Novel 4-(Aryloxy)tetrahydropyridine Analogs of MPTP as Monoamine Oxidase A and B Substrates

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The exceptionally good monoamine oxidase (MAO) substrate properties of several 4-(arylmethyl)-1-methyl-1,2,3,6-tetrahydropyridine derivatives related to the neurotoxin MPTP have prompted studies to evaluate the corresponding properties of tetrahydropyridine derivatives bearing heteroatom-linked groups at C-4. The expected dihydropyridinium metabolites generated from these MAO-A- and MAO-B-catalyzed oxidations of the 4-(aryloxy)tetrahydropyridine analogs were found to undergo rapid hydrolytic cleavage to yield the corresponding arenol and 1-methyl-2,3-dihydro-4-pyridone, a species that could be monitored spectrophotometrically. We have exploited this reaction sequence to probe the active sites of beef liver MAO-B and human placental MAO-A with a variety of 4-(aryloxy)-1-methyl-1,2,3,6-tetrahydropyridine derivatives. The results are discussed in relationship to recently published reports describing the MAO-A vs MAO-B selectivity of various 4-(arylmethyl)tetrahydropyridine derivatives.

Introduction

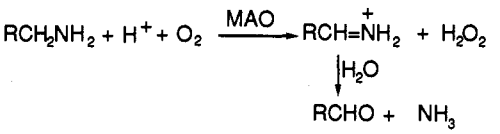
The monoamine oxidases (MAO) A and B are the principal enzymes which catalyze the oxidative deamination of the catecholamine and indolealkylamine neurotransmitters[1,2] as well as a variety of acyclic aliphatic amines.[3,4] The overall stoichiometry of the reaction involves the consumption of 1 mol of oxygen and amine substrate and the formation of 1 mol of hydrogen peroxide and the iminium metabolite derived from the parent amine. Subsequent hydrolysis of the iminium intermediate yields the corresponding aldehyde and ammonia (Scheme 1).

A cyclic tertiary amine substrate of MAO-A and MAO-B is the parkinsonian-inducing nigrostriatal neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (1) which is bioactivated by the B form of the enzyme to generate the dihydropyridinium intermediate $MPDP^+$ (2). Hydrolysis of this cyclic iminium intermediate is reversible, a feature which may contribute to its susceptibility to further oxidation to yield the pyridinium species MPP^+ (3), the ultimate neurotoxin responsible for the neurodegenerative properties of the parent tetrahydropyridine (Scheme 2).[5-7]

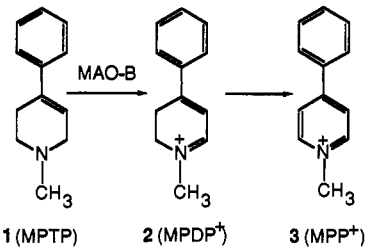
MPTP and structurally related 1-methyl-4-substitutedtetrahydropyridines are the only cyclic amines which are reported to be good substrates for MAO. With the exception of the nature of the C-4 substituent and possibly the replacement of the *N*-methyl group with a proton,[8] essentially all structural variations studied to date lead to dramatic losses in substrate activity.[9,10] These unusual properties together with the general interest in the mechanism by which MPTP destroys nigrostriatal neurons have led us[11-14] and others[9,10,15-17] to examine a variety of MPTP analogs in an effort to characterize those structural features of tetrahydropyridines that lead to good MAO substrate and inhibitor properties.

Structural modifications at the C-4 position of MPTP (replacement of the phenyl group with an arylmethyl group) have been shown to alter the substrate selectivity toward the two forms of the enzyme with the B form of the enzyme showing less tolerance to bulky groups.[18-21]

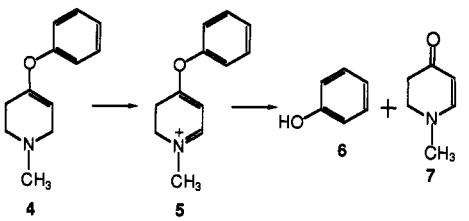
Scheme 1. MAO-Catalyzed Oxidation of Amines



Scheme 2. MAO-B-Catalyzed Oxidation of MPTP (1)



Scheme 3. MAO-B-Catalyzed Oxidation of 1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (4)



The most important determinants appear to be the flexibility and the molecular size of the C-4 substituent. Other structural features, however, are likely to contribute to substrate/enzyme interactions since attempts to formulate reliable models to predict the effects of structural variations of the C-4 substituent on the turnover number (TN) and K_m values for individual substrates have been only partially successful.

The MAO-B-catalyzed oxidation of 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (4) has been shown to form a dihydropyridinium intermediate, 5, that undergoes spontaneous hydrolysis to yield phenol (6) and the amino enone 7 (Scheme 3).[13] Since 7 displays a strong chromophore (λ_{max} 324 nm, ϵ 15 350 M^{-1}),[22] we were able to monitor the rates of increase of absorbance at 324 nm vs time to estimate the initial rates of oxidation of 4 to the

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corresponding dihydropyridinium metabolite 5. In this paper, we present the results of analogous studies using both beef liver MAO-B and human placental MAO-A on a variety of 4-(aryloxy)-1-methyl-1,2,3,6-tetrahydropyridine derivatives which were selected principally to assess how the steric bulk of the C-4 substituent influences substrate properties as measured by TN/K_m.

