivative **30**. In view of the deuterium labeling pattern, the most likely structures of the intermediates leading to the various *N*-ethyl products are the corresponding iminodihydropyridinium species **40** shown in Scheme 4. The pathway leading to **40**, however, remains to be determined.

The *N*-propyl product isolated in the reaction with **6-*d*$_4$** as substrate retained all four of the deuterium atoms present in the d_4 starting material. A relatively strong fragment ion at *m*/*z* 204 indicated the presence of a proton at C6. Furthermore, the base peak at *m*/*z* 175 resulting from loss of Et·, appeared at 30 amu lower than the mass of the parent ion. Consequently, Et· must have contained one deuterium atom and that deuterium atom must have been derived from the C6 position of the starting material. These spectral features are well accommodated by **30-*d*$_4$** that would result from deuterium migration as depicted in Scheme 5. SET initiated oxidation of **6-*d*$_4$** generates the aminyl radical cation **7-*d*$_4$** which, following ring opening, leads to the distonic radical cation **8-*d*$_4$**. Rearrangement of **8-*d*$_4$** yields the more stable allylic radical cation **42-*d*$_4$**. Deprotonation of **42-*d*$_4$** followed by a second electron transfer yields **43-*d*$_4$** which, following $NaBH_4$ reduction, gives **30-*d*$_4$**. Earlier studies on the mass spectral characteristics of the electron ionization generated radical cation **7-*d*$_4$** from **6-*d*$_4$** have established that an analogous migration takes place in the gas phase.[31] This pathway also is consistent with the results obtained with the $NaBD_4$ treated reaction mixture starting from **6**. The sequence **6** → **7** → **8** → **42** would lead to **43** which, upon treatment with $NaBD_4$, would yield **30-*d*$_2$** the mass spectrum of which is consistent with that observed (Table 3).

The available data from these SET experiments are summarized in Scheme 6. *N*-Methyltetrahydropyridines (**A**) undergo allylic ring α-carbon oxidations to yield **B** in both model reactions. Compounds **B** also are formed in the MAO-B catalyzed oxidation of **A**. *N*-Cyclopropyltetrahydropyridines (**C**) also undergo allylic ring α-carbon oxidation to yield tetrahydropyridine products (**D**) under HAT but not under SET reaction conditions. Somewhat indirect evidence suggests that the products obtained in these reactions that are trapped following hydride (or deuteride) reduction are formed from the

Table 3. Key GC-EIMS ions of products obtained form compound **6** and **6-*d*$_4$** following a 10 min reaction under the SET reaction and workup conditions indicated

Substrate	Reaction conditions	Product					
		N-Ethyl product			*N*-Propyl product		
		$M^{\cdot +}$	$(M^{\cdot +}-H.)+$ or $[M^{\cdot +}-D.]+$	$(M^{\cdot +}-Me\cdot)^+$	$M^{\cdot +}$	$(M^{\cdot +}-H\cdot)+$ or $[M^{\cdot +}-D\cdot]+$	$(M^{\cdot +}-Et\cdot)^+$
6	$NaBH_4$ (CH_3CN)	187 (100)	186 (65) [185 (0)]	172 (70)	201 (70)	200 (13) [199 (0)]	172 (100)
6	$NaBD_4$ (CH_3CN)	190 (100)	189 (65) [188 (15)]	175 (70)	203 (70)	202 (35) [201 (15)]	174 (100)
6-d_4	$NaBH_4$ (CH_3CN)	190 (100)	189 (65) [188 (15)]	[188 (15)]	205 (70)	204 (14) [z203 (5)]	175 (100)
6$_4$	NaBH (CD_3CN)	190 (100)	189 (65) [188 (0)]	172 (70)	201 (70)	200 (13) [199 (0)]	172 (100)



Scheme 6. Fate of *N*-methyl- and *N*-cyclopropyltetrahydropyridines in SET, HAT, and MAO-B oxidation conditions.

ring opened intermediate **E**, the same intermediate postulated to account for the inactivation of MAO-B. It may be reasonable to speculate, therefore, that the MAO-B catalyzed ring allylic α-carbon oxidations of certain *N*-cyclopropyltetrahydropyridines do not proceed via the SET pathway but rather via a pathway that does not include the cyclopropylaminyl radical cation intermediate. An alternative explanation would invoke a restricted conformation of the cyclopropylaminyl radical cation in the enzyme active site such that overlap of the half-filled *p*-orbital on the nitrogen atom and the *p*-type orbitals of the cyclopropyl group required for ring opening would be prohibited. We hope to explore this possibility by examining the chemical fate under SET and HAT reaction conditions of rigid *N*-cyclopropyltetrahydropyridinyl analogues that impose such constraints.

Experimental

*Important notice: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (**1**) and related 1,4-disubstituted 1,2,3,6-tetrahydropyridines are known nigrostriatal neurotoxin sand should be handled using disposable gloves in a properly ventilated hood. Detailed procedures for the safe handling of MPTP have been reported.*[32]

