Studies on the Monoamine Oxidase (MAO)-Catalyzed Oxidation of Phenyl-Substituted 1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine Derivatives: Factors Contributing to MAO-A and MAO-B Selectivity

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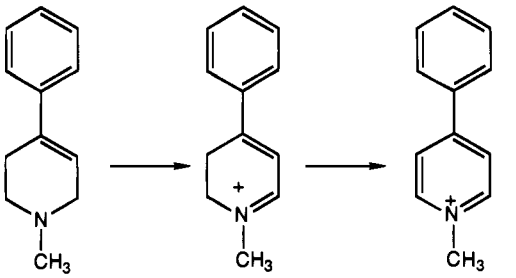
The structural parameters responsible for the substrate and inhibitor selectivities of the monoamine oxidases (MAO) A and B remain poorly understood. This situation has improved somewhat with structure–activity studies that have been performed on nuclear-substituted pargyline derivatives and 4-substituted 1-methyl-1,2,3,6-tetrahydropyridine derivatives. The results of these studies suggest that the active site of MAO-A is sterically more accommodating than the active site of MAO-B. In the present work we have undertaken a more systematic structure–substrate activity analysis with the aid of a series of 4-phenoxytetrahydropyridine analogs substituted at the *para*, *meta*, and *ortho* positions of the phenyl ring with chloro, methoxy, methyl, nitro, and phenyl groups. All of the compounds proved to be good substrates for both MAO-A and MAO-B, and all were better MAO-A substrates than MAO-B substrates. The best defined structural parameter relating to selectivity again was the relatively better MAO-A substrate properties of tetrahydropyridine derivatives bearing bulky C-4 substituents. Attempts to identify stereoelectronic effects related to substrate properties and selectivity with this series of compounds were not successful. Although some structural correlates with substrate activity can be made, overall the present state of knowledge is inadequate to provide good descriptors of structural features that characterize MAO-A and MAO-B substrates.

Introduction

The outer mitochondrial membrane-bound flavoenzymes monoamine oxidase A and B (MAO-A and MAO-B) catalyze the oxidative deamination of the neurotransmitter amines including dopamine and serotonin.[1,2] Although the primary structures of these enzymes have been established from the corresponding gene sequences,[3,4] relatively little is known regarding the structural features of the active sites which lead to selectivities observed with various substrates[5] and inhibitors.[6,7] One important contribution to this field is a recent quantitative structure–activity relationship (QSAR) study in which various *meta*- and *para*-substituted *N*-benzyl-*N*-methylpropargylamine (pargyline) derivatives were evaluated for their ability to inhibit competitively rat liver mitochondrial MAO-A and MAO-B catalytic activities. Although attempts to formulate correlations that included both the *meta*- and *para*-substituted analogs failed, the authors were able to conclude that the binding site of MAO-B is likely to be present within a cavity of more limited lateral dimensions than that present on the MAO-A surface.[8]

A novel opportunity to investigate the structure–substrate activity relationships of molecules which contribute to MAO-A and MAO-B substrate selectivities has become available with the cyclic tertiary allylamine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**; Scheme 1), a potent nigrostriatal neurotoxin[9] that undergoes MAO-B-catalyzed bioactivation to the corresponding dihydropyridinium and pyridinium metabolites $MPDP^+$ (**2**) and MPP^+ (**3**), respectively.[10] To the best of our knowledge, this is the only known class of cyclic amines which displays good substrate properties with these flavoproteins.

Scheme 1. MAO-B-Catalyzed Bioactivation of MPTP



Some of the global structural features that are associated with MAO substrate properties of MPTP analogs include the following: (1) the six-membered heterocyclic system must contain the double bond at the position β,γ to the nitrogen atom;[11] (2) maximal activity requires an *N*-methyl group;[12] and (3) the 1-methyl-1,2,3,6-tetrahydropyridine ring must bear a C-4 substituent but no other ring carbon substituent.[13,14] In contrast to these restrictions, a wide variety of groups is tolerated at the C-4 position. Dramatic differences have been observed for the rates of the MAO-A- vs MAO-B-catalyzed oxidations of some substrates. MPTP is reported to be a better MAO-B substrate (k_{cat}/K_M = 523 min^{-1} mM^{-1} at 30 °C) than MAO-A substrate (k_{cat}/K_M = 143 min^{-1} mM^{-1} at 30 °C).[15] Introduction of an isopropyl group at the 2 (i.e., *ortho*) position of the phenyl ring leads to an almost 8-fold increase in MAO-A activity but an almost 10-fold decrease in MAO-B activity.[15] On the other hand, replacement of the 4-phenyl group with a benzyl group results in a decrease in MAO-A activity but a 5-fold increase in MAO-B activity.[15]

More recent efforts to characterize factors relating to the selectivity of these two enzymes have focused on a

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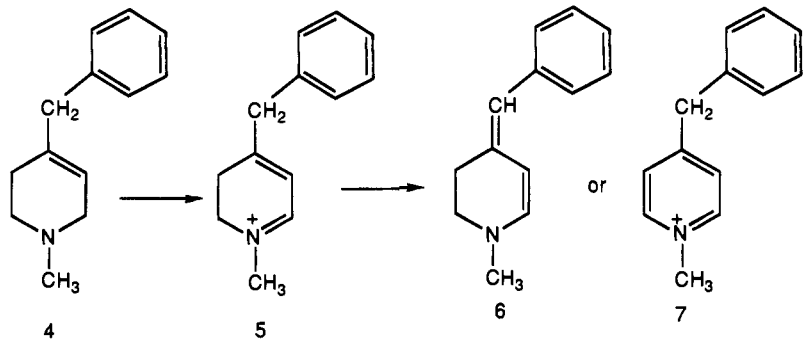
Scheme 2. MAO-B-Catalyzed Oxidation of the 4-Benzyl MPTP Analog **4**



