JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
*J. Phys. Org. Chem.* 2003; **16:** 783–796
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.670

# *Ab initio* study of tautomerism and of basicity center preference in histamine, from gas phase to solution—comparison with experimental data (gas phase, solution, solid state)†

**Ewa D. Raczyńska,[1]* Małgorzata Darowska,[1] Michał K. Cyrański,[2] Mariusz Makowski,[3] Tomasz Rudka,[4] Jean-François Gal[5] and Pierre-Charles Maria[5]**

[1]Department of Chemistry, Agricultural University (SGGW), ul. Nowoursynowska 159c, 02-776 Warsaw, Poland
[2]Department of Chemistry, Warsaw University, ul. Pasteura 1, 02-093 Warsaw, Poland
[3]Faculty of Chemistry, University of Gdańsk, ul. Sobieskiego 18, 80-952 Gdańsk, Poland
[4]Interdisciplinary Department of Biotechnology, Agricultural University (SGGW), ul. Rakowiecka 26/32, 02-528 Warsaw, Poland
[5]Chimie des Matériaux Organiques et Métalliques, Université de Nice–Sophia Antipolis, Parc Valrose, 06108 Nice Cédex 2, France

*Received 20 December 2002; revised 23 April 2003; accepted 26 April 2003*

ABSTRACT: Tautomeric and basicity center preferences for isolated neutral and monoprotonated histamine were studied by means of *ab initio* calculations (HF, MP2 and DFT). The polarizable continuum model (PCM) was applied to the study of the variations of the tautomeric and basicity center preferences in histamine on going from the gas phase to aqueous solution. Twelve solvents of different polarities (from *n*-heptane to water) were chosen and calculations were performed for geometries optimized at the HF/6–31G* level. In low-polarity solvents and in the gas phase the protonation site is identical. A change of the preferred site of protonation takes place in solvents containing heteroatoms (except tetrachloromethane). Under the same conditions, a variation of the tautomeric preference in the monocation occurs. The ring $N^2$-protonated form ($ImH^+$)—favored in gas phase—is also preferred in non-polar solvents (*n*-heptane, benzene, tetrachloromethane). The $ImH^+$ form becomes less important in more polar solvents. In such a case, the chain $N^3$-protonated form ($AmH^+$-$T_1$) predominates. For the neutral histamine, solvation has a relatively small influence on the relative energies (variations are less than 1 kcal mol$^{-1}$), and does not change the tautomeric preference (HA-$T_2$). Calculated basicity parameters were compared with those obtained experimentally in the gas phase and in aqueous solution. In the gas phase, the experimental ('macroscopic') basicity parameter (*PA*) is close to the 'microscopic' *PA* calculated for the *gauche* conformation. In aqueous solution, the microscopic p$K_a$ order is similar to that of the $E_{prot}$ calculated for the *trans* conformation. In the solid state, both forms of histamine (neutral and monoprotonated) prefer the *trans* conformation. Some exceptions occur for complexes with metals. Copyright © 2003 John Wiley & Sons, Ltd.
*Additional material for this paper is available from the epoc website at http://www.wiley.com/epoc*

KEYWORDS: histamine; basicity; tautomerism; medium effects (gas phase, solution, solid state)

## INTRODUCTION

In the course of our studies on the proton-transfer reactions involving compounds containing the formamidine group (>N—CH═N—),[1–5] it has been found that bifunctional amidinamines containing the amidine and alkylamino groups [$R_2N$—CH═N$(CH_2)_n$N$R'_2$] possess particular properties in the gas phase. Two basic groups (the *N*-imino in the amidine group and the *N*-amino in the heteroalkyl chain) separated by a flexible polymethylene chain may chelate the proton,[1c,5] similarly to diamines [$R_2N(CH_2)_nNR'_2$].[6,7] This effect strongly stabilizes the cyclic conformation of the monocation and augments the gas-phase basicity of the bifunctional ligand by 5–20 kcal mol$^{-1}$ (1 cal = 4.184 J) in comparison with the corresponding monofunctional base [$R_2N$—CH═N$(CH_2)_n$H or $R_2N(CH_2)_n$H].

A similar chelation of the proton and a strong increase in the gas-phase basicity [by 11 kcal mol$^{-1}$ in comparison with 4(5)-methylimidazole] have been observed for histamine {2-[4(5)-imidazole]ethylamine}[5,7,8]—a biogenic amine containing the formamidine group in the imidazole ring and the amino group in the heteroalkyl side chain—formed by enzymatic decarboxylation of histidine. The proton is bonded to the ring *N*-imino (the most basic site in the gas phase) and to the chain *N*-amino group by formation of an intramolecular hydrogen bond.[5,8] The

**Correspondence to:* E. D. Raczyńska, Department of Chemistry, Agricultural University (SGGW), ul. Nowoursynowska 159c, PL-02-776 Warsaw, Poland. E-mail: raczynskae@delta.sggw.waw.pl
†This work is dedicated to Prof. Tadeusz M. Krygowski (Department of Chemistry, Warsaw University, Poland).

Copyright © 2003 John Wiley & Sons, Ltd.

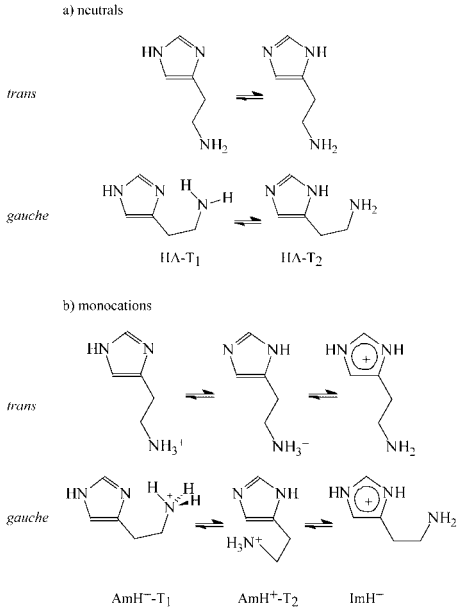



**Figure 1.** The *trans* and *gauche* conformations for (a) neutral histamine and (b) ionic forms of histamine protonated on the imidazole ring ($ImH^+$) and on the amine side-chain ($AmH^+$)

flexible heteroalkyl side-chain has the possibility of adopting a 'scorpio' (*gauche*) conformation,[8a] which stabilizes the monoprotonated form (*gauche*-$ImH^+$ in Fig. 1).

The situation is completely different in solution.[9] For histamine in water, the chain *N*-amino is first protonated and the protonated group has no possibility of interacting with the other basic group in the ring (*N*-imino) owing to strong interactions with water molecules.[9a] The p$K_a$ of histamine (9.8 at 25 °C—value recommended by IUPAC)[9c] is not different from that of 2-phenylethylamine (9.8 at 25 °C).[10] Many reports of IR, Raman and NMR spectra of the monocation have appeared in the literature, but their interpretations have not led to converging conclusions on the structure of histamine in solution. IR and Raman spectra indicated that the monocation prefers only 'essential' (*trans*) conformation (*trans*-$AmH^+$-$T_1$ in Fig. 1).[9e] However, both conformations (*trans*- and *gauche*-$AmH^+$) have been identified in $^1$H NMR spectra.[11]

This complex situation on proceeding from the gas phase to aqueous solution encouraged us to undertake investigations on the structure and proton-transfer reactions in histamine. This bioamine is considered one of the most important mediators of allergy and inflammation.[12] It is a chemical messenger and a neurotransmitter playing a variety of roles in different tissues. The effects are exerted by interaction with histamine receptors, four of which ($H_1$–$H_4$) have been discovered to date.[12–20] All of them are members of the G-coupled receptor family and display seven transmembrane domain structures, with the N-terminus outside the cell and the C-terminus in the cytoplasm. The pharmacology of these receptors differs among animal species.[12–15] Most research has been done on one of the best characterized among them, the $H_2$ receptor. Here, the histamine monocation ($AmH^+$-$T_1$) in the *trans* conformation is the main form in physiological conditions at pH 7.4.[9,13–16] The 4-position of the ring is a requisite for changing selectivity between the $H_1$ and $H_2$ receptors.[15] Methyl group(s) or the hydrogen alone give the $H_2$ agonists. Electron-accepting groups in 4-position suppress this effect by shifting the tautomeric preference from the $AmH^+$-$T_1$ form to the $AmH^+$-$T_2$. Analysis of the $H_3$ receptor indicated that it is less hydrophilic than $H_2$ receptor, and is characterized by moderately negatively charged regions.[19] Here, histamine can take the *gauche* conformation. The $H_4$ receptor, which has been discovered only recently,[20] is one of the least studied. X-ray crystallographic measurements performed on complexes of histamine with several histamine-binding proteins (isozymes, enzymes, nitrophorins and other proteins) indicated that in all cases histamine prefers the *trans* conformation.[21] The complexity of the structures and their experimental quality do not allow a detailed analysis of the tautomeric preference; however, Paesen and co-workers[21c,d] suggested that histamine probably has its dicationic form in the binding center of the investigated histamine-binding protein (Ra-HBP2) owing to the strongly acidic microenvironment of this center.

Although numerous studies on histamine, its analogues, agonists and antagonists have been undertaken with the aim of defining structural characteristics of the specific receptors and to explain their interactions with histamine (more than 50 000 references in the NCBI database[15]), general relations between the structures and the biological activity of histamine have not yet been established. In fact, the high rates of the proton-transfer reactions and the high flexibility of the histamine side-chain make the search for a detailed mechanism of the histamine/receptor interactions difficult. In this regard, progress in modelling the internal and external effects, which influence the conformation and the proton-transfer reactions, will lead to a better understanding of these interactions. The changes in molecular structure induced by a more or less polar medium could be used as a guide towards a more complete picture of the microscopic events that occur when the active molecule approaches the receptor.

In the present investigations, two stable conformations were selected for the neutral and monoprotonated histamine: the 'essential' (*trans*) (found in the solid state[22] and in aqueous solution[9b,e]), and the 'scorpio' (*gauche*) conformation (proposed in the gas phase).[5b,8,23] For isolated molecules, *ab initio* calculations were performed using the HF, MP2 and DFT methods, and tautomeric and basicity center preferences were found.

To study the solvation effect on the prototropic tautomerism in the neutral histamine and its monocation, 12 solvents of different polarities (from *n*-hexane to water) and the polarizable continuum model (PCM)[24] were

Copyright © 2003 John Wiley & Sons, Ltd.

chosen. The PCM is based on the Kirkwood and Onsager model[25] of solute–solvent interactions. Although the Kirkwood and Onsager model only takes into account the physical interactions, also called non-specific solvation,[26] it has been shown that the PCM—similarly as self-consistent reaction field model (SCRF)[27]—gives a fair description of the thermodynamics of the proton-transfer reactions in polyfunctional nitrogen bases in various solvents (e.g. cyclohexane, benzene, chloroform, acetone, water).[4,28–30] Experimental separation of the specific from the non-specific interactions is a difficult task,[26c] especially for ionic systems, for which solvents of low polarity are difficult to use. In various model experiments, one type of interaction or the other may be favoured but never completely eliminated.[26] The success of the Kirkwood and Onsager model is attributed to the following facts: (i) the substrate(s) and product(s) in the tautomeric ($T_1 \rightleftharpoons T_2$) and dissociation reaction ($B_1H^+ + B_2 \rightleftharpoons B_1 + B_2H^+$) are similar from a physical point of view, and (ii) the experimental values of the dielectric function describe—in part—the specific (or chemical) interactions.

