Catalysis & Inhibition Issues Associated with Monoamine Oxidase (MAO). How Unusually Low α-C-H Bond Dissociation Energies May Open the Door to Single Electron Transfer

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Abstract

C-H Bond dissociation energies for a unique selection of tertiary amines that are known substrates or inhibitors of monoamine oxidase have been calculated using density functional theory. These amines are unusual because they are the only tertiary amines that exhibit MAO substrate or inhibitor behavior. The unique structural feature common to these specific compounds is an sp^3-hybridized CH_2 moiety, which is α-both to nitrogen and an C=C or C≡C. The stabilization afforded the resulting radicals by extended delocalization dramatically lowers both the C-H bond strength of the substrate (R-H → R• + H•) and pK_a of the corresponding radical cation ($RH^{\bullet+} \rightarrow R\bullet + H^+$). This interplay of structure and thermodynamics may provide the driving force for an electron transfer mechanism for MAO catalysis and inhibition.

Keywords. Monoamine oxidase (MAO), single electron transfer, C-H bond dissociation energies, MAO catalysis and inhibition.

1. Introduction

Monoamine oxidase A (MAO-A) and -B (MAO-B) catalyze the oxidation of various neurotransmitters, including dopamine, norepinephrine, epinephrine and serotonin. The overall stoichiometry of this reaction is a two-electron α-carbon oxidation, $H_2N\text{-}CH_2R \rightarrow HN{=}CHR$, a reaction that is coupled to the two-electron reduction of the flavin cofactor FAD to $FADH_2$. Several mechanisms have been proposed to account for the initial stages of the mechanism of MAO-catalyzed oxidations:[1-3] Conventional "two-electron/polar" pathways (nucleophilic addition,[4] hydride transfer[5, 6]), and a single electron transfer (SET) pathway that involves paramagnetic intermediates (*vide infra*).[7-9]

Recognizing the hazard of summarizing a large body of literature in a short narrative, it seems fair to say that for the most part, the SET pathway has fallen out of favor. While there are scattered reports in the recent literature advocating for SET,[1, 10-12] most recent studies and reviews have focused almost exclusively on the polar pathways (nucleophilic and or hydride transfer mechanisms).[2, 13-15] The objective of this paper is not to enter into this debate. Based upon our reading of the literature, it seems reasonable to conclude that the emerging consensus favoring non-radical pathways for MAO-catalyzed oxidations may be correct, *for the enzymes' natural substrates*. Rather, this manuscript addresses a glaring anomaly that has not been discussed in recent mechanistic studies--the reaction of MAO with tertiary amines.

Generally, tertiary amines are *not* MAO substrates. This makes sense because steric crowding prevents reaction from occurring via either of the "accepted" polar mechanisms. For one of

these mechanisms, the (multi-step) polar/nucleophilic mechanism depicted in Figure 1a, the problem is obvious. In this mechanism, the nitrogen lone pair of the amine attacks the C_{4a} of the flavin, generating an intermediate which subsequently undergoes elimination to form the products. Nucleophilic addition would be sterically prohibitive for a tertiary amine, which based upon this mechanism, likely explains why tertiary amines are not typically oxidized by MAO.

In the hydride transfer pathway (Figure 2), hydride is transferred to the nitrogen of the flavin directly forming the imine and partially reduced flavin in a single step. Recent computational studies have suggested that the nitrogen of the amine and the C_{4a} carbon of the flavin moiety are in close proximity, and that both the hydride transfer pathway (Figure 2) and a concerted polar/nucleophilic pathway (Figure 2b) proceed through a similarly-structured transition state, differing mainly in the direction of electron flow.[13, 14] Although either or both of these mechanisms are viable for MAO's natural substrates (most of which are primary or secondary amines), steric constraints make the polar/nucleophilic and hydride transfer pathways untenable for tertiary amines.