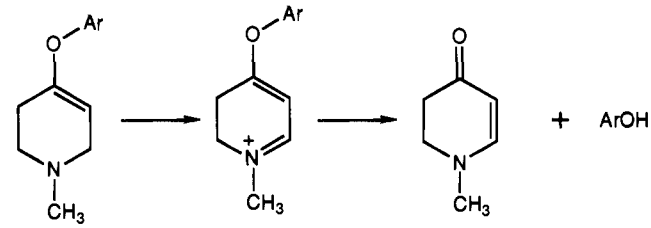
series of "flexible" 4-arylmethyl and 4-arylethyl MPTP analogs[16] and various "tetrahydrostilbazole derivatives", i.e., phenyl-substituted 1-methyl-4-(2-phenylethenyl)-1,2,3,6-tetrahydropyridine derivatives.[17] Results obtained with the flexible arylalkyl analogs are reasonably consistent and suggest that the active site of MAO-A can accommodate bulkier substituents than the active site of MAO-B, a conclusion similar to that reached earlier with a series of pargyline analogs.[8] Other rational structure–substrate activity correlations, however, are difficult to extract from the results obtained in this study. Testa and his colleagues have examined electronic, steric, and lipophilicity parameters of a variety of tetrahydropyridine derivatives in an attempt to identify useful correlations between structure and substrate activity. However, since all of these variables seem to contribute to the substrate properties of these tetrahydropyridine derivatives, it has been difficult to formulate well-defined correlations with any predictive power.[18–20]

The 4-benzyl-1-methyl-1,2,3,6-tetrahydropyridine analog (**4**) has been studied extensively. Although an excellent MAO-B substrate,[15] this compound lacks neurotoxic properties.[12] A reasonable explanation for this behavior is the instability of the intermediate dihydropyridinium metabolite **5** which does not undergo extensive conversion to the neurotoxic pyridinium species **7**, possibly because of the ease of deprotonation of the acidic benzylic protons to form the unstable dienamine system **6** (Scheme 2).[21]

These properties of **4** led us to examine the substrate and neurotoxic potential of the corresponding phenoxy compound **8a** which is structurally related to **4** but lacks the benzylic protons.[22] Compound **8a** proved to be a good substrate for MAO-B but, like the benzyl analog, was not neurotoxic in the mouse model. This lack of neurotoxicity also could be explained in terms of the instability of the dihydropyridinium metabolite **9a** which underwent spontaneous hydrolysis to yield the phenol and the amino enone **10** (Scheme 3).

The UV absorption characteristics of **10** (λ_{max} = 324 nm, ϵ = 15 300 M^{-1}) provide a convenient means of monitoring the rates of the MAO-catalyzed oxidation of 4-(aryloxy)tetrahydropyridine derivatives and obviate the need of synthesizing the individual dihydropyridinium metabolites which otherwise would be required for molar extinction coefficient determinations. We exploited the behavior of this system to evaluate the human placental MAO-A- and beef liver MAO-B-catalyzed oxidation of a series of 4-(aryloxy)-1-methyl-1,2,3,6-tetrahydropyridine analogs that includes, in addition to the 4-phenoxy analog **8a**, the 4-(2,4-dichlorophenoxy)- (**8b**), 4-(4-butylphenoxy)- (**8c**), 4-(4-*tert*-butylphenoxy)- (**8d**), 4-(4-methylphenoxy)- (**8e**), 4-(1-naphthoxy)- (**8f**), 4-(2-naphthoxy)- (**8g**), 4-(9-phenanthroxy)- (**8h**), and 4-(4-phenylphenoxy)- (**8i**) tetrahydropyridines.[23] MAO-A and MAO-B catalyzed the oxidations of all of these aryloxy compounds to the corresponding dihydropyridinium metabolites **9b–h**, respectively, which, as with **9a**, underwent spontaneous hydrolysis to yield the corresponding arenols and 1-methyl-2,3-dihydro-4-pyridone (**10**) as shown in Scheme 3. The selectivities of these substrates as measured by the MAO-A/MAO-B k_{cat}/K_M ratios varied from almost 5 (for the phenylphenoxy compound **8i**) to about 0.6 (for the phenoxy compound **8a**). In general, the substrate behavior of these compounds suggested that the catalytic site of MAO-B is more sensitive to steric interactions with bulky C-4 substituents than the active site of MAO-A. Further inspection of the results from these studies, however, failed to identify other structural features that could be linked to the observed substrate selectivities.

Scheme 3. MAO-A- and MAO-B-Catalyzed Oxidation of 4-(Aryloxy)tetrahydropyridines **5a–i**[a]



[a] a, Ar = C_6H_5; **b**, 2,4-diClC_6H_3; **c**, Ar = 4-(*n*-C_4H_9)C_6H_4; **d**, Ar = 4-(*tert*-C_4H_9)C_6H_4; **e**, Ar = 4-$CH_3C_6H_4$; **f**, Ar = 1-$C_{10}H_7$; **g**, Ar = 2-$C_{10}H_7$; **h**, Ar = 9-$C_{14}H_9$; **i**, Ar = 4-(C_6H_5)C_6H_4.

In an attempt to provide a more systematic analysis of steric and possible electronic parameters that may contribute to enzyme selectivity, we have determined the k_{cat} and K_M values for the MAO-A- and MAO-B-catalyzed oxidations of 1-methyl-1,2,3,6-tetrahydropyridine derivatives bearing a 4-phenoxy group substituted in the *para*, *meta*, and *ortho* positions with chloro, methoxy, methyl, phenyl, and nitro groups. The results of these studies are discussed in terms of current thoughts regarding the structural requirements for MAO-A and MAO-B catalysis.

Experimental Section

Chemistry. All chemicals were reagent or HPLC grade. Proton NMR spectra were recorded on a Bruker WP 270 MHz spectrophotometer. Chemical shifts are reported in ppm relative to 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 on a Hewlett Packard (HP) Model 5890 capillary column gas chromatograph [HP-1 methylsilicone capillary column (24 m × 0.2 mm × 0.33 μm film thickness)] employing helium as the carrier gas (40 mL/min) which was coupled to an HP 5970 EI mass spectrometer controlled by a Chem Station. The following GC temperature program was used, 90 °C for 1 min and then a ramp of 25 °C/min up to 275 °C. Enzyme kinetic studies were performed on a Beckman Model DU-50 spectrophotometer. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses, performed by Atlantic Microlab, Inc., Norcross, GA, were within 0.4% of theoretical values calculated for C, H, and N. The amino enone **10**,[24] 4-chloro-1-methylpyridinium iodide (**11**),[25] and 1-methyl-4-(4-phenylphenoxy)-1,2,3,6-tetrahydropyridine [**8i** (Scheme 3) or **14m** (Scheme 4)][22] were synthesized as described previously.

