236. Conversion of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its 5-Methyl Analog into Pyridinium Salts[1])

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(6.XI.84)

Summary

Monoamine oxidase B metabolizes 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; **1**) first to 1-methyl-4-phenyl-2,3-dihydropyridinium salt ($MPDP^+$; **5**), and then to 1-methyl-4-phenylpyridinium salt (MPP^+; **7**). Chemical synthesis of $MPDP^+$ and its 5-methyl analog **6** was accomplished from the *N*-oxides **3** and **4** of MPTP and its 5-methyl analog, respectively, by a *Polonovski* reaction. Oxidation of $MPDP^+$ to MPP^+ was accomplished with air, and greatly accelerated by Pt catalyst. Reduction of $MPDP^+$ and MPP^+ with $NaBH_4$ afforded MPTP.

The compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; **1**) [1] was found to be highly neurotoxic in man and certain animal species, depleting the substantia nigra of dopamine and causing effects similar to those observed in *Parkinson*'s disease [2–5]. The pyridinium salt **7** (MPP^+), isolated from brain tissue of monkey and characterized by HPLC/MS, is considered to be the major metabolite of MPTP in human and primates [6–8].

The 5-methyl derivative **2** was included in this investigation since it is closly related to analgetics of the prodine family of drugs [9].

Chemistry. – In this communication we report the facile chemical conversion of MPTP (**1**) and its 5-methyl derivative **2** [9] to their pyridinium analoges **7** and **8**, respectively, *via* dihydropyridinium salts **5** and **6**. Although oxidation of tetrahydropyridines to pyridines can be accomplished directly using mercury acetate [10], we considered the approach *via* dihydropyridinium derivatives to be more 'physiological' since the 1-methyl-4-phenyl-2,3-dihydropyridinium salt (**5**, $MPDP^+$) is a likely intermediate metabolite of MPTP [7] [8]. The methodology applied for conversion of tetrahydropyridines to dihydropyridinium salts was based on the *Polonovski* reaction utilized successfully

[1]) Dedicated to Prof. Dr. *H.C. Beyerman* of the Laboratory of Organic Chemistry at the Technical Highschool in Delft, the Netherlands, on the occasion of his 65th birthday.

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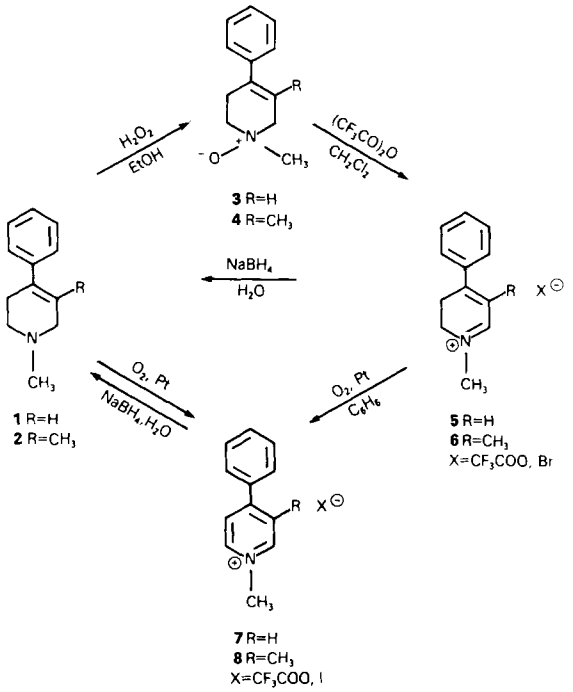