General methods

Reagents and starting materials were obtained from commercial suppliers and were used without further purification. *Tris*(1,10-phenanthroline)iron (III) *tris*(hexafluorophosphate) was prepared by a literature method.[29] The preparations of the tetrahydropyridines **6**,[16] **9**,[14] **10**,[14] **11**,[19] **6**-2,2,6,6-d_4,[27] 1-ethyl-4-phenyl-1,2,3,6-tetrahydropyridine (**29**)[33] and 1-propyl-4-phenyl-1,2,3,6-tetrahydropyridine (**30**)[12] were prepared as reported previously. Free bases of the tetrahydropyridines were extracted with ethyl acetate from saturated aqueous potassium carbonate of the corresponding protonated salts. The combined organic extracts were dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. All reactions were conducted using flame dried glassware under an atmosphere of dry nitrogen. Chromatography refers to flash column chromatography on silica gel unless otherwise noted. Proton NMR spectra were recorded on a Bruker WP 270-MHz spectrometer. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Spin multiplicities are given as s (singlet), brs (broad singlet), d (doublet), t (triplet) or m (multiplet). Coupling constant values (J) are given in hertz (Hz). GC-EIMS analyses were performed at an ionization voltage of 70 eV on a Hewlett Packard (HP) 5890 GC fitted with an HP-1 methyl silicone capillary column (12.5 m×0.2 mm×0.33 mm film thickness) employing helium as the carrier gas (40 mL/min). The ion currents were detected with an HP 5870 mass-selective detector. Data were acquired using an HP 5970 Chemstation. Normalized peak heights are reported as a percentage of the base peak.

The HAT model. Solutions of the tetrahydropyridine free bases **6** and **9–11** (0.114 mmol) and CuCl (1.1 mg, 10%) in acetonitrile (5 mL) were prepared. Nitrogen was bubbled gently through the solution and *t*-butyl peroxybenzoate (43 μL, 0.228 mmol) was added at room temperature. Timed aliquots (0.1 mL) (1, 2, 3, 5 min) of the reaction mixtures were treated with an excess of $NaBH_4$ or $NaBD_4$ in CH_3OH and 5 min later the solvent was evaporated under reduced pressure. A saturated solution of potassium carbonate was added and organic compounds were extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and analyzed by GC-EIMS. Ethyl acetate extracts of potassium carbonate only treated aliquots also were examined by GC-EIMS.

The SET model. To a solution of the tetrahydropyridine free bases **6**, **6-d_4** and **9–11** (0.0125 mmol) and pyridine (5 μL, 0.07 mmol) in 10 mL of acetonitrile was added at room temperature $Fe^{3+}(Phen)_3(PF_6^-)_3$ (51 mg, 0.05 mmol). Aliquots (0.5 ml) of the reaction mixture were treated with an excess of $NaBH_4$ or $NaBD_4$ in CH_3OH following which the solvents were removed under reduced pressure. A saturated solution of aqueous potassium carbonate was added and the organic compounds were extracted into diethyl ether. The extract was dried over $MgSO_4$ and analyzed by GC-EIMS. Alternatively, a 0.5 mL aliquot (5 min) was treated with saturated potassium carbonate and the organic compounds directly extracted with diethyl ether and analyzed by GC-EIMS.

Characterization of 1-ethyl-4-phenyl-1,2,3,6-tetrahydropyridine (29) formed from 6 in the SET model study. To a solution of **6** free base [from 144.6 mg of the corresponding oxalate salt (144.6 mg, 0.5 mmol)] and pyridine (0.2 mL, 2.5 mmol) in 200 ml of acetonitrile was added $Fe^{3+}(Phen)_3(PF_6^-)_3$ (2 g, 2 mmol) at room temperature. After 20 min, the mixture was added to a solution of $NaBH_4$ (189 mg, 5 mmol) in CH_3OH (100 mL) at 0 °C. After stirring for 1 h, the solvent was evaporated under reduced pressure, the solid residue was stirred with diethyl ether (100 mL) and the red reduced iron complex was removed by filtration. A solution of saturated K_2CO_3 (100 mL) was added to the ether filtrate. The aqueous layer was extracted an additional three times with diethyl ether (80 mL each) and the combined organic extracts were dried over $MgSO_4$ and solvent evaporated under reduced pressure. The residue was chromatographed with CH_2Cl_2 employing a CH_3OH gradient from 0 to 5%. The 1-ethyl-4-phenyltetrahydropyridine **29** was obtained as a yellow oil. The ^1H NMR spectrum (CD_3Cl) δ 1.18 (t, 3H, CH_3, $J = 7.2$ Hz), 2.55–2.63 (m, 4H, $C_{1'}$, C3), 2.74–2.78 (m, 2H, C_2), 3.19–3.22 (m, 2H, C_6), 6.05–6.07 (m, 1H, C_5) and GC-EIMS spectrum (free base, *m*/*z*, %) 187 ($M\cdot^+$, 100), 186 (46), 172 (76), 158 (15), 130 (26), 129 (43), 128 (26), 115 (43), 110 (37), 91 (26), 77 (15) were identical to the corresponding spectra of an authentic synthetic standard.[33]

Characterization of the 1-ethyl-d_2-4-phenyl-1,2,3,6-tetrahydropyridine-6-d_1 (29a-d_3) formed from 6 in the SET model study. The same procedure just described was followed except that $NaBD_4$ was used instead of $NaBH_4$. Compound **29a-d_3** was obtained as a yellow oil: ^1H NMR (CD_3Cl) δ 1.18 (s, 3H, CH_3), 2.60–2.62 (m, 2H, C_3), 2.73–2.76 (m, 2H, C_2); 3.16–3.19 (m, 1H, C_6), 6.06–6.07 (m, 1H, C_5); GC-EIMS analysis (free base, *m*/*z*, %) 190 ($M\cdot^+$, 100), 189 (70), 188 (28), 175 (76), 174 (28), 161 (9), 131 (33), 130 (48), 129 (30), 116 (33), 115 (22), 113 (39), 92 (13), 91 (20), 77 (15).

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