Notes

Synthesis and Toxicity toward Nigrostriatal Dopamine Neurons of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Analogues

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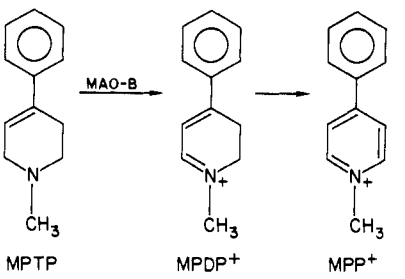
Six compcunds having structural features in common with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were synthesized and tested in mice for the ability to produce a prolonged decrease in nigrostriatal dopamine (DA) and DA metabolites. The compounds that were prepared and tested include the ester elimination products of the analgetic drugs α-prodine and trimeperidine. None of the compounds in this study, except for MPTP, produced significant neurotoxic effects in the mouse model. The study shows that minor changes in the tetrahydropyridine ring of MPTP result in a marked decrease in neurotoxicity.

The selective neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 1) toward dopaminergic neurons in the nigrostriatum of humans,[1,2] other primates,[3,4] and several animal species.[5-8] has been well documented. The neurotoxicity of this substance requires its metabolism (Scheme I) to 1-methyl-4-phenyl-2,3-dihydropyridinium ($MPDP^+$) by MAO-B and perhaps the subsequent oxidation of $MPDP^+$ to 1-methyl-4-phenylpyridinium (MPP^+).[9-11] Selective inhibitors of MAO-B have been shown to prevent the conversion of MPTP to MPP^+ and to block totally the neurotoxic effects of MPTP both in vivo[12,13] and in vitro.[11,14] The identity of the ultimate toxogenic metabolite remains unknown. $MPDP^+$ is an electrophilic species and could react with nucleophiles within the cell, or it could serve as an oxidizing agent to produce other toxic agents such as the *o*-quinone form of dopamine.[11,15] On the other hand, MPP^+ is more toxic than MPTP itself when studied in cell culture[14] and MPP^+ has been shown to accumulate in the nigrostriatal cells of primates after they have been treated with MPTP.[13,16] Interestingly, rats, mice, and guinea pigs do not retain MPP^+ in their striata to the same extent as primate, and MPTP is much less toxic in these nonprimate animals.[16] It remains unexplained as to why MPTP is not toxic to adrenal chromaffin cells, which make large quantities of MPP^+, or why, of the various dopamine neuronal tracts in the CNS, only the cells of the nigrostriatal tract concentrate MPP^+ and only these cells are destroyed after MPTP treatment.

The discovery of the neurotoxic properties of MPTP has led to the development of a drug-inducible model for Parkinsonism in monkeys.[3,4] Due to as yet undescribed differences in metabolism and/or distribution, it is difficult, if possible at all, to obtain DA reductions equivalent to those obtained in monkeys when treating small laboratory animals with MPTP.[5,7,8,17,18] The discovery of a new compound with increased effectiveness in producing an irreversible Parkinsonism-like syndrome in small animals could prove to be a useful research tool.

There is a need to define the limiting structural components of MPTP that are necessary for the toxic action.

Scheme I



Several drugs are already marketed[19] or are in development[20] that contain the tetrahydropyridine structure as

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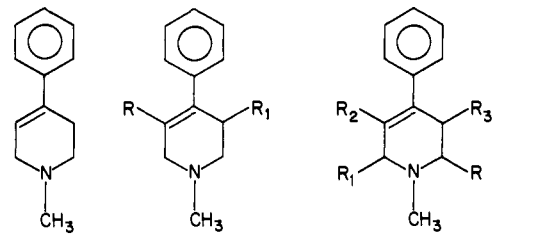
found in MPTP. Other drugs such as α-prodine and trimeperidine contain a 4-(aryloxy)piperidine functional group that can easily undergo an elimination reaction to give structures closely related to MPTP. Even small amounts of these elimination products originating in the manufacture, formulation, storage, or metabolism of these drugs could cause significant but not readily detectable neuronal damage in humans.