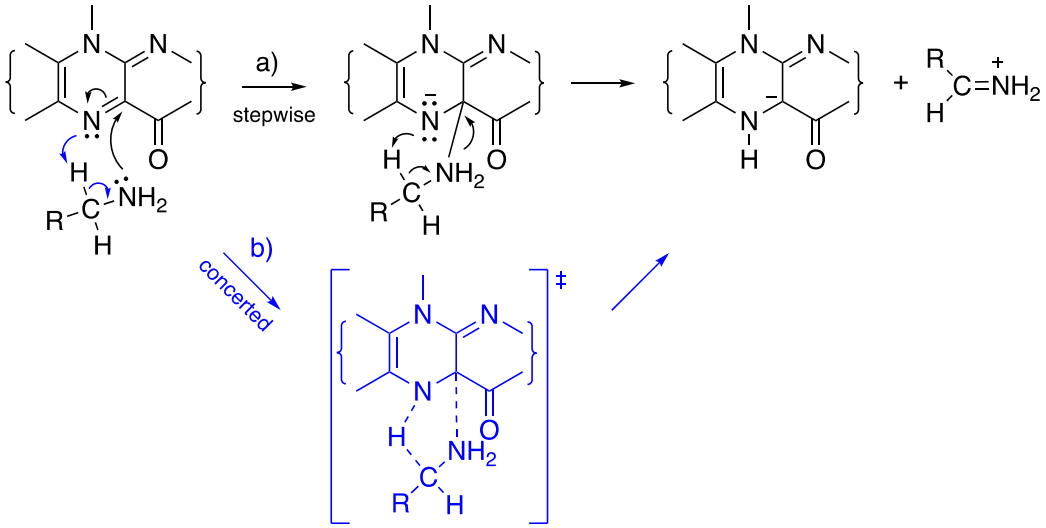


Figure 1. The polar/nucleophilic mechanism for MAO-catalyzed amine oxidations. a) Stepwise (black): The electron pair of the amine attacks the C=N of the flavin moiety, followed by elimination to form the partially reduced flavin and iminium. b) Concerted (blue): All the elements of the stepwise mechanism occur in a single step.

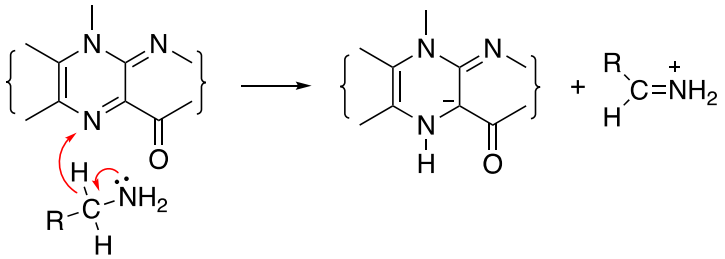


Figure 2. The hydride transfer mechanism: H^- is transferred from the amine to the flavin to form the partially reduced flavin and iminium. It has been suggested that the hydride transfer

mechanism and concerted nucleophilic mechanism (shown in blue in Figure 1b) both occur through the same transition state with the key difference being the direction of electron flow.

Tertiary amines such as the neurotoxin MPTP,[16] and its close structural analogs that are the only reported tertiary amines with good MAO substrate (or inhibitor) properties. Other notable exceptions include propargylic amines such as the MAO inhibitors pargyline and selegiline (deprenyl),[17] and other compounds (such as those reported by Silverman[18]) which that possess analogous functionality (an unsaturated group at the α-position).

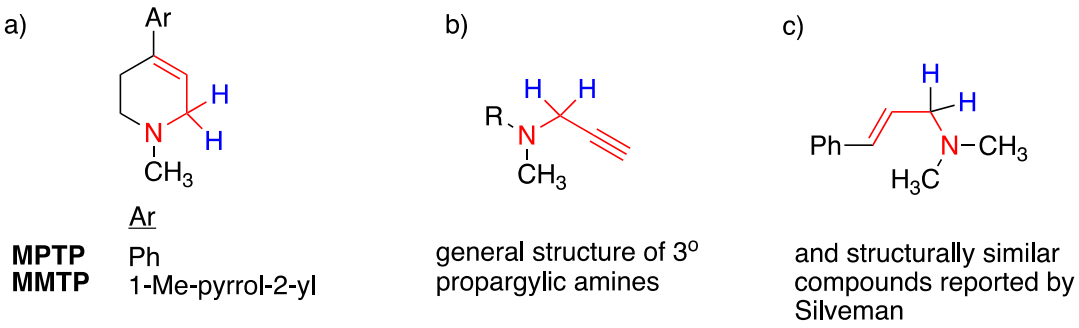


Figure 3. Tertiary amines that are known MAO-B substrates or inhibitors. The critical functionality central to our hypothesis is highlighted in red, and the corresponding reactive hydrogens in blue

