Free Rad. Res. Comms., Vol. 2, No. 2, pp. 107-113
8755-0199/86/0202-0113 $25-00/0

Printed in Great Britain

OXIDATIVE IONIC METABOLITES OF 1-METHYL-4-PHENYL-1, 2, 3, 6-TETRAHYDROPYRIDINE (MPTP): CORRELATION OF ELECTROREDUCTION WITH PHYSIOLOGICAL BEHAVIOR†

JAMES R. AMES[1], NEAL CASTAGNOLI, JR.[2], MICHAEL D. RYAN[3], AND PETER KOVACIC[1]††

[1]Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, [2]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, and [3]Department of Chemistry, Marquette University, Milwaukee, WI 53233

(Received January 22, 1986)

Electrochemical studies (reduction potential and reversibility) were performed on 1-methyl-4-phenylpyridinium (MPP^+) and 1-methyl-4-phenyl-2, 3-dihydropyridinium ($MPDP^+$). MPP^+ gave reduction potentials in the range of -1.09 to -1.11 V in organic solvents in a process which was reversible. The reduction potential of $MPDP^+$ was -0.64 V (irreversible). Possible relationships involving the electrochemical properties, oxy radical formation, and biological activity of these and related iminium species are discussed.

Key words: electroreduction; 1-methyl-4-phenylpyridinium; iminium; charge transfer; physiological activity; MPTP; oxy radicals.

INTRODUCTION

The piperideine derivative 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP, *1*), causes a clinical syndrome indistinguishable from idiopathic Parkinson's disease in man[1-3] and destroys selectively the nigrostriatal neuronal system in monkeys[4, 5]. Recent evidence has established that MPTP is a substrate for brain monoamine oxidase (MAO) which catalyzes the oxidation of the parent drug to the 1-methyl-4-phenylpyridinium species (*2*, MPP^+)[6]. Further investigation of the metabolism of *1* provided evidence for the MAO B catalyzed formation of the intermediate dihydropyridinium species *3*.

Recently, it has been suggested that charge transfer (CT) by iminium *4*, may play a critical role in the mechanism of action of a variety of biologically active compounds[7].

† Presented at the 190th national meeting of the American Chemical Society, Chicago, Ill, MEDI Abstracts, 64 (1985).

†† Author to whom all correspondence should be addressed.

More specifically, electron transfer by the catalytic agent is believed to involve a donor, e.g. protein or DNA, and an acceptor, such as oxygen or a component of the central nervous system. Experimental application has been made to carcinogens (purine iminium)[8] and antibacterial heterocyclic di-N-oxides[9].

The principal objective of the present work was to ascertain the electrochemical characteristics, specifically the reduction potentials and the reversibility of MPP^+ and $MPDP^+$. In addition, the relationship of electrochemical behavior to oxy radical generation and biological activity will be discussed.

MATERIALS AND METHODS

Syntheses of 1-methyl-4-phenyl-2,3-dihydropyridinium perchlorate 3[10] (m.p. 122.5°C) and 1-methylpyridinium iodide (m.p. 115–116°C, lit.[11] m.p. 117°C) followed literature methods. The preparation of 1-methyl-4-phenylpyridinium iodide (*2*) was accomplished by stirring a solution of methyl iodide and 4-phenylpyridine in DMF at 80°C for 5 hr. and then at room temperature for 15 hr.[12] Several crystallizations from methanol gave material melting at 168–169°C. *Anal.* Calc. for $C_{12}H_{12}NI$: C, 48.48, H, 4.04, N, 4.71. Found: C, 48.61, H, 4.17, N, 4.64.

Cyclic voltammetry was performed on an ECO model 550 potentiostat with a PARC model 175 waveform generator. All solutions were degassed with prepurified dinitrogen that was passed through an oxygen-scrubbing system. A platinum flag and a mercury drop (HMDE) were the working electrodes. The reference for all solvents was an IBM aqueous Ag/AgCl electrode in saturated KCl. The supporting electrolyte was tetraethylammonium perchlorate (G.F. Smith Chemical Co.). The organic solvents were obtained from Aldrich Chemical Co. in the highest available purity. Acidic solutions consisted of aqueous perchloric acid.

RESULTS AND DISCUSSION

Compound *3* can exist in equilibrium with its conjugate base *5*[10,13] analogous to the iminium-enamine equilibrium in other systems[14]. Therefore reductions were run with strong acids in order to minimize the amount of *5* present. The reduction potentials were uniformly in the range of -0.63 to -0.65 V in all solvents (Table I). In the absence of added acid (pH 6.9 in H_2O), the values were somewhat more varied and more negative, -0.69 to -0.90 V. All reactions occurred irreversibly. Cyclic voltammetry data for (*3*) are summarized in Table I.

