Studies on Semirigid Tricyclic Analogues of the Nigrostriatal Toxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Raymond G. Booth,[†,‡] Anthony Trevor,[‡,§] Thomas P. Singer,[‡] and Neal Castagnoli, Jr.*[‡,§]

Departments of Pharmaceutical Chemistry and Pharmacy, School of Pharmacy, and Department of Pharmacology, School of Medicine, University of California, San Francisco, California 94143-0446. *Received April 18, 1988*

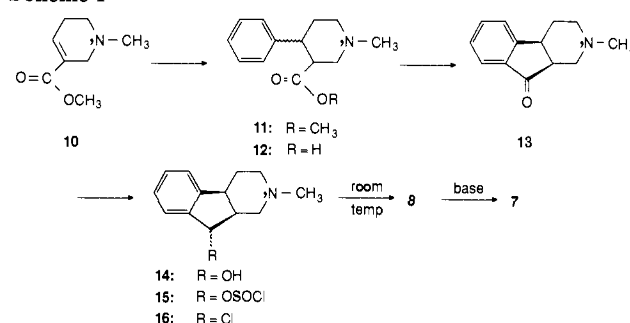
The tetrahydro-β-carboline derived from the condensation of *N*-methyltryptamine and formaldehyde, a semirigid tricyclic analogue of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that has been detected in the brains of normal laboratory rats, is biotransformed in a monoamine oxidase B (MAO-B) catalyzed reaction to the corresponding dihydro compound at a rate that is approximately 0.5% of that observed with MPTP. The corresponding tetrahydroindenopyridine in which the double bond β,γ to the nitrogen atom retains allylic character is a somewhat better MAO-B substrate. The steric bulk of the nitrogen and methylene bridges in addition to ring strain present in the proposed carbon-centered radical intermediates derived from these types of tricyclic structures may contribute to their relatively poor MAO-B substrate properties. Although no MPTP-like neurotoxic properties were observed following acute administration of the test compounds to mice, we speculate that the chronic accumulation of β-carbolinium type metabolites could contribute to the rate of nigrostriatal cell loss associated with idiopathic Parkinson's disease.

Extensive studies have established that the nigrostriatal toxicity of the Parkinsonian-inducing piperidiene derivative 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 1) is mediated by metabolites that are formed by the action of monoamine oxidase B (MAO-B), which catalyzes the oxidation of the parent tertiary amine to the corresponding 1,2-dihydropyridininium species $MPDP^+$ (2).[1] Subsequent transformation of this unexpected two-electron oxidation product yields the pyridinium metabolite MPP^+ (3), which is reported to accumulate in the terminals of nigrostriatal neurons[2] and to undergo active transport into mitochondria[3] where it inhibits NADH oxidation.[4] The MAO-B-catalyzed oxidation of MPTP to $MPDP^+$ has been well characterized kinetically.[5]

The discovery that this simple nitrogen heterocycle can produce neurochemical and neuropathological changes that are remarkably similar to idiopathic Parkinson's disease has led to speculation that the etiology of this disease may be related to neuronal injury caused by endogenous and/or environmental MPTP-like compounds.[6-8] Much interest has focused on 2-methyl-1,2,3,4-tetrahydro-β-carboline (4) because of its structural analogy to MPTP, its potential in vivo formation via a Pictet-Spengler type condensation between *N*-methyltryptamine and formaldehyde,[9] and its presence in the brains of normal laboratory rats.[10] Although this indole derivative may lower striatal levels of dopamine in mice,[11,12] it does not appear to be neurotoxic in monkeys.[6,13] On the basis of results obtained with colorimetric assays and rat mitochondrial preparations, Gibb et al.[14] have suggested that 4 is not an MAO-A or MAO-B substrate. The lack of MPTP-like neurotoxicity observed in the primate studies, therefore, may reflect insufficient production of the dihydro-β-carbolinium and β-carbolinium species 5 and 6, respectively. Consistent with this view, recent studies in our laboratory have shown that an intrastriatal perfusion of the β-carbolinium 6 does indeed cause an irreversible lesion of dopamine-containing neurons in rats.[15]

In an effort to provide additional documentation of the structural features required of compounds with potential MPTP-like activity, we have examined the MAO-B substrate and inhibitory properties of the tetrahydro-β-carboline 4 using enzyme purified from beef liver mitochondria. We also have attempted to assess the importance of the aromatic character of the 4a,9a double bond

Scheme I



* Address correspondence to this author at Harvey W. Peters Center, Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212.

† Department of Pharmaceutical Chemistry.

‡ Department of Pharmacy.

§ Department of Pharmacology.