In 2020, based on the results of a biomimetic study using 5-ethyl-3-methyllumiflavinium (Fl^+) perchlorate as a model for flavin moiety in the active site and the tertiary amine MMTP (a non-toxic MPTP derivative), we offered a modification to the single electron transfer mechanism to explain why this pathway may become important for these particular tertiary amines (Figure 4).[19] Specifically, based upon redox potentials, and as many have argued previously to discount the SET mechanism, electron transfer between the flavin moiety in MAO (MAO-Fl) and an amine is thermodynamically unfavorable. We suggest, however, that it is likely reversible. The overarching hypothesis is that the SET pathway is always present and available, but becomes important when the resulting radical cation is particularly acidic.

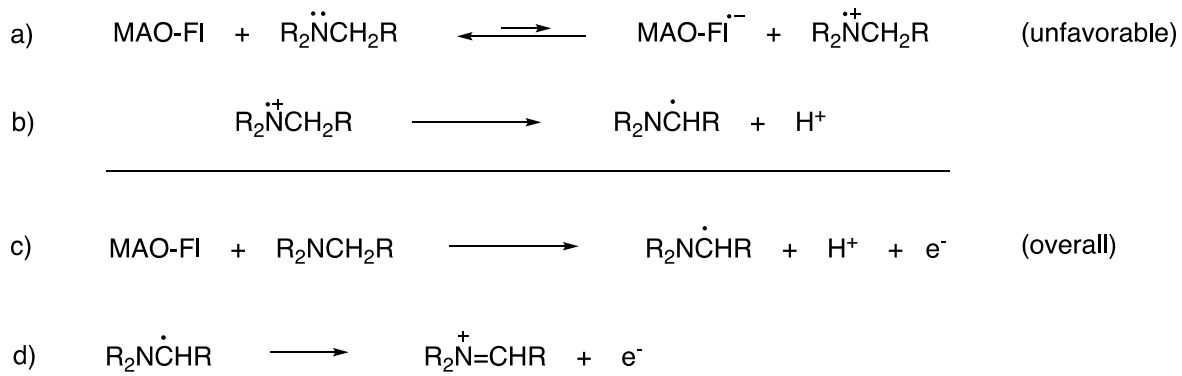


Figure 4. Modified single electron transfer (SET) hypothesis. a) Electron transfer between the flavin moiety and an amine is thermodynamically unfavorable. b) For some substrates, the

radical resulting from deprotonation is very stable and the pK_a of the radical cation is exceptionally low. c) The overall result of coupling and unfavorable electron transfer (a) and a very favorable proton transfer (b) drives the overall proton coupled electron transfer (PCET) process. d) The final oxidation of the α-amino alkyl radical to imminium is expected to be very favorable.[20]

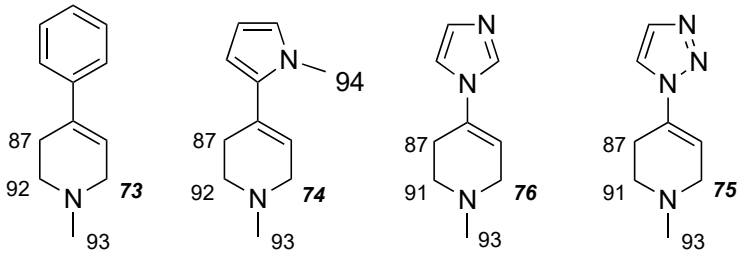
We believe that the α-C=C in **MPTP** (and derivatives) and perhaps the α-C≡C in the propargylic amine inhibitors dramatically lowers the pK_a values of the corresponding aminyl radical cations. For MPTP specifically, based upon radical stability, we estimated that the pK_a is reduced from *ca.* 8 for a "normal" tertiary amine radical cation to as low as -5 for **MPTP$^{\bullet+}$**.[19] In essence, the (unfavorable) SET process is driven by an extremely favorable deprotonation step in the context of LeChatlier's principle. This coupling of an electron transfer event with a favorable proton transfer is referred to a proton coupled electron transfer (PCET), and may occur in a stepwise or concerted manner.