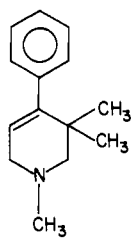
In an effort to evaluate the structure–activity relationships (SAR) of compounds closely related to MPTP, we report here the syntheses and evaluations for toxic effects on CNS dopaminergic neurons of six compounds. Compounds **2** and **3** are the elimination products formed by treatment of α-prodine (or its alcohol precursor) with acid. Compounds **4** and **5** are the elimination products formed from trimeperidine or its diastereomers (promedols). Compound **6** was prepared for its potential to evaluate the relative importance of the dihydropyridinium species vs. the pyridinium species in the toxicity of these agents. It was reasoned that this compound might be converted to the dihydropyridinium species by MAO but the 3,3-dimethyl substitution would prevent aromatization to the pyridinium metabolite. Compound **7** was included as an "open-ring" analogue of MPTP. Neurotoxicological evaluations of these compounds would reveal the SAR of structures closely related to existing drugs, lead potentially to the discovery of compounds with different interspecies toxicity than MPTP, and provide some insight into the toxic mechanism of MPTP.



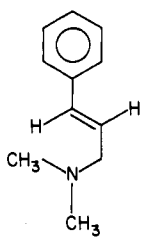
1, MPTP

2: R=CH_3, R_1=H
3: R=H, R_1=CH_3

4: R, R_2=CH_3; R_1, R_3=H
5: R, R_2=H; R_1, R_3=CH_3



6



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Chemistry. Compounds **2** and **3** were obtained by refluxing a mixture of α- and β-prodinol in 36% HCl and purification of the isomeric elimination products by column chromatography. Compounds **4** and **5** were prepared by similar acid treatment of trimeperidine alcohol and separation of the isomers by column chromatography. The alcohol precurser to **6** was prepared by the method of Katvalyan and Mistryukov.[21] When heated under reflux in aqueous HCl, this alcohol afforded the desired tetrahydropyridinium compound. Compound **7** was prepared by treating cinnamyl bromide with dimethylamine as described by Mitch and Cromwell.[22]

Results and Discussion

Neurotoxicological evaluations were carried out in mice, and the results are given in Table I. The drug treatment regimen used in this study is similar to that used by Heikkila et al.[5,6] and the MPTP-induced 75–80% loss of DA from nigrostriatal tissue at both 1 and 9 days after the last injections compares favorably in the two studies. While the mouse is not as sensitive to the toxic effects of MPTP as the primate, similar neurochemical results appear to be achievable. There is an irreversible loss of DA neurons in the nigrostriatum, while other DA and other neurotransmitter systems seem not to be chronically altered.[5] Thus, the ability of a test compound to decrease chronically the levels of DA and its metabolites in the mouse striatum appears to be a valid assay for the evaluation of MPTP-like neurotoxicity. Toxicity is demonstrated by the sustained depletion of DA and does not require the appearance of a Parkinsonian-like syndrome in the test animals.

The failure of any of the test drugs at high doses (equal to 30 mg kg^{-1} day^{-1} of MPTP·HCl for 10 consecutive days) to effect a decrease in striatial DA content is somewhat surprising. We have direct evidence (HPLC–EC analysis of brain tissue)[23] that compounds **1**, **2**, and **6** reach comparable brain concentrations in mice at short time periods (10–30 min) after administration. Thus, distribution is not likely to be an important factor in the relative toxicities of these agents. In a separate experiment, using female mice, the control animals and the **1**- and **6**-treated animals had striatal DA, HVA, and DOPAC contents statistically equal to the equivantly treated groups listed in Table I. Thus, there does not appear to be a sex difference in the toxicity of MPTP. A plausible explanation for the lack of toxicity is that none of the compounds are substrates for MAO-B. This explanation would be in agreement with the report that a number of *p*-phenyl- or *N*-alkyl-substituted MPTP analogues had toxicities that paralleled their activities as substrates for MAO.[24] To evaluate this hypothesis, the properties of these compounds as substrates or inhibitors of MAO-B will be part of our future research efforts.

In conclusion, it may be stated that the addition of one or more methyl groups at various positions of the tetrahydropyridine ring of MPTP results in compounds that show no effect on striatal DA, HVA, or DOPAC content when administered to mice in high doses. In addition, compound **7**, which may be considered as an open-ring analogue of **1**, showed no toxicity in this assay. While it cannot be stated for certain that the MPTP analogues are not toxic at any dose, their failure at the dose level employed in this study to cause any decrease in striatal DA content indicates that they are much less toxic than MPTP.

In view of the known differences in metabolism and toxicity in mice vs. primates,[16] confirmation of these results in monkeys should be obtained. Such studies would serve to validate the usefulness of the mouse in the evaluation of MPTP-type neurotoxicity.

