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Tautomeric conjugate acids of 2-aminopyrroles: effect of substituents, solvation and cosolute

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Abstract. The tautomeric preferences of the conjugated acids of 2-aminopyrrole derivatives have been examined both in the gas phase and in aqueous solution by using a combination of quantum mechanical, self-consistent reaction field and Monte Carlo–free-energy perturbation methods. The results show that the nature of substituents, the solvent and the presence of cosolute are relevant factors in modulating the relative stability between the tautomeric conjugate acids protonated at the heterocyclic ring and at the exocyclic amino nitrogen. Thus, attachment of electron-withdrawing groups to the ring, solvation in polar solvents, and the presence of negatively charged cosolutes tend to favor protonation at the exocyclic amino nitrogen. Nevertheless, none of these factors alone suffice to change the tautomeric preference for the ring-protonated forms. The results point out that the concerted occurrence of the three factors is necessary to shift the tautomeric preference towards the conjugated species protonated at the exocyclic nitrogen.

Keywords: 2-Aminopyrroles – Tautomerism – Solvation – Cosolute

Introduction

Amino-substituted pyrroles are important building blocks for the synthesis of nitrogen heterocycles of biological interest [1], which explains the interest in developing readily available synthetic sources of these compounds [2, 3]. Efficient synthetic procedures for amino-substituted pyrroles need profound knowledge of the chemical reactivity of these compounds. Unfortunately, to obtain such knowledge is difficult owing to the large number of factors that modulate the reactivity of these compounds. Tautomerism and protonation are of particular relevance, since different protonation/tautomeric states seem to be involved in different synthetic routes. For example, the behavior of 2-amino heterocycles as enamines or as aromatic amines might be related to the tautomeric preference of ring-protonated forms versus protonation at the exocyclic amino group [1].

The possibility that 2- and 3-aminopyrroles can exist as either the amino or the imino tautomer or as an equilibrium mixture of tautomers has been examined in previous studies [4]. Both experimental and theoretical data show that the amino/imino tautomeric equilibrium is determined by a delicate balance between the effects played by substituent and solvation. Thus, whereas 2-aminopyrrole is the main species in a variety of solvents [5], its 1-alkyl derivatives are predicted to exist as amino tautomers in chloroform but as a mixture of amino and imino forms in water [5]. Likewise, it has been observed that 1-(triphenylmethyl)-3-aminopyrrole is found to exist exclusively as the imino tautomer in chloroform [6]. The balance between substituent and solvation effects makes difficult the assignment of the preferred tautomer in certain cases, such as 1-acetyl-2-amino-4,5-dimethylpyrrole, which was firstly described to exist as the imino tautomer [7], but subsequent ^{1}H and ^{13}C NMR data indicated the preference of the amino tautomer [8].