The objective of this work is to estimate the bond strengths of several compounds that are either MAO substrates or inhibitors in the context of our SET-based hypothesis for the MAO-catalyzed oxidation of these unique tertiary amines.

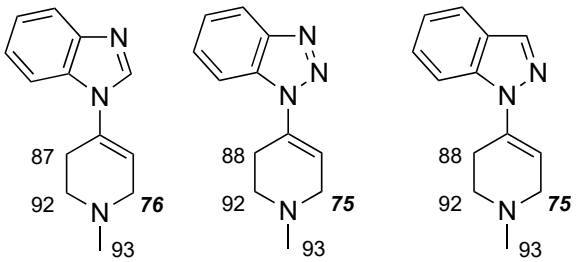
2. Results

Values of the C-H bond dissociation energies for were estimated using an extension of a previously described procedure.[21, 22] Briefly, for each compound (R-H), a conformational search was performed to identify the lowest energy conformation(s). The starting geometries of the corresponding radicals (R•) were generated by removing hydrogen from the pertinent position of each compound. After an initial geometry optimization at the AM1 level to clean up the structures, geometries were optimized at the DFT/B3LYP/6-31G(d) level, and energies obtained by a single point calculation at the DFT/B3LYP/ccPVTZ level. The energy difference (ΔE) between R-H and R• was used to estimate the C-H BDE. This was accomplished by using a "calibration curve" (plot of C-H BDE vs ΔE based upon thirty compounds with C-H BDE's ranging from 79 to 130 kcal/mol. A broad range of compounds was utilized to construct this calibration curve, and included hydrocarbons, alcohols, ethers, amines and more. (The specific compounds used to construct the calibration curve and other pertinent information is provided in the supporting information.) With the calibration curve in hand, BDEs were estimated for all of the compounds listed in Figure 5.

a) MPTP and related tetrahydropyridines:

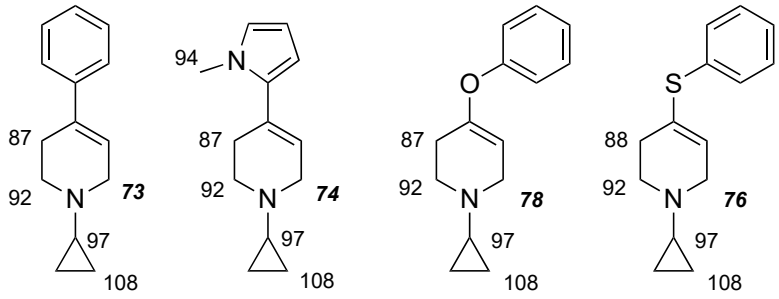


1 (MPTP) **2 (MMTP)** **3** **4**



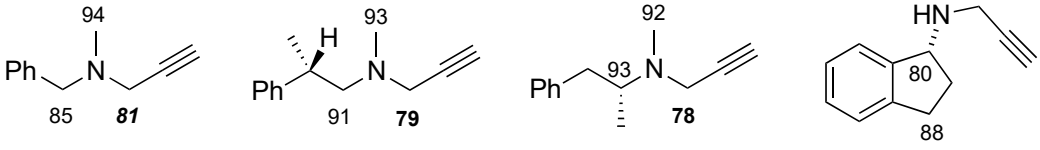
5 **6** **7**

b) N-cyclopropyl derivatives of MPTP and related compounds



8 **9** **10** **11**

c) Propargyl amines:



12 (pargyline) **13** **14** (L-deprenyl) **15** (rasagiline)

Figure 5. Estimates of the C_{sp3}-H bond dissociation energies (kcal/mol) at various positions of MAO substrates, inhibitors, and related compounds.

3. Discussion

3.1. MAO Substrates: MPTP and related tetrahydropyridines. The calculations reveal that for all the tetrahydropyridines studied all of which are MAO substrates (Figure 5a), the C-H BDE of

the CH_2 moiety that is both α- both to the nitrogen and C=C is 75 (± 1) kcal/mol, and much weaker than any of the other C-H bonds in these molecules. Moreover, the BDE does not vary significantly with the nature of the aromatic ring.