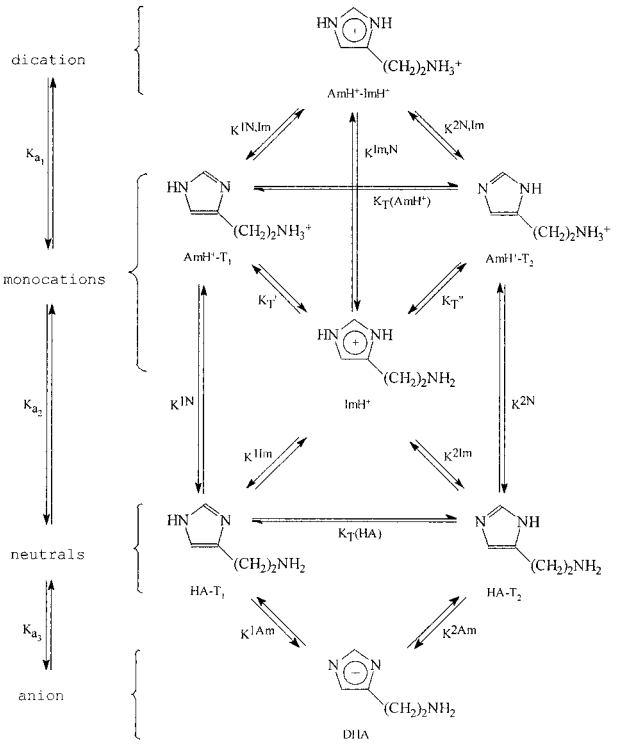
Geometries optimized at the HF/6–31G* level were used in the PCM method. Changes in the tautomery and basicity center preference were investigated on going from gas phase to aqueous solution. Calculated basicity parameters were compared with experimental data obtained in the gas phase and in aqueous solution. The variation of the tautomerism and basicity center preferences observed in solid state are also discussed.

## RESULTS AND DISCUSSION

### Proton-transfer reactions

Through the study of agonists and antagonists of histamine receptors,[15] it has been concluded that the transfer of the proton plays an important role in the interactions of histamine with specific receptors. Three binding sites, which form a proton-transfer system, have been proposed in the literature for interactions of histamine (*trans*-$AmH^+$-$T_1$) with the $H_2$ receptor: site I binding the $NH_3^+$ group in the side-chain, site II binding the ring NH group and site III binding the ring *N*-aza atom (see Fig. 6 in Ref. 16a). A model was constructed with hydroxyl anion, ammonia and ammonium at sites I, II and III, respectively. It has been shown that the proton transfer in the amidine moiety of the imidazole ring depends strongly on some kind of interaction of the monocation with a negatively charged group.[16a] Further studies on the catalytic triad of serine proteases model (Ser, His, Asp) led to a model structure for binding sites corresponding to Asp, Asp and Thr residues, respectively.[17] The same behavior has been observed for the appropriate agonists and antagonists (cimetidine, impromidine, burimamide, metiamide).[14–16] The presence of the tautomeric amidine moiety in the imidazole ring has not been found obligatory for the $H_1$ receptor.[13–15] Most of its agonists and antagonists possess the aromatic ring(s) with the aza group (five- or six-membered ring) and the alkylamino group in the side-chain (mepyramine, tripelenamine, chlorpheniramine, 2-aminoethylpyridine).[13–15] Studies with mutant $H_1$ receptors showed that antagonists bind to specific amino acid residues in the transmembrane domains 3 and 5.[18] Substitution of histamine by methyl groups changes its activity. For example, $\alpha$-methylhistamine displays a selective activity toward the $H_3$ receptor, which is especially pronounced with the *R*-enantiomer.[13,14b,15] Designed agonists and antagonists of the $H_3$ receptor include imetit, clobenpropit, iodophenpropit, thioperamide and immepip.[14b,15] There are also reports of activation of the $H_4$ receptor by both an $H_3$ agonist and antagonist (*R*)-$\alpha$-methylhistamine and clobenpropit.[15]

From the chemical point of view, histamine (HA) is a polyfunctional compound, containing three nitrogen atoms: the amino ($N^1$) and the imino ($N^2$) nitrogens in the imidazole ring and the amino ($N^3$) nitrogen in the side-chain. Two nitrogens ($N^2$ and $N^3$) are potential basic sites and one nitrogen ($N^1$) bears an acidic hydrogen. Similarly to 4(5)-substituted imidazoles, histamine exhibits a prototropic tautomerism. Two tautomeric forms are thus possible for the neutral histamine (HA-$T_1$ and HA-$T_2$). The acid–base equilibria (Scheme 1) are therefore more complicated for histamine[13,29] than for monofunctional nitrogen derivatives such as primary amines or




**Scheme 1.** Proton-transfer reactions in histamine

Copyright © 2003 John Wiley & Sons, Ltd.

pyridines.[10,31] Monoprotonation of the tautomeric mixture of HA leads to the corresponding mixture of three different protonated tautomers ($ImH^+$, $AmH^+$-$T_1$ and $AmH^+$-$T_2$). Many researchers considered only two (instead of three) monocationic forms, $AmH^+$-$T_1$ and $AmH^+$-$T_2$, that are preferred in aqueous solution. [9d,16a,b,32] Recent gas-phase basicity measurements for free histamine indicated that the $ImH^+$ form should not be omitted. Moreover, it should be considered as favoured in the gas phase.[5a,b,8] For these reasons, we have considered three protonated tautomers. Diprotonation of the tautomeric mixture of histamine leads to only one dication ($AmH^+$-$ImH^+$). For the sake of completeness, we have also considered the deprotonation of the ring NH group, which leads to one anion (DHA).[9,13,16]

In aqueous solution, histamine exists as an equilibrium mixture of seven species: two neutral (HA-$T_1$ and HA-$T_2$) and five ionic forms (one dication, $AmH^+$-$ImH^+$, three monocations, $AmH^+$-$T_1$, $AmH^+$-$T_2$ and $ImH^+$, and one anion, DHA). Between the corresponding pairs of these species, nine microscopic dissociation reactions and four tautomeric equilibria may be considered. Similar acid–base and tautomeric equilibria may be present in the gas phase. However, the direct observation of all these proton-transfer reactions (Scheme 1) in the gas phase is not possible with current gas-phase techniques, as is possible in solution at different pH using various techniques (potentiometry, IR, Raman, NMR).[9,11] It is noteworthy that the gas-phase structures of both neutral histamine tautomers have been investigated by microwave spectrometry[23] and the gas-phase basicity of histamine has been measured by ion cyclotron resonance mass spectrometry.[5a,8b] Therefore, the quantum-chemical treatment of the complete set of structures is very helpful (i) for examining each neutral and protonated histamine species and (ii) for assessing each equilibrium connecting the species.

## Selected conformations

Since the ethylamino side-chain in histamine is very flexible, different conformations are possible for its neutral and ionic forms.[5b,8,9d,e,13,23,29] Rotation may take place around three single bonds: C(ring)—C(chain), C(chain)—C(chain) and C(chain)—N(chain). The presence of three H-bond donor or acceptor groups in the histamine skeleton and the charge in the ionic forms is supposed to influence strongly the conformation of the side-chain. In particular, we expect large changes in the rotational angles of the most stable conformers on going from the neutral to ionic forms and from the gas phase to solution.

Two stable conformations were selected: 'essential' (*trans*) and 'scorpio' (*gauche*) conformations for both the neutral and protonated forms of histamine (Fig. 1; Table E1 and more details on their geometric parameters are given in supplementary material available at the epoc website at http://www.wiley.com/epoc). The main reasons for this selection are as follows. First, the *trans* conformation has been found in the solid state for free histamine, its ionic forms (mono- and dication) and its complexes with various proteins.[21,22] It has also been identified in solution.[9,11] The *gauche* conformation has mainly been observed in the gas phase.[5,8,23] There are also some reports on its presence in solution.[9d,11] Moreover, the $AmH^+$-$T_1$ tautomer in the *trans* ('essential')[9b] conformation has been proposed to be a crucial structure for histamine activity with the $H_2$ receptor.[15,16] As for the role of the side-chain and the intramolecular hydrogen bond formation in the *gauche* ('scorpio') conformation, it has been observed that $\alpha$-methylhistamine displays a selective activity with the $H_3$ receptor.[13,14b,15]

## Aromaticity of the imidazole ring

Aromaticity of the imidazole ring is one of the very important structural properties of histamine, that influences its biological activity. The mechanism of interactions of histamine with specific receptors is not yet well known; however, the structures of other compounds, which cause reactions similar to that caused by histamine, have been described.[14,15] Most of the agonists and antagonists of the histamine receptors contain the imidazole or other aromatic ring with (or even without) the aza group. This ring may interact with the binding site of the histamine receptor. Depending on the type of receptor ($H_1$, $H_2$, $H_3$ or $H_4$), its pocket is less or more hydrophobic, hence the interactions of the aromatic (hydrophobic) fragment with the receptor are more or less important. This is the main reason why the aromaticity of the imidazole ring was considered in this paper.

In all calculated structures, the imidazole fragment is highly planar with no difference for the protonated ($AmH^+$-$T_i$, $ImH^+$) and unprotonated tautomers (HA-$T_i$): the mean least-squares deviation from the best plane does not exceed 0.0037 Å and most often it is smaller than 0.001 Å. The same was observed for the experimental geometries of the ring in the histamine free base and in all its salts and complexes.[13,22,33,34]

Importantly, neither the conformational changes of the side-chain nor the protonation leading to the $AmH^+$ and $ImH^+$ structures affect the cyclic $\pi$-electron ring structure appreciably. Table 1 presents the quantitative descriptors of aromaticity: HOMA (Harmonic Oscillator Model of Aromaticity) [the HOMA is a geometry-based index defined as follows: $\mathrm{HOMA} = 1-\alpha/n \sum (d_{opt})-d_i)^2$, where $n$ is the number of bonds taken into account, $\alpha$ represents a normalization constant (fixed to give HOMA = 0 for the non-aromatic system and HOMA = 1 for the system with all bonds equal to the optimal value), $d_{opt}$ is the optimum bond length (assumed to be realized when full delocalization of $\pi$-electrons occurs) and $d_i$ are the running bond

Copyright © 2003 John Wiley & Sons, Ltd.

**Table 1.** HOMA and NICS(1) calculated for histamine species by the GIAO/HF/6–31 + G* method[66]

| Tautomer[a] | Conformation | HOMA | NICS(1) |
|---|---|---|---|
| HA-$T_1$ | *trans* | 0.83 | −10.5 |
| | *gauche* | 0.83 | −10.4 |
| $AmH^+$-$T_1$ | *trans* | 0.85 | −10.5 |
| | *gauche* | 0.83 | −10.4 |
| HA-$T_2$ | *trans* | 0.82 | −10.4 |
| | *gauche* | 0.85 | −10.3 |
| $AmH^+$-$T_2$ | *trans* | 0.84 | −10.5 |
| | *gauche* | 0.86 | −10.3 |
| $ImH^+$ | *trans* | 0.78 | −10.1 |
| | *gauche* | 0.80 | −10.1 |

[a] Geometries taken from Table E1 (supplementary material).

lengths in the ring][35] and NICS (Nucleus Independent Chemical Shift) (the NICS is a magnetic index defined as the negative value of the absolute magnetic shielding computed in the center of the ring; a negative values of NICS points to an aromatic system)[36] calculated for geometries from Table E1 (supplementary material). It is expected[37] that among many easily accessible quantitative definitions of aromaticity,[38] these two models are the most efficient for an accurate description of stabilization energies due to cyclic $\pi$-electron delocalization. Recently, it has been shown[39] that NICS calculated 1 Å above the ring center [denoted NICS(1)] serves much better as a descriptor of the $\pi$-electron delocalization than NICS calculated at the center of the ring.

