Structure of a Neurotoxin that Induces Parkinsonism Symptoms: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine Hydrochloride

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Abstract: The X-ray crystal structure of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been elucidated. A comparison of the structural relationship of his agent, which induces Parkinsonism by the selective destruction of nigrostriatal dopamine neurons, to the crystal structure of dopamine is presented.

The observation that MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), an artifact in illicit samples of synthetic heroin, induces chronic and irreversible symptoms of Parkinsonism in addicted individuals (1) has generated considerable recent interest. It would appear that the Parkinsonism induced is a result of the selective destruction of nigrostriatal dopamine neurons. It has also been reported that MPTP produces its effects *via* the formation of an active metabolite involving the generation of MPP+ (*N*-methyl-4-phenyl-pyridine) through the actions of oxidases, particularly monoamine oxidase (2, 3, 4). Other metabolites of MPTP have also been reported (5). In an effort to better understand the selectivity of action of MPTP at dopamine neurons, the X-ray crystal structure of MPTP.HCL was investigated.

Materials and Methods

A single colorless needle-like crystal of MPTP.HCL recrystallized from ethanol/hexane with dimensions 0.05 × 0.07 × 0.26 mm was mounted on an Enraf-Nonius CAD-4 diffractometer with a Mo target X-ray tube (λ = 0.71073 Å) and a graphite crystal monochrometer. The compound was found to crystallize in space group $P2_1$ with unit cell dimensions of a = 6.950 (3), b = 6.53 (1), c = 12.176 (6) Å, β = 96.85 (3)° and V = 549.1 Å^3. Three dimensional intensity data were collected in the ω: 2$\ominus$ scan mode at 100 K. A total of 1058 reflections were collected to a 2$\ominus$ maximum of 50°. Data were corrected for Lorentz and polarization effects. Absorption as a function of psi was observed to be minimal and therefore not corrected. Four standard reflections measured every two hours during data collection showed no significant change in intensity.

The structure was solved by direct methods using the MULTAN (6) series of programs which revealed the location of 12 atoms on the initial E map. A subsequent Fourier map revealed the location of the remaining two non-hydrogen atoms. All hydrogen atoms were calculated on the basis of sp^2 or sp^3 geometry and a C-H bond distance of 0.95 Å. The structure was refined by least squares minimization of the function $\Sigma\ w(F_o-F_c)^2$ with anisotropic thermal parameters for all non-hydrogen atoms, and hydrogen atom positions and temperature factors fixed (B = 5.0 Å^2). Using 894 independent observed reflections with I>0 led to a final R = 8.2% and R_w = 6.3% (7). The corresponding R and R_w values using 655 reflections with I>3σ(I) were 4.9% and 6.0%, respectively.

All computer programs used for data collection and refinement are part of the CAD4-SDP package (8). Scattering factors were taken from the *International Tables for X-ray Crystallography* (9) and included corrections for anomalous scattering contributions.

Results and Discussion

Final fractional coordinates are given in Table I. The numbering system for the molecule may be found in Figure 1. Bond lengths and bond angles are listed in Table II (10). MPTP crystallizes as the hydrochloride salt with two molecules in the unit cell. The nitrogen atom is protonated and hydrogen bonded to the chloride ion(N-Cl, 3.011(3) Å). The chloride ion is 3.947 (4) Å from the next neighboring MPTP molecule with a N-Cl-N angle of 174.9(1)°. A unit cell packing diagram is plotted in Figure 2. The molecules pack in the unit cell such that the phenyl rings are stacked with a separation of 3.475(2) Å. The atoms in the phenyl ring have exceptionally high temperature factors for a compound whose data were collected at 100 K. This high thermal motion is an indication of either loose packing of the molecules in

Table I. Table of Positional Parameters and their Estimated Standard Deviations.

Atom	X	Y	Z	B(Å2)
CL	0.8327 (2)	0.000	0.6054 (1)	3.01 (2)
N	0.2679 (5)	−0.000 (2)	0.6179 (3)	2.07 (7)
C1	0.3099 (6)	−0.020 (2)	0.5012 (4)	2.4 (1)
C2	0.3424 (9)	−0.1865 (9)	0.6800 (5)	2.0 (1)
C3	0.3027 (9)	−0.177 (2)	0.7931 (5)	4.5 (2)
C4	0.2911 (6)	0.015 (2)	0.8514 (4)	2.7 (1)
C5	0.301 (1)	0.190 (1)	0.7976 (6)	4.6 (2)
C6	0.347 (1)	0.182 (1)	0.6743 (5)	4.6 (2)
C7	0.2614 (6)	−0.004 (3)	0.9718 (4)	4.8 (2)
C8	0.232 (1)	0.196 (2)	1.0202 (7)	8.9 (3)
C9	0.1785 (9)	0.238 (1)	1.1260 (5)	4.1 (2)
C10	0.1880 (8)	0.062 (2)	1.1897 (5)	7.0 (4)
C11	0.236 (1)	−0.115 (1)	1.1508 (5)	4.3 (2)
C12	0.256 (1)	−0.162 (2)	1.0339 (6)	8.2 (2)

Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter.

