J|A|C|S
ARTICLES
Published on Web 08/10/2002

Site of Protonation of Nicotine and Nornicotine in the Gas Phase: Pyridine or Pyrrolidine Nitrogen?

Jérôme Graton,[†] Michel Berthelot,[†] Jean-François Gal,[§] Sandrine Girard,[‡,#] Christian Laurence,*[,†] Jacques Lebreton,[‡] Jean-Yves Le Questel,[†] Pierre-Charles Maria,[§] and Petr Nauš[⊥]

Contribution from the Laboratoire de Spectrochimie (EA 1149), Laboratoire de Synthèse Organique (CNRS UMR 6513), and Laboratoire d'Analyse Isotopique et Electrochimique des Métabolismes (CNRS UMR 6006), Université de Nantes, 2, rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France, Chimie des Matériaux Organiques et Métalliques, Université de Nice-Sophia Antipolis, 06108 Nice Cedex 2, France,and Department of Organic Chemistry, Charles University, Hlavova 2030, 128 40 Praha 2, Czech Republic

Received December 14, 2001

Abstract: The gas-phase basicities (GBs) of nornicotine, nicotine, and model pyrrolidines have been measured by FT-ICR. These experimental GBs are compared with those calculated (for the two sites of protonation in the case of nicotine and nornicotine) at the B3LYP/6-311+G(3df,2p)//B3LYP/6-31G(d,p) level, or those estimated from substituent effects on the GBs of 2-substituted pyrrolidines, 2-substituted *N*-methylpyrrolidines, and 3-substituted pyridines. It is found that, in contrast to the Nsp^3 protonation in water, in the gas phase nornicotine is protonated on the pyridine nitrogen, because the effects of an intramolecular CH···Nsp^3 hydrogen bond and of the polarizability of the 3-(pyrrolidin-2-yl) substituent add up on the Nsp^2 basicity, while the polarizability effect of the 2-(3-pyridyl) substituent on the Nsp^3 basicity is canceled by its field/inductive electron-withdrawing effect. The same structural effects operate on the Nsp^3 and Nsp^2 basicities of nicotine, but here, the polarizability effect of the methyl group puts the pyrrolidine nitrogen basicity very close to that of pyridine. Consequently, protonated nicotine is a mixture of the Nsp^3 and Nsp^2 protonated forms.

1. Introduction

Nicotine (**1**, Figure 1) is an agonist of the important neurotransmitter acetylcholine (**2**).[1] Both interact with a wide range of biological binding sites, including those in acetylcholine esterases[2,3] and nicotinic acetylcholine receptors.[4] One essential element of the nicotinic pharmacophore[5] is a quaternized amino nitrogen atom.[6,7] Nicotine protonated at the pyrrolidine nitrogen seems, therefore, to be the active species at physiological pH.

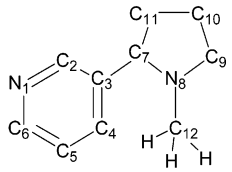


Figure 1. Numbering scheme used for nicotine and related species in this work.

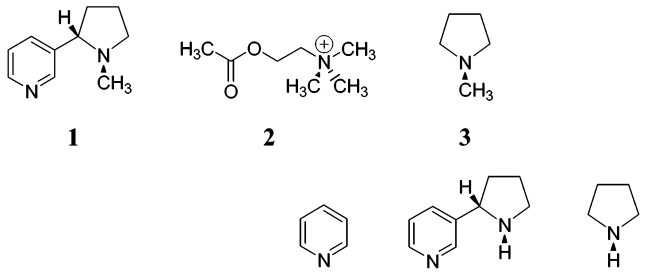
Despite its great biochemical interest, determination of the site of protonation of nicotine has received little attention. Nicotine has two acceptor sites, the N atoms of the pyridine and pyrrolidine rings, giving rise to two pK_a values, 3.10 and 8.01 at 298 K in water,[8] the attribution of which is discussed below. *N*-Methylpyrrolidine (**3**, $pK_a = 10.46$)[9] is a much stronger Brønsted base than pyridine (**4**, $pK_a = 5.20$)[10] in water. On going from *N*-methylpyrrolidine to nicotine, the basicity of the pyrrolidine nitrogen is decreased by the electron-withdrawing

* Address correspondence to this author. E-mail: laurence@chimie.univ-nantes.fr.
[†] Laboratoire de Spectrochimie (EA 1149), Université de Nantes.
[‡] Laboratoire de Synthèse Organique (CNRS UMR 6513), Université de Nantes.
[#] Laboratoire d'Analyse Isotopique et Electrochimique des Métabolismes, Université de Nantes.
[§] Université de Nice-Sophia Antipolis.
[⊥] Charles University.

(1) Beers, W. H.; Reich, E. *Nature* **1970**, *228*, 917.
(2) Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
(3) Harel, M.; Shalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Nirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9031.
(4) For recent reviews on structure−activity relationships for nAChR ligands, see: (a) Lloyd, G. K.; Williams, M. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 461. (b) Holladay, M. W.; Dart, M. J.; Lynch, J. K. *J. Med. Chem.* **1997**, *40*, 4169. (c) Latli, B.; D'Amour, K.; Casida, J. E. *J. Med. Chem.* **1999**, *42*, 2227. (d) Nielsen, S. F.; Nielsen, E. O.; Olsen, G. M.; Liljefors, T.; Peters, D. *J. Med. Chem.* **2000**, *43*, 2217. (e) Mullen, G.; Napier, J.; Balestra, M.; De Cory, T.; Hale, G.; Macor, J.; Mack, R.; Loch, J., III; Wu, E.; Kover, A.; Verhoest, P.; Sampognaro, A.; Phillips, E.; Zhu, Y.; Murray, R.; Griffith, R.; Blosser, J.; Gurley, D.; Machulskis, A.; Zongrone, J.; Rosen, A.; Gordon, J. *J. Med. Chem.* **2000**, *43*, 4045.
(5) Sheridan, R. P.; Nilakantan, R.; Scott Dixon, J.; Venkataraghavan, R. *J. Med. Chem.* **1986**, *29*, 899.
(6) Dougherty, D. A.; Stauffer, D. A. *Science* **1990**, *250*, 1558.
(7) Kearney, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 9907.
(8) Barlow, R. B.; Hamilton, J. T. *Br. J. Pharmacol. Chemother.* **1962**, *18*, 543.
(9) Jones, F. M.; Arnett, E. M. *Prog. Phys. Org. Chem.* **1974**, *11*, 263.
(10) Sawada, M.; Ichihara, M.; Yukawa, Y.; Nakachi, T.; Tsuno, Y. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2055.

10.1021/ja017770a CCC: $22.00

effect of the 2-(3-pyridyl) substituent (vide infra), while on going from pyridine to nicotine, the basicity is slightly increased by the weak electron-donating effect of the 3-(*N*-methylpyrrolidin-2-yl) substituent (vide infra). However, the pK_a sensitivity to these substituent effects[10,11] does not allow the basicity order of the Nsp^3 and Nsp^2 nitrogens to be inverted. For these reasons, it is considered that, in water, the first protonation, with pK_a = 8.01, occurs on the nitrogen atom of the *N*-methylpyrrolidine ring in a nicotine molecule. The same conclusion can be drawn for nornicotine (**5**) when their pK_a's of 3.50 and 9.12[12] are compared to the pK_a's of pyrrolidine (**6**) (11.31)[9] and pyridine. Crystal stuctures of the monoprotonated forms of nicotine as iodide[13] or salicylate[14] salts do, indeed, show that the proton is attached to the pyrrolidine ring.



1 **2** **3** **4** **5** **6**

However, the site of protonation in solution of a base bearing more than one basic center depends not only on their relative intrinsic strengths but also on the different stabilizations by the solvent of the different protonated forms. The question has been reviewed in a book,[15] and more details are given in refs 16−21. Dramatic medium effects on the site of deprotonation have also been recently described.[22] For this reason, we tried to determine, 10 years ago,[23] the intrinsic basic strengths of nicotine and nornicotine in the gas phase. By comparing their experimental gas-phase basicities (GB), measured by Fourier transform ion cyclotron resonance (FT-ICR), to those calculated by the semiempirical AM1 method or estimated empirically from substituent effects, we observed[23] that the two basic sites present in nicotine and nornicotine are much closer in strength in the gas phase than in water. Although we reserved judgment on the quantitative difference, we concluded that nornicotine and nicotine seemed to be protonated preferentially on the pyrrolidine nitrogen. In a recent theoretical study, Elmore and Dougherty[24] have investigated in the gas phase (and in aqueous solutions) the conformational preference(s) of nicotine in three protonation states: unprotonated, singly protonated on the pyrrolidine nitrogen, and doubly protonated. However, the pyridine nitrogen was not considered as a first protonation site; therefore, these data do not allow the determination of the preferred protonation site of nicotine in the gas phase.