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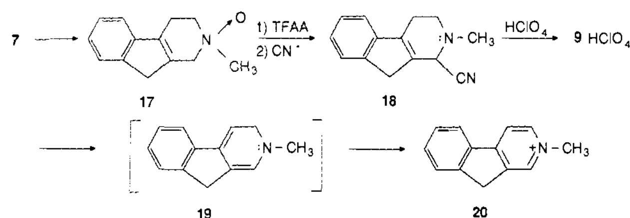
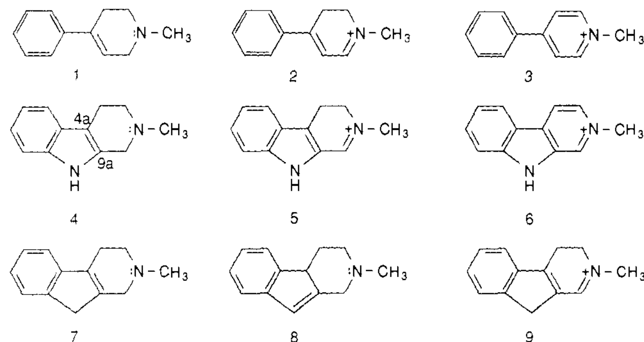
Scheme II



present in tetrahydro-β-carbolines as opposed to the allylic character of the 4,5 double bond present in MPTP by examining the MAO-B substrate and inhibitory properties of 1,2,3,4-tetrahydro-2-methyl-9*H*-indeno[2,1-*c*]pyridine (**7**). The corresponding properties of the isomeric 2,3,4,4a-tetrahydroindenopyridine system **8**, which was obtained as a side product in the synthesis of **7**, and of the dihydroindenopyridinium compound **9**, the potential MAO-B oxidation product of **7**, also have been examined. The nigrostriatal toxicity of **7** and **8** have been assessed in the mouse.



Results

Chemistry. The tetrahydro-β-carboline **4** was prepared by $NaBH_4$ reduction of the β-carbolinium compound **6**.[16] Synthesis of the tetrahydroindenopyridine **7** (Scheme I) followed a pathway reported previously by Plati et al.[17] for the preparation of related systems. Cyclization of piperidinecarboxylic acid **12** obtained by treatment of arecoline (**10**) with phenylmagnesium bromide followed by hydrolysis of the corresponding ester **11** gave a single tricyclic ketone **13**. The cis stereochemistry of this product was established by ^1H NMR spectroscopy as summarized in the Experimental Section. Reduction of **13** with $LiAlH_4$ gave a mixture of diastereomeric alcohols **14**, which could be separated by fractional crystallization. Their essentially identical FTIR and ^1H NMR spectra precluded the possibility of making stereochemical assignments.

Room-temperature treatment of the diastereomeric alcohols **14** with thionyl chloride led to a mixture of products from which it was possible to isolate a monochloro species, one of the two possible 9-chlorohexahydroindenopyridine derivatives **16**, and a product with UV (λ_{max} 250 nm) and mass spectral characteristics expected for a tetrahydroindenopyridine system. Proton NMR analysis revealed a one-proton singlet at δ 7.0 ppm which identifies this compound as the $\Delta^{9,9a}$ olefin **8** that would form via elimination of the thioester **15**. Compound **8** rearranged to **7** upon treatment with base. Compound **7** was prepared more efficiently by heating the crude mixture obtained in the thionyl chloride reaction with base followed by purification of the allylamine as its hydrochloride salt. As required, no olefinic proton signal was observed in the ^1H NMR spectrum of this product.

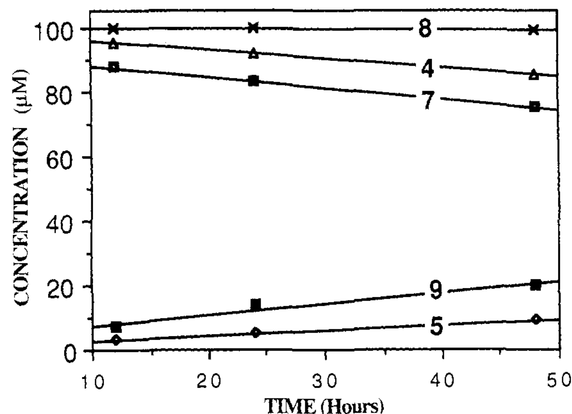


Figure 1. Incubation mixtures of **4**, **7**, and **8** (100 μM) containing 0.2 unit of MAO-B were scanned (500–200 nm) every 12 h for 48 h. The rates of disappearance of substrates **4** and **7** corresponded to the rates of appearance of products **5** and **9**. Compound **8** was stable for 48 h.