by *Husson et al.* [11]. Thus oxidation of **1** and **2** with H_2O_2 in EtOH yielded *N*-oxides **3** and **4**, respectively, isolated in crystalline form as dihydrates. Reaction of the *N*-oxides with trifluoroacetic anhydride in CH_2Cl_2 afforded dihydropyridinium trifluoroacetates **5** and **6** which, after conversion to bromides, could be isolated in crystalline form. Oxidation of **5** and **6** to the pyridinium salts **7** and **8**, respectively, was observed when solutions of these compounds in CH_2Cl_2 were stirred in the presence of air. In contrast, solutions in H_2O seemed to be quite stable. The oxidation of **5** and **6** to **7** and **8**, respectively, could be accomplished much more efficiently by using Pt as a catalyst. Thus, by stirring **5** or **6** at 60–80°C in benzene or toluene with freshly prepared Pt (from PtO_2) in the presence of air, these compounds were converted to the corresponding pyridinium salts in a few hours. Using this method, it was also possible to convert MPTP (**1**) directly to MPP^+ (**7**). It was also found that addition of **3** to a CH_2Cl_2 solution of **5** oxidizes this last compound to **7** whereas **3** was reduced to **1**. Reduction of an aqueous solution of **5** with $NaBH_4$ afforded MPTP (**1**), also obtained from **7** using the same method. It is evident from the results obtained that **1**, **5**, and **7** (and similarly **2**, **6**, and **8**) are related to each other through oxidation and reduction. Dihydropyridinium species **5** may for this reason be considered to be an intermediate metabolite of MPTP which because of its chemical reactivity has so far not been isolated.

Addition of nucleophiles to compounds **5** and **6** will be discussed in the next paper.

Biological Evaluations. – Since $MPDP^+$ has been postulated as an intermediate in the *in vivo* metabolism of MPTP to MPP^+ [8], we determined whether this dihydro derivative is formed when MPTP is added to human and bovine liver monoamine oxidase B (amine: oxygen oxidoreductase, *EC 1.4.3.4, MAO B*). We also tested

Table 1. *Specific Activities of Human and Bovine Liver* MAO B *using Benzylamine, MPTP* (**1**) *and MPDP*$^+$ (**5**) *as Substrates*[a])

	Initial rates of reaction, nmol/min/mg protein ± S.D.			
	Substrates: Products			
Enzyme source	Benzylamine: Benzaldehyde	MPTD: MPDP$^+$	MPDP$^+$: MPP$^+$	MPTP: MPP$^+$
Human liver mitochondrial extract	9.92 ± 0.42	0.95 ± 0.04	0.21 ± 0.02	0.221 ± 0.04
Purified bovine liver *MAO B*	221 ± 4	34.3 ± 0.9	8.02 ± 0.53	6.69 ± 0.42

[a]) Initial rates of reaction were determined over an 8–10 min period at 25 °C, using 100 μM of substrate in each reaction. Human liver mitochondrial enzyme reactions ($N = 6$ per assay), which oxidized benzylamine, MPTP, and MPDP$^+$, used 10–48, 240–480, and 240–480 μg of enzyme, respectively. Partially purified bovine liver enzyme reactions ($N = 3$ per assay), which oxidized benzylamine, MPTP, and MPDP$^+$, used 0.9–3.0, 9–30, and 9–30 μg of enzyme, respectively. Under the assay conditions used, the response was linear for at least 30 min.

MPDP$^+$ and MPTP *N*-oxide as inhibitors of dihydropteridine reductase (NADH:6,7-dihydropteridine oxidoreductase, *EC 1.6.99.10*), using highly purified preparations of human liver enzyme and rat striatal synaptosomes, because we had previously found that hydroxylated derivatives of MPTP and its corresponding piperidine and pyridine derivatives are potent inhibitors of this enzyme [12] [13].

MPDP$^+$ Formation. Triton X-100 extracts of human liver mitochondria and bovine liver *MAO B* purified by the method of *Salach* [14], which was judged to be about 80% pure when examined by SDS gel electrophoresis [15], were used as sources of enzyme. MPTP, MPDP$^+$, and MPP$^+$ exhibit markedly different UV spectral characteristics, and MPDP$^+$ and MPP$^+$ were determined by measuring the increase in absorbance at 340 and 290 nm, respectively. *Table 1* shows that *MAO B* from both sources catalyzed the conversion of MPTP to MPDP$^+$ and MPP$^+$ at rates which were dependent upon the concentration of enzyme in the preparation. *This result confirms that MPDP$^+$ is formed as an intermediate in the metabolism of MPTP.* MPTP was converted to MPDP$^+$ by *MAO B* at approximately 12% the rate observed with benzylamine, a commonly studied substrate [14]. Also, MPDP$^+$ is a substrate for *MAO B*, but the conversion of MPDP$^+$ to MPP$^+$, the final product of MPTP metabolism, occurs at a 5-fold slower rate than the conversion of MPTP to MPDP$^+$. These results suggest that MPTP may be a better substrate than MPDP$^+$, and because of its reactivity, MPDP$^+$ may follow multiple metabolic pathways.

