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Experimental (FT-ICR) and theoretical (DFT) estimation of the basic site preference for the bidentate molecule 2-(β-aminoethyl)-pyridine: similarity with histamine†

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ABSTRACT: The gas-phase basicity of 2-(β-aminoethyl)-pyridine (AEP)—an agonist of the histamine H_1 receptor—containing two potential basic sites (the ring N-aza and the chain N-amino) was obtained from proton-transfer equilibrium constant measurements using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR). Comparison of the experimental gas-phase basicity found for AEP with those reported for monobasic model compounds indicates that the ring N-aza is the favoured site of protonation, as with histamine. The gas-phase basicity of AEP is lower than that of histamine by only 1.3 kcal mol^{-1}. DFT(B3LYP)/6–31G* calculations performed for AEP, histamine and their protonated forms confirm this interpretation. The energy barriers calculated at the DFT(B3LYP)/6–31G* level for internal transfer of the proton (ITP) between the ring N-aza and the chain N-amino in AEP and histamine are extremely low, and vanish when thermal corrections are applied to obtain the enthalpies or Gibbs energies of activation for the proton transfer. This indicates that the quantum-chemical ITPs in AEP and histamine have a single-well character, similar to that proposed for the previously studied dibasic nitrogen ligand, N^1,N^1-dimethyl-N^2-β-(2-pyridylethyl)-formamidine, where two potential nitrogen basic sites (both nitrogen atoms in sp^2 hybridization) are separated by the ethylene group. Enlarging the basis set to 6-311G(2d,p) has no influence on this finding. A change of the basicity centre preference in AEP from the ring N-aza to the chain N-amino on going from the gas phase to aqueous solution was predicted using the polarizable continuum model applied to the DFT(B3LYP)/6–31G* optimized geometries of the N-aza and N-amino monoprotonated forms. This behaviour is similar to that observed for histamine. Copyright © 2005 John Wiley & Sons, Ltd.
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KEYWORDS: 2-(β-aminoethyl)-pyridine; histamine; basic site preference; internal transfer of the proton; solvent effect

INTRODUCTION

Proton-transfer reactions in polyfunctional—and therefore polybasic—nitrogen compounds (biomolecules and their models, agonists or antagonists) that may interact with specific biological receptors are usually complex processes and difficult to characterize by experimental methods. The presence of basic and/or acidic functions separated by flexible chains facilitates the formation of intramolecular hydrogen bonds and may change the course of the proton transfer reactions. Depending on their environment, these functions also may form intermolecular hydrogen bonds. Computational studies performed in parallel to experiments are highly desirable, because they give insights on internal (gas-phase) and external (solvation) effects that influence the proton transfer.[1,2] They are also good tools in identification of the favoured site of protonation/deprotonation. Such information is essential for compounds of biological importance and for their models (agonists or antagonists) because they help to understand the interactions of bioactive molecules in living organisms.

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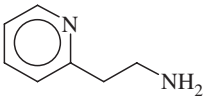
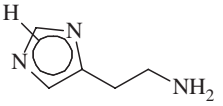
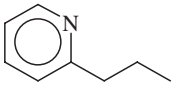
2-(β-Aminoethyl)-pyridine (AEP) is a bifunctional nitrogen ligand with a flexible conformation. Being an agonist of histamine receptors, it shows a physiological action similar to histamine (HA), binding with high affinity and specificity to the histamine H_1 receptor.[3] Both AEP and HA belong to the family of aminazines. They contain two potential basic sites, the ring N-aza and the chain N-amino separated by an ethylene group. Depending on the environment, one of the sites may be favoured in the protonation reaction. Due to the flexibility of the side-chain, the bases may also adopt various conformations, the *trans* (so called 'essential') or the *gauche* ('scorpio') conformation. In addition, HA bears an acidic amino group (intra-annular NH), which adds to the complexity of its proton-transfer pathways[4] as well as of its biological activity.[5]



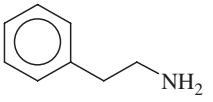
AEP



HA



NPP



PEA

To understand similarities and differences between AEP and HA in binding with the H_1 receptor, we have undertaken structural and physicochemical studies for both ligands.[6] Although it is well known that acid–base properties are one of the principal factors that govern the interactions of biomolecules in living organisms, to our knowledge there is no information for AEP on the gas-phase basicity and its basic centre preference. Acid–base equilibria and acid–base properties are reported in the literature solely for HA.[4,6a] This situation encouraged us to perform detailed investigations of the potential sites of protonation for AEP in the gas phase and to compare it with those for HA.