For comparison, trimethyl amine $(CH_3)3N$) has a C-H BDE of 93 kcal/mol. This difference suggests that the additional resonance stabilization afforded by the double bond in these tetrahydropyridines corresponds to a radical stabilization energy on the order of 18 kcal/mol. The pK_a of $(CH_3)_3N\bullet+$ is 8.0. We assume that the oxidation potentials of $(CH_3)_3N$ and the tetrahydropyridines are similar, which seems plausible given the nitrogen atom is insulated from the C=C by the sp^3 hybridized CH_2 group in the latter. Accordingly, this difference 18 kcal/mol difference in radical stabilization energy translates to about 13 pK_a units, suggesting the radical cations derived from these tetrahydropyridines have pK_as on the order of -5 (Figure 6).

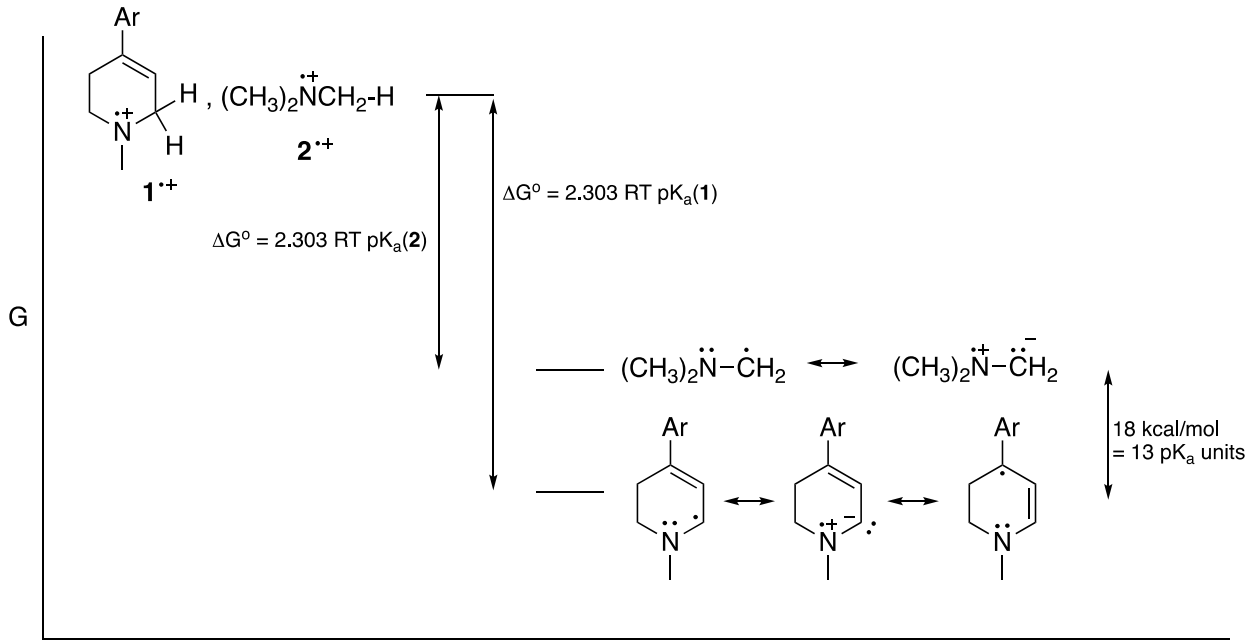


Figure 6. Estimating the pK_a of the tetrahydropyridine radical cations ($\mathbf{1}^{\bullet+}$): Radicals derived from the tetrahydropyridines are stabilized by ca. 18 kcal/mol (compared to $(CH_3)_2NCH_2\bullet$) because of enhanced resonance. This energy difference corresponds to 13 pK_a units, meaning the pK_a of $\mathbf{1}^{\bullet+}$ may be as low as -5.