Preparation of the 3,4-dihydroindenopyridinium species **9** (Scheme II) was achieved via initial hydrogen peroxide oxidation of **7** to yield the corresponding *N*-oxide **17**. Treatment of **17** with trifluoroacetic anhydride (TFAA) led to the formation of a product that displayed UV spectral characteristics (λ_{max} 360 nm) expected for the dihydroindenopyridinium species **9**. The crude product was isolated via its cyano adduct **18**, which upon treatment with ethanolic perchloric acid yielded the crystalline perchlorate salt of **9**. Unlike $MPDP^+$ (**2**), which undergoes autoxidation[18] and, at concentrations greater than 100 μM, disproportionation,[18] the corresponding methylene-bridged indenopyridinium species **9** proved to be remarkably stable. A 100 μM solution of **9** in pH 7.2 phosphate buffer showed no evidence of decomposition at 25 °C after 15 h and a 10 mM solution was stable for at least 4 h. The oxidation of **9** to the corresponding indenopyridinium species **20** was accomplished in benzene with chloranil although the reaction proceeded slowly (Scheme II). The enhanced stability of **9** compared to that of **2** in part may be due to the ring strain present in the dihydroindenopyridine free base **19**, which is likely to be an intermediate in the oxidation of **9** to **20**.

Pharmacology. The spectral features of tetrahydro-β-carboline **4** (λ_{max} 274 nm) and tetrahydroindenopyridine **7** (λ_{max} 250 nm) vs the dihydro species **5** (λ_{max} 360 nm)[19] and **9** (λ_{max} 360 nm) were exploited to develop a UV-based spectrophotometric assay for the MAO-B-catalyzed oxidation of **4** and **7** to **5** and **9**, respectively. This assay also was used to assess the substrate properties of the $\Delta^{9,9a}$ isomer **8** (λ_{max} 250 nm). Incubation mixtures containing **4**, **7**, or **8** and MAO-B were scanned over a 48-h period and substrates showed a loss of absorbance due to the chromophores of the tetrahydro species and a concomitant increase of the chromophores corresponding to the oxidation products. Figure 1 shows that at the end of 48 h, the tetrahydro-β-carboline **4** was about 15% metabolized to the dihydro-β-carbolinium **5** while the tetrahydroindenopyridine **7** was about 30% metabolized to the dihydroindenopyridinium **9**. As reported for the oxidation of MPTP to $MPDP^+$,[1] pargyline (10^{-6} M), an MAO inhibitor, inhibited both oxidations by over 95%. The isomeric tetrahydroindenopyridine **8** was stable over the

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Table I. Interaction of MPTP and Tricyclic Analogues with MAO-B

compd	initial rates of oxidation,[a] nmol min^{-1} mL^{-1}	inhibn: K_i,[b] μM
MPTP	25.87	100
4	0.10	400
7	0.21	75
8	0.00	65

[a] Incubations consisting of 3 mM substrate and 0.1 unit of enzyme were monitored spectrophotometrically at the λ_{max} corresponding to the dihydro products. [b] Refers to inhibition of the MAO-B-catalyzed oxidation of benzylamine (0.17–3.3 mM) by test compounds (0–0.5 mM).

48-h incubation period. The dihydroindenopyridinium compound **9** (data not shown) also was incubated with MAO-B and its conversion to the corresponding indenopyridinium species **20** (λ_{max} 305 nm) was monitored spectrophotometrically. A 100 μM solution of **9** showed no evidence of decomposition during a 12-h period. A slow increase in absorbance at 305 nm was observed at high (500 μM) concentrations of **9**. This transformation, however, does not appear to be MAO-B catalyzed since the rate of oxidation was essentially identical in the presence and absence of enzyme and pargyline. This behavior contrasts with the reported MAO-B substrate properties of $MPDP^+$.[5]

The initial rates of oxidation of 3 mM MPTP, **4**, and **7** to their corresponding dihydro analogues by 0.1 unit of MAO-B are shown in Table I. These studies establish that MAO-B catalyzes the oxidation of the tetrahydro-β-carboline **4** to the dihydro-β-carbolinium **5** at about 0.5% of the rate observed for the corresponding conversion of MPTP to $MPDP^+$. Oxidation of the tetrahydroindenopyridine **7** to the corresponding dihydroindenopyridinium **9** proceeded at about twice the rate observed with compound **4**.

Table I also shows the K_i values for the competitive inhibition by **7** and **8** of the MAO-B-catalyzed oxidation of benzylamine, which are similar to that of MPTP, and by **4**, which is somewhat higher. Preliminary evidence (data not shown) indicates that **7** also inhibits MAO-B in a time- and concentration-dependent manner, reminiscent of the mechanism-based inactivation observed with MPTP.[20] The tetrahydroindenopyridine isomer **8** proved to be a pure competitive inhibitor as expected for a non-MAO-B substrate.

The effects of acute administration of MPTP, **7**, and **8** on striatal dopamine levels in mice are summarized in Table II. Consistent with several reports,[21] significant reductions in dopamine were observed at 24 h, 1 week, and 1 month after the last injection of MPTP. In contrast, the isomeric tetrahydroindenopyridine analogues **7** and **8** produced a transient rise in dopamine levels at 24 h followed by a return to control values at the 1-week and 1-month time points.