The reversible reduction potentials for MPP^+ were -1.09 V (DMF), -1.11 V (DMSO), and irreversible at -1.36 V (H_2O) (Tables II and III). The diffusion coefficient was calculated to be 5.3×10^{-6} cm^2s^{-1} (DMF) (eq.1) using benzil[15] ($D = 1.1 \times 10^{-5}$ cm^2s^{-1}) as the reference. The independence of peak potential vs. scan rate, constant value of the current function (Table III), and ΔEp of 64 mV vs. the theoretical value (56.5/n mV)[16] for a one electron reduction verify a reversible, diffusion-controlled, one electron process. The more negative potential for *2* vs. *3* is probably due to the greater stability associated with the aromatic pyridinium nucleus[17].

The parent N-methylpyridinium salt gave reduction potentials of -1.28 V (acetonitrile)[18] and -1.27 V (DMF)[19], in essential agreement with our results: -1.27 V (DMF), -1.33 V (DMSO) (Table II). The change in the positive direction for *3*

TABLE I
Cyclic voltammetry of $MPDP^{+}$ [a-c]

Solvent	$[H^+]$mM	Ep_1 (V)	Ep_2 (V)
DMF	——	−0.90	−1.15
	1.34	−0.63	——
DMSO	——	−0.69	−1.13
	1.34	−0.66	——
CH_3CN	——	−0.74	——
	1.34	−0.65	——
H_2O	pH 6.9	−0.74	−0.86
	d	−0.65	——

[a]100 mV/s, tetraethylammonium perchlorate (0.1M), substrate (0.5mM), vs. Ag/AgCl; aqueous and acidic solutions, HMDE; organic solutions, Pt flag.
[b]Irreversible.
[c]Controls with acid were performed to validate acidic reduction potentials.
[d]pH 2, buffer (HCl/Cl^-).

TABLE II
Cyclic voltammetry of pyridinium ions[a]

Pyridinium Ion	Solvent	E½ (V)
3	DMF[a-f]	−1.09
	DMSO[a-f]	−1.11
	H_2O[c,g]	−1.36
N-Methylpyridinium	DMF[c,g]	−1.27
		−1.28[h]
		−1.27[i]
	DMSO[c,g]	−1.33
	H_2O[c,d,g]	−1.56

[a]100 mV/s, tetraethylammonium perchlorate (0.1 M), substrate (0.25–0.50 mM), vs. Ag/AgCl.
[b]Pt flag electrode.
[c]HMDE.
[d]Duplicate runs were made.
[e]No difference with excess acid, $[H^+] = 1.34$ mM.
[f]Reversible at all scan rates (10–200 mV/s).
[g]Irreversible, Ep values.
[h]Ref. 18.
[i]Ref. 19.

(E1/2 = −1.09 V, DMF) is in accord with the inductive, polar, and resonance effects of the phenyl substituent. For both the N-methylpyridinium salt and *2* the reduction potentials were significantly more negative in aqueous solution, −1.56 V and −1.36 V, respectively.

The pyridinium salts (*6*), which inhibit the growth of influenza virus[20,21] gave reduction potentials of −0.27 to −0.52 V. There was partial reversibility corresponding to the first wave. The very negative (−1.71 V) reduction potential[22] for *7*,

TABLE III
Parameters from Cyclic Voltammetry of MPP^+ [a]

υ(V/s)	$-Ep_c$(V)	$-E½$(V)	ΔEp(mV)	$ip_c(A \times 10^{-3})$	$ip_c/(\upsilon)^{½}C$
0.010	1.12	1.09	7011.55	0.29	11.60
0.020	1.13	1.10	7011.55	0.39	11.03
0.050	1.13	1.10	6011.55	0.65	11.63
0.100	1.13	1.08	6011.55	0.95	12.02
0.200	1.13	*1.10*	*6011.55*	1.28	*11.45*
		1.09 (av.)	64[b] (av.)		11.55[c,d] (av.)

[a] DMF, Pt flag, $[MPP^+] = 0.25$ mM.
[b] ± 4.9
[c] $\pm 3.19 \times 10^{-1}$
[d] $A/(V/s)^{1/2}M$.

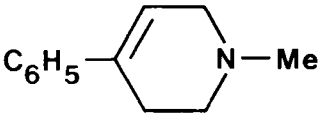
$$\frac{(ip/(\upsilon)^{½}C)_{MPP^+}}{(ip/(\upsilon)^{½}C)_{benzil}} = \frac{(D_{MPP^+})^{½}}{(D_{benzil})^{½}} \qquad \text{(eqn 1)}$$

analog of *1*, indicates that MPTP is not likely to be involved in CT processes requiring a bioreduction step.

