25. Electronic and Conformational Effects on the Lipophilicity of Isomers and Analogs of the Neurotoxin 1-Methyl-4-phenylpyridinium (MPP$^+$)

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A number of isomers and analogs of the neurotoxin 1-methyl-4-phenylpyridinium (MPP$^+$) were examined for their lipophilic behavior. Their partition coefficients in the 1-octanol/H$_2$O system were measured by centrifugal partition chromatography (CPC), which, being much faster and markedly more precise than the classical shake-flask method, proved a very promising alternative for assessing lipophilicity. A smaller-than-expected lipophilicity was shown by the *ortho*-isomer of MPP$^+$ (M2PP$^+$) and is explained in terms of electronic effects and rigidity, as revealed by UV and NMR spectroscopy, and conformational analysis. Implications of lipophilicity in modulating the *in vivo* dopaminergic neurotoxicity of the examined compounds are also discussed.

Introduction. – The discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces symptoms very similar to those observed in *parkinsonism* [1–3] has led to vigorous research aimed at elucidating the molecular mechanisms responsible for its toxicity. MPTP, which causes degeneration of the nigrostriatal system, must be activated, before it exerts its neurotoxic actions. The toxication route involves oxidation in the brain by type B monoamine oxidase (MAO-B), and to a lesser extent by MAO-A, to a dihydropyridinium intermediate (1-methyl-4-phenyl-2,3-dihydropyridinium, MPDP$^+$) which then spontaneously oxidizes to form 1-methyl-4-phenylpyridinium ion (MPP$^+$), or disproportionates to form MPTP and MPP$^+$ [4–9]. MPP$^+$ is the neurotoxin which causes cell death.

It is documented that MPP$^+$ is transported and accumulated in dopaminergic neurons *via* the dopamine (DA) uptake system and then concentrated in the matrix of mitochondria [10] [11] where it inhibits NADH-dehydrogenase [12–17], leading to cessation of oxidative phosphorylation, ATP depletion, and ultimately to neuron death [18]. Some recent papers suggested that the concentration of MPP$^+$ inside mitochondria can occur *via* a passive transport mechanism driven by the transmembrane electrochemical potential [19] [20], and evidence were reported for its carrier-independent entry into cells and subcellular organelles [21]. Charge delocalization was invoked as the property that could permit to the MPP$^+$ ion to reach a sufficient degree of liposolubility and to cross a variety of cell membranes along concentration and charge gradients.

Moreover, an interesting attempt to gain a better understanding in the structure-toxicity relationships of two large series of MPTP and MPP$^+$ analogs [22] led to the following conclusions: *1*) only permanently charged compounds show neurotoxic effects; *2*) with few exceptions, hydrophilic substituents decrease toxicity; *3*) toxic potency is enhanced

by lipophilic groups, but increased steric bulk around the N-atom tends to decrease activity. However, no physicochemical parameter, especially partition data, were reported in order to substantiate these hypotheses. We, therefore, undertook to measure the lipophilicity of a number of isomers and analogs of MPP$^+$ in order to obtain some insight into structure-toxicity relationships. For this purpose, we used centrifugal partition chromatography (CPC), also known as centrifugal counter-current chromatography (CCCC), a novel technique displaying numerous advantages over the traditional shake-flask (SF) and reversed-phase HPLC methods [23–25]. Indeed, CPC is far less time-consuming and much more precise and accurate than the SF method. Over RP-HPLC, it has the advantage of not employing a solid support, thus eliminating any adsorption phenomenon and allowing the direct obtention of partition coefficients rather than capacity factors.

The partition coefficients of compounds **1–10** (*Table 1*) were compared with calculated values and then analyzed in terms of the structural factors influencing them.
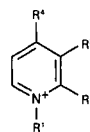
Table 1. *Structure and Lipophilicity Parameters of the Investigated MPP$^+$ Analogs*

	Abbreviation	R^1	R^2	R^3	R^4	log P_{exp}[a])	log P_{calc}[b])
1	MPP$^+$	Me	H	H	Ph	–2.28	–0.81
2	M3PP$^+$	Me	H	Ph	H	–2.46	–0.81
3	M2PP$^+$	Me	Ph	H	H	–3.00	–0.60
4	4'-MeMPP$^+$	Me	H	H	4'-methylphenyl	–1.90	–0.31
5	4'-OMeMPP$^+$	Me	H	H	4'-methoxyphenyl	–2.14	–0.89
6	EPP$^+$	Et	H	H	Ph	–2.08	–0.28
7	PPP$^+$	Pr	H	H	Ph	–1.83	0.25
8	i-PPP$^+$	i-Pr	H	H	Ph	–1.98	0.03
9	*N*-Methylquinolinium					–2.71	–1.31
10	*N*-Methylisoquinolinium					–2.75	–1.52

