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Hydrogen-Bond Accepting Strength of Protonated Nicotine

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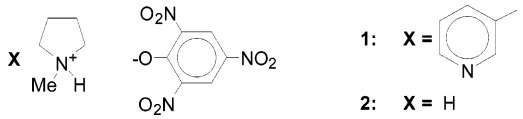
Recent crystal structures of nicotine bound to the acetylcholine binding protein (AChBP) ended a long debate confirming that the pyridine nitrogen of nicotine is indeed hydrogen-bonded to receptor residues through a bridging water molecule. Here, we describe the first direct experimental evaluation of the hydrogen-bond affinity of the nicotinium pyridine nitrogen. The equilibrium constant of its association with a phenol is 1 order of magnitude greater than the association of the acetylcholine carbonyl oxygen.

Nicotine, the main tobacco alkaloid, is a psychoactive drug presenting a wide array of biological activities, some positive, such as enhancement of cognitive performances,[1] others negative, such as addiction liability.[2] The functional importance of the central nervous system nicotinic acetylcholine receptors (nAChRs) and their implication in numerous pathologies have led to a great number of investigations aimed at their structural characterization.[3,4] Furthermore, the need to design therapeutic agents with high affinity and improved pharmacology and toxicity profiles have induced several research groups to expand significant efforts in the development of novel therapeutic agents and identification of pharmacophores.[5]

The two key features of the nAChRs pharmacophores models are a quaternized nitrogen atom and a hydrogen-bond (HB) acceptor site.[6] Recently, the resolution of the three-dimensional structure of a soluble acetylcholine binding protein (AChBP) homologous to the ligand binding domain of the nAChRs[7] and of its complexes with nicotine and carbamylcholine[8] have allowed to rationalize the interpretation of the results of more than 30 years of research on nAChRs. In these experimental structures and in the theoretical three-dimensional models elaborated for other nAChRs isotypes,[9,10] the ligand's HB abilities are recognized as a prerequisite for high affinity binding. Despite their well-established importance, the investigations devoted to nAChRs ligand HB properties are scarce. In an early study, the sp^2 pyridine nitrogen of the nicotine neutral form has been identified as the only HB acceptor site.[11] In a recent work,[12] we have found that, in solution, both nitrogens (pyridine Nsp^2 and pyrrolidine Nsp^3) of nicotine are involved in HB interactions, 90% of the hydrogen-bonded complexes being formed on the pyridine nitrogen. The bifunctional property of neutral nicotine is crucial for understanding its transport and molecular recognition in nonprotonic media. However, the charged nicotine is the form which is interacting with nAChRs, since it is predominant at the physiological pH (the pK_{a1} of nicotine is 8.05)[13] and the form which possesses the ammonium element of the pharmacophore. An experimental characterization of the pyridine nitrogen HB strength in the protonated nicotine therefore appears to be an important issue.



We present here the first experimental determination of the HB affinity of the pyridine nitrogen in the monoprotonated form **1** of nicotine. The picrate ion has been selected as the most convenient counterion since the salt, apart from making the synthesis and the purification of the crystals relatively easy, also allows the measurement of reliable equilibrium constants.

Several studies have shown that the minima of the electrostatic potential on the molecular surface, $V_{s,min}$, can be used to locate the preferred sites of HB interactions,[14] and numerous family dependent relationships between the HB-accepting strength and $V_{s,min}$ have been set up.[12,15] The electrostatic potential surface of the ion pair, represented in Figure 1, reveals that the two ions appear as potential HB-accepting sites. The respective contributions of the picrate anion nitro groups and of the nicotinium cation Nsp^2 nitrogen can be assessed through the examination of $V_{s,min}$ and equilibrium constant values of association on selected model compounds. For *N*-methylpyrrolidinium picrate **2**, where the picrate anion nitro groups are the only HB-accepting centers, these values are respectively $V_{s,min} = -161$ kJ mol^{-1} and $K = 3$ dm^3 mol^{-1} (vide infra). The corresponding values for the Nsp^2 nitrogen of pyrimidine are $V_{s,min} = -133.6$ kJ mol^{-1} and $K = 12$ dm^3 mol^{-1}.[16] These data indicate that, despite the greater absolute values of $V_{s,min}$ (−167.1 and −141.3 kJ mol^{-1}) on the picrate anion oxygen nitro groups compared to the nicotinium cation Nsp^2 nitrogen (−135.1 kJ mol^{-1}), the Nsp^2 nitrogen is predicted as the preferred HB-acceptor site of nicotinium picrate (NP).