The HOMA values do not differentiate between HA-$T_1$ and HA-$T_2$ tautomers and their protonated forms $AmH^+$-$T_1$ and $AmH^+$-$T_2$, indicating in all cases the high $\pi$-electron delocalization in the imidazole fragment (HOMA is in the range 0.82–0.86). This is in excellent agreement with the HOMA mean value based on experimentally determined histamine derivatives,[13] where it amounts to 0.85 ($\pm$0.05) with no significant changes due to protonation or complexation. The high and not differentiated aromaticity is also nicely supported by NICS(1), which is highly negative, and varies insignificantly from −10.3 ppm (for the *gauche* conformer of HA-$T_2$ and $AmH^+$-$T_2$) to −10.5 ppm (for the *trans* conformer of HA-$T_1$, $AmH^+$-$T_1$ and $AmH^+$-$T_2$). A small lowering of aromaticity is observed for $ImH^+$ in both the *trans* and *gauche* conformations. HOMA = 0.78 in the former case and 0.80 in the latter, while NICS(1) drops to −10.1 in both cases. For comparison, the protonation of pyridine results in a greater decrease in the aromatic character [ca 1 ppm as indicated by NICS(1)]. Hence it may be concluded that the changes in aromaticity are very subtle. This means that the cyclic $\pi$-electron delocalization stabilizes the imidazole fragment of histamine and its protonated derivatives in a very similar way.

## Tautomeric preferences in gas phase

Extended *ab initio* calculations (including thermal corrections) were performed using the HF method and the 6–31G* basis set.[40] This basis set gives almost the same relative energies between histamine tautomers as those with diffuse functions (e.g. HF/6–31++G**).[9d,29a,41] The use of the second-order Möller–Plesset perturbation[42] and the density functional B3LYP[43] does not lead to large changes in $\Delta E$ (Table 2).[9d,23,29a,41,44] The differences in the relative energies are not larger than 1 kcal mol$^{-1}$ for neutral histamine, and 3 kcal mol$^{-1}$ for the monocation. These differences, however, do not affect qualitatively the calculated tautomeric preferences.

The *ab initio* calculations (Tables 2 and 3) predict that the $T_2$ tautomer is favoured (by 2–2.6 kcal mol$^{-1}$) for the most stable *gauche* conformation in the isolated neutral histamine. This result is in qualitative agreement with the conclusions of a study of the rotational spectrum recorded for neutral histamine ($\Delta G = 0.7$ kcal mol$^{-1}$ between the *gauche* tautomers; $\Delta G$ derived from their mole fractions in the jet after expansion from 130 °C).[23]

**Table 2.** Relative energies (kcal mol$^{-1}$) between the neutral histamine tautomers ($\Delta E$)[a] and monocations [$\Delta E$(1–2)[b] and $\Delta E$(1–3)[c]] calculated using the HF, MP2 and DFT methods on geometries optimized at the HF/6–31G* level

| | *trans* | | | *gauche* | | |
|---|---|---|---|---|---|---|
| Method | $\Delta E$ | $\Delta E$(1–2) | $\Delta E$(1–3) | $\Delta E$ | $\Delta E$(1–2) | $\Delta E$(1–3) |
| HF/6–31G*//6–31G* [d,e] | −0.8[f] | −11.5 | 3.6 | 2.3 | −19.7 | 3.2 |
| HF/6–311++G**//6–31G* [d] | −0.5 | −11.7 | 3.9 | 2.0 | −19.4 | 2.9 |
| MP2/6–31G*//6–31G* [d] | −0.9 | −12.0 | 3.6 | 2.6 | −21.5 | 3.2 |
| MP2/6–311++G**//6–31G* [d,e] | −0.9 | −11.9 | 1.2 | 2.5 | −20.4 | 0.2 |
| DFT/6–31G*//6–31G* [e] | −0.7 | −10.9 | 3.6 | 2.1 | −21.0 | 1.5 |
| DFT/6–311++G**//6–31G* [e] | −1.1 | −11.3 | 4.5 | 2.0 | −20.7 | 2.0 |

[a] $\Delta E = E$(HA-$T_1$) − $E$(HA-$T_2$).
[b] $\Delta E$(1–2) = $E$($AmH^+$-$T_1$) − $E$($AmH^+$-$T_2$).
[c] $\Delta E$(1–3) = $E$($AmH^+$-$T_1$) − $E$($ImH^+$).
[d] As in Ref. 29a.
[e] This work.
[f] For 4(5)-methylimidazole $\Delta E = -0.2$ kcal mol$^{-1}$ [45] (M. Darowska and M. Makowski, unpublished results).

Copyright © 2003 John Wiley & Sons, Ltd.

**Table 3.** Thermodynamic parameters ($\Delta E$, ΔZPVE, $\Delta H^\circ$, $\Delta G^\circ$ in kcal mol$^{-1}$)[a] and tautomeric equilibrium constants (p$K_T$) for tautomerization process in the *trans* and *gauche* conformations of the neutral histamine and its monocation (Fig. 1) calculated at the HF/6–31G*//6–31G* level (at 298.15 K and 1 atm)

| | | Thermodynamic parameter | | | | |
|---|---|---|---|---|---|---|
| Conformation | Pair of tautomers | $\Delta E$ | ΔZPVE | $\Delta H^\circ$ | $\Delta G^\circ$ | p$K_T$ |
| *trans* | HA-$T_1$, HA-$T_2$[b] | −0.8 | −0.2 | −0.9 | −0.9 | −0.7 |
| | $AmH^+$-$T_1$, $AmH^+$-$T_2$[c] | −11.5 | 0.1 | −11.4 | −10.9 | −8.0 |
| | $AmH^+$-$T_1$, $ImH^+$[c] | 3.6 | 1.1 | 4.7 | 4.5 | 3.3 |
| *gauche* | HA-$T_1$, HA-$T_2$ | 2.3 | −0.3 | 2.1 | 1.7 | 1.3 |
| | $AmH^+$-$T_1$, $AmH^+$-$T_2$[c] | −19.7 | 0.0 | −19.7 | −19.0 | −13.9 |
| | $AmH^+$-$T_1$, $ImH^+$[c] | 3.2 | 0.7 | 3.9 | 3.8 | 2.8 |

[a] $\Delta E$ (relative Gibbs energies), ΔZPVE (relative zero point energies), $\Delta H^\circ$ (relative enthalpies), $\Delta G^\circ$ (relative free energies).
[b] For 4(5)-methylimidazole the following values were found[45b] (M. Darowska and M. Makowski, unpublished results): $\Delta E = -0.2$, $\Delta H^\circ = -0.1$, $\Delta G^\circ = -0.25$ and p$K_T = -0.2$.
[c] As in Ref. 29b.

In the monocationic mixture, the $ImH^+$ tautomer predominates for the same most stable *gauche* conformation. There is no experimental evidence on the gas-phase tautomeric preference in the monocationic mixture. However, the exceptionally high basicity ($GB$ = 229.9 kcal mol$^{-1}$)[7] determined independently in two laboratories for histamine by means of Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry[5a,8b] can only be explained by the chelation effect of the proton by two basic nitrogens ($N^2$ and $N^3$), among which the ring $N^2$ is the most basic site. This chelation is possible in the *gauche* conformation of the $ImH^+$.[5b,8] The $AmH^+$-$T_2$ tautomer is the less stable for both the *trans* and *gauche* conformations (by more than 10 kcal mol$^{-1}$ in comparison with the $AmH^+$-$T_1$), hence it can be neglected in the gas phase. Only two tautomers, $ImH^+$ and $AmH^+$-$T_1$, and the proton transfer between the basic nitrogens can be considered in the gas phase. The *gauche* conformation of both tautomers is more stable than the *trans* conformation by ca 12 kcal mol$^{-1}$ owing to possible intramolecular hydrogen bonding between the free and protonated basic functions.

The same conclusion has been derived previously on the basis of other quantum-chemical calculations (RHF with use of different basis sets from STO-3G to 6–311++G**).[29a] The good agreement between *ab initio* calculations and the experimental results may be explained by the fact that the proton is transferred between atoms of the same element, from the amino to the imino nitrogen atom.[29] The ZPVE and other thermal corrections are almost the same for individual pairs of tautomers, hence these corrections are close to zero for the proton-transfer process, particularly for the prototropic tautomerism in the imidazole ring ($T_1 \rightleftharpoons T_2$). The same behaviour has been found for 4(5)-methylimidazole[45] (M. Darowska and M. Makowski, unpublished results). For the proton transfer between the ring and chain nitrogen atoms, the ZPVE is slightly larger (ca 1 kcal mol$^{-1}$) owing to the difference in the bonding properties of nitrogen atoms.

## Tautomeric preferences on going from gas phase to solution

For a better understanding of the effects of solvation on the position of the tautomeric equilibria, when the gas-phase species are transferred into a solvent, the PCM method was applied to geometries optimized at the HF/6–31G* level and to solvents of different polarities (from *n*-heptane to water). The calculated relative energies between the neutral tautomers ($\Delta E$) and separately between the ionic forms [$\Delta E$(1–2) and $\Delta E$(1–3)] in gas phase and 12 solvents are given in Tables 4 and 5, respectively. For comparison, relative energies between 4- and 5-methylimidazoles obtained in the same conditions are also listed in Table 4. The variations of the

**Table 4.** Relative total energies between neutral histamine (HA) and 4(5)-methylimidazole (MI) tautomers ($\Delta E$ in kcal mol$^{-1}$)[a] in gas the phase and solution calculated using the PCM model

| | | | $\Delta E$ | | |
|---|---|---|---|---|---|
| Phase | $\varepsilon_r$[b] | $(\varepsilon_r-1)/(2\varepsilon_r+1)$[c] | MI[d] | HA-*trans*[d] | HA-*gauche*[d] |
| Gas[e] | 1.000 | 0.000 | −0.2 | −0.8 | 2.3 |
| *n*-Heptane | 1.920 | 0.190 | 0.1 | −0.5 | 2.2 |
| $CCl_4$ | 2.228 | 0.225 | 0.1 | −0.5 | 2.1 |
| Benzene | 2.247 | 0.227 | 0.1 | −0.5 | 2.1 |
| $CHCl_3$ | 4.900 | 0.361 | 0.3 | −0.3 | 1.9 |
| THF | 7.580 | 0.407 | 0.4 | −0.2 | 1.8 |
| $CH_2Cl_2$ | 8.930 | 0.420 | 0.4 | −0.2 | 1.8 |
| MeCOMe | 20.700 | 0.465 | 0.4 | −0.1 | 1.7 |
| EtOH | 24.550 | 0.470 | 0.4 | −0.1 | 1.7 |
| MeOH | 32.630 | 0.477 | 0.5 | −0.1 | 1.7 |
| $MeNO_2$ | 38.200 | 0.481 | 0.5 | −0.1 | 1.7 |
| DMSO | 46.700 | 0.484 | 0.5 | −0.1 | 1.7 |
| $H_2O$ | 78.390 | 0.490 | 0.5 | 0.0 | 1.6 |

[a] $\Delta E = E(T_1) - E(T_2)$ as in Ref. 29a.
[b] Relative dielectric permittivity (dielectric constant),[26c] values as in the GAMESS program for the PCM method.[64]
[c] Kirkwood and Onsager function.[25]
[d] Geometries optimized at the HF/6–31G* level.
[e] Taken from Table 2.

Copyright © 2003 John Wiley & Sons, Ltd.