Experimental Section

Chemistry. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. (*R*)-(−)-Deprenyl and clorgyline were obtained from Research Biochemicals Inc., Natic, MA. All other chemicals were reagent, enzyme, or HPLC grade. ^{1}H NMR spectra were recorded on a Bruker WP 270 MHz spectrophotometer; chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), or m (multiplet). Gas chromatography–electron ionization mass spectrometry (GC-EIMS) was performed using a Hewlett Packard (HP) 5890 capillary column gas chromatograph coupled via direct inlet to an HP 5970B mass selective detector controlled by an HP 59970C MS ChemStation. Except where noted, the capillary column used was an HP-1 (12.5-m × 200-mm × 0.33-mm film thickness). UV–vis spectra and enzyme kinetic studies were performed on a Beckman DU Series 50 spectrophotometer. Microanalyses, performed by Atlantic Microlab, Inc., Norcross, GA, were within 0.4% of the theoretical values calculated for C, H, and N. The amino enone 7,[22] 4-chloro-1-methylpyridinium iodide (8),[23] and 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (4)[13] were synthesized as described previously.

General Procedure for the Synthesis of 4-(Aryloxy)-1-methylpyridinium Iodide Salts. A solution containing the arenol (2 mmol), freshly distilled triethylamine (3 mmol), and 4-chloro-1-methylpyridinium iodide (2 mmol) in 25 mL of dry acetone was stirred under nitrogen overnight. The solvent was concentrated, and the residue was treated with saturated $NaHCO_3$. The aqueous solution was extracted with dichloromethane (2 × 25 mL), and the combined organic extracts were dried (Na_2SO_4) and filtered. Diethyl ether was added dropwise to the filtrate to afford the essentially pure pyridinium iodide which was recrystallized from dichloromethane/diethyl ether unless otherwise indicated.

1-Methyl-4-(α-naphthoxy)pyridinium iodide (9·I) was obtained in 41% yield: mp 199–200 °C; ^{1}H NMR (DMSO-d_6) δ 9.3 (d, 2 H, C-2 and C-6), 7.2–8.1 (m, 9 H, C-3, C-5, and ArH), 4.6 (s, 3 H, NCH_3); UV (methanol) λ_{max} 279 nm (ε 6050 M^{-1}); GC-EIMS (temperature program, 40 °C for 1 min followed by a ramp of 20 °C/min for 12 min) showed a single peak with t_R = 5.25 min and *m/z* 221 ($M - 15^{\cdot+}$, 100), 115 (68), 51 (40). Anal. ($C_{16}H_{14}NOI$) C, H, N.

1-Methyl-4-(β-naphthoxy)pyridinium iodide (10·I) was obtained in 64% yield: mp 230–233 °C; ^{1}H NMR (DMSO-d_6) δ 8.8 (d, 2 H, C-2 and C-6), 7.9–8.1 (m, 4 H, C-3, C-5, and ArH), 7.4–7.6 (m, 5 H, ArH), 4.22 (s, 3 H, NCH_3); UV (methanol) end absorption only; GC-EIMS (temperature program, 40 °C for 1 min followed by a ramp of 20 °C/min for 12 min) showed a single peak with t_R = 5.46 min and *m/z* 221 ($M - 15^{\cdot+}$, 100), 115 (60), 51 (41). Anal. ($C_{16}H_{14}NOI \cdot 0.25\ H_2O$) C, H, N.

4-(4-Butylphenoxy)-1-methylpyridinium iodide (11·I) was obtained in 50% yield: mp 98–100 °C; ^{1}H NMR ($CDCl_3$) δ 9.3 (d, 2 H, C-2 and C-6), 7.0–7.4 (m, 6 H, C-3, C-5, and ArH), 4.5 (s, 3 H, NCH_3), 2.8 (m, 2 H, butyl CH_2), 1.7 (m, 2 H, butyl CH_2), 1.4 (m, 2 H, butyl CH_2), 1.0 (m, 3 H, butyl CH_3); UV (methanol) end absorption only; GC-EIMS (temperature program, 50 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 7.34 min and *m/z* 227 ($M - 15^{\cdot+}$, 26), 184 (100), 107 (20). Anal. ($C_{16}H_{20}NOI$) C, H, N.

4-(4-*tert*-Butylphenoxy)-1-methylpyridinium iodide (12·I) was obtained in 53% yield: mp 187–189 °C; ^{1}H NMR ($CDCl_3$) δ 9.2 (d, 2 H, C-2 and C-6), 7.0–7.6 (m, 6 H, C-3, C-5, and ArH), 4.5 (s, 3 H, NCH_3), 1.4 (s, 9 H, *tert*-butyl CH_3); UV (ethanol) λ_{max} 243 nm (ε 15 275 M^{-1}); GC-EIMS (temperature program, 50 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 6.90 min and *m/z* 227 ($M - 15^{\cdot+}$, 26), 212 (100), 51 (34). Anal. ($C_{16}H_{20}NOI$) C, H, N.

