(3.70 g, 0.016 mol) in 5 N HCl (60 mL), and the mixture was warmed on a steam bath for 20 min. It was then cooled and made basic with dilute NaOH solution, and the mixture was shaken with $CHCl_3$ and filtered. The $CHCl_3$ layer of the filtrate was separated, dried (Na_2SO_4), and evaporated. The residue was crystallized from ethyl acetate/petroleum ether (bp 60-80 °C) to give **27**: yield 2.25 g (69%); mp 91-93 °C. Anal. ($C_{11}H_{13}N_3O$) C, H, N.

[4-[2-(1*H*-Imidazol-1-yl)ethoxy]phenyl]urea (28). A solution of KCNO (0.30 g, 0.0037 mol) in water (1 mL) was added to a solution of **27** (0.60 g, 0.0029 mol) in 1 N HCl (3.0 mL). The mixture was allowed to stand at room temperature for 15 min, and the precipitate was filtered off, washed with water, and crystallized from water to give **28**: yield 0.60 g (82.5%); mp 199-201 °C. Anal. ($C_{12}H_{14}N_4O_2$) C, H, N.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzenesulfonamide (29). PCl_5 (2.08 g, 0.01 mol) was added cautiously to chlorosulfonic acid (2.91 g, 0.025 mol), and the mixture was cooled to 0 °C. **1** (1.88 g, 0.01 mol) was added portionwise, and then the mixture was heated on a steam bath for 10 min and cooled. It was poured onto a mixture of crushed ice and excess concentrated NH_3 solution, and the gummy solid was filtered off and sucked dry. The solid was chromatographed on silica gel. Elution with $CHCl_3$/MeOH (4:1) gave a gum that crystallized on trituration with a little EtOH. The solid was crystallized from EtOH to give **29**: yield 0.50 g (19%); mp 147.5-148.5 °C; 60-MHz ^1H NMR (Me_2SO-d_6) 4.37 (m, 2 H, CH_2), 4.45 (m, 2 H, CH_2), 6.95 (s, 1 H, imidazole H^5), 7.14 (d, J = 8.9 Hz, 2 H, benzene CH), 7.28 (s, 2 H, SO_2NH_2), 7.30 (s, 1 H, imidazole H^4), 7.74 (s, 1 H, imidazole H^2), 7.79 (d, J = 8.9 Hz, 2 H, benzene CH). Anal. ($C_{11}H_{13}N_3O_3S$) C, H, N.

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Registry No. **1**, 30170-83-3; **2** maleate, 75912-61-7; **3**, 75912-58-2; **4**·HCl, 75912-62-8; **5**, 75912-57-1; **6** fumarate, 75912-66-2; **7**, 75912-59-3; **8** fumarate, 75912-68-4; **9**, 74226-04-3; **9**·HCl, 97315-33-8; **10**·HCl, 75922-17-7; **11**·HCl, 75912-72-0; **12**·HCl, 75912-73-1; **13**·HCl, 75912-75-3; **14**·HCl, 74226-22-5; **15**, 75912-95-7; **16**, 97315-34-9; **17**·HCl, 75922-18-8; **18**, 75912-78-6; **19** fumarate, 75912-80-0; **20**, 75912-99-1; **21**, 75912-76-4; **21** $^1/_2$ fumarate, 97315-35-0; **22**, 75912-77-5; **23**, 75912-90-2; **24**, 75912-91-3; **25**, 75912-69-5; **26**, 75912-70-8; **27**, 75912-83-3; **28**, 75912-85-5; **29**, 75912-82-2; **30**, 288-32-4; **33**, 2301-25-9; **34**, 97315-36-1; **35**, 97315-37-2; **36**, 97315-38-3; **37**, 97315-39-4; **38**, 97315-40-7; **39**, 74226-00-9; 4-$HOC_6H_4CH_2CONH_2$, 17194-82-0; 4-$CH_3C_6H_4SO_2O(CH_2)_2Cl$, 80-41-1; 3-$HOC_6H_4CN$, 873-62-1; 4-$HOC_6H_4CO_2C_2H_5$, 120-47-8; $Br(CH_2)_3Br$, 109-64-8; 4-Cl-$(CH_2)_2OC_6H_4CONH_2$, 36616-30-5; 4-$Cl(CH_2)_2OC_6H_4NO_2$, 3383-72-0; CH_3NH_2, 74-89-5; $(CH_3)_2NH$, 124-40-3; CH_3CO_2H, 64-19-7; $C_6H_5CO_2H$, 65-85-0; NH_3, 7664-41-7; $ClSO_3H$, 7790-94-5; 2-$HOC_6H_4CO_2C_2H_5$, 118-61-6; 4-$HOC_6H_4CH_2CO_2C_2H_5$, 17138-28-2; 4-$HOC_6H_4OCH_2CO_2C_2H_5$, 20872-28-0; TxA_2 synthetase, 60832-04-4; PGI_2 synthetase, 65802-86-0; cyclooxygenase, 39391-18-9; steroid 11β-hydroxylase, 9029-66-7.