**Table 5.** Relative total energies between monoprotonated histamine tautomers [$\Delta E$(1–2) and $\Delta E$(1–3) in kcal mol$^{-1}$][a] in the gas phase and solution calculated using the PCM model and geometries optimized at the HF/6–31G* level

| | $\Delta E$(1–2) | | $\Delta E$(1–3) | |
|---|---|---|---|---|
| Phase | *trans* | *gauche* | *trans* | *gauche* |
| Gas[b,c] | −11.5 | −19.7 | 3.6 | 3.2 |
| *n*-Heptane | −7.7 | −14.2 | 1.4 | 1.5 |
| $CCl_4$[d] | −6.9 | −13.2 | 1.0 | 1.1 |
| Benzene[d] | −6.9 | −13.2 | 1.0 | 1.1 |
| $CHCl_3$[d] | −4.1 | −9.2 | −0.5 | −0.2 |
| THF[d] | −3.3 | −7.9 | −1.0 | −0.6 |
| $CH_2Cl_2$ | −2.8 | −7.4 | −1.2 | −0.8 |
| MeCOMe[d] | −1.7 | −6.1 | −1.6 | −1.2 |
| EtOH | −1.8 | −5.9 | −1.7 | −1.3 |
| MeOH | −1.6 | −5.6 | −1.8 | −1.4 |
| $MeNO_2$ | −1.6 | −5.6 | −1.8 | −1.4 |
| DMSO | −1.6 | −5.5 | −1.8 | −1.4 |
| $H_2O$[c] | −1.3 | −5.2 | −2.0 | −1.6 |

[a] $\Delta E(1\text{–}2) = E(\text{AmH}^+\text{-T}_1) - E(\text{AmH}^+\text{-T}_2)$, $\Delta E(1\text{–}3) = E(\text{AmH}^+\text{-T}_1) - E(\text{ImH}^+)$ as in Ref. 29a,b.
[b] Taken from Table 2.
[c] As in Ref. 29a.
[d] As in Ref. 29b.

dipole moments of histamine species in selected solvents are listed in Table E2 (supplementary material).

In the case of the neutral histamine tautomers (HA-$T_1$ and HA-$T_2$), the $\Delta E$ calculated for the *trans* conformation varies from −0.8 kcal mol$^{-1}$ in the gas phase to 0.0 kcal mol$^{-1}$ in aqueous solution. This change is small, but the equalization of the total energies of individual tautomers indicates that the interactions of functional groups in neutral histamine with water molecules reduce drastically the small effect of the side-chain manifested in gas phase. Similar changes are found for the *gauche* conformation of the neutral histamine. The calculated $\Delta E$ varies by 0.7 kcal mol$^{-1}$ on going from gas phase ($\Delta E = 2.3$ kcal mol$^{-1}$) to aqueous solution ($\Delta E = 1.6$ kcal mol$^{-1}$). This suggests that the variation of the $\Delta E$ does not depend on the conformation of the ethylamino side-chain in histamine, and that interactions of the functional groups in neutral *gauche*-histamine with solvent molecules decrease the intramolecular differences in the transmission of the internal effects to the same degree as for the *trans* conformation. Similar changes in the relative energy in 4(5)-methylimidazoles ($\Delta E$ varies by 0.7 kcal mol$^{-1}$ on going from the gas phase to aqueous solution) show additionally that interactions of neutral histamine and 4(5)-methylimidazole with solvent molecules may be similar.

Small differences in the total energies between the individual tautomers of the neutral histamine in the gas phase and also in aqueous solution (see details in the supplementary material) indicate that both tautomers $T_1$ and $T_2$ in the *trans* and *gauche* conformations (with a slight preference for the $T_2$ tautomer) may be present in quantities which could be identified by experimental techniques. This may explain the discrepancies in the interpretation of the NMR spectra of neutral histamine, in which only conformational or only tautomeric differences have been considered instead of both the tautomeric and rotational differences.[46] The general tautomeric preference ($T_2$) in the neutral histamine mixture found by the PCM method in aqueous solution [similar to that observed for 4(5)-methylimidazole] is in agreement with an empirical estimation ($\Delta G = 0.2$ kcal mol$^{-1}$, $pK_T = 0.15$) based on the Hammett equation found for 4(5)-substituted imidazoles.[13,47] The $T_2$ tautomer has also been found to be favoured in the solid state.[22a]

For the different monocationic histamine species protonated at the side-chain ($AmH^+$) or at the imidazole ring ($ImH^+$), larger variations of the relative total energies were observed than for the neutral histamine. This is due to a higher polarity (larger dipole moment $\mu$ values, Table E2 in supplementary material) for the ionic forms, particularly the $AmH^+$-$T_2$, than for the neutral tautomers.

The $\Delta E$(1–2) value calculated between the $T_1$ and $T_2$ tautomers of the $N^3$-amino protonated histamine ($AmH^+$) varies by 10.2 and 14.5 kcal mol$^{-1}$ for the *trans* and *gauche* conformations, respectively, on going from the gas phase to aqueous solution, i.e. from −11.5 to −1.3 kcal mol$^{-1}$ for the *trans* conformation and from −19.7 to −5.2 kcal mol$^{-1}$ for the *gauche* conformation. The variations are very large owing to interactions of the charged ethylamino side-chain in the $AmH^+$ with the polar solvent molecules.[29a,b] The reaction field, modeled by PCM, reduces the transmission of the electronic field originating in the positive charges, and thus leads to a strong attenuation of the intramolecular differences in the transmission of the internal effects in both tautomers. The different variation of the $\Delta E$(1–2) for the *trans* (10.2 kcal mol$^{-1}$) and *gauche* conformation (14.5 kcal mol$^{-1}$) on going from gas phase to aqueous solution indicates that the solvation effects depend slightly on the conformation of $AmH^+$.

Smaller changes are found for the relative energies between $AmH^+$-$T_1$ and $ImH^+$tautomers for both the *trans* and *gauche* conformations of the monoprotonated histamine. This is due to a smaller dipole moment (Table E2 in supplementary material) of the $ImH^+$ than of the $AmH^+$-$T_2$.[29a,b] The calculated $\Delta E$(1–3) varies by 5.6 and 4.8 kcal mol$^{-1}$, respectively, on going from the gas phase to aqueous solution, i.e. from 3.6 to −2.0 kcal mol$^{-1}$ for the *trans* conformation and from 3.2 to −1.6 kcal mol$^{-1}$ for the *gauche* conformation. These variations indicate that the difference between the basicity of the ring *N*-imino and the chain *N*-amino groups is not very large and relatively weakly dependent on the conformation of the protonated forms.

A change in the sign of $\Delta E$(1–3) on going from the gas phase (positive) to aqueous solution (negative) indicates that the favoured site of protonation is changed by solvation. The ring *N*-imino is only favoured in the gas

Copyright © 2003 John Wiley & Sons, Ltd.

phase and solvents of low dielectric constants ($\varepsilon_r < 2.4$, e.g. *n*-heptane, benzene and $CCl_4$). The chain *N*-amino predominates in more polar solvents ($\varepsilon_r > 4.5$). Such kinds of solvents, interacting by their dipole moment with histamine species, reduce the polarizability effect of the imidazole ring, decrease the basicity of the *N*-imino group and, in consequence, change the favored site of protonation. This behavior is common for both conformations (*trans* and *gauche*). The monocation prefers the $ImH^+$-H form in the gas phase and solvent of low dielectric constant and the $AmH^+$-$T_1$ form in more polar solvents (for more details, see the supplementary material). A similar change in basicity order has been observed earlier for monofunctional $sp^2$ and $sp^3$ nitrogen bases (e.g. pyridines and amines) on going from the gas phase to aqueous solution.[48] Exceptionally, proton sponges with a rigid structure preserve their high basicity both in the gas phase and in solution.[49]

## Thermodynamic basicity parameters in the gas phase

The thermodynamic basicity parameters ($E_{prot}$, *PA* and *GB*) calculated for the stable *trans* and *gauche* conformations of isolated histamine at the HF/6–31G*//6–31G* level (Table 6) correspond to the partial acid–base equilibria given in Scheme 1. The comparison indicates that the *GB* of the ring *N*-imino (the favored site of protonation in the gas phase) in the HA-$T_1$ tautomer is larger than that of the chain *N*-amino by ca 4–5 kcal mol$^{-1}$ for both conformations. The difference seems to be almost independent on the conformation of the heteroalkyl side-chain. This indicates that the intramolecular interaction possible between the ring *N*-imino (H-bond acceptor) and the chain $NH_3^+$ (H-bond donor) in *gauche*-$AmH^+$-$T_1$ is similar to that between the ring $NH^+$ (H-bond donor) and the chain $NH_2$ (H-bond acceptor) in *gauche*-$ImH^+$.

**Table 6.** Microscopic thermodynamic basicity parameters (in kcal mol$^{-1}$) for *trans*- and *gauche*-histamine calculated at the HF/6–31G*//6–31G* level (at 298.15 K)

| Conformation | Microscopic reaction | Thermodynamic parameter | | |
|---|---|---|---|---|
| | | $-E_{prot}$ | *PA* | *GB* |
| *trans* | HA-$T_1$→$ImH^+$[a] | 242.3 | 231.5 | 226.8 |
| | HA-$T_2$→$ImH^+$[b] | 243.0 | 232.5 | 227.9 |
| | HA-$T_1$→$AmH^+$-$T_1$ | 238.7 | 226.9 | 222.3 |
| | HA-$T_2$→$AmH^+$-$T_2$ | 228.0 | 216.5 | 212.5 |
| *gauche* | HA-$T_1$→$ImH^+$ | 253.6 | 242.6 | 237.1 |
| | HA-$T_2$→$ImH^+$ | 251.3 | 240.5 | 235.3 |
| | HA-$T_1$→$AmH^+$-$T_1$ | 250.4 | 238.7 | 233.2 |
| | HA-$T_2$→$AmH^+$-$T_2$ | 228.4 | 217.0 | 212.5 |

[a] For 4-methylimidazole the following values were found: $-E_{prot} = 243.2$, $PA = 232.2$ and $GB = 227.5$.
[b] For 5-methylimidazole the following values were found: $-E_{prot} = 243.3$, $PA = 232.4$ and $GB = 227.4$.

The interactions stabilize the *gauche*-monocations and increase *GB* by 10–11 kcal mol$^{-1}$ in comparison with the *trans*-monocations. The experimental *GB* of histamine (229.9 kcal mol$^{-1}$) is about 10 kcal mol$^{-1}$ larger than that of 4(5)-methylimidazole (220.1 kcal mol$^{-1}$).[5b,7] The *GB* value calculated at the HF/6–31G*//6–31G* level for 4-methylimidazole (227.5 kcal mol$^{-1}$) is also about 10 kcal mol$^{-1}$ lower than that calculated for the *gauche* conformer of HA-$T_1$ (237.1 kcal mol$^{-1}$) protonated at the ring *N*-imino. The *GB* value calculated for 4-methylimidazole is close to that obtained for the *trans*-HA-$T_1$ (226.8 kcal mol$^{-1}$) protonated at the ring *N*-imino. If we consider that the methyl substituent in 4(5)-methylimidazole and the ethylamino group in histamine exert both (i) a similar polarizability effect ($\sigma_\alpha = -0.35$ and $-0.52$, respectively) and (ii) a negligible field effect ($\sigma_F = 0.00$ and 0.04, respectively)[50] (calculated according to the method given in note 17 in Ref. 1c), we can conclude that the calculations at the HF/6–31G*//6–31G* level reproduce fairly well the effect of the internal hydrogen bonding stabilization in the *gauche* conformation.

A different situation is found for the HA-$T_2$ tautomer. The difference between the *GB* values of two basic groups, the ring *N*-imino and the chain *N*-amino is considerably larger and equal to ca 15 and 23 kcal mol$^{-1}$ for the *trans* and *gauche* conformations, respectively. This means that the intramolecular interaction possible in the *gauche*-$ImH^+$ between the basic function in the chain [the N-amino (H-bond acceptor)] and the acidic function in the ring [the NH-amino (H-bond donor)] is considerably stronger than the interaction of the chain $NH_3^+$ with the $\pi$-electrons of the imidazole ring in the *gauche*-$AmH^+$-$T_2$. The difference between these interactions is ca 7 kcal mol$^{-1}$.

The microscopic *PA* values calculated for the most reasonable acid–base equilibria in the gas phase, *gauche*-HA-$T_1$→*gauche*-$ImH^+$ (242.6 kcal mol$^{-1}$), *gauche*-HA-$T_2$→*gauche*-$ImH^+$ (240.5 kcal mol$^{-1}$) and *gauche*-HA-$T_1$→*gauche*-$AmH^+T_1$ (238.7 kcal mol$^{-1}$), can be compared with that obtained experimentally (239.0 kcal mol$^{-1}$).[7] Considering that protonation occurs on the most stable form, and at the imino nitrogen (*gauche*-HA-$T_2$→*gauche*-$ImH^+$), the deviation from the experimental value is only 1.5 kcal mol$^{-1}$. Therefore, we can conclude that the HF/6–31G*//6–31G* level is sufficient for the study of the proton-transfer reactions in polyfunctional nitrogen ligands and gives reasonable differences between the experimental and computed *PA* values, which lend support to the conformational preferences obtained for the neutral and ionic forms.