General Procedure for the Synthesis of 4-(Substituted phenoxy)-1-methylpyridinium Iodide Salts. A solution of 4-chloro-1-methylpyridinium iodide (**11**; 80 mmol), the substituted phenol (**12a–o**; 80 mmol), and freshly distilled triethylamine (12 mmol) in 100 mL of acetone was stirred at room temperature under nitrogen for the indicated time. The solvent was evaporated, and the residue was treated with saturated sodium bicarbonate. The aqueous solution was extracted with dichloromethane (3 × 60 mL), and the combined organic extracts were washed with saturated aqueous NaCl and dried ($MgSO_4$). Evaporation of the solvent gave crude pyridinium product (**13a–o**) which was purified further by recrystallization from ethanol/ether unless noted otherwise.

1-Methyl-4-(4-chlorophenoxy)pyridinium iodide (13a): obtained after a 24 h reaction period in 65.5% yield; mp 163–164 °C (from CH_2Cl_2/Et_2O); ^1H NMR ($CDCl_3$) δ 9.13 (d, 2H, C2 and C6), 7.39 (d, 2H, C3 and C5), 7.53 and 7.18 (dd, 4H, Ph-H), 4.46 (s, 3H, N-CH_3); GC–EIMS t_R 6.99 min, *m/e* 205 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{12}H_{11}ClINO$) C, H, N.

1-Methyl-4-(3-chlorophenoxy)pyridinium iodide (13b): obtained after a 24 h reaction period in 66.4% yield; mp 164–165 °C (from CH_2Cl_2/Et_2O); ^1H NMR ($CDCl_3$) δ 9.25 (d, 2H, C2 and C6), 7.09–7.52 (m, 6H, C3, C5, and Ph-H), 4.59 (s, 3H, N-CH_3); GC–EIMS t_R 6.81 min, *m/e* 205 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{12}H_{11}ClINO$) C, H, N.

1-Methyl-4-(2-chlorophenoxy)pyridinium iodide (13c): obtained after a 24 h reaction period in 62.3% yield; mp 170–171 °C (from CH_2Cl_2/Et_2O); ^1H NMR ($CDCl_3$) δ 9.31 (d, 2H, C2 and C6), 7.28–7.60 (m, 6H, C3, C5, and Ph-H), 4.60 (s, 3H, N-CH_3); GC–EIMS t_R 6.78 min, *m/e* 205 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{12}H_{11}ClINO$) C, H, N.

1-Methyl-4-(4-methoxyphenoxy)pyridinium iodide (13d): obtained after a 24 h reaction period in 69.2% yield; mp 159–160 °C; ^1H NMR ($CDCl_3$) δ 9.21 (d, 2H, C2 and C6), 7.30 (d, 2H, C3 and C5), 6.67–7.45 (m, 4H, Ph-H), 4.55 (s, 3H, N-CH_3), 3.85 (s, 3H, OCH_3); GC–EIMS t_R 7.52 min, *m/e* 201 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{13}H_{14}INO_2$) C, H, N.

1-Methyl-4-(3-methoxyphenoxy)pyridinium iodide (13e): obtained after a 48 h reaction time in 70.1% yield; mp 168–169 °C; ^1H NMR ($CDCl_3$) δ 9.19 (d, 2H, C2 and C6), 6.67–7.45 (m, 6H, C3, C5, and Ph-H), 4.57 (s, 3H, N-CH_3), 3.84 (s, 3H, OCH_3); GC–EIMS t_R 7.78 min, *m/e* 201 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{13}H_{14}INO_2$) C, H, N.

1-Methyl-4-(2-methoxyphenoxy)pyridinium iodide (13f): obtained after a 24 h reaction period in 61.9% yield; mp 151–152 °C; ^1H NMR ($CDCl_3$) δ 9.19 (d, 2H, C2 and C6), 7.03–7.40 (m, 6H, C3, C5, and Ph-H), 4.57 (s, 3H, N-CH_3), 3.80 (s, 3H, OCH_3); GC–EIMS t_R 6.94 min, *m/e* 201 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{13}H_{14}INO_2$) C, H, N.

1-Methyl-4-(4-methylphenoxy)pyridinium iodide (13g): obtained after a 24 h reaction period in 54.0% yield; mp 164–165 °C; ^1H NMR (DMSO-d_6) δ 8.80 (d, 2H, C2 and C6), 7.49 (d, 2H, C3 and C5), 7.20 and 7.38 (dd, 4H, Ph-H), 4.20 (s, 3H, N-CH_3), 2.35 (s, 3H, CH_3); GC–EIMS t_R 6.28 min, *m/e* 185 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{13}H_{14}INO$) C, H, N.

1-Methyl-4-(3-methylphenoxy)pyridinium iodide (13h): obtained after a 24 h reaction period in 49.1% yield; mp 159–160 °C; ^1H NMR (DMSO) δ 8.79 (d, 2H, C2 and C6), 7.51 (d, 2H, C3 and C5), 7.09–7.47 (m, 4H, Ph-H), 4.20 (s, 3H, N-CH_3), 2.36 (s, 3H, CH_3); GC–EIMS t_R 6.23 min, *m/e* 185 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{13}H_{14}INO$) C, H, N.

1-Methyl-4-(2-methylphenoxy)pyridinium iodide (13i): obtained after a 24 h reaction period in 55.8% yield; mp 161–162 °C; ^1H NMR (DMSO) δ 8.80 (d, 2H, C2 and C6), 7.45 (d, 2H, C3 and C5), 7.23–7.41 (m, 4H, Ph-H), 4.20 (s, 3H, N-CH_3), 2.11 (s, 3H, CH_3); GC–EIMS t_R 6.03 min, *m/e* 185 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{13}H_{14}INO$) C, H, N.