Ionic species are generally in different states of aggregation in non-hydroxylic solvents. However, cryoscopic[17] and dielectric studies[18,19] conducted on a series of ammonium picrates have shown that, unlike other ammonium salts, picrates of tertiary amines are mostly present as ion pairs in apolar solvents such

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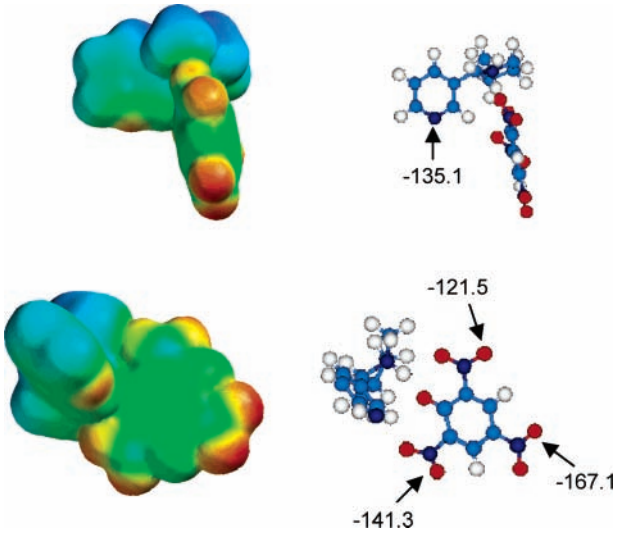

Figure 1. Different views of the electrostatic potential map at the molecular surface of nicotinium picrate (B3LYP/6-31+G**). Locations and energies ($V_{s,min}$/kJ mol^{-1}) of the most negative points are indicated by the arrows.

TABLE 1: Dielectric, Refractometric and Densitometric Data of Dichloromethane Solutions of NP

C, mmol dm^{-3}	ϵ^{20} [a]	n_d^{20} [b]	d_4^{20}
0	8.8920	1.42236	1.3244
3.31	8.9580	1.42272	1.3252
6.68	9.0041	1.42274	1.3257
16.87	9.1594	1.42356	1.3263
33.67	9.4033	1.42488	1.3274

[a] Measured at 2 MHz. [b] A correction of 5% has been made in the calculations to take into account the atomic polarization.

as benzene.[20] It was also found that these ion pairs are the dominant species in weakly polar solvents. To determine the structure of NP in the solvent selected for the equilibrium constant measurements, we have measured its dipole moment in dichloromethane. This dipole moment is calculated using Onsager's equation[21] adapted for diluted solutions in weakly polar solvents by Ménard and Chabanel.[22] From the variations of the permittivity, refractive index, and density with the concentration of diluted solutions of NP in dichloromethane (Table 1), a dipole moment of 9.65 D is found. This value, combined with the excellent linearity of the concentration dependence of the permittivity (correlation coefficient r = 0.9994), indicates that the ion pairs are the predominant species at the concentrations used for the IR determination of the HB accepting strength.