Dihydropteridine Reductase Inhibition. The neurochemical effect of MPDP$^+$ was evaluated by determining the inhibition of dihydropteridine reductase. This enzyme regenerates tetrahydrobiopterin, which is an essential cofactor for tyrosine hydroxylase and tryptophan hydroxylase during dopamine and serotonin synthesis, respectively [16].

Rat striatal synaptosomes were prepared by the method of *Gray & Whittaker* [17] with slight modification [18]. Human liver dihydropteridine reductase was purified by *DEAE-Sephacel* chromatography and naphthoquinone-*Sepharose* affinity chromatography as previously described [18]. Dihydropteridine reductase activity was measured spectrophotometrically according to the method of *Nielson et al.* [19].

Table 2. K_i *and* I_{50} *Values of 1-Methyl-4-phenyl-2,3-dihydropyridinium Bromide (***5***, X = Br) and MPTP* N*-Oxide (***3***) as Inhibitors of Dihydropteridine Reductase*[a])

Inhibitor	Enzyme source	Incubation time (temperature)	I_{50} [mol/l]	K_i[b]) [mol/l]
$MPDP^+$ (**5**, X = Br)	Human liver	10 min (25°C)	2.8×10^{-4}	2.9×10^{-4}
	Rat striatal synaptosomes	10 min (25°C)	3.3×10^{-4}	3.7×10^{-4}
		19 h (4°C)	2.5×10^{-4}	
MPTP *N*-oxide (**3**)	Human liver	10 min (25°C)	1.3×10^{-3}	
	Rat striatal synaptosomes	10 min (25°C)	5.2×10^{-3}	
		19 h (4°C)	1.2×10^{-3}	

[a]) Human liver enzyme (6.6 mU or 14 ng of protein) or rat striatal synaptosomes (5.9 mU or 142 μg of P_2 protein) were incubated with various concentrations of the inhibitor (7 concentrations for obtaining I_{50} values and 4 for obtaining K_i values). Residual enzyme activity was assayed at 50 μM of each substrate to obtain I_{50} values and at different $qDMPH_2$ concentrations (20 to 50 μM) while the concentration of NADH was kept constant (50 μM) to obtain K_i values.

[b]) K_i values were obtained by plotting the inhibitor concentration *vs.* the reciprocal of the apparent maximal velocity. *Lineweaver-Burk* plots indicated noncompetitive inhibition.

Table 2 summarized I_{50} and K_i values for $MPDP^+$ and I_{50} values for MPTP *N*-oxide as inhibitor of dihydropteridine reductase. $MPDP^+$ inhibits the enzyme noncompetitively with K_i values of 290–370 μM. These results indicate that $MPDP^+$ is approximately 5 times more potent than MPTP *N*-oxide and 10 to 40 times more potent than MPTP and MPP^+ respectively, as an inhibitor of dihydropteridine reductase [12] [13]. Moreover, its inhibitory potency may be enhanced by a long period of incubation, as indicated by a lower I_{50} value obtained after 19 hours of incubating $MPDP^+$ with synaptosomal preparations. The physiological significance of the inhibition of dihydropteridine reductase by $MPDP^+$ remains to be established.

Additional details on the biological activities of $MPDP^+$, MPP^+ and their 5-methyl analogs will be reported later.