Discussion

The MAO catalytic pathway is thought to involve the formation of an α-carbon-centered radical intermediate.[22] The unexpected and exceptionally good substrate properties of MPTP may be related in part to the stabilization

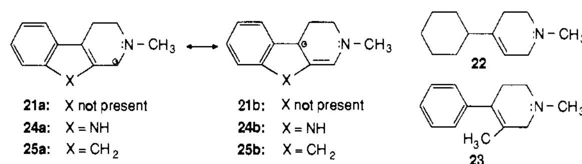
Table II. Striatal Levels of Dopamine in Mice (ng/mg of Tissue)[a]

time after last injection	control (NaCl)	MPTP	7	8
4 hours	16.3 ± 1.8	4.8 ± 0.5	6.5 ± 1.3	4.3 ± 0.4
24 hours	18.0 ± 3.6	6.5 ± 1.0	24.5 ± 4.5	30.3 ± 1.8
1 week	20.1 ± 2.3	2.7 ± 1.7	18.2 ± 1.7	16.4 ± 2.7
1 month	19.0 ± 3.6	5.0 ± 1.3	21.9 ± 3.8	19.2 ± 4.6

[a] C-57 black mice (six per data point) were injected intraperitoneally with the test compound (100 $\mu mol/kg$) in 50 μL of normal saline every hour for 4 h. Control values were obtained with vehicle only.

of the radical intermediate **21** through delocalization of the unpaired electron (**21a** ↔ **21b**). This proposal is consistent with the observations that the allylic double bond is required for substrate activity[23] and that only the allylic position undergoes oxidative attack.[18] The possible further stabilization of intermediate **21** by the phenyl group, however, does not appear to be significant since recent results indicate that 1-methyl-4-cyclohexyl-1,2,3,6-tetrahydropyridine (**22**) also is a good MAO-B substrate.[24]

Using a reliable assay, a purified enzyme preparation, and control incubations in the presence of pargyline, we now have obtained evidence that the tetrahydro-β-carboline **4** and the methylene bridged analogue **7** are MAO-B substrates. The relatively slow turnover of these tricyclic compounds compared to that of MPTP may reflect their poor substrate binding properties. Brossi et al.[25] have reported that the 5-methyl analogue **23** of MPTP is not oxidized in the presence of MAO-B and therefore the steric bulk of the nitrogen and methylene substituents are likely to hinder effective binding of **4** and **7** to the enzyme. It also should be noted that ring strain may limit the resonance stabilization of the corresponding carbon-centered radicals (**24a** ↔ **24b**) and (**25a** ↔ **25b**), which may



contribute to the poor MAO-B substrate properties of **4** and **7**. The lack of substrate properties of the isomeric $\Delta^{9,9a}$-tetrahydropyridine **8** and the dihydroindenopyridinium system **9** may be rationalized by analogous arguments although in the case of **9** other factors including the ring strain present in the dihydropyridine free base **19** may also be important. In contrast to the nitrogen bridge in the tetrahydro-β-carboline **4**, it appears that the electronic and/or hydrophobic character of the methylene bridge in **7** and **8** allows for a tighter competitive inhibitory binding to the enzyme.

In view of the relatively slow rate that compound **7** undergoes MAO-B-catalyzed oxidation and the lack of oxidation of compound **8**, it is not surprising that these compounds do not display MPTP-type neurotoxic prop-

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erties in the mouse model. These results agree with the lack of toxicity of compound **4** in the mouse reported by Perry.[12] The initial increase in striatal dopamine levels observed with the tetrahydroindenopyridines **7** and **8** may reflect their in vivo MAO-inhibitory properties. A possible generalization of these results suggests that the incorporation of the MPTP moiety into fused ring structures is unlikely to produce molecules with good MAO-B substrate and MPTP-type neurotoxic properties. On the other hand, in view of the chronic, cummulative, and more subtle nature of the factors that are likely to contribute to idiopathic Parkinson's disease, the MAO-B substrate properties of the tetrahydro-β-carboline **4** may be significant relative to the potential neurotoxicity and Parkinsonian-inducing properties of these types of compounds.