1-Methyl-4-(4-phenylphenoxy)pyridinium iodide (13·I) was obtained in 62% yield from methanol/diethyl ether: mp 258–260 °C; ^{1}H NMR (DMSO-d_6) δ 8.9 (d, 2 H, C-2 and C-6), 7.4–8.0 (m, C-3, C-5, and ArH), 4.3 (s, 3 H, NCH_3); UV (pH 7.4 sodium phosphate buffer) λ_{max} 250 nm (ε 22 125 M^{-1}). Anal. ($C_{18}H_{16}NOI$) C, H, N.

4-(2,4-Dichlorophenoxy)-1-methylpyridinium iodide (14·I) was obtained in 64% yield: mp 184–185 °C; ^{1}H NMR (DMSO-d_6) δ 8.8 (d, 2 H, C-2 and C-6), 7.6-8.0 (m, 5 H, C-3, C-5, and ArH), 4.3 (s, 3 H, NCH_3); UV (methanol) end absorption only; GC-EIMS (temperature program, 50 °C/min for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 6.94 min and *m/z* 239 ($M - 15^{\cdot+}$, 34), 204 (44), 162 (26), 78 (82), 51 (100). Anal. ($C_{12}H_{10}Cl_2NOI$) C, H, N.

4-(9-Phenanthroxy)-1-methylpyridinium iodide (15·I) was obtained in 41% yield: mp 235–236 °C; ^{1}H NMR (DMSO-d_6) δ 8.9–9.1 (2d, 2 H, 2 ArH), 8.8 (d, 2 H, C-2 and C-6), 7.7–8.1 (m, 9 H, C-3, C-5, and 7 ArH), 4.2 (s, 3 H, NCH_3); UV (methanol) λ_{max} 250 nm (ε 55 250 M^{-1}); GC-EIMS (temperature program, 100 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 8.59 min and *m/z* 271 ($M - 15^{\cdot+}$, 100), 165 (99), 51 (46). Anal. ($C_{20}H_{16}NOI$) C, H, N.

General Procedure for the Synthesis of the Oxalate Salts of 4-(Aryloxy)-1-methyl-1,2,3,6-tetrahydropyridine Derivatives. Sodium borohydride (0.93 mmol) was added in portions to a stirred solution of the appropriate 4-(aryloxy)-1-methylpyridinium iodide (0.78 mmol) in 15 mL of dry methanol at 0 °C. The mixture was stirred for an additional 30 min, and the solvent subsequently was removed under reduced pressure. The residue was taken up in 10 mL of water and the solution extracted with diethyl ether (3 × 20 mL). The combined ether layers were dried (Na_2SO_4), filtered, and concentrated to 50% of the original volume. Treatment of this solution with oxalic acid in 5 mL of diethyl ether precipitated the crude oxalate salt which was recrystallized from methanol/diethyl ether unless noted otherwise.

1-Methyl-4-(α-naphthoxy)-1,2,3,6-tetrahydropyridine oxalate (16·(COOH)$_2$) was obtained in 84% yield: mp 141–142 °C; ^{1}H NMR (DMSO-d_6) δ 7.3–8.0 (m, 7 H, ArH), 5.6 (s, 1 H, C-5), 3.6 (s, 2 H, C-6), 3.4 (t, 2 H, C-2), 2.9 (s, 3 H, NCH_3), 2.6 (s, 2 H, C-3); UV (methanol) λ_{max} 281 nm (ε 5100 M^{-1}); GC-EIMS (temperature program, 40 °C for 1 min followed by a ramp of 20 °C/min for 11 min) showed a single peak with t_R = 7.47 min and *m/z* 239 ($M^{\cdot+}$, 10), 96 (100). Anal. ($C_{18}H_{19}NO_5 \cdot 0.25H_2O$) C, H, N.

1-Methyl-4-(β-naphthoxy)-1,2,3,6-tetrahydropyridine oxalate (17·(COOH)$_2$) was obtained in 88% yield: mp 186–188 °C; ^{1}H NMR (DMSO-d_6) δ 7.3–8.0 (m, 7 H, ArH), 5.6 (s, 1 H, C-5), 3.6 (s, 2 H, C-6), 3.4 (t, 2 H, C-2), 2.9 (s, 3 H, NCH_3), 2.6 (s, 2 H, C-3); UV (methanol) λ_{max} 274 nm (ε 4300 M^{-1}); GC-EIMS (temperature program, 40 °C for 1 min followed by a ramp of 20 °C/min for 11 min) showed a single peak with t_R = 7.64 min and *m/z* 239 ($M^{\cdot+}$, 14), 96 (100). Anal. ($C_{18}H_{16}NO_5$) C, H, N.