Experimental Part

General. Melting points were determined on a *Fisher-Johns* apparatus and are corrected. UV spectra (λ_{max} (ε) in nm) were measured using a *Hewlett-Packard-8450A* UV/VIS spectrometer. ^{1}H-NMR spectra were recorded using a *Varian-HR-220* or *Varian-XL-300* spectrometer with TMS (= 0 ppm) as the internal standard. ^{13}C-NMR spectra were recorded using a *JEOL-FX-100* spectrometer with TMS (= 0 ppm) as the internal reference. Chemical ionization mass spectra (CI-MS; *m/z*) were obtained on a *Finnigan-1015D* spectrometer with a model *600* data collection system. Thermospray LC/MS spectra were performed by Dr. *D.J. Liberato,* Laboratory of Theoretical and Physical Biology, NICHD, NIH, Bethesda, Maryland. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation, Laboratory of Chemistry, NIADDK, NIH, Bethesda, Maryland. Compound **1** was purchased as the free base from *Aldrich Chemical Co., Inc.* and 3-methyl-4-phenylpyridine was a gift from Dr. *Kenner C. Rice,* Laboratory of Chemistry, NIADDK, NIH, Bethesda, Maryland. Silica gel *60* (15–40 μm) was from *E. Merck Co.,* Darmstadt, West Germany.

1,5-Dimethyl-4-phenyl-1,2,3,6-tetrahydropyridine (**2**). To a solution of 3-methyl-4-phenylpyridine [20] (5.0 g, 30 mmol) in 40 ml of acetone were added 6.0 g (42 mmol) of CH_3I, and the mixture was kept at room temperature overnight. The crystalline *1,3-dimethyl-4-phenylpyridinium iodide* was filtered, washed with acetone, and dried to yield 6.33 g (68%) of yellow crystals, m.p. 140–141°. This was dissolved in 200 ml of H_2O, cooled with an ice/H_2O bath, and $NaBH_4$ (1.5 g, 40 mmol) was added in small portions with stirring. After stirring 1 h, it was extracted with Et_2O. The combined Et_2O extracts were dried over Na_2SO_4 and evaporated to dryness. The product was purified over a short silica gel column, converted to the hydrochloride salt and crystallized from $(i\text{-}Pr)_2O$/EtOH to yield 3.6 g (79%) of **2** as colorless crystals, m.p. 187–189° ([9]: 189–190°).

Reduction of **5** and **7** (see below), when similarly executed, afforded MPTP (**1**) quantitatively.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine N-*Oxide* (**3**). To a solution of MPTP (**1**; 5.26 g, 30 mmol) in EtOH (25 ml) was added 30% H_2O_2 (3.5 ml), and the mixture was stirred at 50° for 2 h. An additional portion of 30% H_2O_2 (3.5 ml) was then added, and it was stirred for another 6 h. The excess H_2O_2 was decomposed by stirring with 10% Pd/C in a stream of N_2. The mixture was then filtered, the filter-cake washed with EtOH, the filtrate evaporated under reduced pressure, taken up in benzene/EtOH and evaporated to dryness. The residue was crystallized from acetone to yield 6.4 g (94%) of **3**·$2H_2O$ as colorless crystals, m.p. 136–139° (dec.). UV (H_2O): 242 (16200). ^{1}H-NMR ($CDCl_3$): 7.42 (*m*, 5 arom. H); 6.00 (br. *s*, H–C(5)); 4.08 (br. *s*, 2H–C(6)); 3.54 (*t*, J = 6, 2H–C(2)); 3.29 (*s*, 3H, CH_3N); 3.10 (*m*, H–C(3)); 2.70 (*m*, H–C(3)). MS (CI): 190 (M^+ + 1). Anal. calc. for $C_{10}H_{15}NO \cdot 2H_2O$ (275.29): C 63.97, H 8.50, N 6.22; found: C 64.22, H 8.10, N 5.95.

1,5-Dimethyl-4-phenyl-1,2,3,6-tetrahydropyridine N-*Oxide* (**4**). Compound **4** was prepared in 80% yield from **2** in a manner similar to that described above. The product was crystallized from acetone as the dihydrate, m.p. 112–114°C. UV (H_2O): 231 (9300). ^{1}H-NMR ($CDCl_3$): 7.29 (*m*, arom.); 3.95 (*q*, J = 17, 2H–C(6)); 3.54 (*m*, 2H–C(2)); 3.29 (*s*, 3H, CH_3N); 2.91 (*m*, H–C(3)); 2.56 (*m*, H–C(3)); 1.65 (*s*, 3H, CH_3). MS (CI): 204 (M^+ + 1). Anal. calc. for $C_{13}H_{17}NO \cdot 2H_2O$ (239.32): C 65.25, H 8.85, N 5.85; found: 65.08, H 8.60, N 5.81.