We report here revised conclusions of our preliminary investigation. They are supported by (i) new GB measurements on nicotine **1**, nornicotine **5**, pyrrolidine **6**, *N*-methylpyrrolidine **3**, and a series of model pyrrolidines **7**−**12**, (ii) GB calculations

R = H **7** **8** **9**

R = Me **10** **11** **12**

13 **14** **15**

at the B3LYP/6-311+G(3df,2p) level on molecules **1**, **3**−**15**, and (iii) the use of Taft−Topsom methodology[25] for the interpretation and prediction of gas-phase basicities. In this method, the substituent effect on GB, δGB, is described by the linear structure−energy relationship (1) in terms of substituent

$$\delta\mathrm{GB} = \rho_F\sigma_F + \rho_R\sigma_R + \rho_\alpha\sigma_\alpha \qquad (1)$$

constants, σ, and reaction constants, ρ, corresponding to three assumed additive interaction mechanisms, called field/inductive (F), resonance (R), and polarizability (α). For example, Abboud et al. have obtained[26] eq 2, through a multiple regression analysis, for the gas-phase basicity of 3-substituted pyridines.

$$\delta\mathrm{GB} = -95.4(\pm5.0)\sigma_F - 68.2(\pm6.3)\sigma_R - 17.6(\pm5.0)\sigma_\alpha \qquad (2)$$

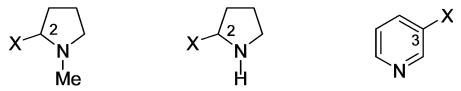
The classical method of establishing σ values was through substituent effects on experimental rate and equilibrium constants.[27] Topsom has proposed[28] quantum mechanical calculations of the three substituent constants, based on the variations, upon substitution, of electronic charges (σ_F, σ_R) or directional polarization potentials (σ_α). These calculations have been recently validated by Exner et al.[29,30] Since the substituents of this work (and future ones needed for correlations between the structure of nicotinic ligands and their activity) are not included (phenyl apart) in the Hansch et al. review,[31] we have performed ab initio calculations of their σ_F, σ_R, and σ_α constants.

The choice, and thereafter the synthesis and measurement, of model pyrrolidines **7**−**12** began with our σ calculations of

(11) Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pK$_a$ prediction for organic acids and bases*; Chapman and Hall: London, 1981.
(12) Fujita, T.; Nakajima, M.; Soeda, Y.; Yamamoto, I. *Pest. Biochem. Phys.* **1971**, *1*, 151.
(13) Barlow, R. B.; Howard, J. A. K.; Johnson, A. *Acta Crystallogr., Sect. C* **1986**, *42*, 853.
(14) Kim, H. S.; Jeffrey, G. A. *Acta Crystallogr., Sect. B* **1971**, *27*, 1123.
(15) Stewart, R. *The Proton: Applications to Organic Chemistry*; Academic Press: New York, 1985; Chapter 5.
(16) Bagno, A.; Scorrano, G. *J. Phys. Chem.* **1996**, *100*, 1536.
(17) Bagno, A.; Scorrano, G. *Acc. Chem. Res.* **2000**, *33*, 609.
(18) Bagno, A.; Terrier, F. *J. Phys. Chem. A* **2001**, *105*, 6537.
(19) Maria, P.-C.; Leito, I.; Gal, J.-F.; Exner, O.; Decouzon, M. *Bull. Soc. Chim. Fr.* **1995**, *132*, 394.
(20) Castano, O.; Notario, R.; Hori, K.; Abboud, J.-L. M. *Struct. Chem.* **1996**, *7*, 321.
(21) Lamsabhi, M.; Alcami, M.; Mó, O.; Bouab, W.; Essefar, M.; Abboud, J.-L. M.; Yanez, M. *J. Phys. Chem. A* **2000**, *104*, 5122.
(22) Notario, R.; Abboud, J.-L. M.; Cativiela, C.; Garcia, J. Y.; Herreros, M.; Homan, H.; Mayoral, J. A.; Salvatella, L. *J. Am. Chem. Soc.* **1998**, *120*, 13224.
(23) Berthelot, M.; Decouzon, M.; Gal, J.-F.; Laurence, C.; Le Questel, J.-Y.; Maria, P.-C.; Tortajada, J. *J. Org. Chem.* **1991**, *56*, 4490.
(24) Elmore, D. E.; Dougherty, D. A. *J. Org. Chem.* **2000**, *65*, 742.
(25) Taft, R. W.; Topsom, R. D. *Prog. Phys. Org. Chem.* **1987**, *16*, 1.
(26) Abboud, J.-L. M.; Catalan, J.; Elguero, J.; Taft, R. W. *J. Org. Chem.* **1988**, *53*, 1137.
(27) Hammett, L. P. *Physical Organic Chemistry*, 2nd ed.; Mc-Graw Hill: New York, 1970.
(28) Topsom, R. D. *Prog. Phys. Org. Chem.* **1987**, *16*, 125.
(29) Exner, O.; Ingr, M.; Èárský, P. *J. Mol. Struct. (THEOCHEM)* **1997**, *397*, 231.
(30) Čársky, P.; Nauš, P.; Exner, O. *J. Phys. Org. Chem.* **1998**, *11*, 485.
(31) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165.

the 3-pyridyl group which operates in nicotine **1** and nornicotine **5**. This substituent was found (vide infra) to be rather strongly electron-withdrawing by the field/inductive mechanism. We selected 3-fluorophenyl (compounds **8** and **11**) and 3-trifluoromethylphenyl (compounds **9** and **12**) in order to bracket 3-pyridyl with substituents without significant proton-acceptor sites. In addition, the phenyl substituent (compounds **7** and **10**) fills the gap, on the field/inductive scale, between 3-fluorophenyl and hydrogen, chosen as the reference substituent ($\sigma_F = 0$).

These models help to understand the GB of the pyrrolidine nitrogens of nicotine and nornicotine through the series of 2-substituted-*N*-methylpyrrolidines **a** and 2-substituted pyrrolidines **b**, respectively. The series **c** of 3-substituted pyridines,



a **b** **c**

useful for the GBs of the pyridine nitrogens of nicotine and nornicotine, has already been well studied in the literature.[26] Our work here consists of understanding the electronic effects of the 3-(pyrrolidin-2-yl) and 3-(*N*-methylpyrrolidin-2-yl) substituents.

The experimental GB measurements, the theoretical GB calculations, and the correlation analysis of substituent effects in the various series **a**−**c** consistently predict and explain that, in the gas phase, the site of protonation of nornicotine is not the pyrrolidine nitrogen but the pyridine nitrogen, and that the two nitrogens of nicotine are very close in basic strength.

2. Experimental Section

2.1. Materials. Nicotine **1**, *N*-methylpyrrolidine **3**, and pyrrolidine **6** were commercially available. They were dried over activated basic aluminum oxide and distilled. Nornicotine **5**, 2-substituted pyrrolidines **7**−**9**, and 2-substituted-*N*-methylpyrrolidines **10**−**12** were synthesized using the protocol described by Jacob.[32] The compounds were obtained as racemic mixtures, and their final purification consisted of a distillation. For compounds **1**, **3**, **5**−**12**, purity was checked by gas chromatography. The other reference bases for GB measurements were commercial compounds.

2.2. FT-ICR Measurements. Proton-transfer equilibrium measurements (eqs 3 and 4) were conducted on an electromagnet Fourier transform ion cyclotron resonance spectrometer as described previously.[23] Reviews of this technique are given in refs 33−35.

$$\mathrm{Ref} + \mathrm{BH}^+ \overset{K}{\leftrightarrow} \mathrm{RefH}^+ + \mathrm{B} \quad (3)$$

$$\Delta\mathrm{GB} = -RT \ln K \quad (4)$$

For the base B under study, of unknown GB, and for the reference compound Ref, variable pressure ratios differing by at least a factor of 3 were used. Relative (to N_2) sensitivities S_r of the Bayard Alpert gauge were estimated using the Bartmess and Georgiadis equation[36] (eq 5).

$$S_r = 0.36\,\alpha + 0.30 \quad (5)$$

The molecular polarizability α was taken as α(ahc), calculated using the atomic hybrid component (τ) approach of Miller.[37]

Equilibrium constants were obtained at an ICR cell temperature of 338 K. Literature GBs of reference compounds (GB(Ref) in Table 1) refer to the standard temperature of 298.15 K. As explained previously,[38] temperature corrections are minor as compared to other experimental uncertainties, and the absolute GB(B)s reported in Table 1 do not include such temperature corrections. These values are referenced to the most recent NIST compilation.[39]

To obtain an internally consistent set of data, all compounds were linked together by overlapping ΔGB measurements (15 intervals to be determined, 28 measurements). GB(B)s reported in Table 1 were obtained by a step-by-step procedure going from the least to the most basic compound.

The consistency of the results was checked by optimizing simultaneously all the relative basicities, by a multiple linear regression procedure, as used by Chen and co-workers,[40] following the Free and Wilson approach.[41] Each experiment (ΔGB) is described by a series of presences (1) or absences (0) of GB intervals, no constant term being included in the model. Two dubious experiments were excluded (nornicotine/pyrrolidine and 2-phenyl-*N*-methylpyrrolidine/*N*-methylpyrrolidine; see Table 1, footnote *e*). This treatment (15 optimized intervals, 26 measurements) led to a model explaining 99.5% of the variance in the experiments. The agreement with the step-by-step addition was within the experimental uncertainties.

3. Computational Methods

All ab initio and density functional theory calculations were performed using the Gaussian 94 and Gaussian 98[42] suites of programs.