1-Methyl-4-(4-nitrophenoxy)pyridinium iodide (13j): obtained after a 24 h reaction period in 37.1% yield; mp 213–215 °C; ^1H NMR ($CDCl_3$/DMSO-d_6) δ 9.18 (d, 2H, C2 and C6), 8.43 (d, 2H, C3 and C5), 7.45–7.53 (m, 4H, Ph-H), 4.50 (s, 3H, N-CH_3); GC–EIMS t_R 8.05 min, *m/e* 216 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{12}H_{11}IN_2O_3$) C, H, N.

1-Methyl-4-(3-nitrophenoxy)pyridinium iodide (13k): obtained after a 2 day reaction period in 6.4% yield; mp 183–184 °C; ^1H NMR ($CDCl_3$/DMSO-d_6) δ 9.17 (d, 2H, C2 and C6), 7.51–8.32 (m, 6H, C3, C5, and Ph-H), 4.48 (s, 3H, N-CH_3); GC–EIMS t_R 7.87 min, *m/e* 216 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{12}H_{11}IN_2O_3$) C, H, N.

1-Methyl-4-(2-nitrophenoxy)pyridinium iodide (13l): obtained after a 3 day reaction period in 29.7% yield; mp 193–195 °C; ^1H NMR ($CDCl_3$/DMSO-d_6) δ 9.13 (d, 2H, C2 and C6), 7.47–8.27 (m, 6H, C3, C5, and Ph-H), 4.45 (s, 3H, N-CH_3); GC–EIMS t_R 7.77 min, *m/e* 216 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{12}H_{11}IN_2O_3$) C, H, N.

1-Methyl-4-(3-phenylphenoxy)pyridinium iodide (13n): obtained after a 48 h reaction period in 79.7% yield; mp 141–142 °C; ^1H NMR (DMSO-d_6) δ 8.83 (d, 2H, C2 and C6), 7.31–7.78 (m, 11H, C3, C5, and Ph-H), 4.23 (s, 3H, N-CH_3); GC–EIMS t_R 9.43 min, *m/e* 247 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{18}H_{16}INO$) C, H, N.

1-Methyl-4-(2-phenylphenoxy)pyridinium iodide (13o): obtained after a 48 h reaction period in 81.8% yield; mp 174–175 °C; ^1H NMR (DMSO-d_6) δ 8.72 (d, 2H, C2 and C6), 7.32–7.62 (m, 11H, C3, C5, and Ph-H), 4.11 (s, 3H, N-CH_3); GC–EIMS t_R 6.86 min, *m/e* 247 (M − CH_3I)$^{\cdot+}$. Anal. ($C_{18}H_{16}INO$) C, H, N.

General Procedure for the Synthesis of the Oxalate Salts of 4-Substituted 1-Methylphenoxy-1,2,3,6-tetrahydropyridine Derivatives 14a–o. Sodium borohydride (1.0 mmol) was added in portions to a stirred solution of the appropriate 1-methyl-4-(substituted phenoxy)pyridinium iodide (0.67 mmol) in 15 mL of dry methanol at 0 °C. The mixture was stirred for an additional 30 min at 0 °C, and then the solvent was removed under reduced pressure. The residue in 10 mL of water was extracted with ethyl acetate (2 × 20 mL). The combined ethyl acetate layers were washed with saturated aqueous NaCl, dried (Na_2SO_4 and $MgSO_4$), filtered, and evaporated. The residue in 15 mL of dry diethyl ether was treated with oxalic acid (0.87 mmol) in 5 mL of dry diethyl ether, and the precipitated oxalate salt was recrystallized from methanol/diethyl ether unless noted otherwise.

Oxalate salt of 1-methyl-4-(4-chlorophenoxy)-1,2,3,6-tetrahydropyridine (14a): obtained in 77.4% yield; mp 182–183 °C; ^1H NMR (DMSO-d_6) δ 7.40 and 7.10 (dd, 4H, Ph-H), 4.93 (unresolved, 1H, C5), 3.61 (unresolved, 2H, C6), 3.32 (t, 2H, C2), 2.77 (s, 3H, N^+-CH_3), 2.51 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 223 (ε = 8200 M^{-1}); GC–EIMS t_R 7.22 min, *m/e* 223 ($M^{\cdot+}$, weak), 96 (100). Anal. ($C_{14}H_{16}ClNO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(3-chlorophenoxy)-1,2,3,6-tetrahydropyridine (14b): obtained in 83.1% yield; mp 145–146 °C; ^1H NMR (DMSO-d_6) δ 7.05–7.43 (m, 4H, Ph-H), 5.03 (unresolved, 1H, C5), 3.63 (unresolved, 2H, C6), 3.32 (t, 2H, C2), 2.88 (s, 3H, N^+-CH_3), 2.51 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 212 (ε = 8700 M^{-1}); GC–EIMS t_R 7.14 min, *m/e* 223 ($M^{\cdot+}$, weak), 96 (100). Anal. ($C_{14}H_{16}ClNO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(2-chlorophenoxy)-1,2,3,6-tetrahydropyridine (14c): obtained in 83.1% yield; mp 150–151 °C; ^1H NMR (DMSO-d_6) δ 7.17–7.56 (m, 4H, Ph-H), 4.69 (unresolved, 1H, C5), 3.59 (unresolved, 2H, C6), 3.33 (t, 2H, C2), 2.76 (s, 3H, N^+-CH_3), 2.57 (unresolved, 2H, C3); UV

(phosphate buffer) λ_{max} = 212 (ϵ = 9200 M^{-1}); GC–EIMS t_R 7.04 min, *m/e* 223 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{14}H_{16}ClNO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(4-methoxyphenoxy)-1,2,3,6-tetrahydropyridine (14d): obtained in 75.5% yield; mp 163–164 °C; ^1H NMR (DMSO-d_6) δ 6.93–7.03 (m, 4H, Ph-H), 4.60 (unresolved, 1H, C5), 3.75 (s, 3H, OCH_3), 3.58 (unresolved, 2H, C6), 3.32 (t, 2H, C2), 2.77 (s, 3H, N^+-CH_3), 2.55 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 224 (ϵ = 6300 M^{-1}); GC–EIMS t_R 7.70 min, *m/e* 219 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{15}H_{19}NO_6$) C, H, N.

