pure fluoride: mp 116–118 °C; $[\alpha]^{28}_D$ = +3.0 (c 1, EtOAc); IR (KBr) 1846 cm^{-1} (COF); ^{1}H NMR ($CDCl_3$) δ 2.95 (dq, 2, β-CH_2), 4.20 (t, 1, OCH_2CH), 4.40 (m, 2, CH_2O), 4.79 (m, 1, α-CH), 5.95 (d, 1, NH), 6.75 (s, 1, NH), 7.05–7.80 (m, 23, aryl).

Anal. Calcd for $C_{38}H_{31}FN_2O_4$: C, 76.24; H, 5.22; N, 4.68. Found: C, 76.15; H, 5.43; N, 4.52.

FMOC-Gln(Trt)-F. Obtained as described above for the Asn analog in 81.7% yield: mp 132–134 °C; $[\alpha]^{28}_D$ = −7.2 (c 1, EtOAc); IR (KBr) 1847 cm^{-1} (COF); ^{1}H NMR ($CDCl_3$) δ 2.10 and 2.20 (2 m, 2, β-CH_2), 2.38 (t, 2, γ-CH_2), 4.20 (t, 1, OCH_2CH), 4.35 (m, 2, CH_2O). 4.55 (m, 1, α-CH), 5.65 (d, 1, NH), 6.68 (s, 1, NH), 7.1–7.75 (m, 23, aryl).

Anal. Calcd for $C_{39}H_{33}FN_2O_4$: C, 76.45; H, 5.43; N, 4.57. Found: C, 76.62; H, 5.49; N, 4.32.

Acknowledgment. We are indebted to the National Science Foundation (NSF CHE-9003192) and the National Institutes of Health (GM-09706) for support of this work. The National Science Foundation is also thanked for grants used to purchase the high-field NMR spectrometers used in this research.

Evidence for a Hydrogen Atom Transfer Mechanism or a Proton/Fast Electron Transfer Mechanism for Monoamine Oxidase

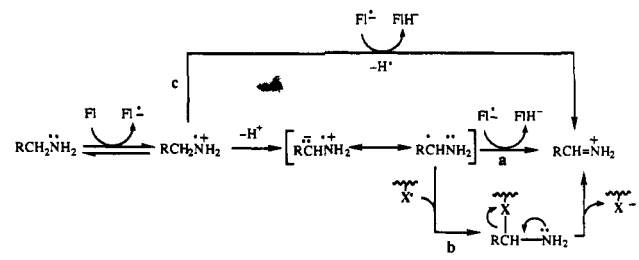
Richard B. Silverman* and Yury Zelechonok

Department of Chemistry, Department of Biochemistry, Molecular Biology and Cell Biology, and the Institute for Neuroscience, Northwestern University, Evanston, Illinois 60208-3113

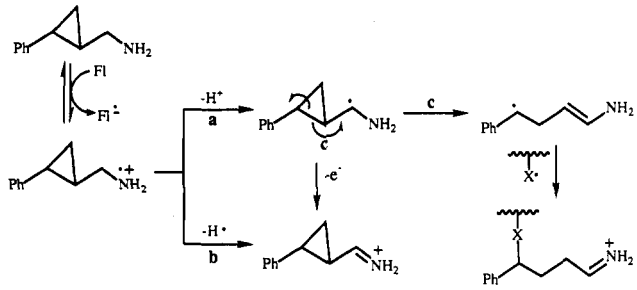
Received July 13, 1992

Monoamine oxidase (EC 1.4.3.4, MAO) has been known for over 60 years,[1] yet its mechanism for amine oxidation is still unclear. On the basis of investigations with mechanism-based enzyme inactivators[2] the most reasonable family of mechanisms for this enzyme is shown in Scheme I. Studies with cyclopropylamines[3–13] and cyclobutylamines[14,15] are consistent with an initial one-electron transfer mechanism from the amine to the flavin to give the amine radical cation. The next step in the mechanism, either proton/electron transfer (Scheme I, pathway a or b) or hydrogen atom transfer (pathway c), is debatable. Nelson and Ippoliti[16] have indicated that