3.1. Conformations. Electrostatic Potentials. The determination of theoretical GBs required a preliminary investigation of the conformation of experimentally studied compounds to find their most stable structure. Twenty-two species were considered at the HF/6-31G(d,p)//HF/6-31G(d,p) level: 10 unprotonated (**1**, **3**, **5**−**12**), 10 Nsp^3 protonated (**1**, **3**, **5**-**12**), and 2 Nsp^2 protonated (**1**, **5**).

A conformational analysis around the C3−C7 bond between the two rings has been made for nicotine and nornicotine at the HF/6-31G(d,p) level with a step of 10°. The three protonation states, unprotonated and singly protonated on the Nsp^3 and Nsp^2 atoms, were investigated. For all the compounds, frequency calculations were performed on the HF/6-31G(d,p) optimized geometries to check that the computed structures were true minima.

The electrostatic potential on the molecular surface, V_S, is calculated at the B3LYP/6-31G(d,p) level for nicotine and nornicotine in neutral and protonated states. The molecular surface was defined[43] by the 0.001 electron/bohr^3 contour of the electronic density.

3.2. GB Calculations. Starting from the optimized structures corresponding to the minima obtained at the HF/6-31G(d,p) level for compounds **1**, **3**, **5**−**12** in their various protonated states, the geometries were fully optimized at the B3LYP/6-31G(d,p) level. The series **c** of 3-substituted pyridines **4**, **13**−**15** was also investigated. We chose the

(32) Jacob, P., III. *J. Org. Chem.* **1982**, *47*, 4165.
(33) Marshall, A. G.; Hendrickson, C. L.; Jackson, G. S. *Mass Spectrom. Rev.* **1998**, *17*, 1.
(34) McMahon, T. B. *Int. J. Mass Spectrom.* **2000**, *200*, 187.
(35) Gal, J.-F.; Maria, P.-C.; Raczinska, E. D. *J. Mass Spectrom.* **2001**, *36*, 699.
(36) Bartmess, J. E.; Georgiadis, R. *Vacuum* **1983**, *33*, 149.
(37) Miller, K. J. *J. Am. Chem. Soc.* **1990**, *112*, 8533.
(38) Decouzon, M.; Gal, J.-F.; Gayraud, J.; Maria, P.-C.; Vaglio, G.-A.; Volpe, P. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 54.
(39) Hunter, E. P.; Lias, S. G. *J. Phys. Chem. Ref. Data* **1998**, *27*, 413.
(40) Chen, L.-Z.; Flammang, R.; Maquestiau, A.; Taft, R. W.; Catalan, J.; Cabildo, P.; Claramunt, E. M.; Elguero, J. *J. Org. Chem.* **1991**, *56*, 179.
(41) Free, S. M.; Wilson, J. W. *J. Med. Chem.* **1964**, *7*, 395.
(42) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Menucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.. Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A.9; Gaussian, Inc.: Pittsburgh, PA, 1998.
(43) Bader, R. F. W.; Carroll, M. T.; Cheeseman, J. R.; Chang, C. *J. Am. Chem. Soc.* **1987**, *109*, 7968.

Table 1. Relative and Absolute Gas-Phase Basicities (kJ/mol) for Compounds **1**, **5**, **7**−**12**

compd	base (B)	reference compd (Ref)	GB(Ref)[a]	ΔGB[b] (338 K)	GB(B)[c]
9	2-(3-trifluoromethylphenyl)pyrrolidine	3-methylpyridine	911.6	+1.84 ± 0.39	
		pyrrolidine	915.3	−3.26 ± 0.36	912.7 ± 1.2
8	2-(3-fluorophenyl)pyrrolidine	pyrrolidine	915.3	+1.79 ± 0.64	917.1
12	2-(3-trifluoromethylphenyl)-*N*-methylpyrrolidine	pyrrolidine	915.3	+11.21 ± 0.49	
		di-*n*-propylamine	929.3	−1.51 ± 0.19	
		N-methylpyrrolidine	934.8	−5.91 ± 0.34	927.7 ± 1.4
7	2-phenylpyrrolidine	di-*n*-propylamine	929.3	+4.24 ± 0.27	
		N-methylpyrrolidine	934.8	−1.32 ± 0.19	933.5 ± 0.1
5	nornicotine	pyrrolidine	915.3	+14.06 ± 0.33[d,e]	
		di-*n*-propylamine	929.3	+3.20 ± 0.41	
		7	933.5	−1.18 ± 0.36	
		N-methylpyrrolidine	934.8	−2.51 ± 0.04[d]	932.4 ± 0.1 (931.0)
1	nicotine	di-*n*-propylamine	929.3	+3.14 ± 0.13[d]	
				+2.51 ± 0.31	
		5	932.4	+0.17 ± 0.04	
		7	933.5	−0.86 ± 0.57	
		N-methylpyrrolidine	934.8	−1.63 ± 0.17[d]	932.6 ± 0.5 (932.6)
11	2-(3-fluorophenyl)-*N*-methylpyrrolidine	di-*n*-propylamine	929.3	+8.41 ± 0.53	
		N-methylpyrrolidine	934.8	+1.91 ± 0.09	
		diisopropylamine	938.6	−0.27 ± 0.07	937.6 ± 1.0
10	2-phenyl-*N*-methylpyrrolidine	*N*-methylpyrrolidine	934.8	> +11.6	
		diisopropylamine	938.6	+9.88 ± 0.92	
		triethylamine	951.0	−2.54 ± 0.62	
		quinuclidine	953.8[f]	−5.40 ± 0.38	948.5 ± 0.1
	quinuclidine	triethylamine	951.0	+2.79 ± 0.06	953.8 (952.5)
	piperidine	pyrrolidine	915.3	+5.44 ± 0.22	
		di-*n*-propylamine	929.3	−8.84 ± 0.31	920.6 ± 0.2 (921.0)
	N-methylpyrrolidine	di-*n*-propylamine	929.3	+5.43 ± 0.15	934.7 (934.8)

[a] Absolute gas-phase basicities (Gibbs energies at 298.15 K for the reaction $RefH^+ \rightarrow Ref + H^+$) from ref 39 unless otherwise stated. [b] Gibbs energies for the reaction $Ref + BH^+ \leftrightarrow RefH^+ + B$ determined during this work unless otherwise stated; quoted uncertainties correspond to the standard deviation for three or four measurements. [c] Absolute gas-phase basicities (Gibbs energies at 298.15 K for the reaction $BH^+ \rightarrow B + H^+$). No temperature correction applied. When indicated, quoted uncertainties (standard deviations) correspond to the overlap quality of experiments involving the different reference compounds. For the nicotine/di-*n*-propylamine couple, results of two experiments (conducted in 1991 and 2001) have been averaged. Values in parentheses are NIST values, ref 39. [d] Measured in 1991, ref 23. [e] Not taken into account for the current GB reevaluation for nornicotine. Overlap with other references is not satisfactory, the gap in basicity between nornicotine and pyrrolidine being probably too large. [f] Reevaluated GB, this work.

B3LYP density functional theory method[44] since it has been shown to yield reliable geometries and vibrational frequencies[45] and gas-phase basicities in good agreement with experimental values.[46] The harmonic vibrational frequencies were calculated on the B3LYP/6-31G(d,p) optimized geometries to yield estimates of the vibrational and thermal contributions to the enthalpy and entropy of the species at 298.15 K and 1 atm by using a scaling factor of 0.9804.[47] The final energies were obtained using a 6-311+G(3df,2p) basis set which reproduces well the basicity of bases containing first-row atoms.[46] These quantum chemistry data were converted into theoretical *absolute* gas-phase basicities, GB, using standard thermochemistry corrections. GBs corresponding to the differences in Gibbs energies between products and reactant at 298 K, reaction 6, were evaluated from electronic

$$BH^+ \rightarrow B + H^+ \qquad (6)$$

energies (E_{elec}), zero-point energies (ZPE), vibrational energies [E_{vib}(298 K)], translational−rotational energies (*RT*/2 per degree of freedom), the *PV* work term, and the entropy contribution to ΔG by using eqs 7−10:

$$\Delta H(298\,K) = E_{elec}(B) - E_{elec}(BH^+) + ZPE(B) - ZPE(BH^+) + E_{vib}(B, 298\,K) - E_{vib}(BH^+, 298\,K) + {}^3/_2(RT) + 3RT - 3RT + \Delta nRT \qquad (7)$$

$$\Delta H(298\,K) = \Delta E_{elec} + \Delta ZPE + \Delta E_{vib}(298\,K) + {}^5/_2(RT) \qquad (8)$$

$$\Delta S(298\,K) = S(H^+) + S(B) - S(BH^+) \qquad (9)$$

$$GB = \Delta G(298\,K) = \Delta H(298\,K) - T\Delta S(298\,K) \qquad (10)$$

At 298.15 K, $S(H^+) = 108.9\ J\ K^{-1}\ mol^{-1}$.[48]

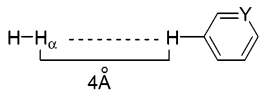
The theoretical *relative* gas-phase basicities, ΔGB, have been computed from the isodesmic equilibrium of proton exchange (eq 3) between the various monoprotonated forms of bases B **1**, **3**−**15** and a reference compound.