Experimental Section

IR spectra were taken on a Meckman Acculab 2 spectrophotometer. NMR spectra were taken on a Hitachi Perkin-Elmer

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Table I. Effects of Prolonged High-Dose Treatment with MPTP and Structurally Related Analogues on Nigrostriatal DA, DOPAC, and HVA Content in Mice

treatment[a] agent	assay time,[b] days	n	content in sriata,[c] μg/g of tissue		
			DA	DOPAC	HVA
normal saline	1	8	14.15 ± 0.50	1.01 ± 0.19	1.28 ± 0.10
normal saline	9	5	15.23 ± 0.85		
MPTP·HCl (1)	1	10	3.16 ± 0.28	0.31 ± 0.03	0.61 ± 0.03
MPTP·HCl (1)	9	5	4.34 ± 0.32		
2·HCl	1	10	13.68 ± 0.64	1.22 ± 0.10	1.26 ± 0.05
2·HCl	9	5	13.85 ± 0.77		
3·HCl	1	9	13.21 ± 0.48	1.11 ± 0.04	1.14 ± 0.04
3·HCl	9	5	13.35 ± 1.02		
4·Fumarate	1	9	15.22 ± 0.64	1.18 ± 0.08	1.27 ± 0.06
4·Fumarate	9	5	14.49 ± 0.86		
5·HCl	1	10	14.42 ± 0.63	1.36 ± 0.10	1.39 ± 0.10
5·HCl	9	5	14.65 ± 0.14		
6·HCl	1	9	17.28 ± 0.45	1.31 ± 0.08	1.39 ± 0.08
6·HCl	9	5	17.28 ± 0.88		
7·HCl	1	8	13.60 ± 0.51	1.37 ± 0.15	1.11 ± 0.05
7·HCl	9	5	14.56 ± 0.75		

[a] Male Swiss mice weighing 25–40 g were injected ip with the test agents at a dose of 0.072 mmol/kg on days 1 and 2 and 0.142 mmol/kg on days 3–12. All drugs were prepared as 0.14 M solutions in sterile normal saline. [b] Assay time is the number of days between the time of sacrifice of the mice and the administration of the last injection of the test agent. [c] The values listed (±SEM) are not corrected for recovery of the chemicals in the assay system. The recoveries are DA >95%, DOPAC ~80%, and HVA ~75%.

R-24 spectrometer with $CDCl_3$ (Me_4Si internal standard) for free-base forms or D_2O (DDS internal standard) for the amine salt forms as solvents. All spectral data were in agreement with the assigned structures. Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were determined in the Microanalytical Laboratory of the University of Groningen and are within ±0.4% of the theoretical values in each case. TLC analyses were done on 5 × 10 cm silica gel plates (E. Merck No. 5719), developed in CH_2Cl_2–CH_3OH–NH_4OH (100:5:1), and visualized with UV light and I_2.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine Hydrochloride (MPTP·HCl, 1). This compound was prepared by reacting 1-methyl-4-piperidinone with PhLi to give 1-methyl-4-phenyl-4-piperidinol, mp 106–108 °C. Heating this piperidinol under reflux in 10% HCl for 4 h and standard workup procedure gave a nearly quantitative yield of 1·HCl; the compound was recrystallized from ethanol; mp 250 °C (lit.[25] mp 241–243 °C).

1,3-Dimethyl- and 1,5-Dimethyl-4-phenyl-1,2,3,6-tetrahydropyridinium Chlorides 2·HCl and 3·HCl. A mixture of α- and β-prodinol hydrochloride (0.7 g, 2.80 mmol) was heated under reflux in 36% HCl solution for 4 h. Analysis of the reaction mixture by TLC showed no starting material and two products with higher R_f values. Separation of these products was achieved by liquid chromatography on a 3 × 40 cm column, which was packed with 40–63 μm silica gel (Merck) and eluted with CH_2Cl_2–MeOH–NH_4OH (100:4:0.75). The NMR of the first compound to elute from the column showed a single vinyl proton at δ 5.7 (t), a methyl at δ 0.95 (d), and all of the remaining absorptions expected for compound **3**.

The free base of **3** as an oil was dissolved in anhydrous Et_2O and treated with Et_2O·HCl solution, and the Et_2O was decanted from the white semisolid material that precipitated. Recrystallization of this material from anhydrous EtOAc afforded pure **3**·HCl (208 mg), 205–206 °C (lit.[26] mp 206 °C). The second compound from the column had the NMR spectral absorptions expected for **2** (no vinyl absorption and singlet 3-methyl peak at δ 1.5). Treatment of **2** in anhydrous Et_2O with Et_2O·HCl afforded a white precipitate that was isolated by filtration, washed with Et_2O (2 × 5 mL), and dried in vacuo to give **2**·HCl (190 mg), mp 182–183 °C (lit.[27] mp 187–189 °C).

