tert-Butoxyl as a Model for Radicals in Biological Systems: Caveat Emptor

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The *tert*-butoxyl radical (^{t}BuO$^\bullet$) has been used as a chemical model for C−H bond cleavage in enzyme-catalyzed oxidations such as those catalyzed by cytochrome P450,[1−3] methane monooxygenase,[3,4] and monoamine oxidase.[5,6] The general idea in such studies is to probe the "similarity" between the chemistry of ^{t}BuO$^\bullet$ and the enzyme, "similarity" being assessed in terms of regiochemistry (i.e., which C−H bond is cleaved) and kinetic isotope effects (the effect on rate associated with the replacement of hydrogen by deuterium). A common (radical) mechanism is assumed to be operating if the enzyme and ^{t}BuO$^\bullet$ behave "similarly".

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) is a tertiary amine of enormous biological significance. Oxidation products arising from MPTP lead to a parkinsonian syndrome in humans. Monoamine oxidase B (MAO-B) is the principal brain enzyme that catalyzes the oxidation of MPTP.[7−9] The initial events in the oxidation of amines by MAO are suspected to involve either homolytic[10] or electron-transfer pathways,[11] illustrated in Scheme 1 for MPTP.

Initially, the objective of this study was to measure the absolute rate constant for hydrogen abstraction from MPTP (by ^{t}BuO$^\bullet$), to provide insight into the mechanism of MAO-B catalysis. Our initial results led to a more general study of the rates and activation parameters associated with the reaction of ^{t}BuO$^\bullet$ with tertiary amines. Experimentally, absolute rate constants for the reaction of ^{t}BuO$^\bullet$ with amines were determined by laser flash photolysis (LFP). In this approach, ^{t}BuO$^\bullet$ was generated by irradiating di-*tert*-butyl peroxide (DTBPO) with a Nd:YAG laser (2 ns pulse) in the presence of an amine.[12]

Photolysis of DTBPO/MPTP (benzene solvent) gave rise to a transient species (λ_{max} 385 nm) assigned to the MPTP-derived radical **3**. Similar spectra were obtained for MPTP-d_2, -d_4, and -d_7.) By measuring the observed rate constant for formation of this species as a function of amine concentration, absolute rate constants for hydrogen abstraction from MPTP and deuterium-substituted-MPTP by ^{t}BuO$^\bullet$ were determined. The results are summarized in Table 1.

Scheme 1

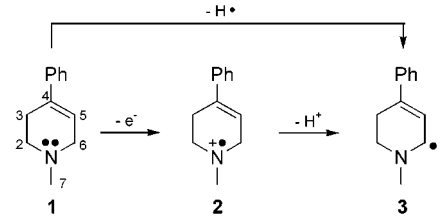
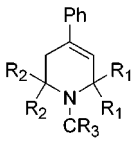


Table 1. Absolute Rate Constants and H/D Isotope Effects for Reaction of ^{t}BuO$^\bullet$ with MPTP (and Deuterated Derivatives)



MPTP ($R_1 = R_2 = R_3 = H$)
MPTP-d_2 ($R_1 = D$; $R_2 = R_3 = H$)
MPTP-d_4 ($R_1 = R_2 = D$; $R_3 = H$)
MPTP-d_7 ($R_1 = R_2 = R_3 = D$)

substrate	k (M^{-1} s^{-1})	k_H/k_D
MPTP	2.27 (±0.06) × 10^8	
MPTP-d_2	1.60 (±0.14) × 10^8	1.41 (±0.13)[a]
MPTP-d_4	1.21 (±0.20) × 10^8	1.31 (±0.25)[b]
MPTP-d_7	1.07 (±0.15) × 10^8	1.13 (±0.25)[c]

[a] d_2 vs d_0. [b] d_4 vs d_2. [c] d_7 vs d_4.