3.3. Ab Initio Calculations of Substituent Constants. The σ substituent constants relevant to this study are unknown (except for the phenyl substituent). We therefore calculated them and recalculated the phenyl one for consistency. We closely followed the original methods developed by Topsom et al.[28,49−51] and Hehre et al.[52] in the

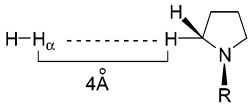
(44) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648. (b) Becke, A. D. *J. Chem. Phys.* **1992**, *96*, 2155.
(45) Llamas-Saiz, A. L.; Foces-Foces, C.; Mó, O.; Yáñez, M.; Elguero, J. *J. Comput. Chem.* **1995**, *16*, 263. Florián, J.; Johnson, B. G. *J. Phys. Chem.* **1994**, *98*, 3681. Bauschlicher, C. W. *Chem. Phys. Lett.* **1995**, *246*, 10. Martell, J. M.; Goddard, J. D.; Eriksson, L. A. *J. Phys. Chem. A* **1997**, *101*, 1927. Cui, Q.; Musaev, D.; Svensson, M.; Seiber, S.; Morokuma, K. *J. Am. Chem. Soc.* **1995**, *117*, 12366. Ricca, A.; Bauschlicher, C. W. *J. Phys. Chem.* **1995**, *99*, 5922. Ziegler, T. *Chem. Rev.* **1991**, *91*, 651.
(46) Smith, B. J.; Radom, L. *J. Am. Chem. Soc.* **1993**, *115*, 4885. Smith, B. J.; Radom, L. *Chem. Phys. Lett.* **1994**, *231*, 345. González, A. I.; Mó, O.; Yáñez, M.; León, E.; Tortajada, J.; Morizur, J.-P.; Leito, I.; Maria, P.-C.; Gal, J.-F. *J. Phys. Chem.* **1996**, *100*, 10490. Amekraz, B.; Tortajada, J.; Morizur, J.-P.; González, A. I.; Mó, O.; Yáñez, M.; Leito, I.; Maria, P.-C.; Gal, J.-F. *New J. Chem.* **1996**, *20*, 1011.
(47) Foresman, J. B.; Frisch, A. *Exploring Chemistry with Electronic Structure Methods*, 2nd ed.; Gaussian, Inc.: Pittsburgh, PA, 1995.
(48) Levine, I. N. *Physical Chemistry*, 3rd ed.; McGraw-Hill: New York, 1988; Chapter 22.
(49) Topsom, R. D. *Acc. Chem. Res.* **1983**, *16*, 292.
(50) Marriott, S.; Topsom, R. D. *J. Am. Chem. Soc.* **1984**, *106*, 7.
(51) Marriott, S.; Topsom, R. D. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1045.

years 1983−1987. We have used their basis sets, which may appear of "low level" now, because (i) they give a rather good agreement between theory and experiment,[28−30,49−52] (ii) higher basis sets would need a recalibration of the substituent scales, e.g., the coefficients of eqs 11 and 12 (vide infra), and (iii) higher basis sets do not change significantly the relative values on the scales for most substituents with first-period atoms.[29,30]

The determination of the substituent field effect, σ_F,[28,49,50] uses the polarization of the hydrogen molecule by an isolated H−X substituted molecule at a predetermined position (4 Å; 1 Å = 0.1 nm), as shown by models **d** and **e**. The Mulliken charges of the hydrogen atom, $Q_{H\alpha}$,



d (Y = aza, CH, CF, CCF_3)

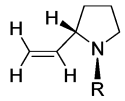


e (R = H, Me)

calculated at the HF/6-31G*//HF/6-31G* level, establish σ_F values according[50] to eq 11.

$$\sigma_F = -35.5[Q_{H\alpha}(H_2\cdots HX) - Q_{H\alpha}(H_2\cdots H_2)] \quad (11)$$

The resonance effect of substituents, σ_R,[28,49,51] is calculated (HF/4-31G//HF/4-31G) as the difference between the total π-electron Mulliken population ($\sum\Delta Q_\pi$) on the two carbon atoms in the substituted ethylene CH_2=CHX and the parent ethylene (system **f**, eq 12[29]).



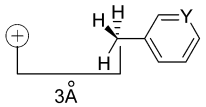
f (R = H, Me)

$$\sigma_R = 4.167\sum\Delta Q_\pi - 0.06083 \quad (12)$$

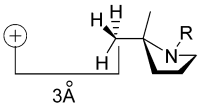
The substituent polarizability scale, σ_α, is defined from the directional electrostatic polarization potential[53] (PP in kcal mol^{-1}, eq 13), calculated

$$PP = 2\sum_i\sum_j \frac{\langle\varphi_i|H'|\varphi_j\rangle\langle\varphi_j|H'|\varphi_i\rangle}{\epsilon_i - \epsilon_j} \quad (13)$$

at the HF/3-21G//HF/3-21G level for molecules CH_3X and CH_4. The polarization is caused by the generation of a positive charge at a predetermined position (3 Å), as shown by models **g** and **h**. In eq 13,



g (Y = aza, CH, CF, CCF_3)



h (R = H, Me)

φ is a Hartree−Fock molecular orbital and ϵ the orbital energy at the optimum geometry. The pair of indices *i* and *j* represent a single electron excitation $i \rightarrow j$, and the H' matrix elements are evaluated for structures **g** and **h**. Values of σ_α are given by eq 14. Two conformations of close

$$\sigma_\alpha = PP(CH_3X) - PP(CH_3H) \quad (14)$$

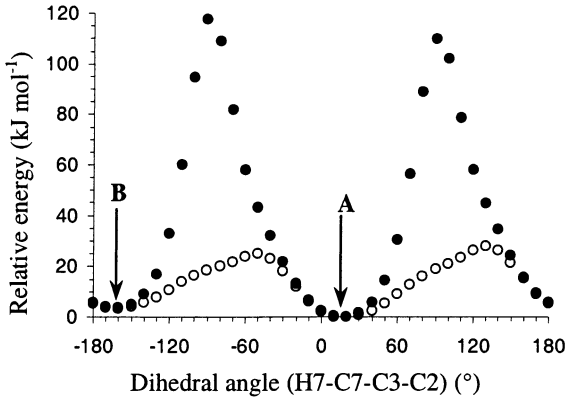


Figure 2. Rotational profiles around the C3−C7 bond of unprotonated nicotine (●) and nornicotine (○) calculated at the HF/6-31G(d,p) level. Conformation **A** is the most stable in the two cases.

energy are found for *N*-methyl-2-methylpyrrolidine and 2-methylpyrrolidine (model **h**). However, the effect on σ_α is small, so we have retained the average of the two conformations.

4. Results

4.1. Conformational Analysis and in Vacuo Structures. Several experimental[54−56] and ab initio computational[24] studies of nicotine conformation have been made. The nicotine conformation is characterized by (i) the relative positions of the methyl and pyridyl substituents on the pyrrolidine ring—cis or trans (in this study, we have only considered the trans stereoisomer since it is found as the most stable structure),[24,54] (ii) the pyrrolidine ring conformation, and (iii) the relative position of the two rings. We are not aware of any conformational studies on nornicotine and on the model pyrrolidines **7**−**12**. The geometries of *N*-methylpyrrolidine **3** and pyrrolidine **6** are known from microwave and electron diffraction experiments.[57,58]

(a) Unprotonated Structures. For all the unprotonated structures **1**, **3**, **5**−**12**, the pyrrolidine ring is always found in the envelope conformation with the nitrogen atom out of the plane and the amino hydrogen or *N*-methyl group in the equatorial position. Previous studies[24,54−56] have shown the presence of two conformers for nicotine, in which the pyridine and pyrrolidine rings are roughly perpendicular to one another. In this work, we confirm the presence of these rotamers from calculations made at the HF/6-31G(d,p) level for nicotine and find that nornicotine behaves similarly. The HF/6-31G(d,p) rotational profiles around the C3−C7 bond of nicotine and nornicotine (Figure 2) show the location of two minima, **A** and **B** (Figure 3). These results confirm the AM1 and MMFF94 rotational profiles calculated recently by Elmore and Dougherty.[24] The higher electronic rotational barrier observed for nicotine in Figure 2 (ΔE = 110 kJ mol^{-1}) by comparison with that for nornicotine (ΔE = 25 kJ mol^{-1}) is explained by the presence of the methyl group, which hinders ring rotation. For the model pyrrolidines **7**−**12**, two minima, **A** and **B**, have also been consistently found, **A** being the most stable one, as observed for nicotine and nornicotine.

(52) Hehre, W. J.; Pau, C. F.; Headley, A. D.; Taft, R. W. *J. Am. Chem. Soc.* **1986**, *108*, 1711.
(53) Kahn, S. D.; Pau, C.; Overman, L. E.; Hehre, W. J. *J. Am. Chem. Soc.* **1986**, *108*, 7381.
(54) Whidby, J. F.; Seeman, J. I. *J. Org. Chem.* **1976**, *41*, 1585.
(55) Pitner, T. P.; Edwards, W. B.; Bassfield, R. L.; Whidby, J. F. *J. Am. Chem. Soc.* **1978**, *100*, 246.
(56) Seeman, J. I. *Heterocycles* **1984**, *22*, 165.
(57) (a) Caminati, W.; Scappini, F.; *J. Mol. Spectrosc.* **1986**, *117*, 184. (b) Pfafferott, G.; Oberhammer, H.; Boggs, J. E. *J. Am. Chem. Soc.* **1985**, *107*, 2309.
(58) Caminati, W.; Dell' Erba, A.; Maccaferri, G.; Favero, P. G. *J. Mol. Spectrosc.* **1998**, *191*, 45.