Scheme I



Scheme II



α-deprotonation of an amine radical cation is not as favorable a pathway as hydrogen atom abstraction on the basis of a thermodynamic acidity measurement. However, Dinnocenzo and Banach[17] argued that the thermodynamic acidity is irrelevant and that the true pK_a for amine radical cations, determined by direct measurement, is no more than about 10; consequently, they suggest that α-deprotonation is a favorable pathway. Das and von Sonntag[18] determined the pK_a of trimethylamine radical cation to be 8. On the basis of photochemical amine oxidation reactions Hasegawa et al.[19] also have evidence that the α-deprotonation/electron-transfer pathway is favorable. We were interested in obtaining evidence for one of these pathways as it relates to monoamine oxidase-catalyzed reactions.

Results and Discussion

The approach that we took to differentiate the second step in the MAO oxidation mechanism was based on the cyclopropylcarbinyl radical rearrangement to the 3-butenyl radical, which is well known to occur at an exceedingly rapid rate estimated to be approximately 10^8 s^{-1}.[20] This rate increases when the cyclopropyl ring is substituted with radical-stabilizing groups. For example, the 2-phenylcyclopropylcarbinyl radical opens to the 1-phenyl-3-butenyl radical at a rate of approximately 10^{11} s^{-1}.[21] This is the sort of rate that could be competitive with electron-transfer mechanisms. Consequently, we synthesized *trans*-1-(aminomethyl)-2-phenylcyclopropane hydrochloride (1) to be used in a test for the α-deprotonation/electron transfer mechanism (pathway a) or hydrogen atom transfer mechanism (pathway b) catalyzed by monoamine oxidase (Scheme II). Monoamine oxidase-catalyzed cyclopropyl ring cleavage of 1, which could lead to inactivation of the enzyme as depicted in pathway c (Scheme II), would be evidence in favor of an α-deprotonation pathway leading to the α-carbon radical (2). If no cyclopropyl ring cleavage occurred, it would indicate

(1) Hare, M. L. C. *Biochem. J.* **1928**, *22*, 968–979.
(2) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, FL, 1988; Vols. I and II.
(3) Silverman, R. B.; Hoffman, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 884–886.
(4) Silverman, R. B.; Hoffman, S. J.; Catus, W. B., III. *J. Am. Chem. Soc.* **1980**, *102*, 7126–7128.
(5) Silverman, R. B.; Hoffman, S. J. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 1396–1401.
(6) Silverman, R. B. *J. Biol. Chem.* **1983**, *258*, 14766–14769.
(7) Silverman, R. B.; Yamasaki, R. B. *Chemistry* **1984**, *23*, 1322–1332.
(8) Silverman, R. B. *Biochemistry* **1984**, *23*, 5206–5213.
(9) Silverman, R. B.; Zieske, P. A. *Biochemistry* **1985**, *24*, 2128–2138.
(10) Silverman, R. B.; Zieske, P. A. *J. Med. Chem.* **1985**, *28*, 1953–1957.
(11) Vazquez, M. L.; Silverman, R. B. *Biochemistry* **1985**, *24*, 6538–6543.
(12) Yamasaki, R. B.; Silverman, R. B. *Biochemistry* **1985**, *24*, 6543–6550.
(13) Silverman, R. B.; Zieske, P. A. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 154–159.
(14) Silverman, R. B.; Zieske, P. A. *Biochemistry* **1986**, *25*, 341–346.
(15) Yelekci, K.; Lu, X.; Silverman, R. B. *J. Am. Chem. Soc.* **1989**, *111*, 1138–1140.
(16) Nelson, S. F.; Ippoliti, J. T. *J. Am. Chem. Soc.* **1986**, *108*, 4879–4881.
(17) Dinnocenzo, J. P.; Banach, T. E. *J. Am. Chem. Soc.* **1989**, *111*, 8646–8653.
(18) Das, S.; von Sonntag, C. *Z. Naturforsch.* **1986**, *41B*, 505–513.
(19) Hasegawa, E.; Xu, W.; Mariano, P. S.; Yoon, U.-C.; Kim, J.-U. *J. Am. Chem. Soc.* **1988**, *110*, 8099–8111.
(20) Newcomb, M.; Glenn, A. G. *J. Am. Chem. Soc.* **1989**, *111*, 275–277.
(21) Newcomb, M.; Manek, M. B. *J. Am. Chem. Soc.* **1990**, *112*, 9662–9663.