Studies on the 1-Methyl-4-phenyl-2,3-dihydropyridinium Species 2,3-MPDP$^+$, the Monoamine Oxidase Catalyzed Oxidation Product of the Nigrostriatal Toxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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The nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is biotransformed by brain monoamine oxidase (MAO) to an unstable dihydropyridinium intermediate that reacts with cyanide ion to form an α-cyano-tetrahydropyridine adduct and, in the absence of cyanide ion, undergoes disproportionation to the 1-methyl-4-phenylpyridinium species MPP$^+$ and MPTP. Comparison of the HPLC retention times, diode array UV, and chemical ion mass spectral characteristics of these products with those of synthetic standards led us to propose the 1-methyl-4-phenyl-2,3-dihydropyridinium species 2,3-MPDP$^+$ and 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine as tentative structure assignments for the dihydropyridinium metabolite and the cyano adduct, respectively. Results presented in this paper confirm the first assignment and establish that, although the proposed 6-cyano adduct is initially formed, the product that was isolated from the mitochondrial incubation mixtures of MPTP and sodium cyanide actually is the isomeric 2-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. On the basis of the selective incorporation of deuterium into these products, we provide rational mechanistic interpretations of the disproportionation reaction and the rearrangement of the cyano adducts. These results establish that the MAO-catalyzed bioactivation of MPTP leads to the formation of a variety of reactive molecules that are potentially cytotoxic to nigrostriatal cells.

The cyclic allylamine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) causes the selective degeneration of the dopaminergic nigrostriatal system in a number of species including mice,[1-3] monkeys,[4,5] and humans[6,7] and leads to a Parkinsonian-like syndrome in monkeys and humans. Both in vivo[8] and in vitro[9] studies have led to the characterization of the 1-methyl-4-phenylpyridinium species MPP$^+$ (**3**$^+$) as a principal brain metabolite of the

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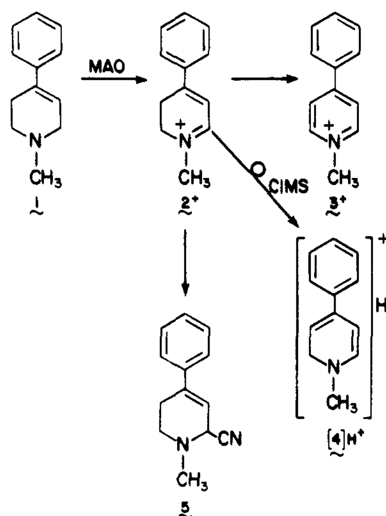
Scheme I. Proposed Brain Metabolic Pathway for the Nigrostriatal Toxin MPTP (1) and the Structure of the Protonated Molecular Ion [4]H$^+$ Observed under CI Mass Spectral Conditions of the Proposed Dihydropyridinium Metabolite



parent drug. Since the metabolic conversion of MPTP to MPP$^+$ is inhibited by pargyline and deprenyl,[9,10] monoamine oxidase (MAO) was suggested and later shown[11] to be the principal brain enzyme that catalyzes the oxidation of MPTP. Furthermore, since the neurotoxicity of MPTP is prevented by these inhibitors,[12] the induced Parkinsonian syndrome caused by this agent is likely to be mediated by the resulting oxidation product(s).