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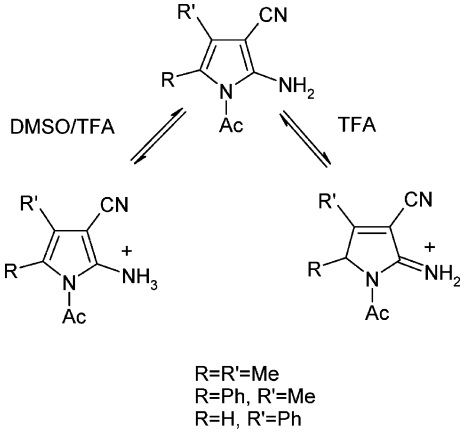
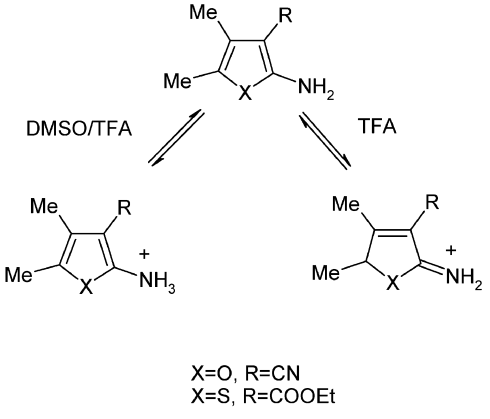
The susceptibility to undergo protonation at different sites can also modulate the reactivity of aminopyrroles. Particularly, tautomerism between protonated species seems to be key for their reactivity. Protonation of 2- and 3-aminopyrroles generally occurs at the heterocyclic ring under different acid conditions, such as trifluoroacetic acid (TFA) or mineral acids [9]. The ring-protonated species has also been found to be involved in tetraphenylborate salts of 2-aminopyrrole and its N-alkyl derivatives [3]. However, one exception to this behavior is 1-acetyl-2-amino-3-cyano-4-*R*-5-*R*′-pyrrole, which undergoes protonation on the exocyclic nitrogen upon addition of two equivalents of TFA to dimethyl sulfoxide (DMSO) [8]. In fact, the species formed in pure TFA and in DMSO–TFA mixtures are tautomeric conjugate acids (Scheme 1). The tautomeric shift between conjugated acids of certain pyrrole derivatives has also been reported for related 2-aminofuran and 2-aminotiophene derivatives under similar solvent conditions (Scheme 2) [8]. We are then facing a general process, which might have a profound impact in heterocyclic chemistry.

The aim of this study is to gain insight into the equilibrium between the tautomeric conjugated acids of 2-aminopyrrole compounds, and how this is modulated by different factors. To this end, quantum mechanical calculations in the gas phase were combined with self-consistent reaction field techniques (SCRF) and Monte Carlo–free-energy perturbation (MC-FEP) simulations in order to discern the differential effect of substituents, solvation and cosolvent. The influence of substituents was examined by considering the relative stability between protonated forms of 2-aminopyrrole and its 1-acetyl, 3-cyano and 1-acetyl-3-cyano derivatives. The influence of solvation was examined by analyzing tautomeric equilibrium in different solvents: water, DMSO and chloroform. Finally, the effect of the TFA molecule as cosolute was also examined. The results point out that a delicate balance of the three factors allows us to rationalize the tautomeric preferences of protonated 2-aminopyrrole derivatives.



Scheme 1



Scheme 2

Methods

Model compounds

To investigate the tautomeric equilibrium between the protonated species of aminopyrrole derivatives, computations were performed for 2-aminopyrrole (I) and for its derivatives substituted at positions 1 and 3 (Fig. 1): 3-cyano (II), 1-acetyl (III) and 1-acetyl-3-cyano (IV). The choice of these substituents allowed us to dissect the effect of each substituent on the equilibrium between tautomeric conjugate acids, and to compare theoretical results with the experimental data available for derivatives of 1-acetyl-2-amino-3-cyanopyrrole (Scheme 1) under different acid conditions. In all cases protonation was considered to occur on the exocyclic nitrogen as well as on carbon positions 3 and 5 of the ring.

Quantum mechanical calculations

To investigate the relative stability of the N-, C3- and C5-protonated species of 2-aminopyrrole derivatives in the gas phase, quantum mechanical computations involving different levels of theory and basis sets were performed. The geometry was fully optimized at

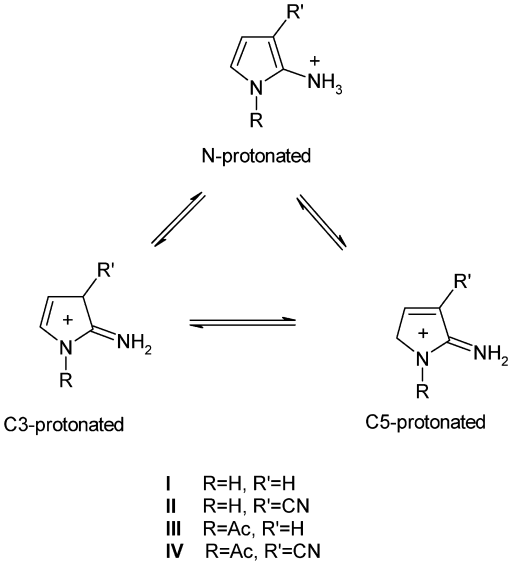


Fig. 1. Representation of the different compounds used to investigate the influence of substituents on the tautomeric equilibrium in the protonated forms of 2-aminopyrrole derivatives