[a]) Measured by CPC. Mean of 3–5 determinations; relative standard deviations were always < 2%, with the exception of **3** (RSD 3%), its log *P* value being close to the lower methodological limit.
[b]) Calculated by CLOGP 3.54 program.

$$\log P_{calc} = f(N^+) + f(1 \text{ aliph. C}) + f(11 \text{ arom. C}) + f(12 \text{ H on C}) + F(\text{fusion})$$
$$= -5.36 + 0.195 + 1.43 + 2.724 + 0.20 = -0.81$$

where (N^+) is the quaternary N-atom in a pyridinium function.

Spectroscopic Measurements. UV spectra were recorded for solns. in phosphate buffer 0.01M (pH 7.40) with a *Philips PU8720* UV/VIS scanning spectrophotometer. NMR spectra were recorded at 25° for solns. in CDCl₃/ (D₆)DMSO 10:1 with a *Varian XL-200* spectrometer. The signal position was recorded with reference to the external standard TMS with an accuracy of ±0.01 ppm. Sample concentrations were in the range 0.04–0.12M. No concentration dependence of NMR signals was observed.

Structural Calculations. Conformational analyses were performed using the *MOPAC 5.0* package (QCPE No. 445 [29]) running on a *Sun SPARC 1* workstation. Molecular and H₂O-accessible surfaces were calculated with the program MOLSV (QCPE No. 509) running on a *Sun SPARC 1* workstation. *Van der Waals* radii were taken from [30], and the H₂O molecule was assumed to be a sphere of radius 1.5 Å. The H₂O-accessible area was calculated by the program as being the ensemble of all points occupied by the centre of the H₂O sphere, when it rolls on the molecular surface.

Results and Discussion. – *Lipophilicity Measurements.* The partition coefficients of the 10 compounds investigated are reported in *Table 1*. The $\log P_{oct}$ value of only one compound, namely MPP⁺, is reported in literature to be −1.05 (SF method) [31]. Our study reveals a much lower lipophilicity, the difference being possible due to some inherent limitations of the SF method [32].

When comparing the $\log P_{oct}$ values of the MPP⁺ analogs among themselves, expected and unexpected findings are seen. Thus, as compared to MPP⁺ itself, the small increases in $\log P_{oct}$ caused by a 4′-Me or 4′-MeO substituents are in line with expectations [28]. The replacement of the *N*-Me group by larger *N*-alkyl groups (compounds **6–8**) leads to limited increase in lipophilicity which reflects the delocalization of the positive charge. Completely unexpected is the behaviour of M3PP⁺ and M2PP⁺, which are slightly and markedly more hydrophilic than MPP⁺, respectively. Understanding of this behavior is not straightforward. It could perhaps be due to a decrease in the H₂O accessible surface area of the most stable conformers. As shown in *Table 2*, the molecular surface area and

Table 2. *Physicochemical Data of MPP⁺ Isomers* **1–3**

		MPP⁺ (**1**)	M3PP⁺ (**2**)	M2PP⁺ (**3**)
UV spectra	λ_{max} [nm] (ε)	224 (20400)	230 (24500)	226 (16800)
		293 (20700)	257 (11100)	281 (7600)
200-MHz NMR data[a]	$\delta(C^1H_3)$ [ppm]	4.60	4.73	4.42
	$\delta(^{13}CH_3)$ [ppm]	48.26	48.59	47.48
	$\delta(^{13}C(1'))$ [ppm]	133.12	132.03	129.56
Molecular surface area [Å²]		207.6	210.1	206.8
H₂O-accessible surface area [Å²]		399.5	398.8	395.4

[a] Only a number of selected chemical shifts are reported. For further details, see *Experimental.*

H₂O-accessible surface area of the three isomers are too much alike to account for the differences in $\log P_{oct}$. Other factors potentially accounting for this difference are electronic and conformational effect, as investigated below.