Gas-phase basicity measurements for AEP were carried out using the same Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer as for other flexible nitrogen ligands.[1c,e] The experimental gas-phase basicity was analysed in AEP and compared with those observed in monobasic model nitrogen bases 2-*n*-propylpyridine (NPP) and β-phenylethylamine (PEA).[7] In parallel, quantum-chemical calculations were performed for the free base AEP and its monocations (the ring N-aza and the chain N-amino protonated form) using density functional theory (DFT)[8] with a combination of the Becke three-parameter hybrid exchange functional with the non-local correlation functional of Lee, Yang and Parr (B3LYP).[9] In DFT calculations, the 6–31G* basis set was used.[10] The justification for the use of the B3LYP functional for predicting the energy barrier for the intramolecular proton transfer in flexible bidentate ligands has been provided in our previous work.[1e] Gouthrie,[11] testing the B3LYP/6–31G* level for a set of 128 organic molecules, showed that the combination of the B3LYP functional with the 6–31G* basis set is the simplest approach for accurate prediction of the entropy values. The overall standard deviation from the best available experimental entropy data was equal to 1.3 cal mol^{-1} K^{-1} (equivalent to 0.4 kcal mol^{-1} in free energy terms at 298.15 K). Finally, the fast convergence (to their complete basis set limits) of Pople's basis sets with the DFT methods is already a well-known fact.[12,13] However, to verify the effect of the basis set increase on the relative thermodynamic properties of the two basic sites and on the energy barrier for the intramolecular proton transfer in our study, calculations were performed using the larger basis set 6-311G(2d,p).[14]

The DFT computations give us the possibility to find the most stable structures for the neutral and protonated forms of AEP. They also support our conclusion (derived on the basis of experimental results) about the favoured site of protonation in the gas phase, predict the relative basicity of the two potential sites and estimate the energy barrier for the internal transfer of the proton (ITP) from the N-aza to the N-amino site. Finally, calculations performed for the monoprotonated forms in seven solvents of different polarities (from cyclohexane to water) using the polarizable continuum model (PCM)[15] applied to geometries optimized at the DFT(B3LYP)/6–31G* level allow the study of the influence of the medium on the proton equilibrium position in AEP when going from the gas phase to aqueous solution. Histamine was studied previously at the HF/6–31G* and PCM//HF/6–31G* levels.[6a] For a more valid comparison of medium effects on AEP and HA protonation, calculations were performed at the DFT(B3LYP)/6–31G* and PCM//DFT(B3LYP)/6–31G* levels for both structures.

EXPERIMENTAL

2-(β-Aminoethyl)-pyridine and the reference bases Et_3N and *n*-Pr_3N for gas-phase basicity (GB) measurements were commercially available (Aldrich). The GB was determined using the same FT-ICR mass spectrometer[16] and the same procedure as described previously.[1c,e] The GB value was obtained from the equilibrium constants for the proton-transfer reaction [Eqn (1)] between AEP (B) and a reference base (Ref) using Eqn (2)

$$BH^+ + Ref \rightleftarrows B + RefH^+ \quad (1)$$

$$GB(B) = GB(Ref) + \Delta G(1) \quad (2)$$

The measurements were carried out at an FT-ICR cell temperature of 338 K.[17] It is important to mention

that literature GBs of reference bases refer to the standard temperature of 298.15 K.[7] However, temperature corrections are minor compared with other experimental uncertainties,[18] and the experimental GB of AEP does not include such temperature corrections.

COMPUTATIONAL DETAILS

The DFT(B3LYP)/6–31G* calculations[8–10] for various thermodynamically stable conformations of AEP, HA and their monocations protonated at the N-aza and N-amino sites were performed using the Gaussian 98 program.[19] The proton affinity (PA) and GB values were estimated as the enthalpy and Gibbs free energy changes of the deprotonation reaction $BH^+ \rightarrow B + H^+$ using Eqns (3) and (4), respectively, as described previously[1e]

$$PA = \Delta_r H_{298} = H_{298}(B) + H_{298}(H^+) - H_{298}(BH^+) \quad (3)$$

$$GB = PA - T\Delta S = G_{298}(B) + G_{298}(H^+) - G_{298}(BH^+) \quad (4)$$

The transition state for the proton transfer between the two possible sites of protonation in the 'scorpio' conformations of the protonated forms was investigated at the DFT(B3LYP)/6–31G* level using the same procedure as in Ref. 1e. The larger basis set of 6–311G(2d,p) was also tested[14] and the anharmonicity of the NH vibration was considered. The anharmonic vibrations were accounted for by using the perturbative scheme. First, analytical harmonic frequencies were computed, followed by numerical differentiation along normal modes to compute zero-point energies and anharmonic frequencies.[20]

RESULTS AND DISCUSSION

Evidence of the favoured basic site based on experimental data

Relative GB measurements performed for dibasic AEP using two reference bases were in good agreement: Et_3N, GB = 227.0 kcal mol^{-1} (1 cal = 4.184 J); *n*-Pr_3N, GB = 229.5 kcal mol^{-1}.[7] The relative basicities measured between AEP and the reference bases (1.19 and −1.34 kcal mol^{-1}, respectively) led to a GB of 228.2 ± 0.1 kcal mol^{-1} for AEP. This experimental result indicates that the GB for AEP for is significantly higher than the GBs for monofunctional 2-alkylpyridines (GB < 222 kcal mol^{-1}) and arylalkyl primary amines (GB < 216 kcal mol^{-1}).[7] It is close to that of HA (GB = 229.5 kcal mol^{-1}), in which both basic sites participate in the protonation reaction as in other flexible bidentate nitrogen ligands.[21] Protonated HA was shown to adopt a 'scorpio' conformation. The close GB values for AEP and HA suggest the formation of an intramolecular hydrogen bond ($N\cdots H–N^+$). This kind of interaction in the gas phase for flexible bidentate nitrogen ligands usually increases the GB value by 5–20 kcal mol^{-1} compared with monofunctional nitrogen bases.[7,21]

To indicate which site is more basic and which is less basic but may participate in the bonding by forming a hydrogen bond, we compared the experimental GB value of AEP with those reported for model compounds NPP and PEA. The two monobasic derivatives contain the same number of heavy atoms as AEP.