We have postulated that the conversion of MPTP to MPP$^+$, a 4-electron oxidation process, proceeds via the 1-methyl-4-phenyl-2,3-dihydropyridinium species, 2,3-MPDP$^+$ (2^+) as outlined in Scheme I.[9] Experimental evidence to support this proposal includes the isolation by HPLC of an unstable brain mitochondrial metabolite of MPTP that displayed an intense CI mass spectral ion at m/z 172, consistent with the protonated molecular ion [4]H$^+$ of the conjugate base derived from 2^+.[13] Synthetic, 2,3-MPDP$^+ClO_4^-$ was shown to have the same HPLC retention volume as this metabolite. Furthermore, the online HPLC diode array UV spectra of the metabolite and the synthetic product were identical as were the CI mass spectra of the two materials isolated by HPLC. The chemical instability of 2,3-MPDP$^+$, which undergoes spontaneous disproportionation to MPP$^+$ and MPTP in pH 7.4 buffer,[13] precluded the possibility of isolating the metabolite for complete chemical characterization. We therefore attempted to trap this intermediate by incubating MPTP with brain mitochondrial preparations in the presence of sodium cyanide.[14] The monocyano adduct that was isolated displayed an intense CI mass spectral ion at m/z 199 (MH$^+$), consistent with the expected 6-cyano adduct **5** that would result from the 1,2-addition of cyanide ion to the putative 2,3-dihydropyridinium metabolite 2^+ (Scheme I). The present paper describes the complete characterization of this cyano adduct and the results of our more detailed studies on the properties of the dihydropyridinium metabolite of MPTP.

Results and Discussion

A preparative scale incubation of MPTP in the presence of the trapping agent sodium cyanide provided a sufficient amount of the crude cyano adduct to obtain a useful ^{1}H NMR spectrum. Comparison of this spectrum with that of synthetic 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (**5**) led to the identification of slight differences in the chemical shifts of the broad signals assigned to the low-field olefinic (δ 6.1) and α-cyanomethine (δ 4.0) protons and the sharp singlet for the N-methyl (δ 2.50) protons compared to the corresponding signals of the synthetic 6-cyano compound (δ 6.0, 4.2, and 2.55, respectively). These observations prompted us to examine more closely the structure assignment of the metabolically generated MPTP cyano adduct.

The availability of synthetic 1-methyl-4-phenyl-2,3-dihydropyridinium perchlorate ($2^+ClO_4^-$) provided an opportunity to study the reaction of the proposed metabolite with sodium cyanide under mitochondrial incubation conditions. After a 45-min reaction period, the ^{1}H NMR spectrum of the organic soluble material displayed signals corresponding principally to those of the 6-cyano product **5** although a second species displaying chemical shifts corresponding to those of the metabolism-generated cyano adduct also was detected. This minor product was the sole compound isolated after an overnight reaction period. Comparable results were obtained when synthetic 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine was stirred in pH 7.4 buffer at 37 °C. These results suggest that the net chemistry recorded in these experiments corresponds to the rearrangement of the piperideine double bond and the concomitant isomerization of the 6-cyano adduct to the 2-cyano adduct.

A homonuclear decoupling experiment on the crystalline material isolated from the overnight reaction mixture established that the two low-field signals (assigned to the olefinic and α-cyanomethine protons) were not coupled to each other. These spectral features led us to assign the structure of the newly formed product as 2-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (**6**, see Scheme III). Additional evidence to support this assignment was obtained by the full characterization of the analytically pure compound. Furthermore, since the ^{1}H NMR spectrum of the metabolically generated cyano product was essentially identical with that of this synthetic product, we now conclude that the compound isolated from the mitochondrial incubation mixture of MPTP and sodium cyanide is not the 6-cyano adduct as previously thought[13] but rather the corresponding 2-cyano adduct **6**.