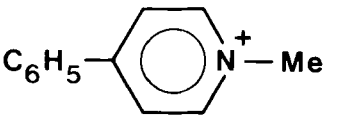
In relation to the mechanism of action of MPTP, both *2* and *6* produce toxic effects. Compound *2* (cyperquat) possesses herbicidal properties[23,24] and may be responsible for the nigrostriatal toxicity of MPTP. Various investigators[25-27] have related the mammalian response induced by MPP^+ to that elicited by diquat and paraquat[28]. Since it is likely that the structurally similar pyridinium herbicides operate by a common mechanism, the reduction potential (-1.09 V) of MPP^+ should then be sufficient to effect CT *in vivo*. Appreciable evidence for participation of oxy radicals in the MPTP system has been presented by a number of groups[26,27,29,30]. Our electrochemical studies indicate that charge transfer may comprise a reasonable route for formation of these radicals. The relevance to the neurological effect would then comprise the selective destruction of the nigrostriatal neuronal cells by the activated oxygen species after site binding by the CT agent. Recent evidence[31] for MPP^+ concentration into dopamine neurons fits nicely into this concept. It is pertinent that oxidative stress mechanisms have been advanced to interpret the toxicities of diverse classes of drugs[30,32-36].

Another conceivable redox pathway consists of the initial oxidation of *5* to a radical iminium entity. Other hypotheses dealing with MPTP action have been suggested[27,37-39].

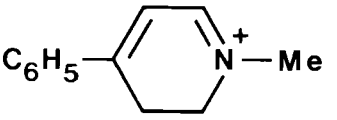
Increasing evidence supports the notion that the iminium species is broadly involved in the action of a variety of important biologically active compounds[7-9,40-42]: carcinogens, herbicides, alkaloids, toxins, and drugs. A recent review deals with iminium ions from xenobiotic metabolism[43], and another describes these cations in the alkaloid domain[44].



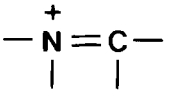
1



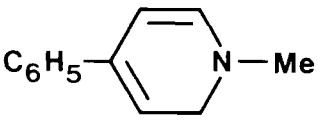
2



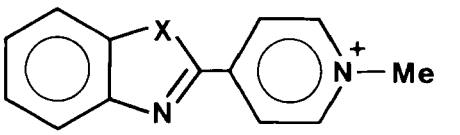
3



4

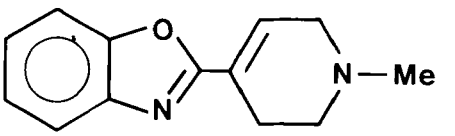


5



X = O, S, NH

6



7

MPTP, Related Heterocycles, and, Relevant Iminium Species.

Acknowledgement

This work was supported by grants from the Urban Research Center, University of Wisconsin-Milwaukee, the Wisconsin Alcoholism and Drug Abuse Research Institute, and DA-30405(UC-SF).