Experimental Section

All synthetic reactions were carried out under a nitrogen atmosphere. Arecoline hydrobromide was purchased from Sigma Chemical Co., St. Louis, MO, and MPTP·HCl from Research Biochemicals, Natick, MA. All other chemicals were of reagent grade or HPLC grade and were purchased from Aldrich Chemical Co., Milwaukee, WI. Proton NMR spectra were obtained at 80 MHz on a Varian FT-80 instrument, at 240 MHz on a custom-built instrument linked to a Nicolet 1180 computer, or at 500 MHz on a General Electric instrument. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) in $CDCl_3$ or 3-(tetramethylsilyl)propionic-*2,3,3,3-d_4* acid, sodium salt (TSP) in the case of D_2O and CH_3OD. Spin multiplicity is given as (s) singlet, (d) doublet, (t) triplet, (q) quartet, or (m) multiplet. Chemical-ionization mass spectra (CIMS) were run on a modified AEI MS 902S at 8 kV with isobutane (ca. 1 Torr) as reagent gas and gas chromatography–electron-ionization mass spectra (GC/EI-MS) on a Kratos MS 25S. Liquid secondary ion mass spectra (LSIMS) were obtained on a Kratos MS 50S. Ultraviolet (UV) spectra were recorded with a Beckman DU-50 spectrophotometer connected to an AT&T PC 6300 computer. Infrared spectra were obtained on a Nicolet-5DX FTIR instrument. Melting points were obtained on a Thomas-Hoover or Fisher-Johns apparatus and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley, CA.

Chemistry. *cis*-1,2,3,4,4a,9a-Hexahydro-2-methyl-9*H*-indeno[2,1-*c*]pyridin-9-one (13). Tricyclic ketone **13** was synthesized in 70% yield according to the method of Plati et al.:[17] mp 64–66 °C (lit.[17] mp 64.5–65.5 °C); CIMS 202 (MH^+, 100). The cis stereochemistry of this product was established by 240-MHz ^1H NMR analysis in $CDCl_3$: δ 1.5 [m, 1 H, C(4)-H], 2.15 [m, 2 H, C(4)-H, C(5)-H], 2.25 (s, 3 H, CH_3), 2.45 [m, 2 H, C(3)-H, C(1)-H], 2.8 [q, 1 H, C(9a)-H], 3.15 [q, 1 H, C(1)-H], 3.4 [q, 1 H, C(4a)-H], 7.4–7.8 (t, d, t, d, 4 H, ArH). Irradiation of H_{9a} signal caused the H_{4a} quartet to collapse to a symmetrical triplet, J = 7.2 Hz, due to the coupling with the C_4 methylene protons which form dihedral angles with H_{4a} of 140° (axial) and 15° (equitorial), consistent with a molecular model in which H_{4a} occupies an axial orientation. Double resonance experiments also established the gem-coupling constant for the C_1 methylene protons as 10.8 Hz (112°) and the symmetrical viscinal coupling constant of 3.6 Hz for each of these protons with H_{9a} as expected for an equitorial orientation for H_{9a} and a dihedral angle of 45° with each of the C_1 methylene protons. Comparable arguments have documented the cis fusion of related ring systems.[26]

***cis*- and *trans*-1,2,3,4,4a,9a-Hexahydro-2-methyl-9*H*-indeno[2,1-*c*]pyridin-9-ol (14).** An ethereal solution of ketone **13** (14 g, 70 mmol) was added slowly to a suspension of $LiAlH_4$ (6 g, 170 mM) in ether with stirring, and the resulting mixture was heated under reflux for 3 h. The reaction was quenched by the addition of 6 mL of H_2O followed by 6 mL of 15% NaOH and 18 mL of H_2O after which the pH was adjusted to 10 with 15% NaOH. Ether extraction provided 12.9 g (91%) of a white powder (mp 110–140 °C) that gave two spots on silica gel TLC [$CHCl_3$–CH_3OH (1:1), R_f = 0.15 and 0.18]. Crystallization from ethyl acetate gave one of the isomeric alcohols as white crystals (mp 144–145 °C; R_f = 0.15) while concentration of the mother liquors yielded the second as large white prisms (mp 124–125 °C; R_f = 0.18). The 240-MHz ^1H NMR ($CDCl_3$) spectra of these compounds were indistinguishable: δ 1.8 (m, 1 H), 2.1 (m, 2 H), 2.25 (s, 3 H, CH_3), 2.5 (m, 2 H), 2.7 (m, 1 H), 3.0 (m, 2 H), 5.08 [d, 1 H, C(9)-H], 7.2–7.5 (m, 4 H, ArH). Both compounds also gave identical CI mass spectra, 204 (MH^+, 100), 186 (43), and FTIR spectra (15 and 3 mM CCl_4), 3300 cm^{-1} (broad, intermolecular bonded OH). Anal. ($C_{13}H_{17}NO$) C, H, N (for each isomer).