A recent paper has described the aluminum oxide catalyzed double-bond rearrangement of several 6-cyano-1,2,3,6-tetrahydropyridine derivatives to the corresponding 2-cyano isomers.[15] The corresponding rearrangement of this 6-cyano analogue occurs spontaneously in solution, presumably because formation of the intermediate 2,3-MPDP$^+$ is energetically favored due to the extended conjugation of this diene system with the phenyl ring. In view of the ease with which this rearrangement occurs, it seemed reasonable to propose that the 2-cyano adduct **6** isolated from the metabolic incubation mixture may be a

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Table I. Rearrangement Studied: Relative Percent Yields and Percent Deuterium Incorporation of the 2- and 6-Cyano Adducts

$[CN^-]$, mM	time, min	isomer distribn[a]		percent deuterium incorpn[b]		
		6-cyano	2-cyano	6-cyano C(6)H	C(2)H	2-cyano C(3)H
		Mitochondrial Studies[c]				
0.5	10	72	28			
	20	51	49			
	30	47	53			
		Chemical Studies[d]				
4.3	35	71	29	33	30	100
	120	50	50	100	35	100
	300	9	91	e	70	100
1.0	35	54	46	30	<25	100
	120	13	87	e	50	100
	360	9	91	e	50	100

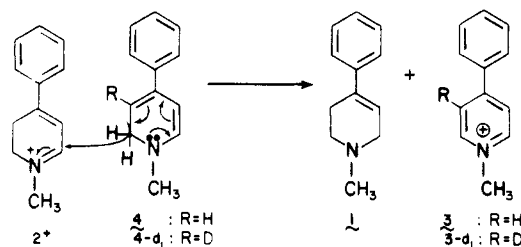
[a] The relative percentages of **5** and **6** were determined by the peak heights of the corresponding NCH_3 signals in the ^1H NMR spectra of extracts of the reaction mixtures. [b] The percent deuterium incorporation was determined by integration of the ^1H NMR spectra. [c] The metabolic mixtures (0.5 mM NaCN, 0.2 mM MPTP, and 5 mg of protein/mL in pH 7.4 phosphate buffer) were incubated at 37 °C for the designated periods. [d] The data were obtained by ^1H NMR analysis of extracts from 37 °C reaction mixtures of 0.77 mM 2,3-MPDP$^+$$ClO_4^-$ and NaCN in pD 7.4 phoshate buffer after the designated reaction times. [e] Not detectable.

type of artifact resulting from the rearrangement of the initially formed 6-cyano adduct **5**. This was an important consideration since the structure of the metabolically generated cyano adduct provides indirect evidence for the structure of the MAO-catalyzed oxidation product of MPTP.

In order to characterize more fully the structure of the unstable MPTP metabolite, we carried out a series of incubations of MPTP with rat brain mitochondrial preparations in the presence of sodium cyanide and examined the ^1H NMR spectra of the organic soluble products following various incubation periods (Table I). After 10 min, the principal cyano adduct present in the isolate proved to be the 6-cyano compound although a small amount of the 2-cyano isomer also was observed. With increasing incubation times, the ratio of the 6-cyano isomer to the 2-cyano isomer decreased, and after 30 min the 2-cyano adduct was the principal isomer present in the incubation mixture. A similar isomer ratio vs. time relationship was observed when 2,3-MPDP$^+$ was incubated with sodium cyanide under conditions similar to those employed in the MPTP/mitochondrial study. These observations are consistent with a kinetic vs. thermodynamic control process in which the 6-cyano adduct is the kinetically preferred product and the 2-cyano adduct the thermodynamically preferred product. Increasing the concentration of the cyanide ion had the expected effect of slowing the rearrangement reaction because of the shift toward **5** in the equilibrium between **5** and **2**$^+$. HPLC coupled with on-line diode array UV spectral analysis of solutions of the 6- and 2-cyano isomers provided additional evidence to support this analysis. The 2-cyano isomer was quite stable in acetonitrile whereas, under the same conditions, the 6-cyano isomer underwent rapid ionization ($t_{1/2}$ = 10 min) to 2,3-MPDP$^+$. The 6-cyano isomer was even more unstable in methanol while no evidence of chemical damage of the 2-cyano isomer was observed after 24 h. The 2-cyano compound in methanol did undergo slow ionization when treated with perchloric acid. The dihydropyridinium species isolated from this solution proved to be the thermodynamically favored 2,3-dihydro isomer **2**$^+$ and not the isomeric 2,5-dihydro species **7**$^+$.