## Partial dissociation constants in aqueous solution

In solution, partial dissociation and tautomeric reactions given in Scheme 1 are described by the so-called partial (microscopic) dissociation ($K^i$) and tautomeric equli-

Copyright © 2003 John Wiley & Sons, Ltd.

brium constants ($K_T$). (Microscopic constants are defined as follows:[31] $K^{1N,Im} = [AmH^+\text{-}T_1][H^+]/[AmH^+\text{-}ImH^+]$, $K^{2N,Im} = [AmH^+\text{-}T_2][H^+]/[AmH^+\text{-}ImH^+]$, $K^{Im,N} = [ImH^+][H^+]/[AmH^+\text{-}ImH^+]$, $K^{1Im} = [HA\text{-}T_1][H^+]/[ImH^+]$, $K^{2Im} = [HA\text{-}T_2][H^+]/[ImH^+]$, $K^{1N} = [HA\text{-}T_1][H^+]/[AmH^+\text{-}T_1]$, $K^{2N} = [HA\text{-}T_2][H^+]/[AmH^+\text{-}T_2]$, $K^{1Am} = [DHA][H^+]/[HA\text{-}T_1]$, $K^{2Am} = [DHA][H^+]/[HA\text{-}T_2]$, and $K_T(AmH^+) = [AmH^+\text{-}T_1]/[AmH^+\text{-}T_2]$, $K_T' = [AmH^+\text{-}T_1]/[ImH^+]$, $K_T'' = [AmH^+\text{-}T_2]/[ImH^+]$ and $K_T(HA) = [HA\text{-}T_1]/[HA\text{-}T_2]$.) Unfortunately, the constants $K^i$ and $K_T$ are exceptionally difficult to determine in direct experiments because (i) the tautomerization reaction in the imidazole ring is a very fast reaction and separation of individual tautomers is impossible[31,51–53] and (ii) all histamine forms are possibly present (in different proportions and different conformations) under physiological conditions, e.g. in aqueous solution at a pH of 7.4, the composition of histamine forms is as follows: 2.4% of the $AmH^+$-$ImH^+$, 81.3% of the $AmH^+$-$T_1$, 15.1% of the $AmH^+$-$T_2$, 0.2% of the $ImH^+$, 0.4% of the HA-$T_1$, and 0.6% of the HA-$T_2$.[13] One can easily measure the so-called macroscopic dissociation constants ($K_{a_i}$).[9a,c] (Macroscopic constants are defined as follows: $K_{a1} = \{[AmH^+\text{-}T_1] + [AmH^+\text{-}T_2] + [ImH^+]\}[H^+]/[AmH^+\text{-}ImH^+]$, $K_{a2} = \{[HA\text{-}T_1] + [HA\text{-}T_2]\}[H^+]/\{[AmH^+\text{-}T_1] + [AmH^+\text{-}T_2] + [ImH^+]\}$ and $K_{a3} = [DHA][H^+]/\{[HA\text{-}T_1] + [HA\text{-}T_2]\}$.)

The relationships between the macro- and micro-constants and between the micro- and tautomeric equilibrium constants are described by Eqns (1)–(14). These relations together with measured dissociation constants for each step of the dissociation reaction and with experimental observations for tautomeric equilibria give the possibility of predicting all partial dissociation constants given in Scheme 1.[13]

$$K_{a1} = K^{1N,Im} + K^{2N,Im} + K^{Im,N} \quad (1)$$

$$1/K_{a3} = 1/K^{1Am} + 1/K^{2Am} \quad (2)$$

$$\begin{aligned} K_{a1}K_{a2} &= K^{1N,Im}K^{1N} + K^{2N,Im}K^{2N} \\ &= K^{Im,N}K^{1Im} + K^{Im,N}K^{2Im} \end{aligned} \quad (3)$$

$$\begin{aligned} 1/(K_{a2}K_{a3}) = {} & 1/(K^{1N}K^{1Am}) + 1/(K^{2N}K^{2Am}) \\ & + 1/(K^{1Im}K^{1Am})[\text{or} + 1/(K^{2Im}K^{2Am})] \end{aligned} \quad (4)$$

$$\begin{aligned} K_{a1}K_{a2}K_{a3} &= K^{1N,Im}K^{1N}K^{1Am} = K^{2N,Im}K^{2N}K^{2Am} \\ &= K^{Im,N}K^{1Im}K^{1Am} = K^{Im,N}K^{2Im}K^{2Am} \end{aligned} \quad (5)$$

$$K_T(AmH^+) = K^{1N,Im}/K^{2N,Im} \quad (6)$$

$$K_T' = K^{1N,Im}/K^{Im,N} = K^{1Im}/K^{1N} \quad (7)$$

$$K_T'' = K^{2N,Im}/K^{Im,N} = K^{2Im}/K^{2N} \quad (8)$$

$$\begin{aligned} K_T(HA) &= K^{2Am}/K^{1Am} = K^{1Im}/K^{2Im} \\ &= (K^{1N,Im}K^{1N})/(K^{2N,Im}K^{2N}) \end{aligned} \quad (9)$$

$$K^{1N,Im}K^{1N} = K^{Im,N}K^{1Im} \quad (10)$$

$$K^{2N,Im}K^{2N} = K^{Im,N}K^{2Im} \quad (11)$$

$$K^{1Im}K^{1Am} = K^{2Im}K^{2Am} \quad (12)$$

$$K_T(HA) = K_T(AmH^+)K^{1N}/K^{2N} \quad (13)$$

$$K_T(AmH^+) = K_T'/K_T'' \quad (14)$$

On the basis of the Hammett equation applied by Charton to 4(5)-substituted imidazoles[47] and Noszál and Rabenstein's NMR experiment (in water as solvent)[54] re-examined according to the equilibria given in Scheme 1,[13] one predicts tautomeric preferences analogous to those for the neutral and monoprotonated histamine [$K_T(HA) = 0.7$, $K_T(AmH^+) = 5.4$, $K_T' = 403.8$ and $K_T'' = 74.8$] to those found for the solid state,[22] i.e. the HA-$T_2$ tautomer for the neutral histamine and the $AmH^+$-$T_1$ for the monocation. Using these $K_T$ and the $K_a$ measured in aqueous solution at 25 °C ($pK_{a1} = 6.1$, $pK_{a2} = 9.8$ and $pK_{a3} = 14.4$, values recommended by IUPAC)[9c] and the relations between the micro- and macroconstants, the partial dissociation constants were obtained (Table 7).[13] The partial $pK^{1Im}$ (7.5) and $pK^{2Im}$ (7.3) corresponding to the basicity of the ring *N*-imino in the HA-$T_1$ and HA-$T_2$ are close to those found for 4- (7.8) and 5-methylimidazole (7.6) on the basis of the measured $pK_a$ (7.4)[10] and the $pK_T$ (0.2) estimated on the basis of the Charton equation.[47]

The order of the partial $pK_a$ values estimated on the basis of experimental data and corresponding to the protonation reactions of the neutral histamine on the ring *N*-imino ($pK^{1Im} = 7.5$, $pK^{2Im} = 7.3$) and chain *N*-amino ($pK^{1N} = 10.1$, $pK^{2N} = 9.2$) follow the order of the energies of protonation obtained for the *trans* conformation using the PCM model (Table 8). The $E_{prot}(aq)$ values calculated for the *trans* conformer of HA-$T_1$ ($-37.7\,kcal\,mol^{-1}$) and HA-$T_2$ ($-37.7\,kcal\,mol^{-1}$) protonated at the ring *N*-imino are close to those for 4- ($-38.9\,kcal\,mol^{-1}$) and 5-methylimidazoles ($-38.5\,kcal\,mol^{-1}$) obtained under the same conditions. The $pK_a$ value corresponding to the protonation of the chain *N*-amino (10.1) in *trans*-HA-$T_1$ is larger by 2.6 $pK_a$

**Table 7.** Microscopic dissociation constants (p*K*) derived for partial acid–base equilibria in aqueous solution (Scheme 1)[a]

| Constant | p*K* | Constant | p*K* | Constant | p*K* |
|---|---|---|---|---|---|
| $pK^{1N,Im}$ | 6.2 | $pK^{1Im}$ | 7.5 | $pK^{2N}$ | 9.2 |
| $pK^{2N,Im}$ | 6.9 | $pK^{2Im}$ | 7.3 | $pK^{1Am}$ | 14.0 |
| $pK^{Im,N}$ | 8.8 | $pK^{1N}$ | 10.1 | $pK^{2Am}$ | 14.2 |

[a] As in Ref. 13.

Copyright © 2003 John Wiley & Sons, Ltd.

**Table 8.** Microscopic energies of protonation in water [$E_{prot}$(aq) in kcal mol$^{-1}$] for *trans*- and *gauche*-histamine calculated at the PCM//HF/6–31G* and the partial p$K_a$ in water predicted from analysis of the equilibria given in Scheme 1 and experimental data (see text)

| Conformation | Microscopic reaction | $-E_{prot}$(aq) | p$K_a$(aq)[a] |
|---|---|---|---|
| *trans*[b] | HA-$T_1$→$ImH^+$ | 37.7[c] | 7.5 |
| | HA-$T_2$→$ImH^+$ | 37.7[c] | 7.3 |
| | HA-$T_1$→$AmH^+$-$T_1$ | 39.7 | 10.1 |
| | HA-$T_2$→$AmH^+$-$T_2$ | 38.4 | 9.2 |
| *gauche*[b] | HA-$T_1$→$ImH^+$ | 41.6 | |
| | HA-$T_2$→$ImH^+$ | 39.9 | |
| | HA-$T_1$→$AmH^+$-$T_1$ | 43.1 | |
| | HA-$T_2$→$AmH^+$-$T_2$ | 36.3 | |

[a] Taken from Table 7.
[b] Noszál and co-workers predicted p$K_a$ = 10.12 and 10.18 for the *trans* and *gauche* conformation, respectively, for the reaction HA→$AmH^+$.[11d]
[c] For 4- ($T_1$) and 5-methylimidazoles ($T_2$), the PCM model gave $-E_{prot}$ = 38.9 and 38.5, respectively; and from the macroscopic[10] p$K_a$ = 7.4 and p$K_T$ = 0.2 estimated on the basis of the Charton equation,[47] one found the microscopic p$K_a$ for $T_1$ and $T_2$ tautomers to be equal to 7.8 and 7.6, respectively.

units (3.5 kcal mol$^{-1}$ at 298.15 K) than that for the ring *N*-imino protonation (7.5). The p$K_a$ value corresponding to the protonation of the chain *N*-amino (9.2) in HA-$T_2$ is larger by 1.9 p$K_a$ units (2.6 kcal mol$^{-1}$ at 298.15 K) than that for the ring *N*-imino protonation (7.3). The PCM model predicts differences in absolute energies of protonation at the $N^3$ and $N^2$ atoms equal to 2 and 0.7 kcal mol$^{-1}$ for the *trans* structures of HA-$T_1$ and HA-$T_2$, respectively. These differences suggest that the *trans* conformations of both the neutral and monoprotonated forms are favored by water solvation.

## Tautomeric and basicity center preferences in the solid state

As mentioned before, free base histamine adopts the HA-$T_2$ form in the solid state.[22a] The crystal field forces the *trans* conformation of the chain residue, allowing the formation of intermolecular hydrogen bonds of moderate strength (N···N distance of 2.851 Å) between the ring $HN^1$ and the chain $N^3$, and leading to an approximately tetrahedral environment of the amino fragment. In fact, in all salts and complexes of histamine, this tendency of the amino fragment is preserved, either by protonation or by metal complexation.