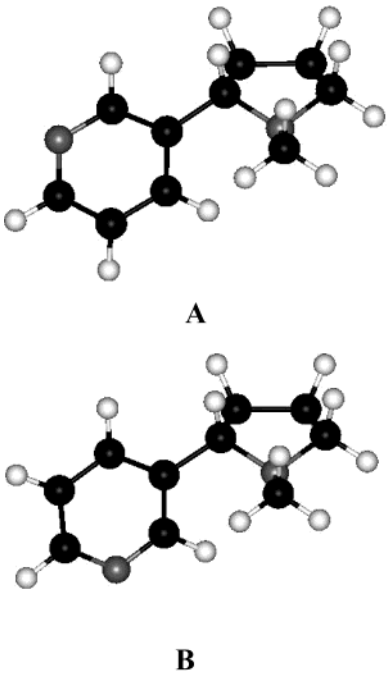


Figure 3. Three-dimensional structures of the two minima **A** and **B** obtained for nicotine at the HF/6-31G(d,p) level. C atoms are shown in black, N atoms in gray, and H atoms in white.

(b) Nsp^3 Protonated Structures. At the HF/6-31G(d,p) level, we observe a difference of the pyrrolidine ring conformation between the 2-substituted pyrrolidines and the 2-substituted *N*-methylpyrrolidines. In the former, the C11 atom adopts an out-of-plane position in the envelope conformation, whereas the C7 atom is out of the plane in the *N*-methylpyrrolidines, as observed by Elmore and Dougherty for nicotine.[24] In contrast, at the B3LYP/6-31G(d,p) level, the C11 atom is in the out-of-plane position in all cases. The rotational profiles around the C3−C7 bond obtained in this protonation state for nicotine and nornicotine are similar to those obtained in their unprotonated form. For all the compounds **1**, **5**, **7**−**12**, the two rings are approximately perpendicular to one another, and the **A** conformation is always the most stable.

(c) Nsp^2 Protonated Structures. Nicotine and nornicotine are the only compounds concerned by this protonated form. The pyrrolidine ring adopts a third kind of envelope conformation in which the C9 atom is out of the plane. The rotational profiles are very close to the ones calculated for the other protonation states: two minima, **A** and **B**, are predicted, but, conversely to the unprotonated and Nsp^3 protonated structures, **B** is the most stable, with $\Delta E = 3.5$ kJ mol^{-1} for nicotine and for nornicotine. We explain this conformational change on Nsp^2 protonation by the formation of a C−H···Nsp^3 intramolecular hydrogen bond in the Nsp^2 protonated form, which is stronger in rotamer **B** than in rotamer **A**: the C2H···Nsp^3 hydrogen bond length (2.41 Å in nicotine, 2.36 Å in nornicotine) in **B** is significantly shorter than the C4H···Nsp^3 one (2.49 Å in nicotine, 2.39 Å in nornicotine) in **A**.

Figure 4 illustrates the three-dimensional structures of nornicotine in its different protonated states computationally optimized at the B3LYP/6-31G(d,p) level.

4.2. Experimental and Theoretical Gas-Phase Basicities. Table 1 presents the experimental relative gas-phase basicities, ΔGB, for compounds **1**, **5**, and **7**−**12**. From the absolute gas-phase basicities of the reference bases,[39] the absolute GB values of nicotine, nornicotine, and their model pyrrolidines **7**−**12** are given in the last column.

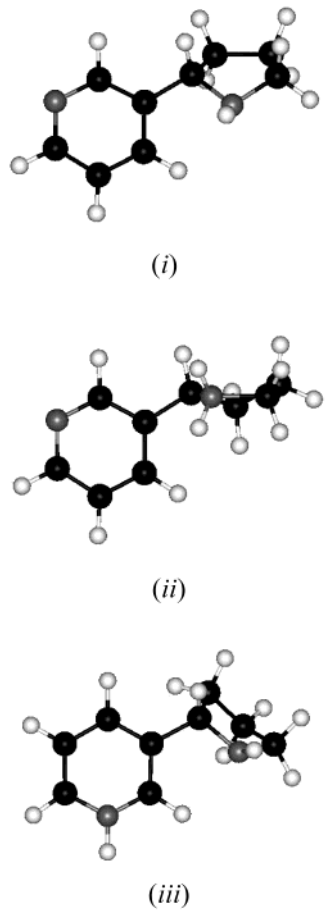


Figure 4. Optimized geometries of nornicotine at the B3LYP/6-31G(d,p) level in (i) unprotonated, (ii) Nsp^3 protonated, and (iii) Nsp^2 protonated states. Conformation **A** is the most stable in the two first cases, whereas conformation **B** is the most stable when the pyridine nitrogen is protonated.

Table 2 compares these experimental GBs, and those from the NIST compilation[39] for 3-substituted pyridines, to the theoretical GBs calculated at the B3LYP/6-311+G(3df,2p)//B3LYP/6-31G(d,p) level. In the series **a** of 2-substituted *N*-methylpyrrolidines, the calculated GBs are smaller than the experimental ones by 1.2−5.2 kJ mol^{-1} (mean relative differences: −0.3%). In contrast, in the series **b** and **c** of 2-substituted pyrrolidines and 3-substituted pyridines, the calculated GBs are larger by 2.5−6.5 kJ mol^{-1} in the series **b** and by 7.1−9.9 kJ mol^{-1} in the pyridine series. Moreover, the mean relative differences are larger than those in the previous series (+0.5% and +0.9%, respectively). For nicotine and nornicotine, two theoretical values are calculated for each protonation site, Nsp^2 and Nsp^3.

4.3. Values of the σ Substituent Constants. Table 3 gives values of σ_F, σ_R, and σ_α parameters, calculated by ab initio methods, for substituents relevant to the series of substituted pyrrolidines **a** and **b** and of substituted pyridines **c**. In nicotine and nornicotine, the 3-(*N*-methylpyrrolidin-2-yl) and the 3-(pyrrolidin-2-yl) substituents alter the pyridine nitrogen gas-phase basicity by the three kinds of electronic effects: field/inductive, resonance, and polarizability (see eq 2).[26] However, in the saturated cyclic amines **a** and **b**, the 2-substituents alter the

Table 2. Experimental and Theoretical (B3LYP/6-311+G(3df,2p)) Absolute Gas-Phase Basicities (kJ mol^{-1}) of 2-Substituted *N*-Methylpyrrolidines, 2-Substituted Pyrrolidines, 3-Substituted Pyridines, Nicotine, and Nornicotine

series	compd	base (B)	GB_{exp}	GB_{theor}	D^a
a	**12**	*N*-methyl-2-(3-trifluoromethylphenyl)pyrrolidine	927.7	922.5	−5.2
	3	*N*-methylpyrrolidine	934.7	933.5	−1.2
	11	*N*-methyl-2-(3-fluorophenyl)pyrrolidine	937.6	932.5	−5.1
	10	*N*-methyl-2-phenylpyrrolidine	948.5	946.2	−2.3
	1	nicotine	932.6	936.6 [b]	+4.0
				926.9[c]	−5.7
b	**9**	2-(3-trifluoromethylphenyl)pyrrolidine	912.7	916.1	+3.4
	6	pyrrolidine	915.3[d]	920.9	+5.6
	8	2-(3-fluorophenyl)pyrrolidine	917.1	923.6	+6.5
	7	2-phenylpyrrolidine	933.5	936.0	+2.5
	5	nornicotine	932.4	939.7[b]	+7.3
				916.1[c]	−16.3
c	**4**	pyridine	898.1[d]	905.2	+7.1
	13	3-methylpyridine	911.6[d]	919.2	+7.6
	14	3-ethylpyridine	915.5[d]	923.6	+8.1
	15	3,5-dimethylpyridine	923.5[d]	933.4	+9.9

[a] $D = GB_{theor} - GB_{exp}$. [b] Pyridine nitrogen protonated form. [c] Pyrrolidine nitrogen protonated form. [d] NIST values, ref 39.

Table 3. Values of Field/Inductive (σ_F), Resonance (σ_R), and Polarizability (σ_α) Substituent Constants Calculated by ab Initio Methods

substituent	σ_F	σ_R	σ_α
H	0	0	0
phenyl	+0.061[a]	*b*	−0.91[c]
3-fluorophenyl	+0.126	*b*	−0.90
3-pyridyl	+0.143	*b*	−0.88
3-trifluoromethylphenyl	+0.166	*b*	−0.92
pyrrolidin-2-yl	−0.034	−0.061[d]	−0.68[e]
N-methylpyrrolidin-2-yl	−0.015	−0.037[f]	−0.82[e]

[a] In agreement with the value 0.06 calculated in ref 50. [b] Not relevant to this study. [c] A value of −0.81 is given in ref 52. [d] $|\sigma_R| = 0.088$ by an IR method (Graton, J., Ph. D. Thesis, Nantes, 2001). [e] Mean value of two conformations of close energy (−0.69 and −0.67; −0.80 and −0.83). [f] The same IR method gives $|\sigma_R| = 0.068$.

pyrrolidine nitrogen GBs only by the field/inductive and polarizability effects.