The results summarized above support our initial proposal that the MAO-catalyzed oxidation of MPTP leads to the formation of 2,3-MPDP$^+$. These results further demonstrate the inherent chemical instability of this metabolite, which already had been indicated by the ease with which it undergoes disproportionation to the pyridinium product **3**$^+$ [4] and MPTP.[13]

Scheme II. Proposed Mechanism for the Disproportionation of 2,3-MPDP$^+$ (**2**$^+$)



Since the neurotoxicity of MPTP apparently is mediated by one or more of the molecular entities that are formed as a consequence of its MAO-catalyzed oxidation, we decided to explore more thoroughly the underlying chemical reaction mechanisms associated with the disproportionation of 2,3-MPDP$^+$ and the rearrangement of the 6-cyano derivative of MPTP that presumably also proceeds via the 2,3-MPTP$^+$ species. In earlier studies we had established that MPTP is converted by mitochondrial incubation mixtures prepared in D_2O to the β-monodeuterio analogue **3**$^+$-d_1 of the 1-methyl-4-phenylpyridinium compound **3**$^+$. Since the formation of this pyridinium product most likely occurs via disproportionation of the 2,3-MPDP$^+$ species,[13,16] we examined the disproportionation of synthetic 2,3-MPDP$^+$$ClO_4^-$ in D_2O. The pyridinium product isolated by extraction of the reaction mixture with methylene chloride was shown to be the same monodeuterio compound (**3**-d_1) that was isolated from the MPTP incubation mixture. On the other hand MPTP, the second of the disproportionation reaction products, was found to contain no deuterium. On the basis of these results, we propose that the disproportionation of 2,3-MPDP$^+$ proceeds through a mechanism in which the conjugate base **4** serves as a hydride donor molecule and the dihydropyridinium species **2**$^+$ as a hydride acceptor molecule (Scheme II). According to this mechanism the reduction of the 2,3-MPDP$^+$ must involve transfer of one of the C-2 protons of **4**, thus resulting in the formation of deuterium-free MPTP.

The incorporation of one deuterium atom in the pyridinium oxidation product can be rationalized for in terms of a rapid equilibration of the free base **4** with the corresponding 2,5-dihydropyridinium conjugate acid **7**$^+$ (Scheme

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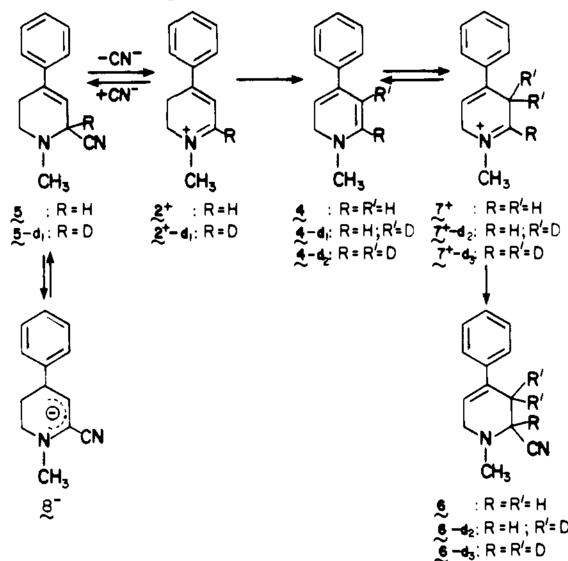
Scheme III. Proposed Mechanism for the Rearrangement of 6-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (5) to 2-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (6) and the Associated Deuterium Incorporation into the Two Products in D_2O Solution at pD 7.4



III). This acid–base equilibration leads to the formation of 1-methyl-4-phenyl-1,2-dihydropyridine-*5*-d_1 (**4**-d_1) via the 1-methyl-4-phenyl-2,5-dihydropyridinium-*5,5*-d_2 intermediate (7^+-*5,5*-d_2). This mechanism also implies that under these reaction conditions the 1,2-dihydropyridine species **4** does not undergo appreciable protonation at C-3 since this would lead to a deuterated 2,3-dihydropyridinium hydride acceptor molecule and the formation of deuterated MPTP. This analysis is consistent with Ingold's rule[17] that predicts the selective protonation of a weakly basic dieneamine such as **4** on the central carbon atom to form the kinetically controlled conjugate acid.[18-21] Finally, it seems likely that the concentration of the deuterium-labeled 2,5-dihydropyridinium species must be quite low since one would expect that this molecule also could serve as a hydride acceptor and therefore precursor to deuterium-labeled MPTP.