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the unit cell or slight disorder. The tetrahydropyridine ring is planar (except for the nitrogen) with a maximum deviation of 0.03 Å from the least-squares plane of the ring. The nitrogen atom is 0.63 Å above this plane. The least-squares plane of the phenyl ring makes a dihedral angle of only 7.8° with the plane of the tetrahydropyridine ring. Although this conformation puts the phenyl ring in the plane of the double bond, it introduces a close contact between H 7 and the *ortho* hydrogen of the phenyl ring. In a structure such as biphenyl, the overcrowding is relieved by a twist to a dihedral angle of about 42° in the gas phase (11). In MPTP, however, such a twist would increase contacts with hydrogens on the other side of the ring. Additionally, the over-crowding is evident in the tilt of the aromatic ring with respect to the C4 bond, as can be seen from the angles C4-C7-C8, 111 (2)°, and C4-C17-C12, 131(2)°.

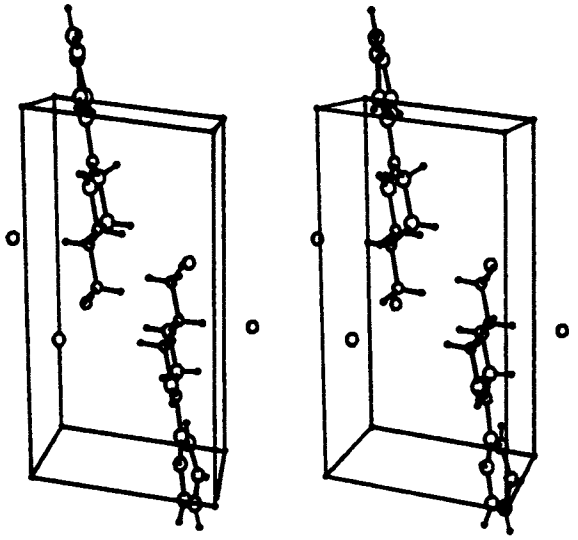

Fig. 2 Stereoscopic diagram showing the packing arrangement of the MPTP molecules and the chloride ions.

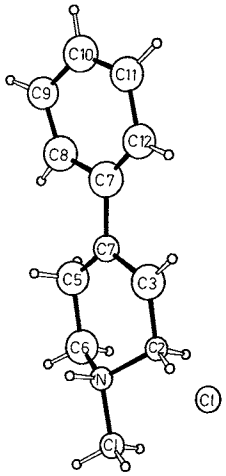

Fig. 1 Labelling system and ORTEP diagram of MPTP plotted using equivalent isotropic temperature factors.

Since MPTP (or its metabolites) are known to cause irreversible neurotoxicity producing symptoms related to Parkinsonism, through a selectivity for dopamine neurons, it seemed reasonable to investigate the possibility that there is a structural similarity between MPTP and dopamine. Comparing the distances between the centroid of the aromatic ring and the nitrogen in each molecule gives 5.15 Å for dopamine and 5.702 (3) Å for MPTP. Alternatively, least-squares minimization of the distances between C7, C4 and N and the comparable atoms in dopamine hydrochloride (C6, C7 and N) yields differ-

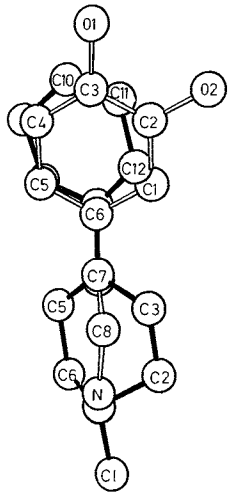

Fig. 3 Overlay diagram of MPTP (black bonds) and dopamine (white bonds) showing the best molecular fit between the crystallographic coordinates of the two molecules.

Table II. Table of Bond Distances in Angstroms.

Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
N	C1	1.491 (5)	C4	C3	1.45 (2)	C8	C9	1.41 (2)
N	C6	1.45 (2)	C4	C7	1.510 (7)	C9	C10	1.39 (2)
N	C2	1.493 (13)	C3	C2	1.44 (2)	C10	C11	1.31 (2)
C6	C5	1.57 (2)	C7	C8	1.46 (3)	C11	C12	1.48 (2)
C5	C4	1.32 (2)	C7	C12	1.28 (3)			

Table of Bond Angles in Degrees

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C1	N	C6	114.6 (9)	C5	C4	C7	125. (1)	C8	C7	C12	117.9 (7)
C1	N	C2	108.5 (9)	C3	C4	C7	115. (1)	C7	C8	C9	127. (2)
C6	N	C2	110.0 (3)	C4	C3	C2	123. (1)	C8	C9	C10	110. (1)
N	C6	C5	111.7 (9)	N	C2	C3	110.7 (9)	C9	C10	C11	121.9 (7)
C6	C5	C4	118. (1)	C4	C7	C8	111. (2)	C10	C11	C12	126. (1)
C5	C4	C3	119.7 (5)	C4	C7	C12	131. (2)	C7	C12	C11	114. (2)

Numbers in parentheses are estimated standard deviations in the least significant digits.

ences at only 0.241, 0.244 and 0.301 Å, respectively (12). Figure 3 shows a diagram of the least-squares overlay of MPTP structure on the structure of dopamine hydrochloride (13). Overlaying the crystallographic coordinates of these atoms results in a good overall fit for the two molecules, even though the rigid conformations observed in the crystal lattice are maintained. On the basis of Dreiding models, it is likely that an even better fit can be obtained, given some flexibility in the tetrahydropyridine ring of MPTP and rotation of the aminoethyl chain of dopamine about the C6–C7 bond. It appears possible, therefore, that the high neurotoxic specificity of MPTP for cells of the substantia nigra may be related to its structural similarity to dopamine. However, recently it has been shown that MPP+ rather than MPTP is accumulated selectively *via* the dopamine neuronal uptake system (14). Thus, the spatial relationships of the crystal structures of MPP+ and dopamine should be of interest. Studies are currently underway to investigate this relationship.

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