Reaction of Alcohols 14 with Thionyl Chloride. A mixture of alochols **14** (2.0 g, 9.9 mmol) and 25 mL of thionyl chloride was stirred at room temperature for 15 min following which the thionyl chloride was removed at reduced pressure to yield a hard white mass. Chromatography on 50 g of silica gel (70–230 mesh) using chloroform with a methanol gradient gave three fractions. The first fraction (0.3 g, 200 mL, $CHCl_3$–CH_3OH, 9.5:0.5) yielded a white solid melting at 227–230 °C dec. Recrystallization from a 1:1 mixture of methanol and ethanol provided the pure **1,2,3,4,4a,9a-hexahydro-9-chloro-2-methyl-9*H*-indeno[2,1-*c*]pyridine (16)** as its hydrochloride salt: mp 294–297 °C dec; 80-MHz ^1H NMR (CH_3OD) δ 2.82 (s, 3 H, CH_3), 3.0–3.7 (m, 8 H), 5.3 [d, 1 H, C(9)-H], 7.3 (m, 4 H, ArH); CIMS 224/222 (MH^+, 33/100), 186 (100). Anal. ($C_{13}H_{17}NCl_2$) C, H, N, Cl. The second fraction (100 mL, $CHCl_3$–CH_3OH, 9:1) gave 0.2 g of a dark green oil with ^1H NMR and CI mass spectral characteristics corresponding to a mixture of the two possible diastereomeric chloro compounds. The third fraction (200 mL, $CHCl_3$–CH_3OH, 9:1) yielded 0.6 g of a white powder melting at 260–265 °C dec. Recrystallization from a 1:1 mixture of methanol and ethanol provided the pure **2,3,4,4a-tetrahydro-2-methyl-1*H*-indeno-[2,1-*c*]pyridine (8)** as its hydrochloride salt: mp 288–291 °C dec; 240-MHz ^1H NMR (D_2O) δ 2.8–2.9 [m, 2 H, C(4)-H_2], 3.0 (s, 3 H, CH_3), 3.4–3.7 [m, 3 H, C(4a)-H, C(3)-H_2], 4.1 [d, 1 H, C(1)-H], 4.5 [d, 1 H, C(1)-H], 7.0 [s, 1 H, C(9)-H], 7.4 (m, 2 H, ArH), 7.6 (m, 2 H, ArH); CIMS 186 (MH^+, 100); UV (CH_3OH) λ_{max} 250 nm (ϵ 11 000). Anal. ($C_{13}H_{16}NCl$) C, H, N.

1,2,3,4-Tetrahydro-2-methyl-9*H*-indeno[2,1-*c*]pyridine Hydrochloride (7·HCl). The diastereomeric alcohols (**14**, 1.0 g, 4.9 mmol) were stirred at room temperature in 15 mL of thionyl chloride for 15 min. The white powder that remained after removal of the thionyl chloride was heated at 50 °C for 18 h in 50 mL of anhydrous THF containing potassium *tert*-butoxide (2.0 g, 17.8 mmol). The reaction mixture was poured into 1.0 L of water and extracted exhaustively with chloroform. The combined extracts were dried over anhydrous sodium sulfate, and the solvent was removed to give 0.7 g of a brown residue. Treatment of this residue in ether with HCl gas precipitated a white powder (0.63 g), which was recrystallized from methanol to yield the desired tetrahydroindenopyridine as its hydrochloride salt: mp 286–290 °C dec; UV (CH_3OH) λ_{max} 250 nm (ϵ 11 000); 240-MHz ^1H NMR (D_2O) δ 2.9 [br s, 2 H, C(4)-H_2], 3.1 (s, 3 H, CH_3), 3.4 [m, 3 H, C(3)-H, C(9)-H_2], 3.8 [br d, 1 H, C(3)-H], 4.1 [d, 1 H, C(1)-H], 4.4 [d, 1 H, C(1)-H], 7.4–7.6 (m, 4 H, ArH); GC/EIMS 185 ($M^{\bullet+}$, 88), 184 (100), 142 (57). Anal. ($C_{13}H_{16}NCl$) C, H, N.

1,2,3,4-Tetrahydro-2-methyl-9*H*-indeno[2,1-*c*]pyridine *N*-Oxide Dihydrate (17·2H$_2$O). A solution of **7** (3.2 g, 17.3 mmol) in 25 mL of ethanol and 25 mL of dichloromethane containing 5 mL of 30% hydrogen peroxide was heated under reflux for 22 h and, after the addition of 0.3 g of 10% Pd/C, for an additional 12 h. The reaction mixture was filtered through Celite and the filtrate was dried by azeotropic distillation in vacuo three times with 4 volumes of anhydrous ethanol and three times with 4 volumes of anhydrous benzene. The light-yellow solid thus obtained was crystallized from benzene to give 2 g (10 mmol, 57%) of pure **17** as its dihydrate: mp 141–143 °C; UV (CH_3OH) λ_{max} 255 nm (ϵ 9000); 500-MHz ^1H NMR (D_2O) δ 2.9 [br s, 2 H, C-(4)-H_2], 3.3 (s, 3 H, CH_3), 3.4 [d, 2 H, C(9)-H_2], 3.6 [t, 2 H, C(3)-H_2], 4.2 [d, 1 H, C(1)-H], 4.5 [d, 1 H, C(1)-H], 7.3–7.6 (m, 4 H, ArH); EIMS 201 ($M^{\bullet+}$,7), 184 (85), 142 (88); LSIMS 202 (MH^+). Anal. ($C_{13}H_{15}NO{\cdot}2H_2O$) C, H, N.