References

1. G.C. Davis, A.C. Williams, S.P. Markey, M.H. Ebert, E.D. Caine, C.M. Reichert and I.J. Kopin, *Psychiat. Res.*, 1979, **1**, 249.
2. J.W. Langston, P. Ballard, J.W. Tetrud and I. Irwin, *Science*, 1983, **219**, 979.
3. J.M. Wright, R.A. Wall, T.L. Perry and D.W. Paty, *N. Engl. J. Med.*, 1984, **310**, 325.
4. R.S. Burns, C.C. Chiueh, S.P. Markey, M.H. Ebert, D.M. Jacobowitz and I.J. Kopin, *Proc. Natl. Acad. Sci.*, 1984, **80**, 4546.
5. J.W. Langston, L.S. Forno, C.S. Rebert and I. Irwin, *Brain Res.*, 1984, **292**, 390.
6. K. Chiba, A. Trevor and N. Castagnoli, Jr., *Biochem. Biophys. Res. Commun.*, 1984, **120**, 574.
7. P. Kovacic, *Kem. Ind.*, 1984, **33**, 473.
8. P. Kovacic, P.W. Crawford, M.D. Ryan and V.C. Nelson, *Bioelectrochem. Bioenerg.*, in press.
9. M.D. Ryan, R.G. Scamehorn and P. Kovacic, *J. Pharm. Sci.*, 1985, **74**, 492.
10. K. Chiba, L.A. Peterson, K.P. Castagnoli, A.J. Trevor and N. Castagnoli, Jr., *Drug Metab. Dispos.*, 1985, **13**, 342.
11. A.B. Prescott, *J. Am. Chem. Soc.*, 1896, **18**, 91.
12. L.W. Hedrich, Ger. Patent 2,223,716, 1973; Chem. Abstr., 1973, **78**, 84262.
13. N. Castagnoli, Jr., K. Chiba and A.J. Trevor, *Life Sci.*, 1985, **36**, 225.
14. S.F. Dyke, in *The Chemistry of Enamines*, (University Press: Cambridge, 1973) pp. 6–9.
15. M.D. Ryan and D.H. Evans, *J. Electroanal. Chem.*, 1976, **67**, 333.
16. A.J. Bard and L.R. Faulkner, in *Electrochemical Methods, Fundamentals and Applications* (Wiley: New York, 1980) p. 229.
17. H. Lund, in *Organic Electrochemistry*, ed. M.M. Baizer and H. Lund (Marcel Dekker: New York, 1985) p. 555.
18. R. Raghavan and R.T. Iwamoto, *J. Electroanal. Chem.*, 1978, **92**, 101.
19. O.N. Chupakhin, V.N. Charushin, I.M. Sosnokin, E.G. Kovalev, G.L. Kalb and I.Ya. Postovskii, *Chem. Hetero. Cmpd.*, 1977, **13**, 562.
20. L. Gergely, F.D. Toth and G. Hadhazy, *Acta Microbiol. Acad. Sci. Hung.*, 1968, **15**, 145; Chem. Abstr., 1968, **69**, 74690.
21. L. Vaczi, G. Hadhazy, K. Hideg, L. Gergely, O.H. Hankovszky and F.D. Toth, *Acta. Virol.* (Prague), Eng. Ed., 1968, **12** 368; Chem. Abstr., 1968, **69**, 57604.
22. V.P. Kadysh, Ya.P. Stradyn', E.S. Lavrinovich and P.P. Zarin, *Chem. Hetero. Cmpd.*, 1975, **11**, 588.
23. I.O. Akobundu, *Weed Res.*, 1981, **21**, 273; Chem. Abstr., 1982, **96**, 99337.
24. J.A. Jagschitz, *Proc. Ann. Meet. Northeast Weed Sci. Soc.*, 1982, **36**, 326; Chem Abstr., 1982, **96**, 157222.
25. A. Barbeau, L. Dallaire, N.T. Buu, F. Veilleux, H. Boyer, L.E. deLanney, I. Irwin, E.B. Langston and J.W. Langston, *Life Sci.*, 1985, **36**, 1125.
26. Symposium on MPTP: A Parkinsonian Syndrome Producing Neurotoxin, Uniformed Services University of the Health Sciences, Bethesda, MD., June 6–7, 1985.
27. R. Lewin, *Science*, 1985, **229**, 257.
28. J.S. Bus and J.E. Gibson, *Environmental Health Perspectives*, 1984, **55**, 37.
29. G.U. Corsini, S. Pintus, C.C. Chiueh, J.F. Weiss and I.J. Kopin, *Eur. J. Pharm.*, 1985, **119**, 127.
30. *Oxidative Stress*, ed. H. Sies (Academic Press: New York, 1985).
31. J.A. Javitch, R.J. D'Amato, S.M. Strittmatter and S.H. Snyder, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 2173.
32. B. Halliwell and J.M.C. Gutteridge, in *Free Radicals in Biology and Medicine* (Clarendon Press: Oxford, 1985).
33. B. Halliwell and J.M.C. Gutteridge, *Molec. Aspects Med.*, 1985, **8**, 89.
34. J.L. Holtzman, *Life Sci.*, 1982, **30**, 1.
35. S.D. Nelson, *J. Med. Chem.*, 1982, **25**, 753.
36. E. Beutler, *Pharmacol. Rev.*, 1969, **21**, 73.
37. J. Poirier, J. Donaldson and A. Barbeau, *Biochem. Biophys. Res. Commun.*, 1985, **131**, 1284.

38. R. Lewin, *Science*, 1984, **225**, 1460.
39. W.J. Nicklas, I. Vyas and R.E. Heikkila, *Life Sci.*, 1985, **36**, 2503.
40. P. Kovacic, *Age*, 1983, **6**, 144.
41. P. Kovacic, M.D. Ryan, *et al.*, unpublished work.
42. J.R. Ames, S. Brandänge, B. Rodriguez, N. Castagnoli, Jr., M.D. Ryan and P. Kovacic, *Bioorg. Chem.*, in press.
43. M. Overton, J.A. Hickman, M.D. Threadgill, K. Vaughan and A. Gescher, *Biochem. Pharm.*, 1985, **34**, 2055.
44. J. Knabe, *Iminium Salts in Organic Chemistry*, Part 2, ed. H. Böhme and H.G. Viehe, *Advances in Organic Chemistry*, ed. E.C. Taylor (Wiley-Interscience: New York) p. 733ff.

Accepted by Dr. B. Halliwell