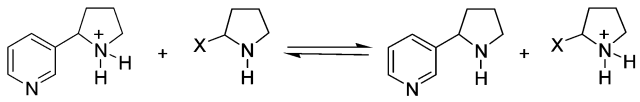
5. Discussion

5.1. Nicotine and Nornicotine Cannot Both Be Protonated on the Pyrrolidine Nitrogen. The most striking feature in the experimental GBs (Table 1) is that the GB of nicotine (932.6 ± 0.5 kJ mol^{-1}) does not differ significantly from the GB of nornicotine (932.4 ± 0.1 kJ mol^{-1}). In contrast, a methyl substitution on the nitrogen of pyrrolidines **b** (**6**−**9**) increases significantly the GBs by 19.4, 15, 20.5, and 15 kJ mol^{-1} on going from the pyrrolidines **b** (**6**−**9**) to the *N*-methylpyrrolidines **a** (**3**, **10**−**12**), as illustrated in Figure 5. Brauman and Blair[59] have already demonstrated that alkyl substituents on sp^3 nitrogen increase consistently the gas-phase basicities of amines. This increased basicity was interpreted[59] as being the result of induced dipole stabilization by the alkyl group of the ion formed on protonation, the so-called polarizability effect. Since nicotine and nornicotine do not show this significant basicity variation (Figure 5) which characterizes a Nsp^3 protonation, we may state that *nicotine and nornicotine are not both protonated on their pyrrolidine nitrogen.* So, two possibilities remain: (i) nicotine and nornicotine are both protonated on their pyridine nitrogens, or (ii) one molecule is protonated on the pyridine nitrogen and the other one on the pyrrolidine nitrogen.

(59) Brauman, J.; Blair, L. K. *J. Am. Chem. Soc.* **1968**, *90*, 5636.

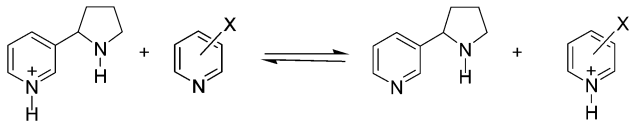
5.2. Nornicotine Is Protonated on the Pyridine Nitrogen. The comparison of theoretical *absolute* GBs (939.7 and 916.1 kJ mol^{-1} for Nsp^2 and Nsp^3 protonation, respectively) shows that the Nsp^2 protonation is favored; their difference of 23.6 kJ mol^{-1} is outside the sum (16.4 kJ mol^{-1}) of the maximum errors of calculation found (Table 2) for the pyrrolidines' protonations (6.5 kJ mol^{-1}) and the pyridines' protonations (9.9 kJ mol^{-1}). Moreover, even after these calculation errors are taken into account, the theoretical GB value for Nsp^2 protonation agrees better with the experimental GB than the theoretical GB value for Nsp^3 protonation.

Because of an expected cancellation of calculation errors, it seems safer to compare *relative* calculated and experimental GBs, ΔGBs, than absolute values. Therefore, we have studied the proton exchange reactions 15−18 between nornicotine and the 2-substituted pyrrolidines, and the proton exchange reactions 19−22 between nornicotine and the 3-substituted pyridines. Table 4 assembles the experimental ΔGBs (from Tables 1 and



X = H (15) ; Ph (16) ; 3-FC_6H_4 (17) ; 3-$CF_3C_6H_4$ (18)

(15)-(18)



X = H (19) ; 3-Me (20) ; 3-Et (21) ; 3,5-Me_2 (22)

(19)-(22)

2), the calculated ΔGBs (from Table 2), and the differences, $\delta\Delta GB$, between experimental and calculated ΔGBs. These results show that, in all cases, the ΔGBs computed for a protonation on the pyridine nitrogen, $\Delta GB(Nsp^2)$, are in close agreement with the experimental values, ΔGB_{exp}, the mean absolute error being only 1 kJ mol^{-1}. In contrast, the ΔGBs calculated under the hypothesis of a protonation on the pyrrolidine nitrogen, $\Delta GB(Nsp^3)$, are far from the experimental

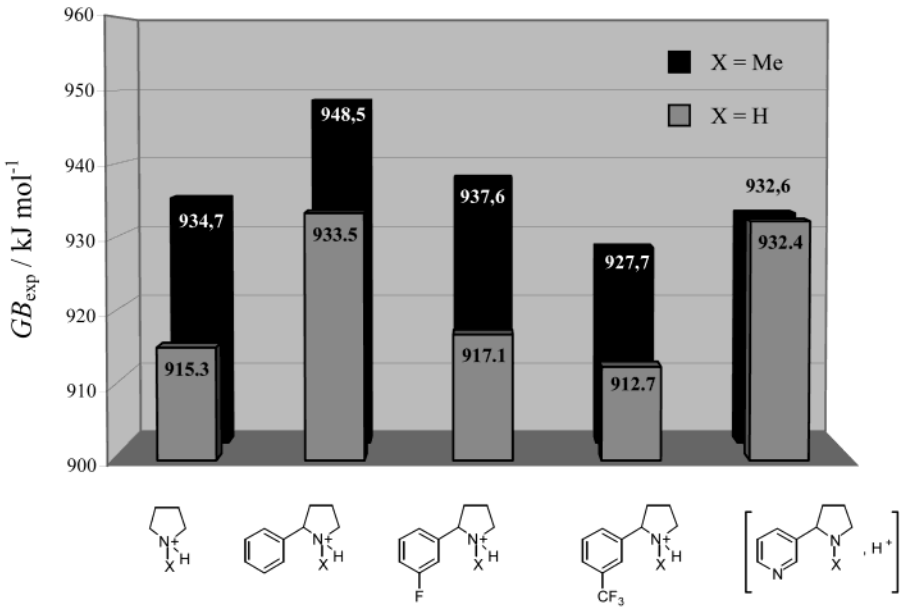


Figure 5. Influence of the methyl substitution of the pyrrolidine nitrogen on the experimental GBs, on going from pyrrolidines (X = H) to *N*-methylpyrrolidines (X = Me), and from nornicotine (X = H) to nicotine (X = Me).

Table 4. Comparison of Experimental, ΔGB_{Exp}, and Theoretical, $\Delta GB(Nsp^3)$ and $\Delta GB(Nsp^2)$, Gibbs Energies (kJ mol^{-1}) for the Proton Exchange Reactions between Nornicotine and 2-Substituted Pyrrolidines (Equilibria 15−18), and Nornicotine and 3-Substituted Pyridines (Equilibria 19−22)

equilibrium	substituted pyrrolidines **b**	ΔGB_{exp}[a]	$\Delta GB(Nsp^3)$[b]	$\delta\Delta GB(Nsp^3)$[c]
15	H	17.1	−4.8	+21.9
16	2-phenyl	−1.1	−19.9	+18.8
17	2-(3-fluorophenyl)	15.3	−7.5	+22.8
18	2-(3-trifluoromethylphenyl)	19.7	0.0	+19.7
equilibrium	**substituted pyridines c**	ΔGB_{exp}[d]	$\Delta GB(Nsp^2)$[e]	$\delta\Delta GB(Nsp^2)$[c]
19	H	34.3	34.5	−0.2
20	3-methyl	20.8	20.5	+0.3
21	3-ethyl	16.9	16.1	+0.8
22	3,5-dimethyl	8.9	6.3	+2.6

[a] $\Delta GB_{exp} = GB_{exp}$(nornicotine) − GB_{exp}(2-substituted pyrrolidine). [b] Theoretical ΔGB in the hypothesis of a nornicotine protonation on the pyrrolidine nitrogen. [c] Difference between experimental and theoretical ΔGB. [d] $\Delta GB_{exp} = GB_{exp}$(nornicotine) − GB_{exp}(3-substituted pyridine). [e] Theoretical ΔGB in the hypothesis of a nornicotine protonation on the pyridine nitrogen.

values (mean absolute error: 21 kJ mol^{-1}). These results definitively support a protonation mechanism on the pyridine sp^2 nitrogen of nornicotine.

How can we explain that nornicotine protonates on the pyridine nitrogen although pyrrolidine is more basic by 17.1 kJ mol^{-1} than pyridine (Tables 1 and 2)? There are several reasons: (i) the field/inductive, resonance, and (mainly) polarizability effects of the 3-(pyrrolidin-2-yl) substituent add up to increase the Nsp^2 basicity from pyridine to nornicotine; (ii) in contrast, the polarizability effect of the 2-(3-pyridyl) substituent is canceled by the field/inductive effect, and the Nsp^3 basicities of pyrrolidine and nornicotine remain close; and (iii) an intramolecular CH···Nsp^3 hydrogen bond stabilizes the Nsp^2 protonated nornicotine.