4-(4-Butylphenoxy)-1-methyl-1,2,3,6-tetrahydropyridine oxalate (18·(COOH)$_2$) was obtained in 74% yield: mp 113–115 °C; ^{1}H NMR (CD_3OD) δ 7.0–7.4 (d, 4 H, ArH), 4.7 (s, 1 H, C-5), 3.8 (s, 2 H, C-6), 3.6 (m, 2 H, C-2), 3.0 (s, 3 H, NCH_3), 2.6–2.9 (m, 4 H, C-3 and butyl CH_2), 1.7 (m, 2 H, butyl CH_2), 1.4 (m, 2 H, butyl CH_2), 1.0 (t, 3 H, butyl CH_3); UV (methanol) end absorption only; GC-EIMS (temperature program, 40 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 7.38 min and *m/z* 245 ($M^{\cdot+}$, 10), 107 (24), 96 (100). Anal. ($C_{18}H_{25}NO_5$) C, H, N.

4-(4-*tert*-Butylphenoxy)-1-methyl-1,2,3,6-tetrahydropyridine oxalate (19·(COOH)$_2$) was obtained in 51% yield: mp 149–151 °C; ^{1}H NMR (DMSO-d_6) δ 7.0–7.5 (m, 4 H, ArH), 4.7 (s, 1 H, C-5), 3.6 (d, 2 H, C-6), 3.3 (t, 2 H, C-2), 2.7 (s, 3 H, NCH_3), 1.7 (s, 9 H, *tert*-butyl CH_2); UV (methanol) end absorption only; GC-EIMS (temperature program, 50 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 7.08 min and *m/z* 245 ($M^{\cdot+}$, 10), 96 (100). Anal. ($C_{18}H_{25}NO_5$) C, H, N.

1-Methyl-4-(4-phenylphenoxy)-1,2,3,6-tetrahydropyridine oxalate (20·(COOH)$_2$) was obtained in 61% yield: mp

212–214 °C; ^{1}H NMR (DMSO-d_6) δ 7.1–7.7 (m, 9 H, ArH), 4.9 (s, 1 H, C-5), 3.6 (s, 2 H, C-6), 3.3 (t, 2 H, C-2), 2.7 (s, 3 H, NCH_3), 2.5 (s, 2 H, C-3); UV (methanol) λ_{max} 252 nm (ε 10 750 M^{-1}); GC-EIMS (24-m column; temperature program, 100 °C for 1 min followed by a ramp of 20 °C/min for 12 min) showed a single peak with t_R = 10.82 min and m/z 264 ($M^{+\cdot}$, 4), 96 (100). Anal. ($C_{20}H_{21}O_5N\cdot0.5H_2O$) C, H, N.

4-(2,4-Dichlorophenoxy)-1-methyl-1,2,3,6-tetrahydropyridine oxalate (21·(COOH)$_2$) was obtained in 74% yield: mp 141–142 °C; ^{1}H NMR (DMSO-d_6) δ 7.2–7.8 (m, 3 H, ArH), 4.8 (s, 1 H, C-5), 3.6 (s, 2 H, C-6), 3.4 (t, 2 H, C-2), 2.8 (s, 3 H, NCH_3), 2.5 (s, 2 H, C-3); UV (methanol) λ_{max} 280 nm (ε 720 M^{-1}); GC-EIMS (temperature program, 50 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 7.10 min and m/z 257 ($M^{+\cdot}$, 4), 96 (100). Anal. ($C_{14}H_{15}Cl_2NO_5$) C, H, N.

1-Methyl-4-(9-phenanthroxy)-1,2,3,6-tetrahydropyridine oxalate (22·(COOH)$_2$) was obtained in 49% yield from 2-propanol: mp 151–152 °C; ^{1}H NMR (DMSO-d_6) δ 8.8–8.9 (m, 2 H, ArH), 7.5–8.2 (m, 7 H, ArH), 4.9 (s, 1 H, C-5), 3.6 (s, 2 H, C-6), 3.4 (t, 2 H, C-2), 2.8 (s, 3 H, NCH_3), 2.5 (s, 2 H, C-3); UV (methanol) λ_{max} 250 nm (ε 40 500 M^{-1}); GC-EIMS (temperature program, 100 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak with t_R = 7.87 min and m/z 289 ($M^{+\cdot}$, 12), 96 (100). Anal. ($C_{22}H_{21}NO_5$) C, H, N.