Comparison of the GB value for NPP (220.8 kcal mol^{-1})[7] with that reported for PEA (215.7 kcal mol^{-1})[7] indicates that in the model bases the ring N-aza in NPP is more basic than the chain N-amino in PEA by ∼5 kcal mol^{-1}. A similar GB difference exists between the model compounds 4(5)-methylimidazole (220.1 kcal mol^{-1})[7] and PEA compared with HA. An additional nitrogen atom introduced in a non-conjugated system usually decreases the basicity of the first one due to its higher electronegativity than that of a carbon atom. Indeed, the estimated total substituent effect (sum of the polarizability, field/inductive and resonance effects) of the ethylamino group (4.6 kcal mol^{-1}) in the series of 2-substituted pyridines is slightly lower than that of the *n*-propyl group (6.1 kcal mol^{-1}). The effect of the *n*-Pr group was estimated on the basis of the experimental GB of unsubstituted pyridine and NPP[7] and that of the $NH_2(CH_2)_2$ group, on the basis of the Taft and Topsom equation[22] recalculated recently[1e] according to data from Ref. 7 for a series of 2-substituted pyridines [$-\delta GB = (9.1 \pm 0.7)\sigma_\alpha + (29.3 \pm 0.8)\sigma_F + (13.9 \pm 0.8)\sigma_R + (-0.1 \pm 0.4)$] and σ_i constants [σ_α −0.52 and σ_F 0.04, estimated according to note 17 in Ref. 23 and the corresponding σ_i for NH_2 taken from Ref. 24; σ_R^+ −0.07, as proposed for other $X(CH_2)_2$ groups in Ref. 24]. The electron-withdrawing field effect of the aza group introduced in the ring of PEA may also reduce the basicity of the chain N-amino. Lack of σ_i constants for the 2-pyridyl$(CH_2)_2$ group makes direct estimation of its effect difficult.[1e] However, the electron-withdrawing effect of the 2-aza group should be attenuated by the two methylene groups and its σ_i constant should not be very different than that of the $Ph(CH_2)_2$ group. Based on these observations one may conclude that the ring N-aza is the preferred site of protonation in AEP, as in HA.

From the substituent effect found for the ethylamino group ($\delta GB = 4.6$ kcal mol^{-1}) and the GB value for the unsubstituted pyridine (GB = 214.7 kcal mol^{-1}), a GB value corresponding to protonation of the ring N-aza in AEP (219.3 kcal mol^{-1}) is estimated. This estimated GB(N-aza) value is lower than the experimental GB of AEP (228.2 kcal mol^{-1}) by ∼9 kcal mol^{-1}. This difference may be assigned to the chelation effect of the proton.

Similar increase of the GB values was observed for HA[6a] and for other flexible bidentate nitrogen ligands (e.g. amidinamines)[21] containing N-sp^2 and N-sp^3 basic sites, of which N-sp^2 is more basic.

Stable conformations found by DFT calculations

To select stable structures of AEP and its monocations, three angles (Φ_1, Φ_2 and Φ_3) were changed systematically by 30° steps. The initial values for the three angles are given in Fig. 1. In this way, nine stable conformations (AEP1–9 given in fig. 1 in *Wiley Interscience*) were found for the neutral AEP and six stable conformations were selected for the monoprotonated forms (AEP-ImH$^+$1–4 and AEP-AmH$^+$1–2, given in fig. 2 in *Wiley Interscience*).

For neutral AEP, the most stable conformation is the *gauche* AEP2 structure. Its electronic energy ($E = -382.261763$ a.u.) and Gibbs energy ($G = -382.131510$ a.u.) correspond to the global minimum. The Gibbs energies of AEP1–9 relative to that of the most stable structure (AEP2) are as follows: 0.32, 0.00, 0.50, 0.61, 0.57, 1.05, 0.81, 0.39 and 1.44 kcal mol^{-1}. This suggests that all nine structures of AEP may be present to some extent, with the AEP2 structure being the most probable in the gas phase. Taking into account the relative Gibbs energies calculated for all stable structures at the DFT(B3LYP)/6–31G* level, their percentage contents are as follows: 15.4, 26.4, 11.4, 9.4, 10.1, 4.5, 6.7, 13.7 and 2.3%. It has been found recently by infrared spectroscopy that their presence may be detected in CCl_4 solution.[6b]

Among AEP monocations, the *gauche* AEP-ImH$^+$1 structure is the most stable one ($E = -382.663704$ a.u., $G = -382.518040$ a.u.). The Gibbs energies of the other N-aza protonated structures (AEP-ImH$^+$2–4) relative to that of AEP-ImH$^+$1 are 9.61, 12.54 and 12.91 kcal mol^{-1}. The differences are sufficient for these three structures to be neglected in the gas phase and their contribution may be $<1 \times 10^{-5}$%. The Gibbs energy of the most stable N-amino protonated structure (*gauche*-AEP-AmH$^+$1) is