The magnitude of the observed isotope effect (k_H/k_D, Table 1) is consistent with hydrogen abstraction by ^{t}BuO$^\bullet$ at C-6 (the allylic position). The observation of a small primary isotope effect at C-6 was expected because this α-C−H bond is the weakest bond in the molecule (ca. 80 kcal/mol). However, the isotope effects at C-2 and C-7 (the *N*-methyl group, Table 1) suggest that ^{t}BuO$^\bullet$ abstracts hydrogen from *all* α-carbons of MPTP, and not only the allylic position. These results were unanticipated because the C-2 and C-7 C−H bonds are at least 10 kcal/mol stronger.[13,14] The above findings are in contrast to the MAO-B- catalyzed pathway, where *only* allylic α-C−H bond cleavage is observed; no isotope effect is observed at either the C-2 or *N*-methyl positions.[15]

Thus, despite large differences in bond strengths, ^{t}BuO$^\bullet$ exhibits little or no selectivity in hydrogen abstractions from MPTP. In fact, ^{t}BuO$^\bullet$ seems to exhibit little or no selectivity in hydrogen abstractions from amines, *in general*. For example, the rate constant for reaction of ^{t}BuO$^\bullet$ with triethylamine[12] and MPTP are nearly identical, *despite the fact that the α-C−H bond in MPTP is weaker by at least 10 kcal/mol*.[14]

It is tempting to explain these results on the basis of the reactivity/selectivity principle. The strength of the O−H bond in *t*-BuOH is 105 kcal/mol, while the α-C−H bonds of most amines lie in the range ca. 80−90 kcal/mol.[13,14] Consequently, the hydrogen abstraction process is exothermic by at least 15 kcal/mol, and because of its high reactivity, ^{t}BuO$^\bullet$ is expected to exhibit low selectivity. However, given the extreme exothermicity of these reactions, several questions arise: Why are these reactions so slow? Why are they *not* diffusion-controlled?

In an attempt to answer these questions, rate constants for reaction of ^{t}BuO$^\bullet$ with several amines were measured over a temperature range of 10−70 °C. Because in most cases, the

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(1) Karki, S. B.; Dinnocenzo, J. P. *Xenobiotica* **1995**, *25*, 711−724.
(2) Manchester, J. I.; Dinnocenzo, J. P.; Higgins, L.; Jones, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 5069−5070.
(3) Choi, S.-Y.; Eaton, P. E.; Hollenberg, P. F.; Liu, K. E.; Lippard, S. J.; Newcomb, M.; Putt, D. A.; Upadhyaya, S. P.; Xiong, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6547−6555.
(4) MacFaul, P. A.; Arends, I. W. C. E.; Ingold, K. U.; Wayner, D. D. M. *J. Chem. Soc., Perkin Trans. 2* **1997**, 135−145.
(5) Franot, C.; Mabic, S.; Castagnoli, N., Jr. *Bioorg. Med. Chem.* **1997**, *5*, 1519−1529.
(6) Franot, C.; Mabic, S.; Castagnoli, N., Jr. *Bioorg. Med. Chem.* **1998**, *6*, 283−291.
(7) Heikkila, R. E.; Manzino, L.; Duvoisin, R. C.; Cabbat, F. S. *Nature* **1984**, *311*, 467−469.
(8) Langston, J. W.; Irwin, I.; Langston, E. B. *Science* **1984**, *225*, 1480−1482.
(9) Chiba, K.; Peterson, L. A.; Castagnoli, K. P.; Trevor, A. J.; Castagnoli, N., Jr. *Drug Metab. Dispos.* **1985**, *13*, 342−347.
(10) Edmondson, D. E. *Xenobiotica* **1995**, *25*, 735−753.
(11) Silverman, R. B. *Acc. Chem. Res.* **1995**, *28*, 335−342.
(12) Griller, D.; Howard, J. A.; Marriott, P. R.; Scaiano, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 619−623.
(13) Wayner, D. D. M.; Clark, K. B.; Rauk, A.; Yu, D.; Armstrong, D. A. *J. Am. Chem. Soc.* **1997**, *119*, 8925−8932.
(14) Dombrowski, G. W.; Dinnocenzo, J. P.; Farid, S.; Goodman, J. L.; Gould, I. R. *J. Org. Chem.* **1999**, *64*, 427−431.
(15) Ottoboni, S.; Caldera, P.; Trevor, A.; Castagnoli, N., Jr. *J. Biol. Chem.* **1989**, *264*, 13684−13688.