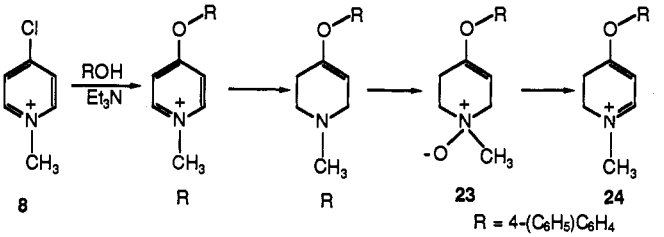
***m*-Chlorobenzoate (CBA) Salt of 1-Methyl-4-(4-phenylphenoxy)-1,2,3,6-tetrahydropyridine *N*-Oxide (23).** A solution of the tetrahydropyridine free base **20** obtained from the corresponding oxalate salt (0.4 g, 1.1 mmol) was stirred with *m*-chloroperoxybenzoic acid (0.208 g, 1.2 mmol) in 10 mL of dichloromethane at 0 °C for 1 h. The residue obtained after removal of the solvent was stirred with diethyl ether to give a white solid which was recrystallized from dichloromethane/diethyl ether to afford **23** as the *m*CBA salt in 56% yield: mp 110–111 °C; ^{1}H NMR ($CDCl_3$) δ 7.0–8.0 (m, 13 H, ArH), 4.67 (t, 1 H, C-5), 3.7–4.3 (complex m, 4 H, C-6 and C-2), 3.6 (s, 3 H, NCH_3), 2.6–3.2 (m, 2 H, C-3). Anal. ($C_{25}H_{24}ClNO_4$) C, H, N.

1-Methyl-4-(4-phenylphenoxy)-2,3-dihydropyridinium Perchlorate (24·ClO$_4$). Trifluoroacetic anhydride (0.31 mL, 2.25 mmol) was added dropwise to a solution of **23** (0.2 g, 0.45 mmol) in 10 mL of dichloromethane at 0 °C following which the reaction mixture was stirred at 0 °C for 30 min. The solvent was evaporated, and the residue was treated with methanolic perchloric acid to afford the crude perchlorate salt of **24** which was recrystallized from methanol/diethyl ether to afford orange needles in 75% yield: mp 145–147 °C; ^{1}H NMR (DMSO-d_6) δ 8.3 (d, 1 H, C-6), 7.3–7.9 (m, 9 H, ArH), 5.4 (d, 1 H, C-5), 4.0 (t, 2 H, C-2), 3.5 (s, 3 H, NCH_3), 3.0 (t, 2 H, C-3); UV (methanol) λ_{max} 256 (ε 18 000), 310 nm (ε 12 600 M^{-1}). Anal. ($C_{18}H_{18}ClNO_5$) C, H, N.

Enzymology Studies. The isolation and purification of MAO-B from beef liver and MAO-A from human placenta were carried out using the methodology reported earlier by Salach[24] with the following variations. The phospholipase A used in our preparations was obtained commercially (Sigma, St. Louis, MO) rather than from the crude venom. We processed only 20% of the mitochondrial fraction obtained from the 5 kg of beef liver MAO-B preparation rather than the entire fraction as reported. Finally, we did not subject the MAO-B preparation to the glucose gradient purification step or the MAO-A preparation to the sephadex purification step. In both cases, however, we obtained highly active and stable preparations which were stored at −15 °C in 50–200-μL aliquots. Solutions of these enzymes at the concentrations used in our studies (0.17 μM for MAO-A and 0.087 μM for MAO-B) were transparent in the UV range of interest. The specific activities of MAO-A (4.2 μM) and B (8.7 μM) were determined spectrophotometrically at 30 °C on a Beckman DU-50 spectrophotometer by estimating the initial (30–120 s) rates of conversion of 2 mM kynuramine to 4-hydroxyquinoline (314 nm, ε 12 300 M^{-1})[23] and 5 mM MPTP (1) to the dihydropyridinium species **2** (343 nm, ε 16 000 M^{-1}),[25] respectively. The turnover numbers for kynuramine (146 min^{-1})[19] and MPTP (204 min^{-1})[26] were taken from the literature.

Substrate Studies with MAO-A and MAO-B. All enzyme assays were performed in duplicate at 37 °C with a Beckman DU-50 spectrophotometer. Kinetic constants were determined using four concentrations of each substrate. Except for those substrates with very low K_m values, concentrations on both sides of the final K_m value were included. Stock solutions (500–62.5 μM) of the test compounds were prepared in 100 mM sodium phosphate buffer, pH 7.4. A 480-μL aliquot (MAO-A experiments) or 495-μL aliquot (MAO-B experiments) of each solution was added to a sample cuvette which was then placed in the spectrophotometer maintained at 37 °C. After a 1-min equilibration period, 20 μL of the MAO-A enzyme preparation (diluted 1:4 from the stock preparation with 20 mM phosphate buffer, pH 7.4, containing 20% w/v glycerol to decrease viscosity) or 5 μL of the MAO-B enzyme preparation was added. The rate of oxidation of each substrate was estimated by monitoring the increment in amino enone absorbance at 324 nm every 3 s for 2 min. The K_m and TN values were calculated from Lineweaver–Burke double-reciprocal plots.