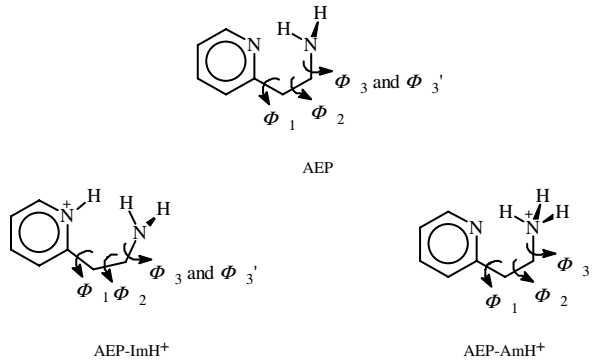


Figure 1. Initial conformations and definitions of the Φ_1, Φ_2 and Φ_3 dihedral angles in AEP and its monocations AEP-ImH$^+$ and AEP-AmH$^+$

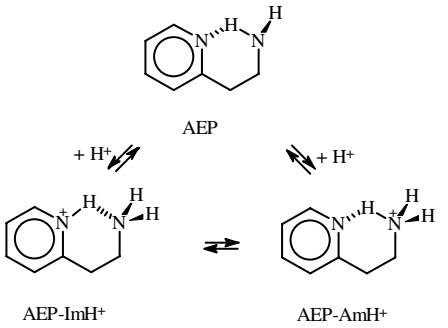


Figure 2. The most stable conformations for AEP and its monocations

larger than that of the most stable ring N-aza protonated form (*gauche*-AEP-ImH$^+$1) by 3.40 kcal mol^{-1}. This means that its contribution is very small (<1% at the DFT level), but taking into account the error of the DFT method (~2 kcal mol^{-1}) this structure might be significant in the gas phase. The Gibbs energy of the *trans*-AEP-AmH$^+$2 structure is still larger than that of *gauche*-AEP-ImH$^+$1 by 17.63 kcal mol^{-1}, and thus it can be neglected in the gas phase.

All the most stable neutral (AEP2) and monoprotonated structures (AEP-ImH$^+$1 and AEP-AmH$^+$1) found at the DFT(B3LYP)/6–31G* level have the *gauche* conformation and they are stabilized by the intramolecular hydrogen bonds between the two functional groups (Fig. 2). In the neutral form, the chain NH_2 group interacts with the ring N-aza, and the distance between the interacting atoms (H···N) is equal to 2.3 Å (1 Å = 0.1 nm). For the monocations, the protonated group interacts with the free basic site to form NH$^+$···N bonds. In the ring N-aza protonated form, the distance between the hydrogen atom of the ring N-azaH$^+$ and the chain N-amino is equal to 1.8 Å. In the chain N-amino protonated form, the distance between the hydrogen atom of the chain N-aminoH$^+$ and the ring N-aza is equal to 1.6 Å.

Similar intramolecular hydrogen bonds between the functional groups stabilize the most stable *gauche* conformations of neutral HA and protonated forms of HA, the ring N-aza (ImH$^+$) and the chain N-amino (AmH$^+$-T$_1$).[6a] The main difference is the prototropic tautomerism that takes place in the imidazole ring (Fig. 3). Therefore, two tautomeric forms (T$_1$ and T$_2$) with the ethylamino group at the 4- and 5-positions occur for the neutral form. Similar intramolecular proton transfer is possible in the chain N-amino protonated form. However, the Gibbs energy of the AmH$^+$-T$_2$ tautomer is by >20 kcal mol^{-1} larger than those of ImH$^+$ and AmH$^+$-T$_1$, and thus this tautomer can be neglected in the mixture of monocationic forms in the gas phase.

Generally, the DFT(B3LYP)/6–31G* results for histamine are quantitatively similar to those found previously at the HF/6–31G* level.[6a] There are only slight qualitative differences in the geometric and thermodynamic

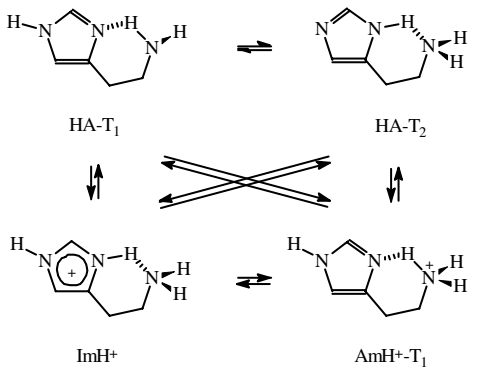


Figure 3. The most stable conformations for HA and its monocations

parameters. The dihedral angles (Φ_1, Φ_2 and Φ_3) and the Gibbs energies are lower at the DFT(B3LYP)/6–31G* level by $<5^\circ$ and 2 kcal mol^{-1}, respectively. The distances between the atoms (NH$\cdots$N or $^+$NH$\cdots$N) of the interacting functional groups in the most stable *gauche* conformations of HA and its monocations are similar to those in AEP. In the neutral HA tautomers (*gauche*-HA-T_1 and *gauche*-HA-T_2) the distances between the hydrogen atom of the chain amino group and the ring N-aza are 2.3 and 2.2 Å, respectively. In the N-aza and N-amino protonated forms of HA, the distances between the hydrogen atom of the protonated group and the nitrogen atom of the free group (the protonated ring N-aza and the free chain NH_2 in *gauche*-AEP-ImH^+1; and the protonated chain NH_3^+ and the free ring N-aza in *gauche*-AmH^+-T_1) are shorter (due to stronger hydrogen bonding) and equal to 1.8 and 1.7 Å, respectively.