The substituent effect of the 2-(3-pyridyl) substituent can be estimated by applying the $\rho\sigma$ equation (1) to the GBs of 2-substituted pyrrolidines. In this series, the GB variations result only from the field/inductive and polarizability effects. The least-squares calculation of regression coefficients ρ_F and ρ_α by means of known GB (Table 1) and σ_F, σ_α values (Table 3) gives eq 23.

$$\text{GB(2-substituted pyrrolidines)} = 915(\pm 2) - 206(\pm 32)\sigma_F - 33(\pm 5)\sigma_\alpha \quad (23)$$

$n = 4$ (**6**−**9**), r (correlation coefficient) = 0.989, s (standard deviation) = 2.4 kJ mol^{-1}

This equation must be used with great caution since it is based on a restricted class of substituents. However, it must be valid inside its definition domain, i.e., for six-membered aromatic substituents (an important class of substituents in the structure−activity relationships of nicotinic ligands).[60−62] The 3-pyridyl substituent pertains to this class; moreover, it is closely bracketed by two substituents obeying eq 23, the 3-FC_6H_4 and 3-$CF_3C_6H_4$ ones. Under these conditions, we may apply eq 23 to nornicotine. By means of the σ_F and σ_α values of the 3-pyridyl

(60) Elliott, R. L.; Ryther, K. B.; Anderson, D. J.; Raszkiewicz, J. L.; Campbell, J. E.; Sullivan, J. P.; Gawey, D. S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 991.
(61) Dukat, M.; Dowd, M.; Damaj, M. I.; Martin, B.; El-Zahabi, M. A.; Glennon, R. A. *Eur. J. Med. Chem.* **1999**, *34*, 31.
(62) Cosford, N. D. P.; Bleicher, L. Herbaut, A.; McCallum, J. S.; Vernier, J. M.; Dawson, H.; Whitten, J. P.; Adams, P.; Chavez-Noriega, L.; Correa, L. D.; Crona, J. H.; Lorrence, S. M.; Menzaghi, F.; Rao, T. S.; Reid, R.; Sacaan, A. I.; Santori, E.; Stauderman, K. E.; Whelan, K.; Lloyd, G. K.; McDonald, I. A. *J. Med. Chem.* **1996**, *39*, 3235.

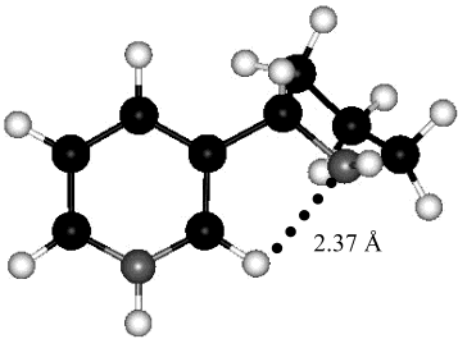


Figure 6. In nornicotine protonated on the pyridine nitrogen, the positive hydrogen of the C2−H bond points to the lone pair of the pyrrolidine nitrogen and establishes a C2−H···Nsp^3 intramolecular hydrogen bond and a five-membered ring.

substituent (Table 3), we find that the field/inductive and polarizability effects of this group cancel each other out almost perfectly, amounting to −29.5 and +29.0 kJ mol^{-1}, respectively. So, the Nsp^3 basicity of nornicotine remains unchanged compared to that of pyrrolidine.

Conversely, the basicity of pyridine (898.1 kJ mol^{-1}) increases by the addition of three kinds of effects of the 3-(pyrrolidin-2-yl) substituent. These field/inductive, resonance, and polarizability effects can be estimated by the $\rho\sigma$ equation (2) for the GBs of 3-substituted pyridines, from the σ values (Table 3) of the 3-(pyrrolidin-2-yl) substituent. They amount to +3.2, +4.2, and +12 kJ mol^{-1}, respectively. They increases GB(Nsp^2) to only 917.5 kJ mol^{-1}. There is still a significant difference of 14.9 kJ mol^{-1} from the experimental value. We have to search for a complementary mechanism enhancing the pyridine nitrogen basicity in nornicotine, not taken into account by the $\rho\sigma$ equation. An examination of the B3LYP/6-31G(d,p) optimized structure of nornicotine protonated on the pyridine nitrogen (Figure 6) reveals the existence of an intramolecular C2−H···Nsp^3 hydrogen bond. This interaction is characterized by a distance d(Nsp^3···H) of 2.37 Å, significantly shorter than the sum (2.65 Å) of the van der Waals radii[63] of nitrogen and hydrogen, and the formation of a five-membered ring. It is made possible by the strong electron-withdrawing effect of the protonated aza group[64] on the ortho C−H bonds. The C2−H hydrogen ortho to the NH^+ group gains a very positive electrostatic potential (V_S = +393 kJ mol^{-1} at the B3LYP/6-31G(d,p) level) compared to the neutral form, where V_S is only +40 kJ mol^{-1}. Since it has been shown by Murray et al.[65−67] and by Platts[68] that the electrostatic potential and the hydrogen bond acidity are well correlated, this CH group obviously becomes a good hydrogen bond donor on Nsp^2 protonation. It seems, therefore, reasonable to attribute the 14.9 kJ mol^{-1} difference to this intramolecular hydrogen bond. This value compares well to a recently calculated[69] energy of 12.6 kJ mol^{-1} for the C−H···Nsp^3 hydrogen bond in the complex of CH_2F_2 with CH_3NH_2.

(63) Rowland, R. S.; Taylor, R. *J. Phys. Chem.* **1996**, *100*, 7384.
(64) Johnson, C. D. *The Hammett Equation*; Cambridge University Press: Cambridge, UK, 1973; p 101.
(65) Murray, J.; Politzer, P. *J. Chem. Res. S* **1992**, 110.
(66) Murray, J. S.; Politzer, P. *J. Org. Chem.* **1991**, *56*, 6715.
(67) Hagelin, H.; Murray, J. S.; Brinck, T.; Berthelot, M.; Politzer, P. *Can. J. Chem.* **1995**, *73*, 483.
(68) Platts, J. A. *Phys. Chem. Chem. Phys.* **2000**, *2*, 973.
(69) Gu, Y.; Kar, T.; Scheiner, S. *J. Mol. Struct.* **2000**, *552*, 17.

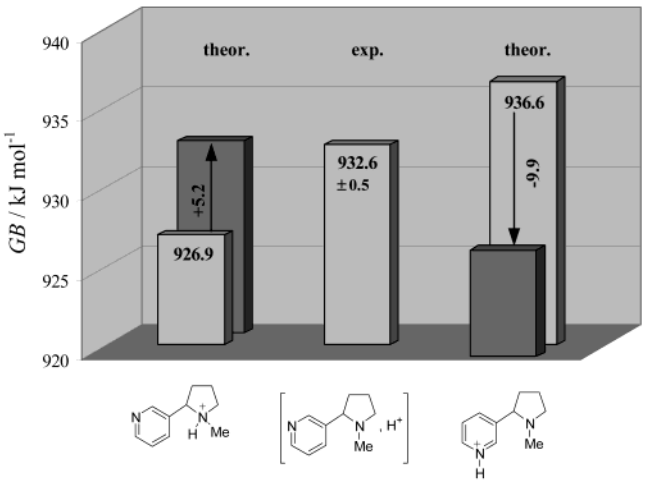
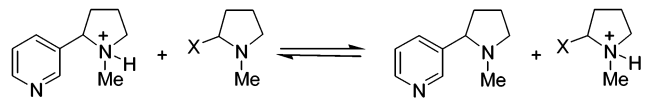


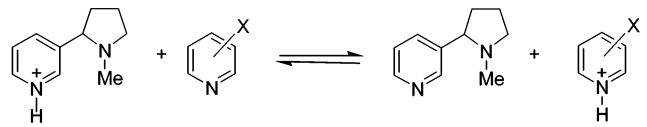
Figure 7. Comparison for nicotine of (i) experimental GB, (ii) theoretical GB for Nsp^2 protonation, with its maximum error of 9.9 kJ mol^{-1}, and (iii) theoretical GB for Nsp^3 protonation, with its maximum error of 5.2 kJ mol^{-1}.

5.3. The Pyridine and Pyrrolidine Nitrogens of Nicotine Are Very Close in Basicity. Figure 7 illustrates how the errors of calculation prevent determination of which of the GBs calculated for the Nsp^2 and Nsp^3 protonations is the more positive, and which agrees better with the experimental GB of nicotine. So, we have again considered the proton exchange reactions 24−27 between nicotine and 2-substituted *N*-methylpyrrolidines, and the reactions 28−31 between nicotine and 3-substituted pyridines. Table 5 compares the relative experi-



X = H (24) ; Ph (25) ; 3-FC_6H_4 (26) ; 3-$CF_3C_6H_4$ (27)

(24)-(27)



X = H (28) ; 3-Me (29) ; 3-Et (30) ; 3,5-Me_2 (31)

(28)-(31)

mental GBs, ΔGB_{exp}, and the theoretical relative GBs for a protonation on the pyridine nitrogen, ΔGB(Nsp^2), and on the pyrrolidine nitrogen, ΔGB(Nsp^3). In the last column, the differences, $\delta\Delta$GB, between the experimental and the theoretical ΔGBs are given. In contrast to the observation for nornicotine, the $\delta\Delta$GB(Nsp^3) and $\delta\Delta$GB(Nsp^2) values are very close to one another, and we cannot simply deduce the protonation site. If we add the theoretical ΔGB(Nsp^3) of proton exchange reactions 24−27 (Table 5) to the experimental GBs of corresponding *N*-methylpyrrolidines (Table 2), we obtain four hypothetical GBs for the Nsp^3 protonation of nicotine; their mean is 930.4 ± 1 kJ mol^{-1}. In the same way, the combination of the theoretical ΔGB(Nsp^2) of proton exchange reactions 28−31 (Table 5) with the experimental GBs of corresponding pyridines (Table 2) leads to four hypothetical GBs for the Nsp^2 protonation of nicotine, of mean 928.4 ± 0.6 kJ mol^{-1}. Thus, the two hypothetical GBs are very close, and close to the experimental value. This suggests