1,2,5-Trimethyl- and 1,3,6-Trimethyl-4-phenyl-1,2,3,6-tetrahydropyridinium Chloride (4·HCl) and Fumarate (5·Fumarate). γ-Promedol alcohol was prepared by the method previously described.[28] Treatment of this piperidinol (1.0 g) by the identical chemical and separation procedures as described for the prodinols gave two dehydration products. The NMR spectrum of the first compound to elute from the column showed two CH_3 absorptions as overlapping doublets centered near δ 1.0 and a single vinyl proton at δ 5.5 (d). Attempts to crystallize this compound as its HCl salt from various solvent systems were not successful. The compound was converted to its fumarate salt and recrystallized from Et_2O to give **5**·Fumarate (240 mg), mp 134 °C. The NMR spectrum of the second compound obtained from the column lacked vinylic proton absorptions and showed one CH_3 as a doublet (δ 1.0) and a second CH_3 as a singlet (δ 1.45). This compound was converted to its HCl salt and recrystallized from EtOAc to give **4**·HCl (320 mg), mp 182–183 °C. Anal. ($C_{14}H_{20}ClN$) C, H, N.

1,3,3-Trimethyl-4-phenyl-1,2,3,6-tetrahydropyridine Hydrochloride (6·HCl). 1,3,3-Trimethyl-4-piperidinone was obtained by the method of Katvalyn and Mistryukov;[19] bp 88–90 °C (20 mmHg) (lit.[19] mp 69 °C (16 mmHg)). This piperidinone (2.3 g, 0.0163 mol) was reacted with an excess of freshly prepared PhLi (0.03 mol) at 0 °C in continuously stirred anhydrous Et_2O solution. After the addition of the PhLi to the piperidinone solutions was completed, the reaction was allowed to warm to room temperature and then refluxed for 3 h. The reaction was maintained in a dry N_2 atmosphere at all times. The reaction was quenched with H_2O (10 mL added slowly followed by 100 mL). The Et_2O layer was separated and extracted with 4 × 20 mL of 3% HCl solution. The combined acidic extracts were made basic with KOH, and the white solid that formed was extracted into Et_2O, dried (Na_2SO_4), evaporated in vacuo to 40 mL, stoppered, and cooled to −20 °C for 3 days. The crystals that formed were isolated by filtration and dried to give 3.19 g (91%) of 1,3,3-trimethyl-4-phenyl-4-piperidinol, mp 121–122 °C. A 1.2-g (0.006-mol) sample of this piperidinol was refluxed in 15 mL of 18% HCl solution under an N_2 atmosphere. After 18 h of reflux, TLC analysis of the reaction mixture showed the remaining presence of 30–40% of the starting material. At this time, 10 mL of 37% HCl was added to the reaction mixture and reflux was contined for 6 h. TLC analysis of the reaction mixture showed no starting material and one higher R_f spot. Workup by making the reaction mixture basic with KOH, extracting with petroleum ether (2 × 30 mL), drying of the combined extracts ($MgSO_4$), filtering, treating the filtrate with Et_2O·HCl solution, and isolating the white solid that formed by filtration gave 1.3 g of **6**·HCl. The product gave white needles from EtOAc; mp 218–219 °C dec. Anal. ($C_{14}H_{20}ClN$) C, H, N.

(*Z*)-3-(Dimethylamino)-1-phenyl-1-propene Hydrochloride (7·HCl). Cinnamyl bromide and dimethylamine were refluxed in Et_2O as previously described.[20] The product was recrystallized

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from 2-butanone as the HCl salt (7·HCl), mp 190–192 °C (lit.[20] mp 189–191 °C).

Neurotoxicological Evaluations. Each of the six test compounds (2–7) and MPTP were injected intraperitoneally into male Swiss mice (CDL, Groningen) weighing 25–40 g. Each animal was dosed with 0.72×10^{-4} mol/kg of each of the compounds on days 1 and 2 followed by 1.44×10^{-4} mol/kg on days 3–12. Failure to give the "half-dose" injections of MPTP resulted in an LD_{70} in this strain of mouse. The injected drugs were all dissolved as their HCl salts, except for 5 which was a fumarate salt, in normal saline solution. In each case, the concentration of compound in the injection solution was 0.14 M. Control animals were injected daily with an equivalent amount (based on body weight) of normal saline solution. Before and during the experiment the animals were maintained in a temperature- and light-controlled animal room and given free access to food and water. The drug solutions used for the injections were prepared at the beginning of the experiment and stored at 5 °C except during the daily injection periods. At the end of the experiment, TLC analyses of each of the solutions vs. pure crystalline materials showed no traces of impurities.