The DFT evidence for the preferred site of protonation

In DFT calculations, AEP was considered as a nitrogen ligand with two basic centres: the ring N-aza and the chain N-amino. As shown above, the lowest Gibbs energy corresponds to the most stable *gauche*-AEP-ImH^+1 structure, indicating that the ring N-aza is the favoured site of protonation in the gas phase, as with HA. The Gibbs energy of the other stable *gauche*-AEP-AmH^+1 structure is larger by 3.40 kcal mol^{-1}. A smaller difference was found for the analogue stable HA monocations: *gauche*-ImH^+ and *gauche*-AmH^+-T_1 (1.74 kcal mol^{-1}).

The proton affinities (PA) calculated for protonation on the two potential basic sites in AEP and HA (the ring N-aza and the chain N-amino) were estimated according to Eqn (3) using the enthalpies calculated at the DFT(B3LYP)/6–31G* level for the most stable *gauche* conformations of the neutral and protonated forms at 298.15 K (Figs 2 and 3). The GB values were calculated according to Eqn (4). Table 1 summarizes the calculated PA, GB and entropy term $T\Delta S$ values for the ring N-aza and chain N-amino sites in AEP and HA. Although the DFT method led to overestimated basicities compared with experimental values, the difference between the GB values (1 kcal mol^{-1}) calculated for the favoured site of protonation (N-aza) in AEP and HA for similar equilibria AEP-ImH^+ $\rightleftarrows$ AEP and ImH^+ $\rightleftarrows$ HA-T_1 is close to that between the experimental GB values (1.3 kcal mol^{-1}). This observation confirms our empirical estimations and indicates that the ring N-aza is a more basic site than the chain N-amino in AEP and HA.

Table 1. Proton affinities (PA), gas-phase basicities (GB) and entropy term ($T\Delta S$) estimated for the most stable *gauche* conformations of AEP (Fig. 2) and HA (Fig. 3) at the DFT(B3LYP)/6–31G* level (in kcal mol^{-1})

Equilibria	PA	GB	$T\Delta S$
AEP-ImH^+ $\rightleftarrows$ AEP	245.0	236.3	8.7
AEP-AmH^+ $\rightleftarrows$ AEP	241.6	232.9	8.7
ImH^+ $\rightleftarrows$ HA-T_1	246.0	237.3	8.7
ImH^+ $\rightleftarrows$ HA-T_2	243.8	235.7	8.1
AmH^+-T_1 $\rightleftarrows$ HA-T_1	244.2	235.6	8.6
AmH^+-T_1 $\rightleftarrows$ HA-T_2	242.1	233.9	8.2

It is interesting to mention that the GB values calculated for the favoured site of protonation (N-aza) for the most stable *trans* conformations of AEP and HA for similar equilibria AEP-ImH^+ $\rightleftarrows$ AEP (224.1 kcal mol^{-1}) and ImH^+ $\rightleftarrows$ HA-T_1 (226.8 kcal mol^{-1}) are lower than those for the corresponding most stable *gauche* conformations by 12.2 and 10.5 kcal mol^{-1}, respectively. The differences in GBs may be assigned to the effect of the conformation change (when going from the *trans* to the *gauche* conformation) and to the effect of proton chelation by the two functions. These increases of the GB values in the most stable *gauche* conformations of AEP and HA are similar to the differences in the estimated GB(N-aza) and the experimental GB values (9–11 kcal mol^{-1}).[6a]

Similar behaviour was observed for flexible diamines, which are good examples of symmetrical Nsp^3–Nsp^3 bidentate nitrogen bases without any constraints on the basic sites.[7,25,26] However, diamines cannot be used as model compounds for the ligands studied here because AEP and HA are unsymmetrical Nsp^2–Nsp^3 bases in which one nitrogen (Nsp^2) is included in the rigid aromatic cycle. This fact may be a reason for the lower entropy contributions when going from B to BH^+ in the case of AEP and HA (1–3 cal mol^{-1} K^{-1}) than in the case of diamines (10–20 cal mol^{-1} K^{-1}).[7] Lack of experimental entropy data for AEP and HA does not allow any comment to be made on the capability of the DFT calculations to reproduce experimental data.

The DFT energy barrier for ITP in the monocation

It is well known that the potential energy surface for the ITP between two potential basic nitrogen sites

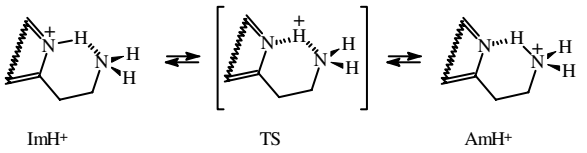


Figure 4. Internal transfer of the proton in monocationic forms of flexible bidentate nitrogen ligands (AEP and HA)

may have a different character: a double or a single well.[1e,27,28] To characterize its shape we examined the energy barrier for proton transfer from the chain N-amino to the ring N-aza in the protonated forms of AEP and HA (Fig. 4). Transition states (see structures in figs 3 and 4 in *Wiley Interscience*) were searched for both ligands at the DFT(B3LYP)/6–31G* level. Harmonic vibrational analysis was performed for the stationary points found. This analysis confirmed the character of first-order saddle points with single imaginary frequencies. The distances between the proton and the chain N-amino in the transition states of AEP (1.362 Å) and HA (1.290 Å) are much closer to the distances in the N-amino protonated forms (1.099 and 1.087 Å for AEP and HA, respectively) than for the N-aza protonated forms (1.751 and 1.824 Å for AEP and HA, respectively), indicating that the transition state geometries are closer to those of the less-stable N-amino forms (see figs 3 and 4 in *Wiley Interscience*).