Table 5. Comparison of Experimental, ΔGB_{Exp}, and Theoretical, $\Delta GB(Nsp^3)$ and $\Delta GB(Nsp^2)$, Gibbs Energies (kJ mol^{-1}) for the Proton Exchange Reactions between Nicotine and 2-Substituted *N*-Methylpyrrolidines (Equilibria 24−27), and Nicotine and 3-Substituted Pyridines (Equilibria 28−31)

equilibrium	substituted *N*-methylpyrrolidines **a**	ΔGB_{exp}[a]	$\Delta GB(Nsp^3)$[b]	$\delta\Delta GB(Nsp^3)$[c]
24	H	−2.2	−6.6	+4.4
25	2-phenyl	−15.9	−19.3	+3.4
26	2-(3-fluorophenyl)	−5.0	−5.6	+0.6
27	2-(3-trifluoromethylphenyl)	4.9	4.4	+0.5
equilibrium	**substituted pyridines c**	ΔGB_{exp}[d]	$\Delta GB(Nsp^2)$[e]	$\delta\Delta GB(Nsp^2)$[c]
28	H	34.5	31.4	+3.1
29	3-methyl	21.0	17.4	+3.6
30	3-ethyl	17.1	13.0	+4.1
31	3,5-dimethyl	9.1	3.2	+5.9

[a] $\Delta GB_{exp} = GB_{exp}$(nicotine) − GB_{exp}(2-substituted *N*-methylpyrrolidine). [b] Theoretical ΔGB in the hypothesis of a nicotine protonation on the pyrrolidine nitrogen. [c] Difference between experimental and theoretical ΔGB. [d] $\Delta GB_{exp} = GB_{exp}$(nicotine) − GB_{exp}(3-substituted pyridine). [e] Theoretical ΔGB in the hypothesis of a nicotine protonation on the pyridine nitrogen.

the coexistence of the Nsp^2 and Nsp^3 protonated forms in the protonated nicotine. Such a coexistence of forms protonated on different sites has already been studied.[20]

Another method for determining the protonation site is the calculation of $GB(Nsp^3)$ by applying the $\rho\sigma$ methodology to the GBs of 2-substituted *N*-methylpyrrolidines **a**. The least-squares calculation of regression coefficients ρ_F and ρ_α by means of known GBs (Table 1) and σ_F, σ_α values (Table 3) gives eq 32.

$$\text{GB(2-substituted } N\text{-methylpyrrolidines)} = 935(\pm 2) - 197(\pm 27)\sigma_F - 29(\pm 4)\sigma_\alpha \quad (32)$$

$$n = 4\ (\mathbf{3}, \mathbf{10}-\mathbf{12}), \quad r = 0.991, \quad s = 2\ \text{kJ mol}^{-1}$$

This equation must be used with the same cautions as eq 22. However, as a local parametrization of the field and polarizability effects of six-membered aromatic substituents, it may be applied to nicotine. With the σ_F and σ_α values of the 2-(3-pyridyl) substituent (Table 3), we find that the field/inductive effect, $\rho_F\sigma_F = -28.2$ kJ mol^{-1}, and the polarizability effect, $\rho_\alpha\sigma_\alpha = +25.5$ kJ mol^{-1}, almost cancel each other out, and we estimate a $GB(Nsp^3)$ value of 932 kJ mol^{-1} for the gas-phase basicity of the pyrrolidine nitrogen of nicotine. This estimated value compares very well with the experimental basicity of nicotine (932.6 kJ mol^{-1}).

However, the estimated gas-phase basicity of the pyridine nitrogen is also close to the experimental basicity of nicotine. This estimated basicity is calculated as follows. To the experimental GB value of pyridine (898.1 kJ mol^{-1}) we add, successively by means of eq 2 and σ values of the 3-(*N*-methylpyrrolidin-2-yl) substituent (Table 3), the polarizability effect $\rho_\alpha\sigma_\alpha$ (+14.3 kJ mol^{-1}), the resonance effect $\rho_R\sigma_R$ (+2.5 kJ mol^{-1}), and the field effect $\rho_F\sigma_F$ (+1.4 kJ mol^{-1}) of this substituent. In the nicotine protonated on the pyridine nitrogen, there is also an intramolecular hydrogen bond, C2−H···Nsp^3. The electrostatic potential on the C2−H hydrogen, $V_S = +402$ kJ mol^{-1}, and the hydrogen bond length, d(N···H) = 2.41 Å, are very close to those calculated for the Nsp^2 protonated nornicotine, for which we had estimated a hydrogen bond energy of 14.9 kJ mol^{-1}. If we retain and add this stabilization energy, we obtain a final $GB(Nsp^2)$ value of 931 kJ mol^{-1}, also in good agreement with the experimental basicity of nicotine (932.6 kJ mol^{-1}).

In conclusion, the GB values estimated for the Nsp^3 and Nsp^2 protonations by the $\rho\sigma$ methodology and the proton exchange reactions 24−31 are very close. Thus, protonated nicotine is likely an equilibrating mixture of Nsp^2 and Nsp^3 protonated forms (reaction 33). The results of proton exchange reactions 24−27 and 28−31 yield a $\Delta G°$ value of −2 kJ mol^{-1} (928.4 minus 930.4, vide supra) for reaction 33, from which an equilibrium constant $K = 2.17$ can be calculated. Thus protonated nicotine might be a ca. 2:1 mixture of the Nsp^3 and Nsp^2 protonated forms. Under these conditions, the global (observed) equilibrium constant of the protonation reaction of nicotine is the sum of the individual constants for the Nsp^2 and Nsp^3 protonation, and we deduce the global GB value from eqs 34 and 35.

(33)

$$K(\text{global}) = K(Nsp^3) + K(Nsp^2) = 3.17\,K(Nsp^2) \quad (34)$$

$$\text{GB(global)} = \text{GB}(Nsp^2) + RT\ln(3.17) = 931.3\ \text{kJ mol}^{-1} \quad (35)$$

The good agreement of this value with the experimental value of 932.6 ± 0.5 kJ mol^{-1} supports the simultaneous existence of two protonation reactions in the ICR apparatus.

Conclusions

Nicotinic agonists have recently been identified[4,70,71] as promising therapeutic agents for a variety of neurodegenerative and neuropsychiatric disorders.[72] Most studies to date[1,4b,5] have selected as elements of nicotinic pharmacophores a protonated sp^3 nitrogen atom and a less basic nitrogen (e.g., the pyridine nitrogen of nicotine). So, proton basicity (aqueous, but also intrinsic, relevant to biological hydrophobic environments) clearly constitutes an important property to be considered in the quantitative structure−activity relationships of nicotinoids. In this work, we have measured the intrinsic basicity of pyrrolidines, nornicotine, and nicotine, determined the protonation site(s) of the nicotines, and established the following structural effects.

(70) *Chem. Br.* **2000**, *36*, 20.
(71) Brennan, M. B. *Chem. Eng. News* **2000**, *78* (March 27), 23.
(72) Aubin, H. J. *Nicotine et troubles neuropsychiatriques*; Masson: Paris, 1997.

In nicotine and nornicotine, the field, resonance, and (mainly) polarizability effects of the pyrrolidinyl substituents add up to an intramolecular hydrogen bond C2—H···Nsp^3 that raises the Nsp^2 basicity from 898 kJ mol^{-1} in pyridine to ca. 930 kJ mol^{-1}. Conversely, the polarizability and field effects of the 2-(3-pyridyl) substituent on the pyrrolidine nitrogen almost cancel each other out, and the Nsp^3 basicity remains close to that of the parents, pyrrolidine (915 kJ mol^{-1}) and *N*-methylpyrrolidine (935 kJ mol^{-1}). So, despite the weaker basicity of pyridine compared to that of pyrrolidine, nornicotine is protonated on the pyridine nitrogen in the gas phase. In nicotine, the polarizability effect of the methyl group allows the pyrrolidine nitrogen to keep a basicity very slightly stronger than the pyridine nitrogen basicity, so that protonated nicotine is nearly a 2:1 mixture of the Nsp^3 and Nsp^2 protonated forms.

Acknowledgment. The authors gratefully acknowledge the IDRIS (Institut du Développement et des Ressources en Informatique Scientifique) and the CINES (Centre Informatique National de l'Enseignement Supérieur) for a grant of computer time and the Conseil Général de Loire-Atlantique for a grant to S.G. and J.G. The authors express their thanks to Dr. R. Robins (LAIEM), co-director of the thesis of S.G.

Supporting Information Available: Cartesian coordinates and Gibbs energies of B3LYP/6-311+G(3df,2p)//B3LYP/6-31G(d,p) optimized neutral and protonated structures (PDF). This material is available free of charge via the Internet at http://www.pubs.acs.org.

JA017770A