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Table 2. Rate Constants and Activation Parameters for Reaction of ^{t}BuO$^\bullet$ with Several Amines

	amine	BDE (kcal/mol)	k_H^{22} (M^{-1} s^{-1})[a]	E_a (kcal/mol)	log A
1	$(CH_2{=}CHCH_2)_3N$	82.6[b]	4.42×10^7	0.076 (±0.43)	7.70 (±0.31)
2	$PhN(CH_2Ph)_2$	85.4[b]	2.78×10^7	1.12 (±0.62)	8.27 (±0.44)
3	$(PhCH_2)_3N$	89.1[b]	4.91×10^7	0.22 (±0.30)	7.85 (±0.21)
4	$(CH_3CH_2)_3N$	90.7[b]	1.59×10^8	2.45 (±0.34)	10.0 (±0.24)
5	$PhN(CH_3)_2$	91.7[b]	1.00×10^8	2.41 (±0.27)	9.78 (±0.19)
6	quinuclidine	95.8[c]	9.26×10^6	2.43 (±0.46)	8.76 (±0.32)

[a] Rate constant at 22 °C, calculated from activation parameters. [b] Literature, ref 14. [c] Calculated, using energies obtained via density functional theory (B3LYP/cc-pVTZ).

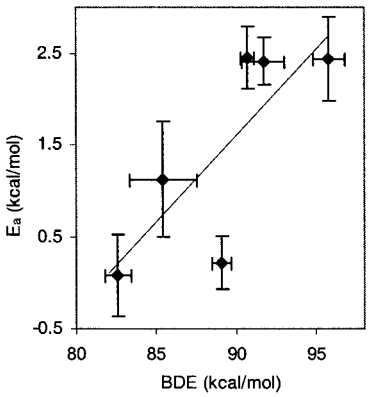


Figure 1. Activation energy for hydrogen abstraction from amines by ^{t}BuO$^\bullet$ as a function of α-C−H bond strength.

radicals resulting from hydrogen abstraction from these amines do not give a strong UV/vis absorption, diphenylmethanol (DPM) was used as a probe.[12] Activation parameters were determined by fitting to the Arrhenius equation.[16] The results of these experiments are summarized in Table 2. (Rate constants at 22 °C, calculated from the activation parameters, compare well to values in the literature (units M^{-1} s^{-1}): $(CH_3CH_2)_3N$, 1.80×10^8;[12] $PhN(CH_3)_2$, 1.4×10^8;[17] quinuclidine, 6.00×10^6).[12] The rate constants do not reveal any sensible structure/reactivity trend: *Substrates with the weakest C−H bond react with ^{t}BuO$^\bullet$ at a lower rate*! However, as illustrated in Figure 1, the activation energies do parallel the strength of the C−H bond.

Clearly, the intrinsic reactivity trend observed in the E_a's is offset by the preexponential (*A*-factor) term in the Arrhenius equation. It is in fact the magnitude of the *A*-factor which is preventing these rate constants from being diffusion controlled. This situation is easy to understand if rather than Arrhenius parameters (E_a, *A*), the discussion is recast in terms of the enthalpy and entropy of activation. A plot of $T\Delta S^\ddagger$ ($T = 298$ K) vs $\Delta H^\ddagger$ (Figure 2) reveals that for all these amines (a) $T\Delta S^\ddagger > \Delta H^\ddagger$ (i.e., at room temperature, the entropy of activation contributes more to the barrier than does the enthalpy of activation), and (b) substrates which have a low enthalpy of activation (because of stabilization of the resulting radical) have a higher cost to "pay" in terms of entropy (reflecting a more highly ordered transition state). There are two considerations pertaining to the entropy of the transition state for these reactions: (1) In the transition state for hydrogen abstraction, the C−H bond must align with the nitrogen lone pair (and the orbitals of an adjacent π-system, when present) for maximum resonance stabilization of the developing radical center (Scheme 2). (2) Most of the surface of ^{t}BuO$^\bullet$ is unreactive, aliphatic bulk. Hence, there are stringent requirements associated with the trajectory of the approach of ^{t}BuO$^\bullet$ to the amine.