Scheme 4. Synthesis of the 4-(Aryloxy)-1-methyl-1,2,3,6-tetrahydropyridine Derivatives **16-22** and the Dihydropyridinium Species **24**



9:	α-$C_{10}H_7$	**16:**	α-$C_{10}H_7$
10:	β-$C_{10}H_7$	**17:**	β-$C_{10}H_7$
11:	$CH_3(CH_2)_3C_6H_4$	**18:**	$CH_3(CH_2)_3C_6H_4$
12:	$(CH_3)_3CC_6H_4$	**19:**	$(CH_3)_3CC_6H_4$
13:	4-$(C_6H_5)C_6H_4$	**20:**	4-$(C_6H_5)C_6H_4$
14:	2,4-$Cl_2C_6H_3$	**21:**	2,4-$Cl_2C_6H_3$
15:	9-$C_{14}H_9$	**22:**	9-$C_{14}H_9$

Results and Discussion

Chemistry. The synthesis of the desired 4-(aryloxy)-1-methyl-1,2,3,6-tetrahydropyridine derivatives **16-22** was achieved by reaction of 4-chloro-1-methylpyridinium iodide (**8**) with the appropriate arenol in the presence of triethylamine to generate the corresponding 4-(aryloxy)-1-methylpyridinium species **9-15** (Scheme 4). The pyridinium intermediates were reduced with sodium borohydride to yield the desired tetrahydropyridine derivatives which were characterized as their oxalate salts. In the case of the 4-(4-phenylphenoxy) derivative **20**, the corresponding dihydropyridinium species **24** also was prepared as a model to examine the hydrolysis of the dihydropyridinium metabolic intermediates. The synthesis of **24** was achieved by *m*-chloroperoxybenzoic acid oxidation of amine **20** followed by treatment of the resulting *N*-oxide **23** with trifluoroacetic anhydride.[16] The final product was purified as its perchlorate salt.

Enzymology. The MAO-A- and MAO-B-catalyzed oxidations of the 1-methyl-4-(aryloxy)-1,2,3,6-tetrahydropyridine derivatives were examined spectrophotometrically. In order to obtain an initial estimate of the substrate properties of these tetrahydropyridine derivatives and to determine if the resulting dihydropyridinium metabolites undergo hydrolysis in a manner similar to that observed with **4** (Scheme 3), a series of scans (400–220 nm) vs time over a 1-h period for each compound at a substrate concentration of 500 μM in the presence of 0.17 μM MAO-A or 0.087 μM MAO-B was obtained. All of the aryloxy compounds appeared to be moderate to excellent substrates for both MAO-A and -B as measured by the generation of the amino enone chromophore at 324 nm. Furthermore, the formation of this chromophore was

Table 1. Kinetic Parameters for the MAO-A- and MAO-B-Catalyzed Oxidation of the (Aryloxy)tetrahydropyridines

4-substituted-1-methyl-1,2,3,6-tetrahydropyridine	MAO-A			MAO-B			
	TN[a]	K_m (mM)	TN/K_m	TN[a]	K_m (mM)	TN/K_m	$SC_{A/B}$[b]
kynuramine	146	0.170	860				
benzylamine				283	0.290	963	
4-phenyl (MPTP, 1)[c]	20	0.140	143	204	0.390	523	0.27
4-benzyl (25)[d]	9	0.066	139	193	0.154	1250	0.11
4-phenoxy (4)	130	0.055	2359	241	0.058	4151[e]	0.57
4-(4-*tert*-butylphenoxy) (19)	111	0.130	855	22	0.056	395	2.17
4-(4-butylphenoxy) (18)	85	0.051	1676	92	0.084	1090	1.54
4-(2,4-dichlorophenoxy) (21)	95	0.029	3290	223	0.167	1334	2.47
4-(4-phenylphenoxy) (20)	173	0.101	1710	103	0.284	363	4.72
4-(α-naphthylmethyl) (26)[d]	433	0.167	2590	89	0.847	105	24.67
4-(α-naphthoxy) (16)	227	0.144	1567	81	0.143	565	2.77
4-(β-naphthylmethyl) (27)[d]	121	0.036	3340	276	0.892	310	10.77
4-(β-naphthoxy) (17)	207	0.077	2685	333	0.274	1214	2.21
4-(9-phenanthrylmethyl) (28)[d]	365	0.480	759	>53			
4-(9-phenanthroxy) (22)	59	0.278	212	>200	>1.000		

[a] The turnover number (TN) is expressed in mol of product formed/min/mol of enzyme. [b] MAO-A/MAO-B selectivity coefficient $((TN/K_m)_{MAO-A}/(TN/K_m)_{MAO-B})$. [c] See ref 25. [d] See ref 19. In our hands, the benzyl compound was too poor a substrate to obtain reliable kinetic values. [e] A value approximately one-tenth this value was reported for this substrate previously by us[13] and may have involved a pipetting error.

completely blocked when MAO-B was inactivated by pretreatment with 30 μM deprenyl, a potent and selective MAO-B inactivator,[27] and when MAO-A was inactivated by 30 μM clorgyline, a potent and selective MAO-A inactivator.[26] The first scan observed with all of these incubations displayed a λ_{max} near 315 nm, but by the second scan (3 min), the λ_{max} had shifted toward the 324-nm chromophore characteristic of the amino enone **7**. These results were taken as evidence for the initial conversion of the substrate molecules to the corresponding dihydropyridinium intermediates which underwent rapid hydrolysis to yield **7** and the corresponding arenols.