Our mechanistic studies on the reaction between 2,3-$MPDP^+$ and cyanide ion have led to similar conclusions. We examined this reaction in D_2O at two different concentrations of cyanide ion in pD 7.4 buffer and analyzed the mixture of cyano adducts by ^1H NMR spectroscopy after various reaction times. The results of these studies are summarized in Table I, which lists the relative amounts of the 6-cyano, **5**, vs. the 2-cyano, **6**, isomers present in the reaction mixtures and the extent to which deuterium has been incorporated into each product. The most important observations are (1) the absence of deuterium at any position of the 6-isomer except at C-6 (**5**-d_1) and (2) the extensive incorporation of deuterium at C-3 of the 2-cyano isomer (**6**-d_2). Consistent with the proposal stated above, these results confirm that protonation of the 1,2-dihydropyridine free base must occur predominantly at C-3, at least under physiological conditions.

We were somewhat surprised to observe the formation of the C-6 monodeuterated 6-cyano product **5**-d_1 and (to a lesser extent) the *tri*deuterated 2-cyano product **6**-d_3. The incorporation of deuterium into **5** is likely to be due to ionization of the weakly acidic C-6 proton of the 6-cyano isomer to form the resonance-stabilized anion 8^-. The partial incorporation of deuterium at C-2 of the 2-cyano isomer would result from the rearrangement of **5**-d_1 via the pathway **2**-d_1 to **4**-d_2 to **7**-d_3 (Scheme III). As predicted by the above analysis, the extent to which the 6-position of **5** undergoes exchange is cyanide ion concentration dependent—the higher the concentration of cyanide ion, the longer the life time of the 6-cyano isomer and the greater the extent of exchange. The exchange of the α-cyanomethine proton appears to occur exclusively with the 6-cyano isomer since, once the rearrangement has occurred, no additional enrichment of deuterium in the 2-cyano isomer is observed (compare the 120 vs. 360 min deuterium incorporation values of the 2-cyano isomer in the experiment at the lower cyanide ion concentration).

The reaction mechanisms summarized above require the intermediate formation of the 1,2-dihydropyridine species **4** and the 2,5-dihydropyridinium species 7^+. Although these species appear to be transient intermediates, their participation in the reactions recorded here for the 2,3-dihydropyridinium metabolite of MPTP generated by brain MAO suggests that consideration be given to their potential interactions with biomacromolecules in the nigrostriatal cells that are destroyed by MPTP.

Experimental Section

All synthetic reactions were carried out under a nitrogen atmosphere. Synthetic 1-methyl-4-phenyl-2,3-dihydropyridinium perchlorate[13] and 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine[22] were prepared as described previously, and 1-methyl-4-phenylpyridinium iodide was obtained from Dr. Sanford Markey, National Institute of Mental Health, Bethesda, MD. All other chemicals were reagent grade or, in the case of solvents, HPLC grade. Proton NMR spectra were obtained at 240 MHz with a custom-built instrument linked to a Nicolet 1180 computer; chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (Me_4Si) in $CDCl_3$ or CD_3CN. Spin multiplicity is given as (s) singlet, (d) doublet, (t) triplet, (q) quartet, or (m) multiplet. HPLC separations were achieved on a Beckman Model 330 liquid chromatographic system. The precolumn (4.6 mm × 5 cm) and analytical column (4.6 mm × 25 cm) were packed with 5 μm Altex ultrasphere-ODS reversed-phase packing material. Stability studies were performed on the above system with an on-line diode array detector (Hewlett-Packard Model 1040A) to monitor the concentrations of MPTP (245 nm), MPP^+ (293 nm), and $MPDP^+$ (345 nm) in the effluent. Low-resolution CI mass spectra were run on a modified AEI MS 902S at 8 kV with isobutane (ca. 1 torr) as reagent gas. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley, CA.