0022-3263/92/1957-6373$03.00/0

that either α-carbon radical formation via α-deprotonation does not occur or that second electron transfer from the α-carbon radical occurs faster than does cyclopropyl ring cleavage.

Incubation of purified mitochondrial MAO with *trans*-1-(aminomethyl)-2-phenylcyclopropane (1) cleanly produced only one product, identified as *trans*-2-phenylcyclopropanecarboxaldehyde, the oxidation and hydrolysis product without ring cleavage (the hydrolysis product of 3). A kinetic analysis showed that 1 is a good substrate for MAO with K_m = 1.41 mM and k_{cat} = 22 min^{-1}.[22] No inactivation of the enzyme occurred even at a concentration of 20 mM of 1 for 3 h. Chemical model studies[23] of the fate of synthesized radical 2 (in the absence of a hydrogen atom donor) showed that no 2-phenylcyclopropanecarboxaldehyde is formed, only cyclopropyl ring cleavage products. These results suggest that either radical 2 is not an intermediate in the MAO-catalyzed oxidation of 1 or, if it is, second electron transfer to the flavin semiquinone occurs at a rate considerably faster than that for the opening of the cyclopropyl ring of 2. The rate of cyclopropyl ring opening in this case, however, should be somewhat slower than that measured for the cleavage of the 2-phenylcyclopropylcarbinyl radical[21] because of the amino group stabilization energy, which has been determined to be about 10 kcal/mol.[24] Also, it has been shown by Laurie et al.[25] that cyclopropylcarbinyl radicals to which radical-stabilizing groups have been attached can be quite stable. Another possible explanation for lack of ring opening is that because of constraints at the active site of the enzyme there is improper overlap between the cyclopropane bond and the carbon radical, thereby slowing the rate of this ring cleavage.

Experimental Section

Reagents and Enzyme. All reagents are from Aldrich Chemical Co., Inc. Mitochondrial monoamine oxidase was isolated from beef liver and assayed as previously reported.[9]

General Methods. Melting points are uncorrected. Elemental analyses were done by Oneida Research Services (Whitesboro, NY).

***trans*-2-Phenylcyclopropanecarboxamide.** *trans*-2-Phenylcyclopropanecarboxylic acid (2.4 g, 14.8 mmol), anhydrous potassium carbonate (4.1 g, 30 mmol), and thionyl chloride (19.0 g 162.8 mmol) were brought to reflux for 1 h. Excess thionyl chloride was removed in vacuo, and then the resulting liquid was added dropwise over 15 min to ammonia hydroxide (10 mL) at 0-5 °C. The amide was extracted with $CHCl_3$ and dried (Drierite), and the solvent was evaporated to give the product (2.2 g, 92%) as a white solid: mp 197-198 °C; ^1H NMR (DMSO-d_6) δ 1.10-1.20 (m, 1 H), 1.40-1.50 (m, 1 H), 1.85-2.00 (m, 1 H), 2.25-2.40 (m, 1 H), 6.80 (s, 1 H), 7.10-7.40 (m, 5 H), 7.61 (s, 1 H). Anal. Calcd for $C_{10}H_{11}NO$: C, 74.53; H, 6.83; N, 8.70. Found: C, 74.01; H, 6.97; N, 8.62.

***trans*-1-(Aminomethyl)-2-phenylcyclopropane (1).** To a solution of *trans*-2-phenylcyclopropanecarboxamide (2.2 g, 13.7 mmol) in THF (20 mL) was added $LiAlH_4$ (0.65 g, 17 mmol), and the mixture was brought to reflux. After 2 h the reaction was quenched with water (5 mL), and filtered, the THF solution was dried, and the solvent was treated with HCl gas. The solvent was removed in vacuo, and the resulting colorless solid was recrystallized twice from 1:1 ethanol-ether to give 1 as colorless crystals (2.0 g, 74%): mp 192-193 °C; ^1H NMR ($CDCl_3$-DMSO-d_6 (4:1), relative to Me_4Si) δ 1.00-1.10 (m, 1 H), 1.45-1.60 (m, 1 H), 1.90-2.10 (m, 1 H), 2.80-3.05 (m, 3 H), 7.05-7.35 (m, 5 H), 8.50 (s, 2 H); ^{13}C NMR ($CDCl_3$-DMSO-d_6 (4:1), relative to Me_4Si) δ 15, 21, 23, 45, 131, 132, 134, 148. Anal. Calcd for $C_{10}H_{14}ClN$: C, 65.40; H, 7.63; N, 7.63. Found: C, 65.26; H, 7.76; N, 7.52.