1-Methyl-4-phenyl-2,3-dihydropyridinium Bromide (**5**). To the solution of **3** (675 mg, 3 mmol) in 20 ml of CH_2Cl_2 cooled to 0° was added $(CF_3CO)_2O$ (1.2 g, 0.85 ml, 6 mmol) dropwise within 15 min under Ar. It was then stirred 1 h at 0°. This was followed by addition of 48% aq. HBr (3 mmol), and the mixture was evaporated to dryness under reduced pressure (oil pump) at 30°. The resulting yellow oil was crystallized from acetone to yield 445 mg (58%) of **5** as yellow hygroscopic crystals, m.p. 148–150°. UV (H_2O): 343 (19200). UV (CH_2Cl_2): 356 (332100). ^{1}H-NMR ($CDCl_3$): 9.32 (*d*, $J_{5,6}$ = 5, H–C(6)); 7.68 (*m*, H–C(2'), H–C(6')); 7.48 (*m*, 3H, H–C(3'), H–C(4'), H–C(5)); 6.92 (*d*, $J_{5,6}$ = 5, H–C(5)); 4.16 (*t*, $J_{2,3}$ = 9, 2H–C(2)); 3.97 (*s*, 3H, CH_3); 3.35 (*t*, $J_{2,3}$ = 9, 2H–C(3)). ^{13}C-NMR ($CDCl_3$): 163.61 (C(5)), 157.76 (C(1')), 134.32 (C(4)), 132.66 (C(4')), 129.20 (C(2'), C(6')), 127.11 (C(3'), C(5')), 113.56 (C(6)), 48.79 (C(2)), 47.42 (CH_3N), 25.34 (C(3)). LC/MS (thermospray): 172 (M^+), 174.

1,5-Dimethyl-4-phenyl-2,3-dihydropyridinium Bromide (**6**). Compound **6** was obtained in 49% yield in a manner similar to that described above as colorless crystals, m.p. 158–160°. UV (H_2O): 331 (13700). UV (CH_2Cl_2): 338 (283200). ^{1}H-NMR ($CDCl_3$): 9.34 (*s*, H–C(6)); 7.43 (*m*, 2H, H–C(2'), H–C(6')); 7.33 (*m*, 3H, H–C(3'), H–C(4'), H–C(5')); 4.00 (*t*, $J_{2,3}$ = 9, 2H–C(2)); 4.0 (*s*, 3H, CH_3N); 3.13 (*t*, $J_{2,3}$ = 9, 2H–C(3)); 2.18 (*s*, 3H, CH_3). ^{13}C-NMR ($CDCl_3$): 167.80 (C(5)), 152.94 (C(1')), 136.76 (C(4)), 130.08 (C(4')), 129.15 (C(2'), C(6')), 127.79 (C(3'), C(5')), 123.50 (C(6)), 48.88 (C(2)), 47.52 (CH_3N), 29.00 (C(3)), 17.06 (CH_3). LC/MS (thermospray): 186 (M^+), 188. Anal. calc. for $C_{13}H_{16}BrN \cdot \frac{1}{2} H_2O$ (275.19): C 56.74, H 6.22, N 5.09; found: C 57.05, H 6.58, N 5.18.

Oxidation of **5** *to* **7** *with Air in the Presence of Platinum Catalyst.* The *N*-oxide **3** (450 mg, 2 mmol) was converted to the dihydropyridinium trifluoroacetate as described previously. The solution was evaporated to dryness under reduced pressure (bath temp. 30°), dissolved in 50 ml of benzene, 200 mg of freshly prepared (from PtO_2) Pt was added, and the mixture was stirred at 50–60° in the presence of air for 2 h. An additional 200 mg of catalyst was added, and it was then stirred for a further 4 h. The catalyst was removed by filtration, the solution evaporated to dryness, and the residue purified by short-column chromatography (silanized silica gel *60*, acetone H_2O). The product was converted to the iodide salt (using I-form of *Dowex 1×8*) and crystallized from acetone to yield 100 mg (17%) of **7** (X = I) as yellow crystals, m.p. 167–169°. UV (H_2O): 293. LC/MS (thermospray): 170 (M^+). This compound was identical in every respect with *1-methyl-4-phenylpyridinium iodide* prepared from 4-phenylpyridine and MeI.