Oxalate salt of 1-methyl-4-(3-methoxyphenoxy)-1,2,3,6-tetrahydropyridine (14e): obtained in 78.6% yield; mp 110–111 °C; ^1H NMR (DMSO-d_6) δ 6.62–7.31 (m, 4H, Ph-H), 4.92 (unresolved, 1H, C5), 3.75 (s, 3H, OCH_3), 3.61 (unresolved, 2H, C6), 3.32 (t, 2H, C2), 2.77 (s, 3H, N^+-CH_3), 2.52 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 215 (ϵ = 7100 M^{-1}); GC–EIMS t_R 7.86 min, *m/e* 219 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{15}H_{19}NO_6$) C, H, N.

Oxalate salt of 1-methyl-4-(2-methoxyphenoxy)-1,2,3,6-tetrahydropyridine (14f): obtained in 79.2% yield; mp 134–135 °C; ^1H NMR (DMSO-d_6) δ 6.93–7.18 (m, 4H, Ph-H), 4.49 (unresolved, 1H, C5), 3.77 (s, 3H, OCH_3), 3.55 (unresolved, 2H, C6), 3.31 (t, 2H, C2), 2.75 (s, 3H, N^+-CH_3), 2.56 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 213 (ϵ = 7300 M^{-1}); GC–EIMS t_R 7.38 min, *m/e* 219 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{15}H_{19}NO_6$) C, H, N.

Oxalate salt of 1-methyl-4-(4-methylphenoxy)-1,2,3,6-tetrahydropyridine (14g): obtained in 68.2% yield; mp 148–149 °C; ^1H NMR (DMSO-d_6) δ 6.94 and 7.18 (dd, 4H, Ph-H), 4.72 (unresolved, 1H, C5), 3.55 (unresolved, 2H, C6), 3.28 (t, 2H, C2), 2.75 (s, 3H, N^+-CH_3), 2.50 (unresolved, 2H, C3), 2.27 (s, 3H, CH_3); UV (phosphate buffer) λ_{max} = 206 (ϵ = 10 400 M^{-1}); GC–EIMS t_R 6.56 min, *m/e* 203 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{15}H_{19}NO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(3-methylphenoxy)-1,2,3,6-tetrahydropyridine (14h): obtained in 77.1% yield; mp 135–136 °C; ^1H NMR (DMSO-d_6) δ 6.82–7.27 (m, 4H, Ph-H), 4.81 (unresolved, 1H, C5), 3.56 (unresolved, 2H, C6), 3.28 (t, 2H, C2), 2.74 (s, 3H, N^+-CH_3), 2.49 (unresolved, 2H, C3), 2.28 (s, 3H, CH_3); UV (phosphate buffer) λ_{max} = 208 (ϵ = 10 200 M^{-1}); GC–EIMS t_R 6.40 min, *m/e* 203 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{15}H_{19}NO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(2-methylphenoxy)-1,2,3,6-tetrahydropyridine (14i): obtained in 81.8% yield; mp 143–144 °C; ^1H NMR (DMSO-d_6) δ 6.97–7.28 (m, 4H, Ph-H), 4.42 (unresolved, 1H, C5), 3.51 (unresolved, 2H, C6), 3.30 (t, 2H, C2), 2.74 (s, 3H, N^+-CH_3), 2.57 (unresolved, 2H, C3), 2.13 (s, 3H, CH_3); UV (phosphate buffer) λ_{max} = 206 (ϵ = 10 100 M^{-1}); GC–EIMS t_R 6.20 min, *m/e* 203 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{15}H_{19}NO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(4-nitrophenoxy)-1,2,3,6-tetrahydropyridine (14j): obtained in 93.9% yield; mp 213–214 °C; ^1H NMR (DMSO-d_6) δ 8.25 and 7.31 (dd, 4H, Ph-H), 5.41 (unresolved, 1H, C5), 3.66 (unresolved, 2H, C6), 3.33 (t, 2H, C2), 2.78 (s, 3H, N^+-CH_3), 2.51 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 304 (ϵ = 9000 M^{-1}); GC–EIMS t_R 8.40 min, *m/e* 234 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{14}H_{16}N_2O_7$) C, H, N.

Oxalate salt of 1-methyl-4-(3-nitrophenoxy)-1,2,3,6-tetrahydropyridine (14k): obtained in 86.7% yield; mp 165–166 °C; ^1H NMR (DMSO-d_6) δ 7.55–8.03 (m, 4H, Ph-H), 5.14 (unresolved, 1H, C5), 3.62 (unresolved, 2H, C6), 3.31 (t, 2H, C2), 2.77 (s, 3H, N^+-CH_3), 2.53 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 216 (ϵ = 6400 M^{-1}); GC–EIMS t_R 8.07 min, *m/e* 234 ($M^{+\cdot}$, weak), 96 (100). Anal. ($C_{14}H_{16}N_2O_7$) C, H, N.

Oxalate salt of 1-methyl-4-(2-nitrophenoxy)-1,2,3,6-tetrahydropyridine (14l): obtained in 85.2% yield; mp 161–162 °C; ^1H NMR (DMSO-d_6) δ 7.36–8.06 (m, 4H, Ph-H), 4.99 (unresolved, 1H, C5), 3.55 (unresolved, 2H, C6), 3.38 (t, 2H, C2), 2.74 (s, 3H, N^+-CH_3), 2.53 (unresolved, 2H, C3); UV (phosphate buffer) λ_{max} = 214 (ϵ = 5800 M^{-1}). Anal. ($C_{14}H_{16}N_2O_7$) C, H, N.

Oxalate salt of 1-methyl-4-(3-phenylphenoxy)-1,2,3,6-tetrahydropyridine (14n): obtained in 74.8% yield; mp 173–174 °C; ^1H NMR (DMSO-d_6) δ 7.06–7.69 (m, 9H, Ph-H), 4.95 (unresolved, 1H, C5), 3.61 (unresolved, 2H, C6), 3.34 (t, 2H, C2), 2.78 (s, 3H, N^+-CH_3), 2.50 (t, 2H, C3); UV (phosphate buffer) λ_{max} = 249 (ϵ = 15 000 M^{-1}). Anal. ($C_{20}H_{21}NO_5$) C, H, N.