3,4-Dihydro-2-methyl-9*H*-indeno[2,1-*c*]pyridinium Perchlorate (9·ClO$_4^-$). The above *N*-oxide (**17**, 0.4 g, 2 mmol) in 50 mL of dichloromethane was stirred with trifluoroacetic an-

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hydride (1 mL, 0.7 mmol) under nitrogen for 1 h at 0 °C and an additional 30 min at room temperature. Removal of the solvent produced a yellow oil, which was dissolved in 10 mL of water and treated with potassium cyanide (0.5 g, 4.4 mmol). The reaction mixture was stirred vigorously and the pH was adjusted quickly to 4 by addition of 10% aqueous sodium carbonate. After stirring of the mixture for an additional 20 min, the pH was adjusted to 10 with aqueous sodium carbonate and the organic layer was separated and discarded. The remaining aqueous phase was extracted with 2 × 25 mL methylene chloride, and the combined organic extracts were dried over sodium sulfate, filtered, and evaporated to a dark green oil. This oil in 30 mL of ethanol was stirred at room temperature with 1 mL of 70% perchloric acid, which caused a light-brown precipitate to form. The product (400 mg) was collected 20 h later and melted at 74–84 °C. Recrystallization two times from methanol provided orange prisms of the perchlorate salt of **9** (100 mg, 0.4 mmol, 20%): mp 142–145 °C; UV (CH_3CN) λ_{max} 360 nm (ϵ 16 700); 500-MHz ^1H NMR (CD_3CN) δ 3.26 [m, 2 H, C(4)-H_2], 3.6 (s, 3 H, CH_3), 3.8 [2 H, C(9)-H_2], 4.0 [m, 2 H, C(3)-H_2], 7.55 (m, 2 H, ArH), 7.75 (m, 2 H, ArH), 8.4 [s, 1 H, C(1)-H]; LSIMS 184 (M^+). Anal. ($C_{13}H_{14}NO_4Cl$) C, H, N, Cl.

2-Methyl-9*H*-indeno[2,1-*c*]pyridinium Chloride Hydrate (20·Cl⁻·H₂O). In a separate reaction the crude dihydroindenopyridinium compound obtained as above from 200 mg (1 mmol) of *N*-oxide **17** was dissolved in 100 mL of benzene containing 1.2 g (5 mmol) of chloranil. The solution was stirred in the dark at room temperature for a total of 70 h. The reaction mixture was extracted with 25 mL of 6 N HCl and the aqueous layer was washed exhaustively with ether to remove remaining chloranil and the corresponding hydroquinone reaction product. Lyophilization of the aqueous layer yielded a yellow-brown powder, which was dissolved in 10 mL of water. After the pH was adjusted to 10 with concentrated ammonium hydroxide, the resulting purple solution was extracted with benzene to remove traces of organic bases. The aqueous layer was lyophilized to a light purple powder, which was dissolved in chloroform and filtered to remove inorganic salts. The purple residue obtained after removal of the chloroform was dissolved in methanol and passed through a 10-g column of Bio-Rad AG 1-X8, 50–100 mesh, chloride form. The methanol was evaporated to leave a purple crystalline material, which was recrystallized from acetone. The product **20**·Cl^- (50 mg, 0.23 mmol, 23%) was somewhat hygroscopic: mp 138–141 °C; UV (CH_3OH) λ_{max} 305 (ϵ 10 000); 500-MHz ^1H NMR (D_2O) δ 4.17 [s, 2 H, C(9)-H_2], 4.36 (s, 3 H, CH_3), 7.6 [t, 1 H, C(6)-H], 7.7 [t, 1 H, C(7)-H], 7.77 [d, 1 H, C(8)-H], 8.1 [d, 1 H, C(5)-H], 8.25 [d, 1 H, C(4)-H], 8.6 [d, 1 H, C(3)-H], 8.77 [s, 1 H, C(1)-H]; EIMS 181 ($M^{\bullet+}$, free base resulting from loss of a C-9 methylene proton, 100), 166 (77), 139 (31), LSIMS 182 (M^+). Anal. ($C_{13}H_{12}NCl \cdot H_2O$) C, H, N.

2-Methyl-1,2,3,4-tetrahydro-β-carboline Hydrochloride (4·HCl). Reduction of 2-methyl-β-carbolinium iodide (mp 230–232 °C, lit.[16] mp 234–236 °C) with $NaBH_4$ provided **4** in 34% yield: mp 209–211 °C (lit.[16] mp 216–217 °C). The hydrochloride salt of **4** was prepared by passing dry hydrogen chloride gas through a solution of the free base in ethanol: mp 239–241 °C. Anal. ($C_{12}H_{15}N_2Cl$) C, H, N.