Incubation of Monoamine Oxidase with 1. Compound 1 (60 μL of a 25 mM solution in 250 mM Tris-HCl buffer, pH 9.0) and 2 μM MAO (60 μL in the same buffer) were incubated at room temperature. After 2 h, 60 μL of $CHCl_3$ and 60 μL of 5% HCl were added. The mixture was shaken well for 5 min and centrifuged, and the organic layer was used for GC analysis on a HP5880A GC with flame ionization detector and a HP cross-linked methyl silicone capillary column (gradient column temperature was from 50 to 250 °C at a rate of 20 °C/min; detector and injector port temperatures were 250 °C). Only *trans*-2-phenylcyclopropanecarboxaldehyde was observed. Neither of the expected ring cleavage products, 4-phenylbutanal (cleavage and reduction) or 2-hydroxy-5-phenyltetrahydrofuran[23] (cleavage and oxidation), was detected.

Attempted Inactivation of Monoamine Oxidase with 1. Compound 1 (final concentration 20 mM) was incubated with MAO (2 μM) in 100 mM sodium phosphate buffer, pH 8.0, at room temperature. Periodically over 3 h aliquots (20 μL) were removed and assayed for remaining enzyme activity.

Acknowledgment. We are grateful to the National Institutes of Health (GM32634) for financial support of this research.

(22) K_m and k_{cat} values for benzylamine, an excellent substrate for MAO B, were determined under these conditions to be 0.18 mM and 162 min^{-1}, respectively.

(23) Zelechonok, Y.; Silverman, R. B. *J. Org. Chem.*, in press.

(24) Burkey, T. J.; Castelhano, A. L.; Griller, D.; Lossing, F. P. *J. Am. Chem. Soc.* **1983**, *105*, 4701-4703.

(25) Laurie, D.; Lucas, E.; Nonhebel, D. C.; Suckling, C. J.; Walton, J. C. *Tetrahedron* **1986**, *42*, 1035-1045.

Highly Regioselective Bromination of 2,3-Dimethylanisole with *N*-Bromosuccinimide

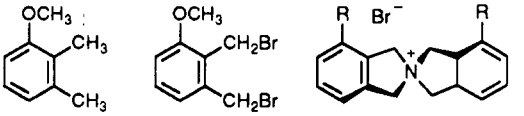
Yuri Goldberg, Corrine Bensimon, and Howard Alper*

Ottawa-Carleton Chemistry Institute, Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Received June 15, 1992

Introduction

The free-radical side-chain bromination of 2,3-dimethylanisole (1) could be a possible route to 2,3-bis(bromomethyl)anisole (2), a known precursor of the spiroindolinium salt 3 (R = OCH_3).[1] Compound 2 was



1 2 3, R = OCH_3, NO_2

prepared previously by multistep synthesis including oxidation of 1 to methoxyphthalic acid, conversion to the anhydride, and reduction of the latter to 2,3-bis(hydroxymethyl)anisole, followed by the treatment with phosphorus tribromide.[1] At the same time, the free-radical bromination of 2,3-dimethylnitrobenzene with NBS did give the corresponding bis(bromomethyl) derivative (although in low yield) which was also converted to salt 3 (R = NO_2).[1] It is for this reason that we examined the reaction of 1 with NBS expecting to obtain a mixture containing reasonable amounts of the desired compound 2. However, the experimental findings revealed rather unexpected results.

Results and Discussion

When a mixture of 1 (2 equiv), NBS (1 equiv) and benzoyl peroxide (0.01 equiv) was heated under reflux in

(1) Brewster, J. H.; Jones, R. J., Jr. *J. Org. Chem.* **1969**, *34*, 354.

0022-3263/92/1957-6374$03.00/0