2-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (6) from Mitochondrial Incubation Mixtures. MPTP (1 mM) was incubated with rat brain mitochondrial fractions (5 mg of protein/mL), prepared as described previously,[13] in 0.1 M potassium phosphate buffer, pH 7.4 (final volume 10 mL) containing 0.5 mM NaCN at 37 °C for 45 min. The incubations were terminated by the addition of an equal volume of cold acetonitrile, and the resulting mixtures were centrifuged to remove protein. The supernatant fractions were allowed to sit at room temperature for 2 h and then were frozen at −20 °C. The upper, clear organic phase was chromatographed (30 × 400 μL injections) with acetonitrile/water (9:1) at a flow rate of 2 mL/min on the HPLC system described above. The fractions containing the cyano

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adduct (retention time 1.8 min) were collected, combined, and extracted with methylene chloride (2 × 40 mL). The combined extracts were dried over Na_2SO_4, and the solvent was removed at 5 °C under vacuum to yield crude **6**: 240-MHz ^1H NMR ($CDCl_3$) δ 2.50 (s, 3 H, NCH_3), 4.0 (m, 1 H, C2), 6.1 (m, 1 H, C5), 7.3–7.5 (m, 5 H, Ar H). The remaining assignments could not be made due to the presence of interfering signals from sample impurities.

2-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (6) from Reaction of NaCN with 2,3-MPDP$^+ClO_4^-$. The perchlorate salt of 2,3-MPDP$^+$ (200 mg, 0.74 mmol) and NaCN (180 mg, 3.7 mmol) were dissolved in 450 mL of 0.2 M phosphate buffer (pH 7.4). This solution was swirled in a water bath at 37 °C for 12 h and then was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated to give a yellow solid. Recrystallization from hot heptane yielded 112 mg (0.56 mmol, 76%) of pure, crystalline product: mp 88 °C; 240-MHz ^1H NMR ($CDCl_3$) δ 2.50 (s, 3 H, NCH_3), 2.74 (br d, 1 H, $C3_{eq}$), 3.0–3.1 (m, 2 H, $C3_{ax}$ and $C6_{eq}$), 3.45 (d of m, 1 H, $C6_{ax}$), 4.0 (m, 1 H, C2), 6.1 (m, 1 H, C5), 7.3–7.5 (m, 5 H, Ar H); CIMS *m/e* 199 (MH)$^+$. Anal. ($C_{13}H_{14}N_2$) C, H, N. Treatment of this product (50 mg, 0.25 mmol) in ethanol (2 mL) with 70% perchloric acid (20 μL) for 5 days at 5 °C led to the formation of crystalline 2,3-MPDP$^+ClO_4^-$ (47 mg, 0.17 mmol, 69%): mp 120–122 °C (lit.[13] mp 122.5 °C).

Rearrangement of 6-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (5) to 2-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (6). A suspension of the 6-cyano isomer **5** (20.7 mg, 0.11 mmoles) in 20 mL of 0.1 M KH_2PO_4 buffer (pH 7.4) was sonicated to facilitate dissolution and then incubated at 37 °C with constant swirling for 18 h. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 mL), and the combined extracts were dried over Na_2SO_4 and evaporated to dryness to yield 17 mg (82% recovery) of the crude 2-cyano isomer which was identified by its ^1H NMR spectrum.

Timed Studies on the Metabolism of MPTP by Brain Mitochondrial Preparations. Timed aliquots (15 mL) were removed from an incubation mixture (45 mL) prepared from brain mitochondrial fraction (5 mg of protein/mL), 0.5 mM NaCN, and 0.2 mM MPTP in pH 7.4 potassium phosphate buffer at 10, 20, and 30 min. The percent metabolism was estimated by analytical HPLC on a 100-μL sample following the procedure described previously.[13] The remaining portion of each aliquot was cooled on ice and then was centrifuged for 5 min at 20000*g* to sediment the protein. Each of the resulting supernatant fractions was extracted with CH_2Cl_2 (2 × 15 mL), and the resulting extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The ^1H NMR data are summarized in Table I.