The monoprotonated structure may be analyzed in histamine hydrobromide[22b] and in two more complex systems [with nickel(II) and calcium],[55,56] where histamine plays the role of a bidentate ligand. In all cases the protonation occurs at the chain $N^3$, but the monocation takes the $AmH^+$-$T_1$ form in the hydrobromide and the nickel complex, whereas the $AmH^+$-$T_2$ tautomeric form is favored in the calcium complex. The conformational preferences are also broad: the side-chain is stabilized in the *trans* conformation in the case of the hydrobromide

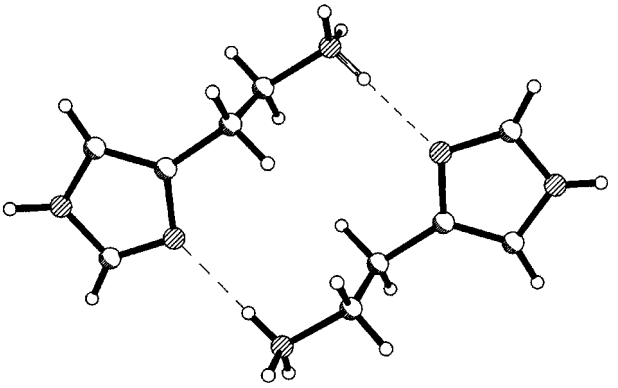


**Figure 2.** Dimer of the $AmH^+$-$T_1$ form in histamine hydrobromide taken from Refs 22b and 55. The N···N distance is equal to 2.835 Å[55]

and the calcium complex, whereas the *gauche* conformation is obtained for the nickel complex. In the latter case, the conformation is a result of an intramolecular hydrogen bond between the $NH_3^+$ group and the NCS fragment.[56] This kind of interaction is expected to stabilize this histamine monocation also in solution. In the case of the hydrobromide, the histamine cations form dimers involving the $NH_3^+$ side-group of one cation and the ring *N*-imino of another one, as shown in Fig. 2.

Structural studies reveal[13] that free base histamine forms complexes with Cu(I),[57] Cu(II),[58] Co(III),[59] Cr(III),[60] Ni(II)[61] and Pd.[62] Apart from monoprotonated histamines described above, in all other systems histamine plays the role of a bidentate ligand which forms a six-membered ring involving the *N*-amino side-chain and the ring *N*-imino, each serving as an electron pair donor to the central metal. The formation of the six-membered ring is possible only for the *gauche*-HA-$T_1$, which is taken by free histamine in almost all complexes with metals. In this conformation, the $\Phi_1$ and $\Phi_2$ angles depend strongly on the kind of the metal center, its oxidation state, the coordination number and the type of other ligands in the complex, resulting in different packing forces. In most cases [except for two copper(II) complexes],[58e,k] the imidazole ring is bound more strongly than the amine site with a mean difference between the N–metal distance $\Delta$ = 0.05 Å. It is surprising that the amino rather than the imidazole site is weakenest since the $NH_2$ group is of high p$K_a$, but not on the basis of the gas-phase basicities. Moreover, the stronger interaction with the imidazole results in a small lowering of the extent of cyclic $\pi$-electron delocalization in the imidazole ring as indicated by aromaticity descriptors. A suggestion has been made[58l] that the histamine chelate with a low-p$K_a$ imidazole binding site cannot provide enough $\sigma$-electron density to the central ion. Therefore, additional electron density may be supplied from the imidazole nucleus via $\pi$-bonds in accord with the electroneutrality principle. However, these explanations did not take into account the intrinsic basicity of the two basic centers,

Copyright © 2003 John Wiley & Sons, Ltd.

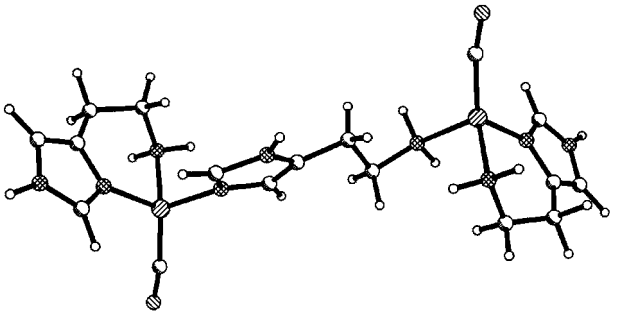


**Figure 3.** Pseudocentrosymmetric macrocomplex of $[Cu_2(HA)_3(CO)_2]^{2+}$ taken from Ref. 57

which is weaker for the amino than for the imidazole nitrogens.

Interestingly, there is one case of a macro complex of copper(I)[57] in which histamine exists in two tautomeric forms, HA-$T_1$ and HA-$T_2$, and takes two conformations, *trans* and *gauche*. The two copper(I) atoms are chelated by *gauche*-HA-$T_1$. The coordination sphere around each copper is completed by the carbon monoxide group and by *trans*-HA-$T_2$, which plays the role of a bridging ligand, as shown in Fig. 3.

In the solid state, it has been found that imidazole fragments can interact with each other[58d] (or possibly with neighboring aromatic fragments)[58g,h] by $\pi$–$\pi$ stacking interactions, which emphasizes that interactions of this kind may also be important in solution, and play an important role in biological systems. There are also well known crystal structures in which histamine can exist as a dication.[13,63] The conformation of the side-chain depends mostly in this case on the presence and strength of the intermolecular hydrogen bond network.[13] Only two of the histamine dications [the dinitrate[63g] and rutheniu-m(IV) complex[63h]] exist in the *gauche* conformation. Structures containing histamine anions have not yet been reported.

The knowledge about tautomeric, conformational and ionic structure preferences in small molecular complexes and salts can be very helpful in the interpretation and deeper analysis of the role of histamine and its interactions in biologically active systems. Recently, Paessen and co-workers published an excellent x-ray structure[21c,d] (at 1.25 Å resolution) of Ra-HBP2 (a high-affinity histamine-binding protein discovered in the saliva of *Rhipicephalus appendiculatus* ticks) complexed by two histamine molecules. Figure 4 shows interactions between protein and histamine in the site of higher affinity (H). This site, which contains four negatively charged residues (Asp-39, Glu-82, Asp-110 and Glu-135), represents a very acidic microenvironment, where histamine in the *trans* conformation is expected to take the dicationic $AmH^+$-$ImH^+$ form. In this form the imidazole fragment interacts strongly with the side-groups of Glu-82 and Asp-39 by hydrogen bonds while the amino fragment interacts with Glu-135 and Asp-110 residues.[21c,d] Importantly, the binding pocket is com-

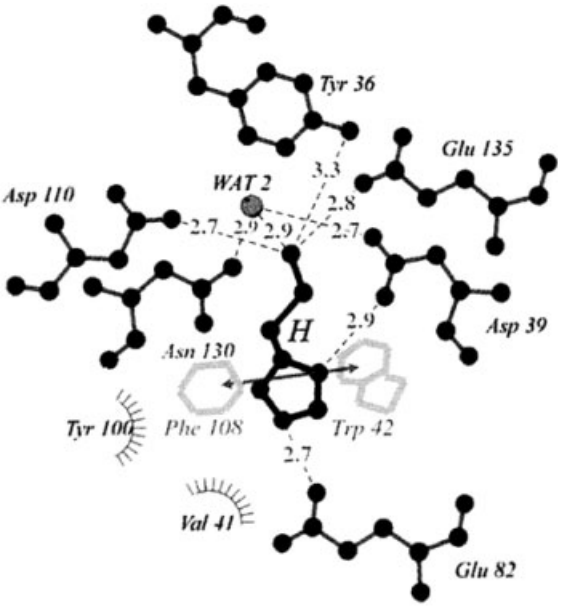



**Figure 4.** Interactions between protein and histamine at one of two binding sites (H) in Ra-HBP2.[21c,d] Reprinted from Paesen GC, Adams PL, Harlos K, Nuttall PA, Stuart DI. Tick histamine-binding proteins: isolation, cloning, and three-dimensional structure. *Molecular Cell*, 1999; **3**: 661–671. Copyright 1999, with permission from Elsevier

pleted by two aromatic fragments of Phe-108 and Trp-42, which are almost parallel to each other, and interact with the imidazole ring by $\pi$–$\pi$ stacking interactions. The significance of these interactions is very high since in a similar protein (Ra-HBP1), modified only by replacing Phe-108 by leucine, the affinity for histamine is appreciably diminished.[21d] Further detailed discussion on the tautomeric and conformational preferences of histamine in the solid state is given in the supplementary material.

## CONCLUSIONS

The comparison of *ab initio* calculations applied to the isolated histamine structures and experimental results obtained in the gas phase allowed the determination of tautomerism and basic center preferences for histamine: (i) the $T_2$ tautomer for the most stable *gauche* conformation of HA as found from the rotational spectrum recorded for neutral histamine[23] and (ii) the $ImH^+$ form for the most stable *gauche* conformation in its monocationic mixture, as derived on the basis of gas-phase basicity measurements of histamine by FT-ICR mass spectrometry;[5a,8b] its exceptionally high *GB* has been explained by a chelation of the proton similar to that observed in bidentate amidinamines.[5b]

*Ab initio* methods predicted also the same basicity center preference in the gas phase (the ring *N*-aza) as did analysis of experimental gas-phase substituent effects. 4(5)-Alkylimidazoles, which are protonated at the *N*-imino atom [e.g. methyl derivative, *GB*(exp) =

Copyright © 2003 John Wiley & Sons, Ltd.

220.1 kcal mol$^{-1}$] are stronger bases than $\beta$-arylethylamines protonated at the *N*-amino atom [e.g. 2-phenylethylamine, $GB(\text{exp}) = 215.7$ kcal mol$^{-1}$].[7] The same preferences are found using the PCM method for nonpolar solvents such as hydrocarbons and $CCl_4$ ($\varepsilon_r < 2.4$).

A change in the conformational and tautomeric preference (from *gauche*-ImH$^+$ to *trans*-AmH$^+$-T$_1$), and of the favored site of protonation (from the ring *N*-aza to the chain *N*-amino) takes place in polar solvents ($\varepsilon_r > 4.5$), e.g. $CHCl_3$, THF, DMSO, alcohols and water. This is derived on the basis of the PCM results and those obtained from an analysis of the partial equilibrium constants in the acid–base equilibria given in Scheme 1. Experimental results obtained in water confirm in part this behavior. There are no doubts that the chain *N*-amino is protonated in water, indicating the preference of the AmH$^+$ form. The measured p$K_a$ of histamine in the first step of protonation (9.8)[9c] is close to that of 2-phenylamine (9.8).[10] 4(5)-Methylimidazole has a basicity lower by 2.4 p$K_a$ units.[10] However, conclusions derived from spectral studies do not agree with regard to the conformational preference. IR and Raman spectra indicated that *trans*-AmH$^+$-T$_1$ is better solvated than the other monocations of histamine.[9d] However, two conformations (*trans* and *gauche*) for the AmH$^+$ of almost equal ratio have been identified in $^1$H NMR spectra.[11] This discrepancy between the IR, Raman and NMR conclusions on the conformational preference may be due to the fact that in NMR studies the tautomerism in the imidazole ring has been omitted, and previously reported results need reexamination.

For neutral histamine, solvation has a relatively smaller influence on the tautomer stability than for the monocation. Variations of the relative energies are $<1$ kcal mol$^{-1}$, and do not change the tautomeric preference. In all phases, gas,[23] solution[13,47] and solid state,[22a] the T$_2$ tautomer predominates in the tautomeric mixture of neutral histamine. The only change for conformational preferences occurs for HA-T$_2$: from *gauche* in the gas phase[23] through a possible mixture of both *trans* and *gauche* forms in solution[11] to *trans* in the solid state.[22a]

The crystal field forces the *trans* conformation for the neutral free base (HA-T$_2$) and its monoprotonated form (AmH$^+$-T$_1$).[13,22] However, in complexes with metal ions, the tautomeric and conformational preferences are very broad. Neutral histamine, playing the role of a bidentate ligand, takes preferentially the *gauche*-HA-T$_1$ form.[57–62] In one Cu(I) complex structure, the *trans*-HA-T$_2$ molecule forms a bridge between two coppers chelated by *gauche*-HA-T$_1$.[57] In the case of the protonated form, *gauche*-AmH$^+$-T$_1$ is found for the Ni(II) complex, but *trans*-AmH$^+$-T$_2$ for the Ca(II) salt.[55,56] This variation shows without any doubt how sensitive the histamine structure is to the environment.