One day after the 12th day of injections, animals were sacrificed by spinal severance and rapidly decapitated, the brains removed, and the striata dissected out and immediately frozen on dry ice. The striatal tissues were weighed, homogenized, and subjected to separation on Sephadex columns, and the DA, DOPAC, and HVA concentrtions were determined by HPLC–EC detection as previously described by Westerink and Mulder.[29] Additional groups of mice were sacrificed 9 days after the last day of injections, and the striata of these animals were analyzed for DA content by the same procedure.

Acknowledgment. We are indebted to Drs. P. S. Protoghese and D. L. Larson of the University of Minnesota for providing the α-prodinol used in this study. This work was funded in part by a grant from the Faculty Research Committee of the University of the Pacific to D.S.F.

Registry No. 1, 28289-54-5; 1·HCl, 23007-85-4; 2, 13515-63-4; 2·HCl, 6672-58-8; 3, 6672-51-1; 3·HCl, 13299-60-0; 4, 3009-16-3; 4·HCl, 36224-30-3; 5, 4986-03-2; 5·fumarate, 99838-20-7; 6, 99156-44-2; 6·HCl, 99838-21-8; 7, 75712-94-6; 7·HCl, 99838-22-9; DA, 51-61-6; Dopac, 102-32-9; PhLi, 591-51-5; 1-methyl-4-piperidinone, 1445-73-4; 1-methyl-4-phenyl-4-piperidinol, 4972-68-3; α-prodinol, 15217-63-7; β-prodinol, 468-59-7; δ-promedol, 36260-34-1; 1,3,3-trimethyl-4-piperidinone, 18436-83-4; 1,3,3-trimethyl-4-phenyl-4-piperidinol, 18436-89-0; cinnamyl bromide, 4392-24-9; dimethylamine, 124-40-3; homovanillic acid, 306-08-1.

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Antipicornavirus Activity of Substituted Phenoxybenzenes and Phenoxypyridines

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Phenoxybenzenes and phenoxypyridines were prepared and tested for the effect of substituents on antipicornavirus activity. The most active compound, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (8), demonstrated broad-spectrum antipicornavirus activity. Compound 8 and several analogues each given orally prior to and during infection protected mice against an otherwise lethal challenge with coxsackievirus A21.

The picornaviruses represent a large family of 20–30-nm single-stranded RNA viruses possessing similar physicochemical properties. They are implicated as the causative agents of several human diseases, including the common cold (rhinoviruses)[1] and primary myocardial disease (coxsackieviruses).[2] The widespread nature of picornavirus diseases, the economic consequences, and the impracticality of vaccine development have stimulated the search for broad-spectrum orally active chemotherapeutic agents.[3] We report here that many simple diphenyl and phenyl pyridyl ethers show considerable activity in cell culture against three representative picornaviruses. Several demonstrated broad-spectrum activity against 20 human rhinovirus serotypes and enhanced the survival rate of mice given the compounds orally prior to an otherwise lethal challenge with coxsackievirus A21.

Chemistry. Five series of diaryl ethers were evaluated and are listed in Tables I–V. All of the compounds were readily available through straightforward nucleophilic aromatic substitution reactions of the appropriate phenol with the appropriate aryl halide in the presence of base. Subsequent functional group transformations, where necessary, gave the desired substituent patterns. All of the starting aryl halides are known in the literature or were available commercially. The (methylsulfonyl)pyridines of Table V were prepared by reaction of phenols with the appropriate bis(methylsulfonyl)pyridine. Preparation of bis(alkylsulfonyl)pyridines is reported separately.[4]

Biological Evaluation. Compounds were evaluated initially for potency by standard tissue culture methods in which the approximate concentration of compound needed to reduce viral cytopathic effect on HeLa cells by 50% (MIC_{50} in the tables) was determined by serial two-fold dilutions in culture medium.[5] The concentration at which compound induced cytotoxicity (Tox) became apparent, as evidenced by cell death or a change in cell morphology or growth rate, is also reported. Rhinovirus

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