3.2. *N*-Cyclopropyl derivatives of MPTP and related compounds. The most compelling argument for single electron transfer in MAO-catalyzed oxidations comes from the behavior of *N*-cyclopropyl compounds. As shown in Figure 7, single electron oxidation of the amine to form the radical cation sets up a competition between deprotonation (k_b[B:]) which results in the usual oxidation products, or ring opening (k_o) which presumably occurs rapidly because of the relief of cyclopropyl ring strain. The *N*-cyclopropyl derivative of MPTP (**8**) is a mechanism-based inhibitor of monoamine oxidase because it is argued that the primary alkyl radical produced by ring opening disrupts the enzyme active site, likely by coupling with the flavin radical anion.

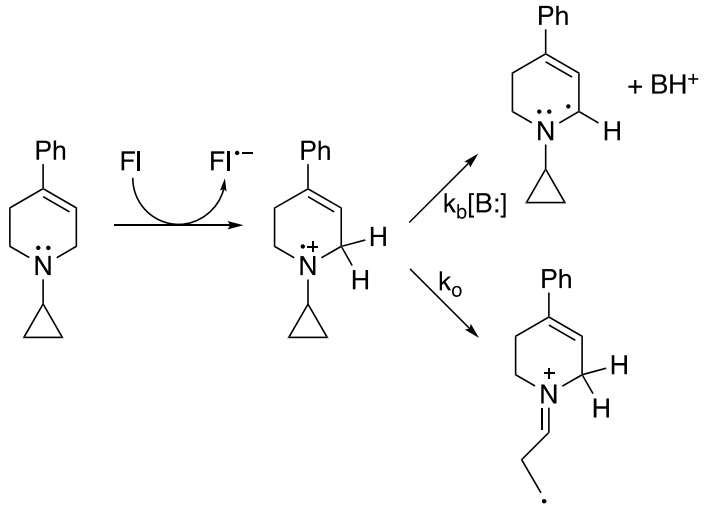


Figure 7. The use of an electron transfer probe to find evidence for electron transfer. Single electron oxidation of the amine by the flavin moiety sets up a competition between deprotonation (normal substrate pathway) and ring opening leading to a 1° radical which disrupts the active site of the enzyme (mechanism-based inhibition).

On the other hand, the *N*-cyclopropyl derivative of MMTP (**9**) is an MAO substrate, implying ring opening does not occur. This discrepancy could be explained by a difference in the rate constants for deprotonation (possibly because of differing pK_as) or differences in the rate of ring opening, i.e., $k_B[B:] > k_o$. The *N*-cyclopropyl compounds were selected for study to ascertain whether the cyclopropyl moiety had any effect on the C-H BDE of the neutrals, and by extension, the pK_as of the corresponding radical cations. As the data in Figure 6 shows, the C-H BDEs of these compounds (and by extension, pK_as of the radical cations) are not significantly impacted by the cyclopropyl group. Any differences in terms of substrate vs. inhibitor properties of the different *N*-cyclopropyl derivatives must be attributed to other factors such as the rate constant for ring opening. (We are currently pursuing experiments to address this issue).

3.3. Propargyl amines. For the propargylic amines, the CH_2 that is α-both to nitrogen and the C≡C have BDEs on the order of 79 (± 1) kcal/mol, with a radical stabilization energy of 10 kcal/mol (relative to trimethylamine.) Invoking the same logic used for the tetrahydropyridines, this suggests that the corresponding radical cations are exceptionally acidic (pKa ≈ -2) compared to tertiary amine radical cations (pKa ≈ 8) in general, though not quite as acidic as is the case for those derived from tetrahydropyridines pKa ≈ -5).

4. Conclusions. The bond strengths of several compounds that are either MAO substrates (tetrahydropyridines) or inhibitors (propargyl amines) have been estimated. All these compounds share a common structural feature, specifically a CH_2 moiety that is α-both to nitrogen and either a C=C or C≡C bond, the latter of which stabilize the resulting radicals by 18 and 10 kcal/mol, respectively (relative to trimethylamine). This added stabilizing afforded the corresponding radicals means that the radical cations derived from these compounds are much

more acidic than typical tertiary amines. Incorporation of an *N*-cyclopropyl on the tetrahydropyridines had no effect on the α-C-H BDE. The thermodynamics of these compounds lends support for the hypothesis that these compounds may react via coupling of an unfavorable single electron transfer with extremely favorable deprotonation (Figure 4).

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Conflict of Interest. The authors declare no conflict of interest.

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