This detailed study of the histamine structure in different environments may help in understanding the mechanism of histamine activity, particularly its interaction with different active regions of variable hydrophilicity and hydrophobicity, and with more or less negatively charged sites in the histamine-specific receptors. Moreover, the $\pi$–$\pi$ stacking interactions observed in the crystal lattice between the imidazole rings of different histamine molecules or between the imidazole ring of histamine and the aryl ring of other species may help in understanding the interactions of histamine with the hydrophobic (aromatic) binding sites of specific receptors.

## COMPUTATIONAL DETAILS

For *ab initio* calculations at the HF level the GAMESS program was used[64] and at the MP2 and DFT levels the Gaussian 94 program[65] was used. Additional computational details are included in the suplementary material.

### Acknowledgment

Calculations were performed at the Interdisciplinary Center for Molecular Modelling (ICM, Warsaw, Poland) and the Informatics Center of the Metropolitan Academic Network (IC MAN, Gdańsk, Poland) at the Technical University of Gdańsk.

## REFERENCES

1. (a) Borgarello M, Houriet R, Raczyńska ED, Drapała T. *J. Org. Chem.* 1990; **55**: 38–42; (b) Decouzon M, Gal J-F, Maria P-C, Raczyńska ED. *J. Org. Chem.* 1991; **56**: 3669–3673; (c) Raczyńska ED, Maria P-C, Gal J-F, Decouzon M. *J. Org. Chem.* 1992; **57**: 5730–5735; (d) Taft RW, Raczyńska ED, Maria PC, Leito I, Gal J-F, Decouzon M, Drapała M, Anvia F. *Pol. J. Chem.* 1995; **69**: 41–53.
2. (a) Raczyńska ED, Taft RW. *Bull. Chem. Soc. Jpn.* 1997; **70**: 1297–1305; (b) Raczyńska ED, Decouzon M, Gal J-F, Maria P-C, Taft RW, Anvia F. *J. Org. Chem.* 2000; **65**: 4635–4640.
3. Raczyńska ED, Mishima M, Mustanir. *Bull Soc. Chim. Jpn.* 1998; **71**: 2175–2179.
4. Makowski M, Raczyńska ED, Chmurzyński L. *J. Phys. Chem. A* 2001; **105**: 869–874.
5. (a) Decouzon M, Gal J-F, Maria P-M, Raczyńska ED. *Rapid Commun. Mass Spectrom.* 1993; **7**: 599–602; (b) Raczyńska ED, Maria P-C, Gal J-F, Decouzon M. *J. Phys. Org. Chem.* 1994; **7**: 725–733; (c) Raczyńska ED, Decouzon M, Gal J-F, Maria P-C, Gelbard G, Vielfaure-Joly F. *J. Phys. Org. Chem.* 2001; **14**: 25–34.
6. (a) Aue DH, Bowers MT. In *Gas Phase Ion Chemistry*, vol. 2, Bowers MT (ed). Academic Press: New York, 1979; chapt. 9; (b) Meot-Ner (Mautner) M, Hamlet P, Hunter EP, Field FH. *J. Am. Chem. Soc.* 1980; **102**: 6393–6399.
7. Hunter EPL, Lias SG. *J. Phys. Chem. Ref. Data* 1998; **27**: 413–656.
8. (a) Tapia O, Cárdenas R, Smeyers YG, Hernández-Laguna A, Rández JJ, Rández FJ. *Int. J. Quantum Chem.* 1990; **207**: 727–740; (b) Hernández-Laguna A, Abboud J-LM, Notario R, Homan H, Smeyers YG. *J. Am. Chem. Soc.* 1993; **115**: 1450–1454.
9. (a) Paiva TB, Tominaga M, Paiva ACM. *J. Med. Chem.* 1970; **13**: 689–692; (b) Farnell L, Richards WG, Ganellin CR. *J. Med. Chem.* 1975; **18**: 662–666; (c) Sjöberg S. *Pure Appl. Chem.* 1997; **69**: 1549–1570; (d) Nagy PI, Durant GJ, Hoss WP, Smith DA. *J. Am. Chem. Soc.* 1994; **116**: 4898–4909; (e) Collado JA, Tuñón I,

Copyright © 2003 John Wiley & Sons, Ltd.

Silla E, Ramírez FJ. *J. Phys. Chem. A* 2000; **104**: 2120–2131; and references cited therein.
10. Perrin DD. *Dissociation Constants of Organic Bases in Aqueous Solution*. Butterworth: London, 1965; Supplement, 1972.
11. (a) Ham NS, Casy AF, Ison RR. *J. Med. Chem.* 1973; **16**: 470–475; (b) Ganellin CR, Pepper ES, Port GNJ, Richards WG. *J. Med. Chem.* 1973; **16**: 610–616; (c) Kovalainen JT, Christiaans JAM, Poso A, Vepsäläinen J, Laatikainen R, Gynther J. *Tetrahedron Lets.* 1999; **40**: 2425–2428; (d) Kraszni M, Kökösi J, Noszál B. *J. Chem. Soc., Perkin Trans. 2* 2002; 914–917.
12. (a) Foye WO, Lemke TL, Williams DA. *Principles of Medicinal Chemistry*. Williams & Wilkins: Baltimore, MD, 1995; (b) Barnes PJ. *Pulm. Pharmacol. Ther.* 2001; **14**: 329–339.
13. Raczyńska ED, Cyrański KM, Darowska M, Rudka T. *Targets Heterocycl. Chem.* 2000; **4**: 327–356, and references cited therein.
14. (a) Watling KJ. *The RBI Handbook of Receptor Classification and Signal Transduction*. RBI: Natick, MA, 1998; (b) Leurs R, Timmerman H. In *TOCRIS*. http://www.tocris.com; (c) Leurs R, Timmerman H (eds). *The Histamine $H_3$ Receptor: A Target for New Drugs*. Elsevier: Amsterdam, 1998.
15. NCBI—National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov.
16. (a) Weinstein H, Chou D, Johnson CL, Kang S, Green JP. *Mol. Pharmacol.* 1976; **12**: 738–745; (b) Topiol S, Weinstein H, Osman R. *J. Med. Chem.* 1984; **27**: 1531–1534; (c) Weinstein H, Mazurek AP, Osman R, Topiol S. *Mol. Pharmacol.* 1986; **29**: 28–33; (d) Mazurek AP, Osman R, Weinstein H. *Mol. Pharmacol.* 1987; **31**: 345–350.
17. (a) Gantz I, Del Valle J, Wang L, Tashiro T, Munzert G, Guo Y, Konda Y, Yamada T. *J. Biol. Chem.* 1992; **267**: 20840–20843; (b) Nederkoorn PH, Vernooijs P, Donne-Op den Kelder GM, Baerends EJ, Timmerman H. *J. Mol. Graph.* 1994; **12**: 242–256.
18. Smit MJ, Hoffmann M, Timmerman H, Leurs R. *Clin. Exp. Allergy* 1999; **29**(Suppl. 3): 19–28.
19. Mazurek AP, Karpińska G. *Z. Naturforsch., Teil C* 1994; **49c**: 471–475.
20. Nakamura T, Itadani H, Hidaka Y, Ohta M, Tanaka K. *Biochem. Biophys. Res. Commun.* 2000; **279**: 615–620.
21. (a) Briganti F, Mangani S, Orioli P, Scozzafava A, Vernaglione G, Supuran CT. *Biochememistry* 1997; **36**: 10384–10392; (b) Briganti F, Iaconi V, Mangani S, Orioli P, Scozzafava A, Vernaglione G, Supuran CT. *Inorg. Chim. Acta* 1998; **275–276**: 295–300; (c) Paesen GC, Adams PL, Harlos K, Nuttall PA, Stuart DL. *Mol. Cell* 1999; **3**: 661–671; (d) Paesen GC, Adams PL, Nuttall PA, Stuart DL. *Biochim. Biophys. Acta* 2000; **1482**: 92–101; (e) Horton JR, Sawada K, Nishibori M, Zhang X, Cheng X. *Structure* 2001; **9**: 837–849; (f) Roberts SA, Weichsel A, Qiu Y, Shelnutt JA, Walker FA, Montfort WR. *Biochemistry* 2001; **40**: 11327–11337; (g) Barbosa JARG, Sivaraman J, Li Y, Larocque R, Matte A, Schrag JD, Cygler M. *Proc. Natl. Acad. Sci. USA* 2002; **99**: 1859.
22. (a) Bonnet JJ, Ibers JA. *J. Am. Chem. Soc.* 1973; **95**: 4829–4833; (b) Prout K, Critchley SR, Ganellin CR. *Acta Crystallogr., Sect. B* 1974; **30**: 2884–2886.
23. (a) Vogelsanger B, Godfrey PD, Brown RD. *J. Am. Chem. Soc.* 1991; **113**: 7864–7869; (b) Godfrey PD, Brown RD. *J. Am. Chem. Soc.* 1998; **120**: 10724–10732.
24. (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.
25. (a) Kirkwood JG. *J. Chem. Phys.* 1934; **2**: 351–361; (b) Onsager L. *J. Am. Chem. Soc.* 1936; **58**: 1486–1493.
26. (a) Koppel IA, Palm VA. In *Advances in Linear Free Energy Relationships*, Chapman NB, Shorter J (eds). Plenum Press: London, 1972; chapt. 5; (b) Kamlet MJ, Abboud J-LM, Taft RW. *Prog. Phys. Org. Chem.* 1981; **13**: 485–630; (c) Reichardt C. *Solvents and Solvent Effects in Organic Chemistry*. VCH: Weinheim, 1988; (d) Kamlet MJ, Abboud J-LM, Abraham MH, Taft RW. *J. Org. Chem.* 1983; **48**: 2877–2887; (e) Krygowski TM, Wrona PK, Zielkowska U, Reichardt C. *Tetrahedron* 1985; **41**: 4519–4527; (f) Abboud J-LM, Notario R. *Pure Appl. Chem.* 1999; **71**: 645–718; (g) Katritzky AR, Wang Y, Sild S, Karelson M. *J. Chem. Inf. Comput. Sci.* 1999; **39**: 684–691; (h) Katritzky AR, Tamm T, Wang Y, Karelson M. *J. Chem. Inf. Comput. Sci.* 1999; **39**: 692–698.
27. (a) Rashin AA, Young L, Topol IA. *Biophys. Chem.* 1994; **51**: 359–374; (b) Bachs M, Luque FJ, Orozco M. *Comput. Chem.* 1994; **15**: 446–454; (c) Fortunelli A, Tomasi J. *Chem. Phys. Lett.* 1994; **231**: 34–39.
28. (a) Schüürmann G, Cossi M, Barone V, Tomasi J. *J. Phys. Chem. A* 1998; **102**: 6706–6712; (b) Tran NL, Colvin ME. *J. Mol. Struct. (Theochem)* 2000; **532**: 127–137; (c) Li H, Hains AW, Everts JE, Robertson AD, Jensen JH. *J Phys. Chem. B* 2002; **106**: 3486–3494, and references cited therein; (d) Pliego JR Jr, Riveros JM. *J Phys. Chem. A* 2002; **106**: 7434–7439; (e) Himo F, Noodleman L, Blomberg MRA, Siegbahn PEM. *J Phys. Chem. A* 2002; **106**: 8757–8761.
29. (a) Raczyńska ED, Darowska M, Rudka T, Makowski M. *J. Phys. Org. Chem.* 2001; **14**: 770–777, and references cited therein; (b) Darowska M, Raczyńska ED. *Pol. J. Chem.* 2002; **76**: 1027–1035; (c) Raczyńska ED, Rudka T, Darowska M. *J. Mol. Struct. (Theochem)* 2001; **574**: 221–232.
30. (a) Topol IA, Tawa GJ, Burt SK, Rashin AA. *J. Phys. Chem. A* 1997; **101**: 10075–10081; (b) Klicić JJ, Friesner RA, Liu S-Y, Guida WC. *J. Phys. Chem. A* 2002; **106**: 1327–1335.
31. King JE. *Acid–Base Equilibria*. Pergamon Press: Oxford, 1965.
32. (a) Durant GJ, Ganellin CR, Parsons ME. *J. Med. Chem.* 1975; **18**: 905–909; (b) Kang S, Chou D. *Chem. Phys. Lett.* 1975; **34**: 537–541; (c) Richards WG, Wallis J, Ganellin CR. *Eur. J. Med. Chem.* 1979; **14**: 9–12; (d) Worth GA, Richards WG. *J. Am. Chem. Soc.* 1994; **116**: 239–250.
33. Krygowski TM, Woźniak K. In *The Chemistry of Amidines and Imidates*, vol. 2, Patai S, Rappoport Z (eds). Wiley: Chichester, 1991; chapt. 2.
34. Allen FH, Davies JE, Galloy JJ, Johnson O, Kennard O, McRae EM, Mitchell GF, Smith JM, Watson DG. *J. Chem. Inf. Comput. Sci.* 1991; **31**: 187–204.
35. (a) Krygowski TM. *J. Chem. Inf. Comput. Sci.* 1993; **33**: 70–78; (b) Krygowski TM, Cyrański MK. *Tetrahedron* 1996; **52**: 1713–1722.
36. Schleyer PvR, Maerker C, Dransfield A, Jiao H, Hommess NJRvE. *J. Am. Chem. Soc.* 1996; **118**: 6317–6318.
37. Schleyer PvR. *Chem. Rev.* 2001; **101**: 1115–1117.
38. Minkin VI, Glukhovtsev MN, Simkin BYa. *Aromaticity and Antiaromaticity—Electronic and Structural Aspects.* Wiley: New York, 1994.
39. Schleyer PvR, Manoharan M, Wang Z, Kiran B, Jiao H, Puchta R, Hommess NJRvE. *Org. Lett.* 2001; **3**: 2465–2468.
40. Hehre WJ, Radom L, Schleyer PvR, Pople JA. *Ab initio Molecular Orbital Theory.* Wiley: New York, 1986.
41. Karpińska G, Dobrowolski JC, Mazurek AP. *J. Mol. Struct. (Theochem)* 1996; **369**: 137–144.
42. (a) Möller C, Plesset MS. *Phys. Rev.* 1934; **46**: 618–622; (b) Pople JA, Brinkley JS, Seeger R. *Int. Quantum Chem. Symp.* 1976; **10**: 1–19.
43. (a) Parr RG, Yang W. *Density Functional Theory of Atoms and Molecules*. Oxford University Press: New York, 1989; (b) Becke AD. *J. Chem. Phys.* 1993; **98**: 5648–5652; (c) Lee C, Yang W, Parr RG. *Phys. Rev. B* 1988; **37**: 785–789.
44. (a) Cruz-Rodríguez Z, Sianz-Díaz CI, Hernández-Laguna A. *Quantum Systems in Chemistry and Physics: Advances Problems and Complex Systems*. Kluwer: Dordrecht, 2000; 367–392; (b) Hernández-Laguna A, Cruz-Rodríguez Z, Gonzalez-Jonte RH, Smeyers YG. *Mol. Eng.* 1998–1999; **8**: 155–180; (c) Hernández-Laguna A, Cruz-Rodríguez Z, Smeyers YG, Abboud J-LM, Tapia O. *J. Mol. Struct. (Theochem)* 1995; **335**: 77–87.
45. (a) Meyer M. *J. Mol. Struct. (Theochem)* 1994; **304**: 45–51; (b) Li G-S, Ruiz-López MF, Zhang M-S, Maigret B. *J. Mol. Struct. (Theochem)* 1998; **422**: 197–204.
46. (a) Byrn SR, Graber CW, Midland SL. *J. Org. Chem.* 1976; **41**: 2283–2288; (b) Reynolds WF, Tzeng CW. *Can. J. Biochem.* 1977; **55**: 576–578; (c) Wasylishen RE, Tomilinson G. *Can. J. Biochem.* 1977; **55**: 579–581.
47. Charton M. *J. Org. Chem.* 1965; **30**: 3346–3350.
48. (a) Arnett EM, Jones FM, Taagepera M, Henderson WG, Beauchamp JL, Holtz D, Taft RW. *J. Am. Chem. Soc.* 1972; **94**: 4724–4726; (b) Taft RW. In *Proton-transfer Reactions*, Caldin E, Gold V (eds). Chapman & Hall: London, 1975, chapt. 2; (c) Arnett EM. In *Proton-transfer Reactions*, Caldin E, Gold V (eds). Chapman &