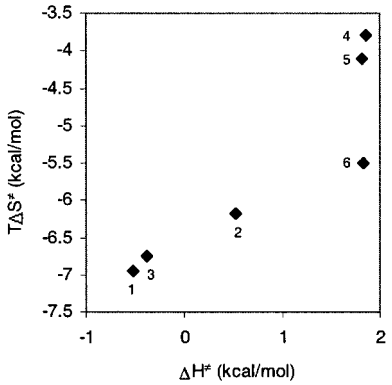
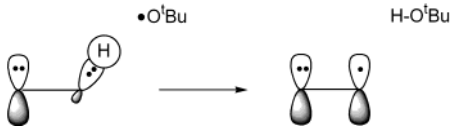


Figure 2. $T\Delta S^\ddagger$ vs $\Delta H^\ddagger$ for hydrogen abstractions from amines by ^{t}BuO$^\bullet$.

Scheme 2



Thus, intrinsic reactivities are masked because the rate constants for hydrogen abstractions from amines by ^{t}BuO$^\bullet$ are governed by entropy considerations. Entropy requirements also explain why these reactions are not diffusion-controlled. This issue is likely not to be restricted simply to amines, but is likely to be important for substrates whose C−H bond strengths are less than ca. 95 kcal/mol. ^{t}BuO$^\bullet$ is not "peculiar" per se, although its bulk and asymmetry will require a more ordered transition state than smaller, more symmetric radicals (e.g., HO•, Cl•), the entropy requirements are not unusually stringent.

Is ^{t}BuO$^\bullet$ a good model for radicals in biological systems? In terms of regiochemistry, the answer is unequivocally no, at least for reactive substrates (i.e., C−H bonds weaker than 95 kcal/mol). For such substrates, the reaction rate and regiochemistry are dominated by entropy considerations—intrinsic reactivity patterns are masked. (It is unlikely the entropy requirements associated with ^{t}BuO$^\bullet$ would in any way serve as an effective model for entropy requirements in an enzyme active site). Many, and perhaps most, substrates of biological significance contain heteroatoms (N, O, S) or sites of unsaturation which stabilize radicals, resulting in weak C−H bonds. Hence, these considerations are not simply restricted to amines. In terms of H/D isotope effects (or trends in isotope effects with a series of substrates), ^{t}BuO$^\bullet$ *may* be a reasonable model (for an extremely reactive radical center). It is likely that the entropy requirements and transition state structure will not change with the substitution of D for H in a substrate. Thus, the magnitude of the isotope (k_H/k_D) should be a reasonable measure of the differences in activation energy for the hydrido and deutero analogues.

Inevitably, as with any model, the usefulness of ^{t}BuO$^\bullet$ requires a thorough understanding of its chemical properties. In the proper context, ^{t}BuO$^\bullet$ can be an effective model for reactive radicals in biological systems. Improperly used, it can lead to confusing and perhaps meaningless results.

Warning: MPTP is a known nigrostriatal neurotoxin and should be handled using disposable gloves in a properly ventilated hood. Detailed procedures for the safe handling of MPTP have been reported.[18]

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(16) Benson, S. W. *The Foundations of Chemical Kinetics*; McGraw-Hill: New York, 1960.

(17) Dinnocenzo, J. P.; Karki, S. B.; Jones, J. P. *J. Am. Chem. Soc.* **1993**, *115*, 7111−7116.

(18) Pitts, S. M.; Markey, S. P.; Murphy, D. L.; Weisz, A.; Lunn, G. *Recommended Practices for the Safe Handling of MPTP In MPTP-A Neurotoxin Producing a Parkinsonian Syndrome*; Academic: New York, 1986; pp 703−716.