Enzyme Assays. MAO-B from beef liver mitochondria was isolated by the method of Salach[27] and traces of heme protein were removed by centrifugation through a sucrose gradient.[28] All assay mixtures (1.0 mL) contained 50 mM sodium phosphate buffer, pH 7.2, with 0.2% (w/v) Triton X-100 (reduced form) and varying amounts of substrate and/or inhibitor (see below). Enzyme activity was determined spectrophotometrically at 250 nm by initial rate measurements (30–120-s reaction periods) with 3.3 mM benzylamine as substrate.[29] One unit of MAO-B is defined as that amount of enzyme that oxidizes 1.0 μmol of benzylamine to benzaldehyde per minute at 30 °C, pH 7.2. Reactions were initiated by the addition of 0.02–0.2 unit of MAO-B. Incubation mixtures containing 0.2 unit of MAO-B and 100 μM **4**, **7**, **8**, or **9** were scanned spectrophotometrically (500–200 nm) every 12 h for 48 h. The initial rate of formation of $MPDP^+$ from 3.0 mM MPTP was monitored at 345 nm while the initial rates of formation of the dihydropyridinium metabolites **5** (from 3.0 mM **4**) and **9** (from 3.0 mM **7**) were monitored at 360 nm in the presence of 0.1 unit of MAO-B. The formation of the indenopyridinium **20** from compound **9** (0.1–0.5 mM) in the presence of 0.2 unit of MAO-B was monitored at 305 nm. All assays were compared to controls which contained substrate, enzyme, and 10^{-6} M pargyline. Lineweaver–Burk plots showed competitive kinetics by **4** (0–500 μM) and **7** and **8** (0–100 μM) for inhibition of benzylamine (0.17–3.3 mM) oxidation by MAO-B (0.02 unit), and K_i values were determined from secondary replots of slope vs concentration of inhibitor.

Animal Neurotoxicity Studies. Eight to 10 week old male C-57 black mice weighing 25–30 g were housed six per cage with free access to food and water in an animal colony room maintained at 23 °C with controlled lighting (lights on 06:00–18:00 h). Treated mice were administered the test compounds (100 μmol/kg per h × 4 h) in 50 μL of normal saline. Control mice were injected with 50 μL of normal saline. Following decapitation, the striata were dissected according to the method of Ricuarte et al.[21] and immediately stored at −80 °C. All samples were assayed within 2 weeks. Dopamine was determined by an HPLC-electrochemical detection method[30] as follows: Tissue samples were spiked with 3 ng/mg of 3,4-dihydroxybenzylamine (DHBA) as an internal standard and then were homogenized with 1.0 mL of cold 0.2 M $HClO_4$. After centrifugation at 13 000 rpm for 15 min, a 500-μL aliquot of supernatant was added to 20 mg of acid-washed Al_2O_3 (pH 3.4) in a 1.5-mL polypropylene tube. To this was added 1.0 mL of 1.0 M Tris buffer (pH 8.6) and the mixture was vortexed for 5 s and then shaken mechanically for 15 min. The supernatant was discarded and the Al_2O_3 was washed with 1.5 mL of cold H_2O. The catechols were eluted by addition of 1.0 mL of cold 1.0 M $HClO_4/NaClO_4$ followed by vortexing for 15 s. The supernatant was filtered through a 3-mm Cameo nylon membrane before injection onto the HPLC column. Samples (10–100 μL) were injected onto an analytical Alltech C-18, 5-μm column. A single piston Altex LC pump, connected to a Bioanalytical System LC-3 amperometric detector (sensitivity set at 5 nA/V), and Shimadzu integrator/printer were used for all assays. The mobile phase (0.06 M methanesulfonic acid, 0.03 M phosphoric acid, 0.05 mM EDTA, pH 3.6) was maintained at a flow rate of 1.5 mL/min. Catechols were oxidized at an applied potential of +750 mV at room temperature. The concentrations of dopamine were determined by peak height ratio analysis (internal standard/dopamine) using linear standard calibration curves prepared from spiked samples (0–150 ng of dopamine/mL) in buffer. Recoveries averaged 20%.

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Registry No. 4, 13100-00-0; 4·HCl, 117897-14-0; 7, 110605-92-0; 7·HCl, 71158-31-1; 8·HCl, 117897-08-2; 9·ClO_4^-, 117897-12-8; 13, 117917-38-1; 14 (isomer 1), 117957-15-0; 14 (isomer 2), 117957-16-1; 16 (isomer 1), 117897-07-1; 16 (isomer 2), 117957-17-2; 17, 117897-09-3; 18, 117897-10-6; 20·Cl^-, 117897-13-9; MAO-B, 9001-66-5; 2-methyl-β-carbolinium iodide, 5667-11-8.

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