Oxalate salt of 1-methyl-4-(2-phenylphenoxy)-1,2,3,6-tetrahydropyridine (14m): obtained in 77.6% yield; mp 155–156 °C; ^1H NMR (DMSO-d_6) δ 7.17–7.46 (m, 9H, Ph-H), 4.66 (unresolved, 1H, C5), 3.50 (unresolved, 2H, C6), 3.21 (t, 2H, C2), 2.65 (s, 3H, N^+-CH_3), 2.41 (t, 2H, C3); UV (phosphate buffer) λ_{max} = 241 (ϵ = 11 000 M^{-1}). Anal. ($C_{20}H_{21}NO_5$) C, H, N.

Enzyme Studies. The isolation and purification of MAO-A from human placenta and MAO-B from beef liver were carried out using the procedures reported by Salach[26] with the following modifications. The phospholipase A used in our preparation was obtained commercially (Sigma, St. Louis, MO) rather than from the crude venom. We did not subject the MAO-A preparation to the Sephadex purification or the MAO-B preparation to the glucose gradient purification step. In both cases, however, we obtained highly active preparations. The specific activity of MAO-A (17 nmol/mL) was established with kynuramine as substrate at 30 °C (k_{cat} = 146 min^{-1}) according to Salach.[26] The specific activity of MAO-B (10 nmol/mL) was established with MPTP as substrate at 30 °C (k_{cat} = 204 min^{-1}) as reported earlier.[23] The MAO-B preparation was found to be stable when stored at −15 °C. The MAO-A preparation was less stable, and its specific activity had to be estimated on a bimonthly basis. Because of its viscosity, the MAO-A preparation was diluted with 3 volumes of phosphate buffer just prior to analysis.

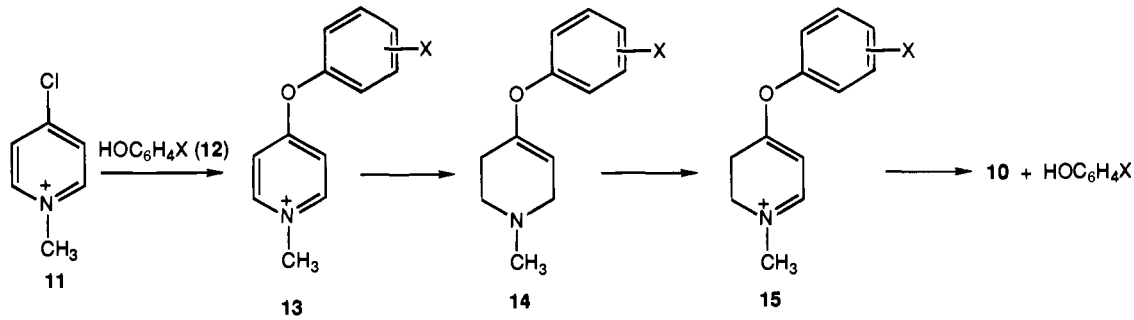
Solutions of the oxalate salts of the 4-(substituted phenoxy)-tetrahydropyridine analogs in phosphate buffer (pH = 7.4, 0.5 mM, final volume 500 μL) in a 1 mL quartz cuvette were treated with 20 μL of the MAO-A preparation (final concentration 0.16 μM) or 5 μL of the MAO-B preparation (final concentration 0.08 μM), and the cuvette was placed in a Beckman Model DU-50 spectrophotometer maintained at 37 °C. The substrate properties were evaluated qualitatively by obtaining a series of scans (450–250 nm) vs time over a 1 h period for each compound.

Kinetic studies were carried out using a Beckman DU-50 spectrophotometer. Solutions of the test compounds (final volume 500 μL, final substrate concentrations 100–2000 μM) in 100 mM sodium phosphate (pH = 7.4) were incubated in the presence of 0.16 μM MAO-A or 0.08 μM MAO-B. The rates of oxidation were obtained by monitoring the increment in amino enone **10** absorbance at 324 nm over a 30–120 s time period. In the case of the 4-(4-nitrophenoxy)tetrahydropyridine analog **14j**, the rates of oxidation were estimated by monitoring the formation of 4-nitrophenol at 400 nm. The absorbtivity of **10** at 400 nm was essentially zero, and no adjustments had to be made in the calculated rates. In case of the 4-(3-nitrophenoxy) analog **14k**, the absorbance of the 3-nitrophenol hydrolysis product at 324 nm (ϵ = 2080 M^{-1}) required the use of an adjusted molar absorbtivity (ϵ = 15 300 − 2080 = 13 270 M^{-1}) for rate calculations. No other interfering or unusual chromophores were observed with the remaining substrates. Repeat determinations on selected substrates performed at different times throughout the course of these studies confirmed the reliability of the kinetic data. The k_{cat} and K_M values were calculated from double-reciprocal plots.

Results and Discussion

Chemistry. Syntheses of the desired substituted 4-phenoxy-1,2,3,6-tetrahydropyridines were achieved by reaction of 4-chloro-1-methylpyridinium iodide (**11**) with the commercially available phenols **12a–o** in the presence of triethylamine to generate the corresponding substituted 4-phenoxy-1-methylpyridinium intermediates **13a–o** (Scheme 4). The pyridinium intermediates were reduced with sodium borohydride to yield the desired tetrahydropyridine derivatives **14a–o** which

Scheme 4. Synthesis and MAO-Catalyzed Oxidation of Phenyl-Substituted 1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine Derivatives[a]



[a] **a**, X = 4-Cl; **b**, X = 3-Cl; **c**, X = 2-Cl; **d**, X = 4-OCH_3; **e**, X = 3-OCH_3; **f**, X = 2-OCH_3; **g**, X = 4-CH_3; **h**, X = 3-CH_3; **i**, X = 2-CH_3; **j**, X = 4-NO_2; **k**, X = 3-NO_2; **l**, X = 2-NO_2; **m**, X = 4-C_6H_5; **n**, X = 3-C_6H_5; **o**, X = 2-C_6H_5.