To place the above qualitative comparisons on a more quantitative basis, the experimental $\log P_{oct}$ values were compared with those calculated by CLOGP software [27] (*Table 1*). The values are much higher than the experimental ones, but a closer look at *Fig. 1* reveals that, with the exception of compound **3** (M2PP⁺), a good linear correlation
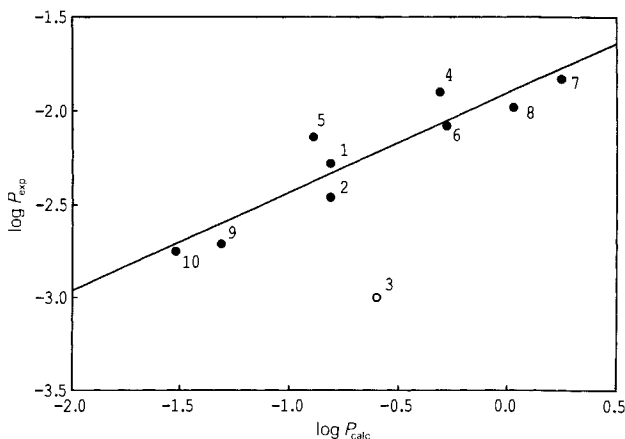
Fig. 1. *Plot of experimental vs. calculated log P values*

exist between the two data set. By omitting compound **3** in the regression analysis, the following equation was obtained:

$$\log P_{\text{exp}} = 0.53 \ (\pm 0.08) \log P_{\text{calc}} - 1.90 \ (\pm 0.07) \tag{1}$$
$$n = 9 \quad r = 0.927 \quad s = 0.136$$

Despite the good linear correlation, the lower-than-unity slope and the markedly negative intercept suggest that the calculation system loses part of its predictive power, at least in absolute values, for ionic solutes where polarity largely predominates over hydrophobicity. In fact, the fragmental value of the N^+ fragment is not correctly parameterized. Based on *Eqn. 1*, a value of *ca.* -7.0 for this fragment can be proposed.

Electronic Structure and Conformational Behavior of MPP$^+$ and Its Regioisomers. Conformational behavior and electronic conjugation are strictly interdependent in the three MPP$^+$ isomers examined. Indeed, it is expected that loss of coplanarity between the Ph and the pyridinium ring results in a diminished electronic conjugation. A comparison of the UV absorption spectra of the compounds in aqueous solution (*Table 2*) shows two absorption maxima. The shift and intensity of the first λ_{max} (293 nm for **1**) are more informative than the second band (224 nm for **1**). Relative to MPP$^+$, M3PP$^+$ shows hypsochromic (-36 nm) and hypochromic effects due to its *meta*-substitution pattern. More interesting is M2PP$^+$, which shows not only a hypsochromic shift (-12 nm) of the first maximum but also a much lower molar absorption coefficient. Hypsochromic and hypochromic effects in the latter case indicate an increase in the torsion angle between the two rings resulting in diminished electronic conjugation.

To determine the charge density on the pyridinium N-atom, ^{1}H- and ^{13}C-NMR spectra of the three MPP$^+$ isomers were recorded, selected chemical shifts being reported in *Table 2*. ^{1}H- and ^{13}C-chemical shifts are indicative of a shielding of the Me group decreasing in the order: M2PP$^+$ > MPP$^+$ > M3PP$^+$. Based on the conjugation deduced from the UV spectra, the deshielding of the Me group should be larger in the *ortho*-isomer (M2PP$^+$) than in MPP$^+$ and M3PP$^+$ (decreased electronic conjugation resulting in a more localized positive charge on the pyridinium N-atom). This apparent anomaly can be explained by considering the diamagnetic anisotropy induced by the ring-current effect

of circulating π electrons. Only in the case of M2PP⁺, due to the more probable non-coplanarity between the two rings, is the Me group held directly above or below the pyridine ring, *i.e.* in the shielding portion of the anisotropy cone.