In the last two decades, we have developed[23] a free energy scale of HB affinity, noted as pK_{HB}, for hundreds of diversified molecular bases B dissolved in carbon tetrachloride using 4-fluorophenol (pFP) as the reference donor (eqs 1−3):

$$4\text{-}FC_6H_4OH + B \leftrightarrows 4\text{-}FC_6H_4OH\cdots B \quad (1)$$

$$K_c = [4\text{-}FC_6H_4OH\cdots B]/[B][4\text{-}FC_6H_4OH] \quad (2)$$

$$pK_{HB} = \log K_c \qquad \Delta G^\circ_{298} = -5.71\ pK_{HB} \quad (3)$$

In the case of the picrate salts, which are sparingly soluble in carbon tetrachloride, we have carried out our measurements in dichloromethane so that conversions of the experimental data toward the pK_{HB} scale in CCl_4 are necessary. The IR spectra of the free and bonded pFP are displayed in Figure 2A and the data corresponding to three independent measurements of the

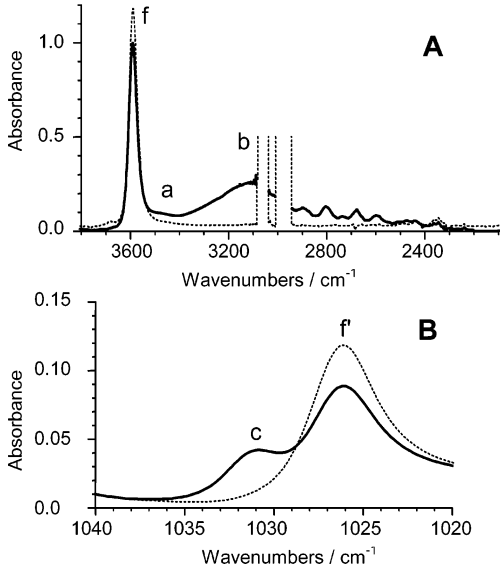

Figure 2. Association 4-fluorophenol (0.03 M)−nicotinium picrate (0.04 M) in dichloromethane. The absorptions of the free donor and acceptor are shown in dashed lines for comparison. (A) OH frequency domain of the donor; *f* is the free pFP absorption; a and b are respectively the absorption of the phenol bonded to the nitro group of the anion and to the pyridine nitrogen of the cation; (1 cm cell). (B) ν_1 frequency domain of the acceptor. The absorption (*f′*) of the acceptor is blue shifted to *c* by association with pFP (0.9 mm cell).

TABLE 2: Total (K_t) and Individual (K_{Nsp^2}) Equilibrium Constants of the 4-Fluorophenol Association on Nicotinium Picrate (Solvent CH_2Cl_2)

C_a° [a], mmol dm^{-3}	C_b° [a], mmol dm^{-3}	A (OH)[b]	K_t, dm^3 mol^{-1}	A (ν_1)[c]	K_{Nsp^2}, dm^3 mol^{-1}
33.07	40.76	0.821	10.3	0.159	9.2
40.17	38.37	0.991	12.5	0.155	9.3
37.51	47.87	0.874	12.3	0.202	10.7

[a] C_a° and C_b° are respectively the initial concentrations of 4-fluorophenol and nicotinium picrate [b] A(OH) is the equilibrium absorbance of the free OH absorption of pFP at 3585 cm^{-1} in a 2.00 mm quartz cell. The temperature is 25 ± 0.1 °C. [c] $A(\nu_1)$ is the equilibrium absorbance of the free ν_1 absorption of the nicotinium ion at 1026 cm^{-1} in a 0.937 mm fluorine cell. The temperature is 25 ± 0.3 °C.

equilibrium constant are presented in Table 2. It can be seen in Figure 2A that, besides the large structured absorption at nearly 3100 cm^{-1} characterizing the association of the phenol on the pyridine nitrogen,[16] there is a second very weak absorption at 3470 cm^{-1} attributed to the association of the pFP molecule to a nitro group.[24] As expected, the NP ion pair appears to be a bifunctional molecule with two coexisting associations corresponding to the two ions. In such a situation where the solutions are diluted, it can be demonstrated[25] that the apparent equilibrium constant calculated from the intensity decrease of the phenol absorption is the sum of the individual equilibrium constants of association. To evaluate these individual contributions, it was then necessary to conduct a second series of measurements on a characteristic absorption of the pyridine ring located at 1026 cm^{-1} (Figure 2B), the intensity of which is only sensitive to the association on the Nsp^2 nitrogen.[25] The spectra of the free (*f′*) and complexed ν_1 absorption (*c*) are presented in Figure 2B and the values of K_{Nsp^2} are given in the last column of Table 2. The strength of the association on the picrate nitro group K_{NO_2} is then evaluated as the difference between K_t and K_{Nsp^2}, amounting to about 1.9 dm^3 mol^{-1}.We have checked the validity of this value by measuring the equilibrium constant of