Table 1. MAO-A-Catalyzed Oxidation of Substituted 1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine Derivatives

	MAO-A			MAO-B			
tetrahydropyridine substrate	K_M (mM)	k_{cat} (min^{-1})	k_{cat}/K_M $(min\ mM)^{-1}$	K_M (mM)	k_{cat} (min^{-1})	k_{cat}/K_M $(min\ mM)^{-1}$	$SC_{A/B}$[a]
MPTP (1)	0.654	31	47	0.191	273	1431	0.03
4-$C_6H_5CH_2$ (**4**)	0.103	28	276[b]	0.180	644	3580	0.08
4-C_6H_5O (**8a**)	0.055	130	2359	0.058	241	4151	0.57
4[c]-Cl (**14a**)	0.063	189	3006	0.254	356	1401	2.15
3-Cl (**14b**)	0.082	358	4368	0.308	224	729	5.99
2-Cl (**14c**)	0.083	204	2458	0.220	181	822	2.99
4-OCH_3 (**14d**)	0.047	73	1550	0.474	354	746	2.08
3-OCH_3 (**14e**)	0.176	492	2797	0.337	243	721	3.88
2-OCH_3 (**14f**)	0.075	57	761	0.313	342	746	1.01
4-CH_3 (**14g**)	0.129	78	602	0.106	214	2019	0.30
3-CH_3 (**14h**)	0.212	431	2034	0.116	202	1747	1.16
2-CH_3 (**14i**)	0.108	270	2501	0.098	175	1782	1.40
4-NO_2 (**14j**)	0.169	82	483	0.443	84	191	2.53
3-NO_2 (**14k**)	0.174	297	1707	0.105	148	1410	1.21
2-NO_2 (**14l**)	0.306	410	1340	0.086	68	786	1.70
4-C_{6H5} (**14m**)	0.101	173	1710	0.284	103	363	4.71
3-C_{6H5} (**14n**)	0.232	1292	5568	0.346	220	637	8.74
2-C_{6H5} (**14o**)	0.426	245	575	0.691	91	132	4.35

[a] $SC_{A/B}$ = the selectivity coefficient = ratio of k_{cat}/K_M for MAO-A to k_{cat}/K_M for MAO-B. [b] The low K_M and k_{cat} values for **4** resulted in low initial rate of oxidation and questionable estimates for the kinetic values recorded here even though the concentration of MAO-A was doubled to 0.32 μM. [c] The numbers indicate the location of the substituent on the 4-phenoxy moiety.

were characterized as their oxalate salts. With the expected exception of the *p*-nitrophenoxy analog **14j**, the UV chromophores of these compounds (λ_{max} = 221–249 nm) were fairly similar. The λ_{max} (304 nm) for **14j** can be interpreted in terms of the relatively extensive delocalization of the oxygen lone pair electrons to the 4-nitro substituent. This depletion of electron density on the enol ether oxygen atom is reflected in the chemical shift value for the C-5 olefinic proton signal which appears at δ 5.41, downfield relative to all of the other analogs (δ 4.49–5.14). The somewhat unusual substrate behavior of **14j** discussed below may be associated with these stereoelectronic effects.

Enzymology. All of the *para*-, *meta*-, and *ortho*-substituted 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine derivatives proved to be substrates for both MAO-A and MAO-B. Initial evidence demonstrating these substrate properties was obtained by examining a series of scans (450–250 nm) vs time over a 1 h period at a substrate concentration of 500 μM in the presence of 0.16 μM MAO-A or 0.08 μM MAO-B. With the exception of the *p*-nitro derivative **14j**, the scans of all incubation mixtures showed the time dependent formation of a new chromophore (λ_{max} near 315 nm) corresponding to the reported UV absorption characteristics of synthetic 1-methyl-4-(4-phenylphenoxy)-2,3-dihydropyridinium perchlorate.[22] This chromophore rapidly shifted to a spectrum with maximal absorption at 324 nm, the λ_{max} of the expected amino enone **10**. These results were taken as evidence for the initial conversion of the substrate molecules to the corresponding dihydropyridinium intermediates **15** (Scheme 4) which subsequently underwent spontaneous hydrolysis to the amino enone **10** and the corresponding phenols.[27] No spectroscopic evidence for the oxidation of the dihydropyridinium intermediates to the corresponding pyridinium species was observed. Incubation mixtures containing the 4-nitrophenyl analog **14j** showed a chromophore with λ_{max} = 400 nm which was assigned to the 4-nitrophenol (λ_{max} = 400 nm, ϵ = 14 300 M^{-1} in pH 7.4 phosphate buffer) liberated upon hydrolysis of the dihydropyridinium intermediate **12j**.

Estimates of the initial oxidation rates for these tetrahydropyridine derivatives as described in the Experimental Section provided linear plots of product formation vs time at all substrate concentrations examined. The k_{cat} and K_M values were calculated by double-reciprocal plots.

The range of k_{cat}/K_M values (Table 1) for the MAO-A-catalyzed oxidations of these phenoxy compounds (483–5568 $min^{-1}\ mM^{-1}$) makes these compounds good to excellent substrates for this form of the enzyme. Analysis of the data summarized in Table 1 demonstrates that all of the *meta*-substituted isomers are

better MAO-A substrates than the corresponding *para*-substituted isomers and, with the marginal exception of the methyl series, are better substrates than the corresponding *ortho*-substituted isomers. The better substrate characteristics of the *meta*-isomers, for the most part, are a consequence of increased k_{cat} values. Particularly impressive are the k_{cat}/K_M values for the *m*-phenylphenoxy analog **14n** (5568 min^{-1} mM^{-1}). The excellent substrate properties of such diverse tetrahydropyridine derivatives as the *m*-phenylphenoxy analog **14n** (k_{cat}/K_M = 5568 min^{-1} mM^{-1}) and the *m*-nitrophenoxy analog **14k** (k_{cat}/K_M = 1707 min^{-1} mM^{-1}) demonstrate the flexibility of the MAO-A active site. Also of interest are the relatively poor substrate properties of the 4-benzyl analog **4** (k_{cat}/K_M = 278 min^{-1} mM^{-1}) relative to the phenoxy analogs including the unsubstituted derivative **8a** (k_{cat}/K_M = 2358 min^{-1} mM^{-1}). This effect of replacing the methylene group in **4** with an oxygen atom may indicate that the electron-releasing properties of the C-4 oxygen atom may facilitate MAO-A catalysis by contributing to the stabilization of the transition state complex involved in the rate-determining catalytic step.