The DFT-calculated thermodynamic quantities for the barriers in the monocations of AEP and HA [ΔE, $\Delta(E+\mathrm{ZPVE})$, ΔH and ΔG, which are the relative electronic energy, the relative sum of the electronic and the zero-point vibrational energies, the relative enthalpy and the relative Gibbs energy] between the transition state (first-order saddle point) and the minimum energy structure of the ring N-aza protonated form are given in Table 2. For comparison, analogous energy differences between the minima corresponding to the N-aza and N-amino protonated forms are also given. One can notice that in both cases, even though the electronic energy of the transition state is higher than that of both the N-amino and N-aza forms, the picture changes upon addition of the zero-point vibrational energy and the thermal terms. The Gibbs energy barriers for proton transfer from the ring N-aza to the chain N-amino in AEP (2.0 kcal mol^{-1}) and HA (1.3 kcal mol^{-1}) are lower than the relative Gibbs energies between the two protonated forms (3.4 and 1.7 kcal mol^{-1}, respectively). Similar behaviour was found when using the 6–311G(2d,p) basis set. The thermochemical data found for the transition state using this basis set (data given in parentheses in Table 2) are not significantly different from those obtained with the 6–31G* basis set. The differences do not exceed 0.2 kcal mol^{-1}.

Table 2. Comparison of thermodynamic properties for the transition state (TS) and the chain N-amino protonated form (N-amino) relative to those of the ring N-aza protonated form calculated for AEP and HA at the DFT(B3LYP)/6–31G* level[a] (in kcal mol^{-1})

	AEP		HA	
Relative property	TS	N-amino	TS	N-amino
ΔE	4.2 (4.3)	3.5 (3.9)	3.3 (3.4)	1.4 (1.8)
$\Delta(E+\mathrm{ZPVE})$	1.8 (1.9)	3.4 (3.6)	1.0 (1.1)	1.8 (2.0)
ΔH	1.6 (1.7)	3.4 (3.5)	0.8 (0.8)	1.7 (1.9)
ΔG	2.0 (2.2)	3.4 (3.6)	1.3 (1.3)	1.7 (2.0)

[a] DFT(B3LYP)/6–311G(2d,p) data in parentheses.

These computations suggest that at 298 K the probability of finding the proton on the N-amino side chain may be negligible for both AEP and HA, and that the proton is located on the ring N-aza. This suggests that the potential energy surface, at least in term of enthalpy or Gibbs energy changes, has a single well character, as in previously studied amidinazine.[1e]

Analysis of the thermochemical data in the anharmonic oscillator approximation performed for the structures optimized at the DFT(B3LYP)/6–311(2d,p) level confirms the observed absence of a barrier. Inclusion of the frequencies anharmonicity into the vibrational contribution to *G* has a stabilizing effect on all structures (N-aza, N-amino and transition state) by 0.4, 0.9 and 2.4 kcal mol^{-1}, respectively, for AEP and by 1.9, 2.5 and 3.7 kcal mol^{-1}, respectively, for HA.

Change of basic site preferences on going from gas phase to aqueous solution

In aqueous solution, ethylamine is more basic than pyridine,[29] but in the gas phase the high polarizability of the aromatic system strongly increases the basicity of the N-aza, which is a stronger base than the N-amino.[7] For investigations of the medium effects on the basic site preferences in AEP, when the gas-phase species are transferred into a solvent, the PCM method[11] was applied to geometries optimized at the DFT(B3LYP)/6–31G* level and to solvents of different polarities (from cyclohexane to water). For comparison, PCM//DFT(B3LYP)/6–31G* calculations were also performed for HA. The calculated relative energies between the most stable *gauche* and *trans* conformations of the ring N-aza and chain N-amino protonated forms of AEP and HA in the gas phase and in seven solvents are listed in Table 3.

Comparison of the results obtained shows similar behaviours for AEP and HA for both conformations (*gauche* and *trans*). A change of the sign of the relative total energies on going from the gas phase (positive) to aqueous solution (negative) is observed. This indicates that the favoured site of protonation is changed by solvation. Generally, the ring N-aza is favoured in the gas phase and in solvents of low dielectric constants (such

Table 3. Relative total energies between the most stable *gauche* and *trans* conformations of the chain N-amino and the ring N-aza protonated forms calculated for AEP and HA (in kcal mol^{-1}) in the gas phase and solution using the PCM method applied to geometries optimized at the DFT(B3LYP)/6–31G* level

	AEP		HA	
Phase	*gauche*	*trans*	*gauche*	*trans*
Gas	3.5	4.5	1.4	4.3
Cyclohexane	1.9	1.0	0.6	2.4
Benzene	1.8	0.6	0.5	2.2
CCl_4	1.8	0.7	0.6	2.2
$CHCl_3$	0.7	−0.2	0.0	1.0
THF	0.4	−1.8	−0.2	0.6
Acetone	−0.1	−2.6	−0.4	0.1
Water	−1.8	−5.3	−2.0	−2.0

as cyclohexane, benzene, CCl_4), whereas in more polar solvents (such as water) the proton prefers the chain N-amino site.