TABLE 3: Equilibrium Constant of the 4-Fluorophenol Association on *N*-Methylpyrrolidinium Picrate (Solvent CH_2Cl_2)

C_a° [a], mmol dm^{-3}	C_b° [a], mmol dm^{-3}	A (OH)[b]	K, dm^3 mol^{-1}
32.83	69.31	0.444	3.3
33.26	68.95	0.443	3.6

[a] C_a° and C_b° are respectively the initial concentrations of 4-fluorophenol and nicotinium picrate. [b] A(OH) is the equilibrium absorbance of the free OH absorption of the phenol at 3585 cm^{-1} for 0.937 mm fluorine cell. The temperature is 25 ± 0.1 °C.

the association of pFP on the model molecule *N*-methylpyrrolidinium picrate **2** where the only active basic center is the picrate anion. Table 3 presents the results given by the intensity decrease of the OH absorption in dichloromethane in two independent experiments. The equilibrium constants determined for the *N*-methylpyrrolidinium picrate are of the same magnitude as the individual values obtained for the nitro groups of the nicotinium picrate, thus confirming the correct separation of the total equilibrium constants in its two components: K_{Nsp^2} and K_{NO_2}. To our knowledge, the only evaluation of the HB affinity of a picrate ion has been realized by Kuntz and Taylor[26] on the phenol-tetrabutylammonium picrate couple in methylene chloride. The reported equilibrium constant, $K = 20$ dm^3 mol^{-1}, indicates a substantial decrease in the HB affinity of the nitro group on passing from the quaternary tetrabutylammonium cation ($K = 20$) to the tertiary *N*-methylpyrrolidinium ion ($K = 3.4$). This large decrease may be attributed to the polarization of the picrate ion induced by the presence of (i) the strong hydrogen bond $N^+H\cdots{}^-O$ and (ii) the smaller distance between the two ions in the latter ion pair.[27] The further decrease in basicity observed going from the *N*-methyl pyrrolidinium cation ($K = 3.4$) to the nicotinium ion ($K = 1.9$) is then the result of the increased HB strength of the donor due to the electron attracting effect of the 3-pyridyl group in alpha position.

Thermodynamic data of hydrogen-bond associations in dichloromethane are very scarce so that the present results on both sites cannot directly be evaluated in the light of similar compounds. However, it has long been established[28] that linear free-energy relationships (lfers) exist between equilibrium constants measured in different solvents, provided that the bases be separated in different families depending on the nature of their accepting group.[23,28] The two families relevant for this study are the polar oxygen bases (carbonyl, sulfonyl, nitro, etc.) on one hand and the Nsp^2 bases on the other. The corresponding lfers[29] between CCl_4 and methylene chloride are respectively eqs 4 and 5 where n is the number of bases in the family, r is the correlation coefficient, and s is the standard deviation.

polar oxygen bases

$$pK_{HB} = 1.092 \log K_{CH_2Cl_2} + 0.866 \qquad n = 15;\ r = 0.997;\ s = 0.09 \quad (4)$$

Nsp^2 bases

$$pK_{HB} = 1.094 \log K_{CH_2Cl_2} + 0.365 \qquad n = 7;\ r = 0.999;\ s = 0.02 \quad (5)$$

Using the mean values of the equilibrium constants determined in dichloromethane (Table 2) and the corresponding eqs 4 and 5, pK_{HB} values of 1.45 ($\Delta G^{\circ}_{298} = -8.3$ kJ mol^{-1}) and 1.19 ($\Delta G^{\circ}_{298} = -6.8$ kJ mol^{-1}) are obtained respectively for the pyridine nitrogen and the nitro oxygen.