The poorest MAO-A substrates in this series are the *p*-nitrophenoxy (**14j**) and *o*-phenylphenoxy (**14o**) analogs (k_{cat}/K_M = 483 and 575 min^{-1} mM^{-1}, respectively). Apparently the steric crowding present in **14o** impacts somewhat on the catalytic efficiency of MAO-A. The poor MAO-A substrate properties of the *p*-nitrophenoxy analog **14j** relative to the out of conjugation *o*-nitrophenoxy analog **14l** (k_{cat}/K_M = 1340 min^{-1} mM^{-1}) are of interest since this behavior is consistent with the possible enhanced catalytic activity being related to the presence of electron-donating groups at C-4. The corresponding values observed for the *p*-methylphenoxy analog **14g** (k_{cat}/K_M = 602 min^{-1} mM^{-1}) and the *o*-methylphenoxy analog **14i** (k_{cat}/K_M = 2501 min^{-1} mM^{-1}), however, render this proposal less tenable.[28]

As was the case with MAO-A catalysis, the poorest MAO-B substrates in this series are the *o*-phenylphenoxy (**14o**) and *p*-nitrophenoxy (**14j**) analogs (132 and 191 min^{-1} mM^{-1}, respectively), suggesting some similarities in steric and electronic interactions of these substrates with both forms of the enzymes. Unlike the behavior observed with MAO-A, a preference for one isomer was not observed with MAO-B. The most consistent observation within this series was the negative impact bulky groups had on the catalytic activity of MAO-B. In contrast to the behavior observed with MAO-A, where the majority of the substituted phenoxy compounds are better substrates than the unsubstituted phenoxy analog **8a**, the k_{cat}/K_M value for the MAO-B-catalyzed oxidation of **8a** is greater than that for all of the phenoxy-substituted compounds.

Estimates of the MAO-A to MAO-B substrate selectivities for each of the compounds examined in this study are listed in Table 1 as selectivity coefficient values ($SC_{A/B}$), i.e., the ratios of the MAO-A k_{cat}/K_M values to the MAO-B k_{cat}/K_M values. In every case except one (the *p*-methylphenoxy analog **14d**), all of the substituted phenoxy derivatives examined in this study are better MAO-A than MAO-B substrates. The exceptional behavior of **14d** is a consequence of its low k_{cat} value for MAO-A, for which we have no explanation. In general, the best selectivity is observed for the *meta*-substituted isomers with the *m*-phenylphenoxy analog **14n** showing the highest selectivity (8.74), again emphasizing the greater capability of the MAO-A active site to accommodate bulky groups attached to the C-4 position of the tetrahydropyridine ring. This difference in susceptibility to negative steric effects between MAO-A and MAO-B catalysis becomes even more apparent when the $SC_{A/B}$ values for the unsubstituted compounds MPTP (0.03), the benzyl analog **4** (0.08), and the phenoxy analog **8a** (0.57) are included in the comparison.

Overall, these results provide additional evidence for the catalytic versatility of both MAO-A and MAO-B. With the possible exception of the negative influence that the nitro group exerts on catalytic activity when located at the *para*-position of the phenoxy moiety, these data suggest that electronic effects are not likely to contribute significantly to the substrate characteristics of these compounds. The only clearly consistent trend that characterizes differences in substrate selectivity appears to be the greater efficiency with which MAO-A catalyzes the oxidation of tetrahydropyridines bearing bulky groups at C-4. Furthermore, it appears that this selectivity applies to phenoxy groups bearing both polar and nonpolar substituents. This conclusion is in general agreement with previous reports employing both 4-(arylmethyl)-[15] and 4-(aryloxy)tetrahydropyridine derivatives.[22]

Although a more formal quantitative structure–reactivity analysis might prove more insightful, a general consideration of the structural features of those compounds displaying exceptionally good MAO-A substrate properties [the 4-phenoxy analog **8a**, the 4-(3-phenylphenoxy) analog **14n**, 4-(2,4-dichlorophenoxy)-1-methyl-1,2,3,6-tetrahydropyridine,[22] 4-(2-isopropylphenyl)-2-methyl-1,2,3,6-tetrahydropyridine,[14] and 1-methyl-4-(2-naphthoxymethyl)-1,2,3,6-tetrahydropyridine (**8g**)[22]] does not point to any obvious factors that would suggest why closely related analogs (the 4-benzyl analog **4**, various MPTP analogs substituted at the 3- and 4-position of the phenyl ring, and MPTP itself)[13] should be poor or nonsubstrates of this enzyme. Further efforts to define better the structural and stereoelectronic features that contribute to MAO-A and MAO-B substrate and inactivator selectivities of tetrahydropyridine derivatives are being pursued with the aid of a variety of derivatives bearing C-4 heteroaromatic systems with characteristic electronic properties.

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(27) Since the initial absorption maxima appeared at wavelengths somewhat less than 324 nm, estimations of the rates of oxidation of the various substrates obtained by monitoring the amino enone **7** at 324 nm may not be accurate. Previous studies have established, however, that essentialy the same kinetic constants are obtained when metabolite formation is monitored at 315 nm (corresponding to the λ_{max} of the dihydropyridinium metabolite) or 324 nm (corresponding to the λ_{max} of the amino enone **7**).[22] Apparently the similar λ_{max} and ϵ values (about 13 000 M^{-1} for 4-(aryloxy)dihydropyridinium systems and 15 000 M^{-1} for **7**) and the fairly rapid rate of hydrolysis of these dihydropyridinium intermediates under the incubation conditions allow some flexibility with regard to the wavelength selected.
(28) The relatively poor substrate properties of the *p*-methyl isomer **14g** compared to the corresponding *m*- and *o*-isomers were confirmed in a repeat experiment which gave essentially the same k_{cat}/K_M values for all three isomers.

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