CONCLUSIONS

Both experimental and theoretical studies of the basic site preferences in the gas phase for the dibasic nitrogen ligand AEP—an agonist of the histamine H_1 receptor—give a convergent answer in favour of the ring N-aza, as with HA.[6a] The chelation effect of the proton derived on the basis of experimental data for AEP and model compounds ($\sim$9 kcal mol^{-1}) is also similar to that observed in HA and other amidinamines.[21]

In the protonated form of AEP, the Gibbs energy barrier value calculated for the internal proton transfer at DFT(B3LYP)/6–31G* and DFT(B3LYP)/6–311(2d,p) levels suggests that the potential energy profile has a single-well character. The proton is located on the ring N-aza site and the hydrogen bond is formed with the chain N-amino site. Similar behaviour is observed for HA at the same level of theory. A change of the favoured site of protonation from the ring N-aza to the chain N-amino is observed for both ligands (AEP and HA) when the gas-phase species are transferred into aqueous solution.

The evident structural similarity of AEP and HA should lead to similar behaviour. In the context of proton transfer, this hypothesis is now validated qualitatively and quantitatively by this joint experimental and theoretical study resting on the GB of these compounds, including the potential energy along the proton coordinates. Furthermore, the two molecules exhibit similarity in terms of the medium effect on the proton location. Because living systems are known to include environments of different polarities, it is believed that this work contributes to a deeper understanding of the causes of specific biological properties of AEP and HA, particularly their mode of interactions with HA-specific receptors.

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REFERENCES

1. (a) Raczyńska ED, Taft RW. *Bull. Chem. Soc. Jpn.* 1997; **70**: 1297–1305; (b) Raczyńska ED, Mishima M, Mustanir. *Bull. Chem. Soc. Jpn.* 1998; **71**: 2175–2179; (c) Raczyńska ED, Decouzon M, Gal J-F, Maria P-C, Taft RW, Anvia F. *J. Org. Chem.* 2000; **65**: 4635–4640; (d) Makowski M, Raczyńska ED, Chmurzyński L. *J. Phys. Chem. A* 2001; **105**: 869–874; (e) Raczyńska ED, Darowska M, Dąbkowska I, Decouzon M, Gal J-F, Maria P-C, Dubin Poliart C. *J. Org. Chem.* 2004; **69**: 4023–4030.
2. (a) Notario R, Abboud J-LM, Cativiela C, Garcia JY, Herreros M, Homan H, Mayoral JA, Salvatella L. *J. Am. Chem. Soc.* 1998; **120**: 13224–13229; (b) Godfrey PD, Brown RD. *J. Am. Chem. Soc.* 1998; **120**: 10724–10732; (c) Rak J, Skurski P, Simons J, Gutowski M. *J. Am. Chem. Soc.* 2001; **123**: 11695–11707; (d) Graton J, Berthelot M, Gal J-F, Girard S, Laurence C, Lebreton J, Le Questel J-Y, Maria P-C, Nauš P. *J. Am. Chem. Soc.* 2002; **124**: 10552–10562.
3. (a) Tran VT, Chang RSL, Snyder SH. *Proc. Natl. Acad. Sci. USA* 1978; **75**: 6290–6294; (b) Eckman DM, Hopkins N, McBride C, Keef KD. *Br. J. Pharmacol.* 1998; **124**: 181–189; (c) Fossati A, Barone D, Benvenuti C. *Pharmacol. Res.* 2001; **43**: 389–392
4. Raczyńska ED, Cyrański MK, Darowska M, Rudka T. *Targets Heterocycl. Syst. Chem. Prop.* 2000; **4**: 327–356.
5. (a) Cooper DG, Vong RC, Durant GJ, Ganellin CR. In *Comprehensive Medicinal Chemistry*, vol. 3, Jammes PG, Taylor JB (eds). Pergamon Press: Oxford, 1990; 323–421; (b) Leurs R, Hoffman M, Wieland K, Timmerman H. *Trends Pharmacol. Sci.* 2000; **21**: 11–12.
6. (a) Raczyńska ED, Darowska M, Cyrański MK, Makowski M, Rudka T, Gal J-F, Maria P-C. *J. Phys. Org. Chem.* 2003; **16**: 783–796; (b) Raczyńska ED, Darowska M, Rudka T. *Polish J. Chem.* 2003; **77**: 1529–1546.
7. Hunter EPL, Lias SG. *J. Phys. Chem. Ref. Data* 1998; **27**: 413–656.
8. Parr RG, Yang W. *Density Functional Theory of Atoms and Molecules*, Oxford University Press: New York, 1989.
9. (a) Becke AD. *J. Chem. Phys.* 1993; **98**: 5648–5652; (b) Lee C, Yang W, Parr RG. *Phys. Rev. B* 1988; **37**: 785–789.
10. (a) Hariharan PC, Pople JA. *Theor. Chim. Acta* 1973; **28**: 213–222; (b) Hehre WJ, Radom L, Schleyer PvR, Pople JA. *Ab Initio Molecular Orbital Theory*, Wiley: New York, 1986.
11. Gouthrie JP. *J. Phys. Chem. A* 2001; **105**: 8495–8499.
12. Jensen F. *J. Chem. Phys.* 2002; **116**: 7372–7379.
13. Boese AD, Martin JML, Handy NC. *J. Chem. Phys.* 2003; **119**: 3005–3013.
14. Krishnan R, Binkley JS, Seeger R, Pople JA. *J. Chem. Phys.* 1980; **72**: 650–654.
15. (a) Miertuš S, Scrocco E, Tomasi J. *Chem. Phys.* 1981; **55**: 117–129; (b) Szafran M, Karelson MM, Katritzky AR, Koput J, Zerner MC. *J. Comput. Chem.* 1993; **14**: 371–377; (c) Cammi R, Tomasi