In our previous work on the nicotinic pharmacophore,[12] we tentatively predicted the HB affinity of the sp^2 nitrogen from the second ionization constant of the nicotinium ion in water and proposed an estimated range of 22−30 dm^3 mol^{-1} for the equilibrium constant. The present experimental determination now yields a value of 28 ± 3 dm^3 mol^{-1} ($1.40 < pK_{HB} < 1.49$) confirming the legitimacy of the estimation. As expected, the positively charged pyrrolidinium substituent is electron-attracting and reduces the association equilibrium constant on the pyridine Nsp^2 site. However, the capital information brought by these experiments is that the HB affinity of this Nsp^2 site is really significant. With the picrate ion as counterion, the electron-attracting effect of the meta pyrrolidinium ion is of medium intensity, comparable to that of a carbomethoxy group,[16] whereas a much stronger deactivation of the nitrogen basicity was expected from a charged substituent.[30]

An independent estimation of the pyridine Nsp^2 nitrogen HB ability of nicotinium picrate can be made from the linear relationship between the experimental Gibbs energy of 4-fluorophenol complexation, ΔG°_{298}, measured in CCl_4 and the calculated density functional (B3LYP/6-31+G**) electronic energy, ΔE_{el}, of hydrogen fluoride complexation in vacuo. In a recent investigation, we have verified the validity of this approach for the association of pFP on the pyridine nitrogen of cotinine, the main metabolite of nicotine.[25] The corresponding correlation is given in eq 6.

$$\Delta G^{\circ}_{298} = -0.603\Delta E_{el} - 25.46 \qquad n = 11;\ r = 0.999;\ s = 0.15 \quad (6)$$

Using the ΔE_{el} value of −52.40 kJ/mol calculated for the association of HF on the pyridine nitrogen of nicotinium picrate, we estimate $\Delta G^{\circ}_{298} = -6.1$ kJ/mol and pK_{HB}(theo.) = 1.08. The difference observed between the theoretical and experimental values (pK_{HB}(exp.) = 1.45) may result in the inability of the lfers, built on neutral species, to predict precisely the effect of a charged one; nevertheless, it confirms the significant HB basicity of the pyridine nitrogen of the protonated active form of nicotine with the picrate counteranion.

To date, numerous quantitative lfers between different kinds of donors have been set up, and among several thousand different donor−acceptor associations, the equilibrium constant of any acceptor with water in CCl_4 can be calculated provided that its pK_{HB} value be known.[31,32] In the present analysis, an equilibrium constant of 2.2 M^{-1} is obtained for the association of water on the pyridine nitrogen of the nicotinium ion leading to a Gibbs free energy of association: $\Delta G^{\circ}_{298} = -1.9$ kJ mol^{-1}.

In this study, we have experimentally determined the HB affinity pK_{HB} value of the pyridine nitrogen of the monoprotonated nicotine with the picrate counterion, so that this basicity can be compared to the numerous entries of the pK_{HB} database. For instance, the carbonyl group of ethyl acetate ($MeCOOCH_2CH_3$; $pK_{HB} = 1.07$)[33] is already less basic than the nicotinium pyridine nitrogen; thus, it can be safely predicted that the carbonyl group of ACh ($MeCOOCH_2CH_2N^+Me_3$) is 1 order of magnitude less basic than the nicotinium nitrogen because it is deactivated by the trimethylammonium substituent. Work is in progress for the experimental determination of the carbonyl HB affinity of acetylcholine picrate. Last, the present investigation on the nicotinium ion also confirms[27,34] that the attractive cation−anion interaction energy is greater with a tertiary ammonium ion than for a quaternary cation so that the binding of nicotine to the receptor through its cationic center is also expected to be stronger than acetylcholine and needs to be further quantified.

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