Disproportionation of 2,3-MPDP$^+ClO_4^-$ in D_2O Buffer. A solution of 2,3-MPDP$^+ClO_4^-$ (20 mg, 0.074 mmol) in 10 mL of 0.1 M phosphate buffer prepared in D_2O (pD 7.4) was incubated at 37 °C with gentle swirling. After 20 h, the clear yellow solution was basified to pH 8 with a 5% K_2CO_3 and then was extracted with ether (3 × 10 mL) and then with CH_2Cl_2 (3 × 10 mL). The ether extracts were combined, dried over Na_2SO_4, and concentrated to yield MPTP. Proton NMR and CI mass spectral analysis established that no deuterium had been incorporated into the product. The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to yield the 1-methyl-4-phenylpyridinium species in which one of the β-pyridinium ring protons was substituted with a deuterium atom: 240-MHz ^1H NMR (CD_3CN) δ 8.61 (br s, 2 H, pyridinium α-H), 8.21 (d, 1 H, pyridinium β-H); CIMS *m/e* 157 (MH − CH_3)$^+$.

Deuterium Incorporation Studies. Solutions (80 mL) prepared in D_2O and containing 0.7 mM 2,3-MPDP$^+ClO_4^-$, 4.3 mM or 1.8 mM NaCN, and 0.2 M phosphate buffer (pD 7.4) were swirled in a 37 °C water bath, and aliquots (15 mL) were removed after 35 min and 1, 2, and 5 h. The aliquots were extracted (15 mL CH_2Cl_2) and the extracts dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Each residue was analyzed by 240-MHz ^1H NMR for deuterium content (see Table I). In a separate experiment, the double-bond rearrangement of 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to the corresponding 2-cyano isomer (as described above) was carried out in D_2O. The following 240-MHz ^1H NMR spectrum ($CDCl_3$) was obtained for the resulting 2-cyano product: δ 2.5 (s, 3 H, NCH_3), 2.9 (m, 0.2 H, $C3_{eq}$), 3.1 (d, 0.2 H, $C3_{ax}$) 3.15 (d, J = 17.7 Hz, 1 H, $C6_{eq}$), 3.48 (dd, J_{gem} = 17.7 Hz, J_{vic} = 1.9 Hz, 1 H, $C6_{ax}$), 4.01 (s, 0.37 H, C2), 6.12 (m, 1 H, C5), 7.3–7.5 (m, 5 H, Ar H).

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Synthesis and Hypoglycemic Activity of N-Alkylated Hydrazonopropionic Acids

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A series of N-alkylated 2-hydrazonopropionic acids have been synthesized and evaluated for their hypoglycemic activity. Most of the compounds exhibit a remarkable blood glucose lowering activity in fasted guinea pigs. Some of the structural variables studied were the effects of branching, unsaturation, or substitution on the alkyl side chain and the effect of nuclear substitution on the aralkyl analogues. From these compounds, 2-[[(*E*)-2-methyl-3-phenyl-2-propenyl]hydrazono]propionic acid (BM 42.304; **42**) was selected for further investigation.

Haeckel and Oellerich recently established in the course of investigations into the effects of hydrazines in perfused guinea pig liver that the addition of these substances to the perfused solution inhibits gluconeogenesis. The hypoglycemic effect of monoamine oxidase inhibitors with a hydrazine structure has already been known for some time, but the observation that the gluconeogenesis-inhibiting effect is due not to the hydrazine itself but to its condensation product with pyruvate, formed in vivo, was new.[1]

Further work on synthetically derived condensation products of this kind in the whole animal showed that the lowering of the blood sugar level was much more pronounced after these products than after the hydrazines. In addition, the inhibitory action on monoamine oxidase was substantially attenuated in the case of the condensation products.[2]

In parallel with the lowering of the blood glucose level, the lactate level underwent an increase. However, the oxidative metabolism of the cells was not inhibited in the

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