J. *J. Comput. Chem.* 1995; **16**: 1449–1458; (d) Pomelli CS, Tomasi J. *Theor. Chem. Acc.* 1997; **96**: 39–43.
16. Decouzon M, Gal J-F, Géribaldi S, Maria P-C, Rouillard M. *SPECTRA 2000* 1989; **17**: 51–57.
17. Gal J-F, Maria P-C, Decouzon M. *Int. J. Mass Spectrom. Ion Processes* 1989; **93**: 87–94.
18. Gal J-F, Maria P-C, Raczyńska ED. *J. Mass Spectrom.* 2001; **36**: 699–716.
19. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Zakrzewski VG, Montgomery JA Jr, Stratmann RE, Burant JC, Dapprich S, Millam JM, Daniels AD, Kudin KN, Strain MC, Farkas O, Tomasi J, Barone V, Cossi M, Cammi R, Mennucci B, Pomelli C, Adamo C, Clifford S, Ochterski J, Petersson GA, Ayala PY, Cui Q, Morokuma K, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Cioslowski J, Oritz JV, Baboul AG, Liu G, Liashenko A, Piskorz P, Komaromi I, Gomperts R, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson BG, Chen W, Wong MW, Andres JL, Gonzalez C, Head-Gordon M, Replogle ES, Pople JA. *Gaussian 98*. Gaussian: Pittsburgh, PA, 1998.
20. (a) Page M, Doubleday C, McIver JW Jr. *J. Chem. Phys.* 1990; **93**: 5634–5642; (b) Miller WH, Hernandez R, Handy NC, Jayatilaka D, Willets A. *Chem. Phys. Lett.* 1990; **172**: 62–68.
21. (a) Raczyńska ED, Maria P-C, Gal J-F, Decouzon M. *J. Phys. Org. Chem.* 1994; **7**: 725–733; (b) Raczyńska ED, Decouzon M, Gal J-F, Maria P-C, Gelbard G, Vielfaure-Joly F. *J. Phys. Org. Chem.* 2001; **14**: 25–34.
22. Taft RW, Topsom RD. *Prog. Phys. Org. Chem.* 1987; **16**: 1–83.
23. Raczyńska ED, Maria P-C, Gal J-F, Decouzon M. *J. Org. Chem.* 1992; **57**: 5730–5735.
24. Hansch C, Leo A, Taft RW. *Chem. Rev.* 1991; **91**: 165–195.
25. Meot-Ner M, Hamlet P, Hunter EP, Fiel FH. *J. Am. Chem. Soc.* 1980; **102**: 6393–6399.
26. Yamdagni R, Kebarle P. *J. Am. Chem. Soc.* 1973; **95**: 3504–3510.
27. (a) Scheiner S. *Acc. Chem. Res.* 1985; **18**: 174–180; (b) Brauman JI. *J. Mass Spectrom.* 1995; **30**: 1649–1651; (c) Scheiner S, Yi M. *J. Phys. Chem.* 1996; **100**: 9235–9241; (d) Pérez P, Contreras R. *Chem. Phys. Letts.* 1996; **256**: 15–20; (e) Lim J-H, Lee EK, Kim Y. *J. Phys. Chem. A* 1997; **101**: 2233–2239; (f) González L, Mó O, Yánez M. *J. Phys. Chem. A* 1998; **102**: 1356–1364; (g) Makowski M, Sadowski R, Augustin-Nowacka D, Chmurzyński L. *J. Phys. Chem. A* 2001; **105**: 6743–6749; (h) Pejov L, Petruševski VM, Rahten A, Jesih A, Skapin T. *J. Mol. Struct.* 2002; **604**: 1–7; (i) Shi T, Wang X, Shi X, Tian Z, Zhu Q. *J. Mol. Struct. (Theochem)* 2002; **578**: 135–143; (j) Perrin CL, Ohta BK. *J. Mol. Struct.* 2003; **644**: 1–12.
28. (a) Cleland WW, Krevoy MM. *Science* 1994; **264**: 1887–1890; (b) Gilli P, Bertolasi V, Ferretti V, Gilli G. *J. Am. Chem. Soc.* 1994; **116**: 909–915; (c) Latajka Z, Bouteiller Y, Scheiner S. *Chem. Phys. Lett.* 1995; **234**: 159–164; (d) Smirnov SN, Golubev NS, Denisov GS, Benedict H, Schah-Mohammedi P, Limbach H-H. *J. Am. Chem. Soc.* 1996; **118**: 4094–4101; (e) Ventura KM, Greene SN, Halkides J, Messina M. *Struct. Chem.* 2001; **12**: 23–31.
29. Perrin DD. *Dissociation Constants of Organic Bases in Aqueous Solution*. Butterworth: London, 1965.