Oxidation of **6** *to* **8** *with Air, in the Presence of Platinum Catalyst.* Using the procedure similar to that described above (toluene was used instead of benzene), **8** (X = I) was obtained in crystalline form, m.p. 138–140°. UV (H_2O): 275. LC/MS (thermospray): 184 (M^+). The compound was identical in every respect with *1,3-dimethyl-4-phenyl-pyridinium iodide* prepared from 3-methyl-4-phenylpyridine and MeI.

The authors thank Dr. *Kenner C. Rice,* of the Section on Medicinal Chemistry, Laboratory of Chemistry, NIADDK, for the gift of 3-methyl-4-phenylpyridine. We would also like to thank Dr. *Daniel J. Liberato,* Laboratory of Theoretical and Physical Biology, NICHD, for performing thermospray LC/MS spectra.

REFERENCES

[1] *A. Ziering, L. Berger, S.D. Heineman & J. Lee,* J. Org. Chem. *12,* 894 (1947).
[2] *G.C. Davis, A.C. Williams, S.P. Markey, M.H. Ebert, E.D. Caine, C.M. Reichert & I.J. Kopin,* Psychiat. Res. *1,* 249 (1979).
[3] *J.W. Langston, P. Ballard, J.W. Tetrud & I. Irvin,* Science *219,* 979 (1983).
[4] *R.S. Burns, C.C. Chiueh, S.P. Markey, M.H. Ebert, D.M. Jacobowitz & I.J. Kopin,* Proc. Natl. Acad. Sci. *80,* 4546 (1983).
[5] *R.E. Heikkila, A. Hess & R.C. Duvoisin,* Science *224,* 1451 (1984).
[6] *S.P. Markey, J.N. Johannessen, C.C. Chiueh, R.S. Burns & M.A. Herkenham,* Nature (London) *311,* 464 (1984).
[7] *J.W. Langston, I. Irwin, E.B. Langston & L.S. Forno,* Neurosci. Lett. *48,* 87 (1984).
[8] *K. Chiba, A. Trevor & N. Castagnoli,* Biochem. Biophys. Res. Commun. *120,* 574 (1984).
[9] *A.F. Casy, A.H. Beckett, M.A. Iorio & H.Z. Youssef,* Tetrahedron *21,* 3387 (1965).
[10] *M. Gerecke & A. Brossi,* Helv. Chim. Acta *47,* 1117 (1964).
[11] *D.S. Gierson, M. Harris & H.P. Husson,* J. Am. Chem. Soc. *102,* 1064 (1980).
[12] *C.W. Abell, R.-S. Shen, W. Gessner & A. Brossi,* Science *224,* 405 (1984).
[13] *W. Gessner, A. Brossi, R.-S. Shen & C.W. Abell,* J. Med. Chem., in press.
[14] *J.I. Salach,* Meth. Enzymol. *53,* 495 (1978).
[15] *V.K. Laemmli,* Nature (London) *227,* 680 (1970).
[16] *S. Kaufman & D.B. Fisher,* in 'Molecular Mechanisms of Oxygen Activation', ed. O. Hayaishi, Academic Press, New York, 1974 pp. 285–369.
[17] *E.C. Gray & V.P. Whittaker,* J. Anat. *96,* 79 (1962).
[18] *R.-S. Shen, R.V. Smith, P.J. Davis & C.W. Abell,* J. Biol. Chem. *259,* 8994 (1984).
[19] *K.H. Nielsen, V. Simonsen & K.E. Lind,* Eur. J. Biochem. *9,* 497 (1969).
[20] *E.A. Harrison & K.C. Rice,* Heterocycles *14,* 813 (1980).