Conformational analysis carried out on compounds **1**, **2**, and **3** confirmed the deductions from the UV and NMR spectra. As seen in *Fig. 2*, M2PP⁺ has limited flexibility compared with its isomers **1** and **2**. Indeed, the rotation barrier around the inter-ring bond always remains below 2.5 kcal/mol for MPP⁺ and M3PP⁺. In contrast, a barrier of *ca.* 9 kcal/mol exists in M2PP⁺, preventing free rotation and coplanarity favorable to conjugation between the two rings.
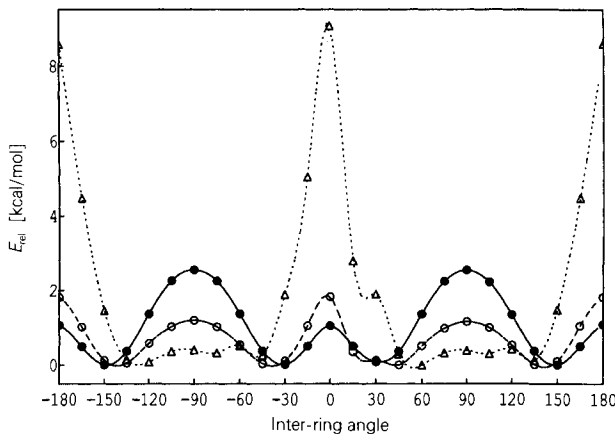
Fig. 2. *Conformational behavior of MPP⁺* (**1**, ●●), *M3PP⁺* (**2**, ○○), *and M2PP⁺* (**3**, △△)

Conformation, Rigidity, and Lipophilicity. Most likely, besides the electronic effects which can explain the difference of 0.2 log P units between MPP⁺ and M3PP⁺, other effects must be considered in explaining the much decreased lipophilicity of M2PP⁺. We believe that the limited flexibility of this isomer could indeed account for this behavior. A number of authors have already noted that rigid compounds are less lipophilic than predicted [33] [34]. It is believed that solvent molecules restrict internal rotation of flexible moieties proportionally to the energy of interaction. Interaction energy and, thus, restriction being greater in H_2O than in most organic solvents, there is much less gain in entropy when dissolving a flexible compound in H_2O than in organic solvent. In contrast, rigid compounds will not display such a decrease in H_2O solubility. In addition, rigid compounds will have less opportunity than flexible compounds for *van der Waals* interactions with octanol.

Confirmation that rigidity *per se* decreases lipophilicity is also seen with N-methyl-quinolinium and N-methylisoquinolinium (**9** and **10**, resp.), whose log P_{oct} values are very close to that of M2PP⁺. That these compounds are not outliers in *Eqn. 1* arises from the fact that delocalization and rigidity of polyaromatics are correctly parameterized in the CLOGP software, in contrast to that of M2PP⁺.

Another confirmation of the role of rigidity can be found in published log P_{oct} values of biphenyls [27], the more rigid 2-chlorobiphenyl being less lipophilic than 4-chloro-biphenyl by 0.23 units.

In Vivo *Toxicological Implications*. The overall toxic activation pathway of MPTP is a complicated and multi-parametric problem. By observing events involving only MPP$^+$, at least three steps should be considered, namely the affinity of MPP$^+$ for the high-affinity dopamine uptake system, its ability to be concentrated into mitochondria, and its inhibitory effect on *Complex I* [8]. The same physicochemical properties could affect any of these steps in different directions. However, the *in vivo* dopaminergic toxicity data can give an overall assessment and were used to rank MPP$^+$ analogs into five groups according to effects on striatal dopamine levels as determined by intracerebral microdialysis: very toxic (*I*), toxic (*II*), moderately toxic (*III*), weakly toxic (*IV*), and nontoxic (*V*) [22]. Within the limits of the small set examined in the present study, it can be shown that as a rule decreased lipophilicity of MPP$^+$ analogs corresponds to decreased neurotoxicity.

Indeed, M2PP$^+$ (group *III*) is a somewhat weaker toxin than M3PP$^+$ (group *II*), while MPP$^+$ is a very toxic compounds (group *I*). This finding parallels the observed variation in lipophilicity. *N*-Methylquinolinium (**9**) and *N*-methylisoquinolinium (**10**), whose log *P* values are close to that of M2PP$^+$, also belong to group *III*. In contrast, compounds **6** and **7**, despite their relatively high lipophilicity, were found to be less toxic than MPP$^+$, presumably because of a decreased affinity for the dopamine uptake system caused by the large *N*-alkyl substituents.

Conclusions. – Reliable lipophilicity data are necessary, if meaningful conclusions in structure-activity relationships are to be reached, and CPC is again proven to be of value in determining partition coefficients with precision and effectiveness. The abnormally low lipophilicity of M2PP$^+$ could be explained in terms of both electronic effects and conformational rigidity. The terms flexibility and rigidity are sometimes used when discussing structural factors influencing pharmacodynamic properties, and the present study once more shows that partition data can encode rigidity in a biologically relevant manner.

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