The MAO-A-catalyzed oxidation of the 4-(4-phenylphenoxy)tetrahydropyridine analog **20** was studied in greater detail with the aid of the synthetic dihydropyridinium species **24**. As with the other 4-(aryloxy)tetrahydropyridine substrates, scans of MAO-A incubation mixtures containing **20** displayed an initial chromophore with λ_{max} 315 nm that shifted with increasing intensity to 324 nm over the course of several minutes. Analogous behavior was observed when a pH 7.4 phosphate-buffered solution of the synthetic dihydropyridinium species **24** was examined spectrophotometrically in the presence of 0.17 μM MAO-A at 37 °C. Complete conversion to the amino enone was estimated to require 6 min. We compared the kinetic characteristics of the MAO-A-catalyzed oxidation of **20** by locking on the dihydropyridinium chromophore (λ_{max} 313 nm, ε 12 600 M^{-1}) and the amino enone (λ_{max} 324 nm, ε 15 350 M^{-1}). The double-reciprocal plots of the resulting initial rates vs concentration gave TN/K_m values of 1789 min^{-1} mM^{-1} (313 nm) vs 1710 min^{-1} mM^{-1} (324 nm). We elected to monitor the reaction at 324 nm since this obviated the need to synthesize each of the intermediate dihydropyridinium species.

The substrate characteristics of the series of aryloxy analogs of MPTP were evaluated by determining the rates of oxidation at various substrate concentrations and a fixed enzyme concentration. An example of the resulting data is provided in Figure 1 which shows the double-reciprocal plot for the MAO-A-catalyzed oxidation of the 4-(4-phenylphenoxy)tetrahydropyridine analog which was constructed from the linear initial rate plots obtained at four substrate concentrations. This plot is typical of those obtained with both MAO-A and MAO-B for all of the substrates. Table 1 summarizes the kinetic parameters

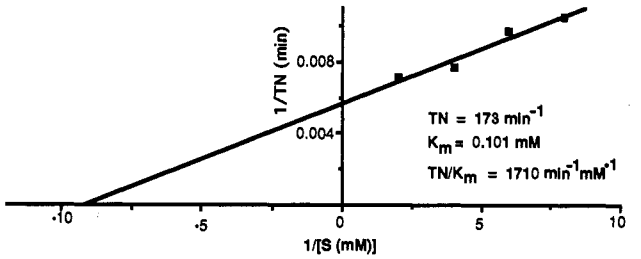


Figure 1. Plot of the 1/TN (mol of product formed/mol of enzyme min^{-1}) vs 1/[*S*, mM] for the MAO-A-catalyzed oxidation of 4-(4-phenylphenoxy)-1,2,3,6-tetrahydropyridine (**20**).

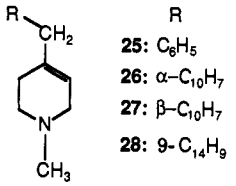
for the oxidation of the aryloxy derivatives by MAO-A and -B determined in these experiments. Kinetic parameters for MPTP, kynuramine, benzylamine, and the (arylmethyl)-1-methyl-1,2,3,6-tetrahydropyridine analogs 4-benzyl- (**25**), 4-(α-naphthyl)- (**26**), 4-(β-naphthyl)- (**27**), and 4-(9-phenanthryl)- (**28**) also are provided for comparisons. The relative substrate properties are expressed in terms of TN/K_m, and the selectivity for MAO-A vs MAO-B is expressed by the A/B selectivity coefficient ($SC_{A/B} = (TN/K_m)_{MAO-A}/(TN/K_m)_{MAO-B}$).

With the exception of the 4-(9-phenanthroxy) analog **22**, a comparison of the TN/K_m values shows that a majority of these compounds are as good as or better MAO-A substrates than kynuramine, the generally accepted preferred substrate for this form of the enzyme. These results also indicate that separation of the phenyl and tetrahydropyridyl moieties of MPTP with an oxygen atom leads to a dramatic improvement in the overall MAO-A substrate properties, particularly in terms of turnover characteristics. Thus, while the TN for the MAO-A-catalyzed oxidation of MPTP is only 20 min^{-1}, the lowest TN in this series of aryloxy compounds was 59 min^{-1}, observed for the bulky 4-(9-phenanthroxy) derivative **22**.

The introduction of a butyl, *tert*-butyl, or phenyl group at the 4-position of the phenyl ring alters turnover characterisitcs to a small extent relative to the unsubstituted compound **4**. The larger *tert*-butyl and phenyl groups, however, do lead to larger K_m values, an effect which presumably reflects unfavorable steric interactions in the active site of this enzyme. Overall, however, all of these groups are well tolerated by MAO-A. Also of interest are the excellent binding characteristics of the 4-(2,4-dichlorophenoxy) derivative **21** (K_m = 29 μM), a compound

which shares structural features present in the MAO-A-selective inactivator clorgyline.