Copyright © 2003 John Wiley & Sons, Ltd. 

Hall: London, 1975, chapt. 3; (d) Arnett EM, Chawla B, Bell L, Taagepera M, Hehre WJ, Taft RW. *J. Am. Chem. Soc.* 1977; **99**: 5729–5738; (e) Taft RW. *Prog. Phys. Org. Chem.* 1983; **14**: 247; (f) Bagno A, Menna E, Mezzina E, Scorrano G, Spinelli D. *J. Phys. Chem. A* 1998; **102**: 2888–2892; (g) Bagno A, Scorrano G. *Acc. Chem. Res.* 2000; **33**: 609–616.
49. Alder RW. *Chem. Rev.* 1989; **89**: 1215–1223.
50. Hansch C, Leo A, Taft RW. *Chem. Rev.* 1991; **91**: 165–195.
51. Elguero J, Marzin C, Katritzky AR, Linda P. *The Tautomerism of Heterocycles*. Academic Press: New York, 1975.
52. Anulewicz R, Wawer I, Krygowski TM, Männle F, Limbach H-H. *J. Am. Chem. Soc.* 1997; **119**: 12223–12230, and references cited therein.
53. Raczyńska ED, Gawinecki R. *Trends Org. Chem.* 1998; **7**: 85–93, and references cited therein.
54. Noszál B, Rabenstein DL. *J. Phys. Chem.* 1991; **95**: 4761–4765.
55. Cole LB, Holt EM. *J. Chem. Soc., Perkin Trans 1* 1986; 151–154.
56. Wojtczak A, Jaskólski M, Kosturkiewicz Z. *Acta Crystallogr., Sect. C* 1983; **39**: 545–547.
57. (a) Pasquali M, Floriani C, Gaetani-Manfredotti A, Guastini C. *Chem. Commun.* 1979; 197–199; (b) Pasquali M, Marini G, Floriani C, Gaetani-Manfredotti A, Guastini C. *Inorg. Chem.* 1980; **19**: 2525–2531.
58. (a) Bonnet JJ, Jeannin Y. *Acta Crystallogr., Sect. B* 1970; **26**: 318–326; (b) Su CC, Chang TY. *J. Coord. Chem.* 1994; **31**: 79–92; (c) Wojtczak A, Jaskólski M, Kosturkiewicz Z. *Acta Crystallogr., Sect. C* 1987; **43**: 645–648; (d) Min KS, Suh MP. *J. Solid State Chem.* 2000; **152**: 183–190; (e) Stocker FB, Fadden P, Dreher S, Britton D. *Inorg. Chem.* 1999; **38**: 3251–3253; (f) Główka ML, Gałecki Z, Kazimierczak W, Maœliñski C. *Acta Crystallogr., Sect. B* 1980; **36**: 2148–2150; (g) Yamauchi O, Odani A, Kohzuma T, Masuda H, Toriumi K, Sato K. *Inorg. Chem.* 1989; **28**: 4066–4068; (h) Zhang F, Yajima T, Masuda H, Odani A, Yamauchi O. *Inorg. Chem.* 1997; **36**: 5777–5784; (i) Scarpellini M, Neves N, Bortoluzzi AJ, Joussef AC. *Acta Crystallogr., Sect. C* 2001; **57**: 356–358; (j) Castan P, Deguenon D, Dahan F. *Acta Crystallogr., Sect. C* 1991; **47**: 2656–2658; (k) Zhang CG, Duan CY, Hu Q, Yan DY. *J. Chem. Crystallogr.* 1999; **29**: 1153–1155; (l) Su CC, Chang TY. *J. Coord. Chem.* 1994; **31**: 79–92.
59. Wojtczak A, Kosturkiewicz Z, Surdykowski A. *Acta Crystallogr., Sect. C* 1990; **46**: 578–581.
60. Sakagami N, Kita E, Kita P, Wiśniewska J, Kaizaki S. *Polyhedron* 1999; **18**: 2001–2007.
61. (a) Bonnet JJ, Jeannin Y. *Bull. Soc. Fr. Miner. Crist.* 1970; **93**: 287–299; (b) Wojtczak A, Jaskólski M, Kosturkiewicz Z. *Acta Crystallogr., Sect. C* 1985; **41**: 1752–1755; (c) Bonnet JJ, Jeannin Y. *Bull. Soc. Fr. Miner. Crist.* 1972; **95**: 61–67.
62. Dahan F. *Acta Crystallogr., Sect. B* 1976; **32**: 2472–2475.
63. (a) Bonnet JJ, Jeannin Y, Laaouini M. *Bull. Soc. Fr. Miner. Crist.* 1975; **98**: 208–213; (b) Veidis MV, Palenik GJ, Schaffrin R, Trotter J. *J. Chem. Soc. A* 1969; 2659–2666; (c) Bonnet JJ, Jeannin Y. *Acta Crystallogr., Sect. B* 1972; **28**: 1079–1085; (d) Yamane T, Ashida T, Kakudo M. *Acta Crystallogr., Sect. B* 1973; **29**: 2884–2891; (e) Cole LB, Holt EM. *Acta Crystallogr., Sect. C* 1990; **46**: 1737–1739; (f) Wojtczak A, Jaskólski M. *Acta Crystallogr., Sect. C* 1989; **45**: 30–33; (g) Wojtczak A, Jaskólski M, Kosturkiewicz Z. *Acta Crystallogr., Sect. C* 1988; **44**: 1779–1781; (h) Efimenko IA, Balakaeva TA, Kurbakova AP, Gorbunova YuE, Mikhailov YuN. *Koord. Khim.* 1994; **20**: 294–299.
64. Schmidt MW, Baldridge KK, Boatz JA, Elbert ST, Gordon MS, Jensen JH, Koseki S, Matsunaga N, Nguyen KA, Su SJ, Windus TL, Dupuis M, Montgomery JA. *J. Comput. Chem.* 1993; **14**: 1347–1363.
65. Frisch MJ, Trucks GW, Schlegel HB, Gill PMW, Johnson BG, Robb MA, Cheeseman JR, Keith TA, Petterson GA, Montgomery JA, Raghavachari K, Al-Laham MA, Zakrzewski VG, Ortiz JV, Foresman JB, Ciosłowski J, Stefanov B, Nanayakkara A, Challacombe M, Peng CY, Ayala PY, Chen W, Wong MW, Andres JL, Replogle ES, Gomperts R, Martin RL, Fox DJ, Binkley JS, DeFrees DJ, Baker J, Stewart JP, Head-Gordon M, Pople JA. *Gaussian 94*. Gaussian: Pittsburgh, PA, 1995.
66. Wolinski K, Hilton JF, Pulay P. *J. Am. Chem. Soc.* 1990; **112**: 8251–8260.

Copyright © 2003 John Wiley & Sons, Ltd. 