The 4-(α-naphthoxy) (**16**) and 4-(β-naphthoxy) (**17**) compounds also display excellent substrate properties for MAO-A (TN/K_m = 1567 and 2685 min^{-1} mM^{-1}, respectively). These values are somewhat lower than the corresponding values for the 4-(α-naphthylmethyl) (**26**) and 4-(β-naphthylmethyl) (**27**) analogs (TN/K_m = 2590 and 3340 min^{-1} mM^{-1}, respectively) reported by Krueger *et al.*[19] On the other hand, the TN/K_m value for the 4-phenoxytetrahydropyridine derivative **4** (2359 min^{-1} mM^{-1}) is 17 times greater than the corresponding value for the 4-benzyltetrahydropyridine derivative **25** (139 min^{-1} mM^{-1}). This difference is principally due to an increase in the TN of the phenoxy compared to the benzyl compound. It would appear, therefore, that relatively subtle changes in the arrangement of the phenyl group at C-4 can influence the nature of the interactions of these types of substrates with the catalytic site of MAO-A. The reason(s) that this difference between aryloxy and arylmethyl analogs is observed only with the phenyl group is not apparent. Additional examples will be sought to clarify this issue.



R

25: C_6H_5

26: α-$C_{10}H_7$

27: β-$C_{10}H_7$

28: 9-$C_{14}H_9$

The aryloxy derivatives also turned out to be excellent MAO-B substrates. TN/K_m values ranged from a low of 363 min^{-1} mM^{-1} for the 4-(4-phenylphenoxy) derivative **20** to over 4000 min^{-1} mM^{-1} for the 4-phenoxy derivative **4**. The majority of these analogs are better MAO-B substrates than MPTP (TN/K_m 523 min^{-1} mM^{-1}), and some are better substrates than benzylamine (TN/K_m 860 min^{-1} mM^{-1}), the preferred MAO-B substrate. A comparison of the TN and K_m values of these oxidations leads to no obvious correlations with the possible exception that the smaller 4-phenoxy derivative **4** is overall a better substrate than the larger aryloxy derivatives examined in this series with MAO-B.

Although substrate hydrophobicities have not been considered, analysis of these results suggests that the B form of the enzyme prefers those substrates with smaller C-4 substituents—$SC_{A/B}$ = 0.27 (4-phenyl), 0.11 (4-benzyl), and 0.57 (4-phenoxy). Tetrahydropyridine derivatives with bulkier groups at C-4 show preference for MAO-A with $SC_{A/B}$ values ranging from 1.54 for the 4-(4-butylphenoxy) derivative to 4.72 for the 4-(4-phenylphenoxy) derivative. The degree of selectivity observed with these substrates is not particularly dramatic and is considerably less than that found with the naphthylmethyl derivatives—10.77 for the β-isomer and 24.67 for the α-isomer. The loss in selectivity observed with the bulkier aryloxy vs arylmethyl derivatives in part is due to the lower K_m values with MAO-B for the aryloxy compounds compared to the arylmethyl compounds.

The results obtained with the 4-(9-phenanthroxy) derivative were particularly surprising. Even though this compound bears the bulkiest substituent in this series, the rate of its MAO-B-catalyzed oxidation at its maximum concentration (500 μM) is at least 200 min^{-1}, which compares favorably with MPTP (TN = 115 min^{-1}) at the same concentration. Due to the limited solubility of **22**, however, we were not able to examine its rates of oxidation at high enough concentrations to determine TN and K_m. The 4-(9-phenanthroxy) derivative also proved to be a very poor MAO-A substrate compared to the other aryloxy derivatives. A comparison of the MAO-A substrate properties of the 4-(9-phenanthrylmethyl) vs 4-(9-phenanthroxy) derivatives shows that, as with the naphthyl derivatives, the arylmethyl derivative (TN/K_m = 759 min^{-1} mM^{-1}) is a better substrate than the aryloxy derivative (TN/K_m = 212 min^{-1} mM^{-1} for MAO-A).

In summary, as with the arylmethyl analogs, MAO-B appears to be more sensitive to steric interactions with bulky C-4 substituents than does MAO-A. On the other hand, the bulky 4-(aryloxy)tetrahydropyridine derivatives proved to be better MAO-B substrates than what might have been predicted by the TN/K_m values reported for the corresponding arylmethyl derivatives. Additional observations of general interest are the excellent MAO-A substrate properties of the 4-phenoxy analog compared to the 4-benzyl analog and the unexpected MAO-B substrate properties of the bulky phenanthroxy analog. These results, together with those described in several recent publications, provide additional evidence for the good to excellent substrate properties of a variety of 4-substituted-1-methyl-1,2,3,6-tetrahydropyridines and clearly demonstrate the catalytic versatility of MAO-A and MAO-B. The nature of the substrate–enzyme interactions that account for the unique substrate properties of these cyclic allyl tertiary amines remains to be fully characterized.

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