the second-order Møller–Plesset (MP2) [10] level with the 6-31G(d,p) [11] basis set, and the minimum-energy nature of the optimized structures was confirmed by inspection of the harmonic vibrational frequencies. Single-point calculations were subsequently performed using 6-31+G(d,p), 6-311++G(d,p) and Dunning's aug-cc-pVDZ or aug-cc-pVTZ [12] basis sets. The influence of the electron correlation on the energy differences between protonated species was examined from calculations carried out at the MP2, fourth-order Møller–Plesset (MP4) with single, double and quadruple excitations, quadratic configuration interaction with single and double excitations (QCISD), and QCISD with perturbative triple excitation [QCISD(T)] levels of theory. The best estimate of the energy difference between protonated species was determined by adding the energy difference between QCISD(T) and MP2 levels of theory with the 6-311++G(d,p) to the relative energy computed at the MP2/aug-cc-pVTZ level (see later). The differences in stability were estimated by correcting the relative energies for zero-point energy, thermal and entropic effects ($P = 1$ atm and $T = 298$ K), which were determined using the scaled vibrational frequencies [13], and the harmonic oscillator-rigid-rotor approximations as implemented in the Gaussian 94 program [14].

Solution calculations

The effect of solvation on the relative stability between the protonated species of 2-aminopyrrole derivatives was examined from continuum SCRF and discrete MC-FEP calculations.

SCRF calculations were performed using the Miertus–Scrocco–Tomasi (MST) model [15, 16, 17, 18], which follows the electrostatic formalism developed in the polarizable continuum model [19]. Calculations were performed to determine the free energy of solvation in water [15], octanol [16], chloroform [17] and carbon tetrachloride [18] using our recent reparameterization of the MST model [16]. For calculations for DMSO, the relative solvation free energy was approximated by considering only the electrostatic term, which is reasonable since the steric term will be similar for the different tautomers. Cavities for the solute in water, octanol, chloroform and carbon tetrachloride were derived using our optimized procedures [15, 16, 17, 18], while that for DMSO was determined by scaling the atomic radii by a factor of 1.4 [20]. On the basis of our previous studies for the solvation of charged compounds [21], calculations were performed at the Hartree–Fock (HF) level with the 6-31+G(d) basis and the atomic radius of the heavy atom the proton is attached to was reduced by a factor of 0.92. MST computations were carried out considering the gas-phase geometries of the tautomeric conjugate acids optimized at both HF/6-31G(d) and MP2/6-31G(d,p) levels, but no relevant differences were observed in the free energies of solvation computed for the two geometries. Though the absolute free energies of solvation can be affected by geometry relaxation in solution, previous studies [22] suggest that the relative free energies of solvation between tautomeric conjugate acids can be assumed to be little influenced by geometry relaxation. Accordingly, no reoptimization of the gas-phase geometry in solution was carried out. Calculations were performed using a locally modified version of the Monster-Gauss [23] program.

MC-FEP simulations were used to estimate the differences in the free energy of solvation between protonated species of compounds I–IV. MC-FEP simulations were carried out only for water and DMSO, which have the greatest influence on the tautomeric equilibrium between conjugated acids. The systems consisted of a single solute immersed in a cubic box containing around 500 water and 265 DMSO molecules. A nonbonded cutoff of 11 Å and periodic boundary conditions were used. The range of solute translations and rotations was adjusted to have an acceptance ratio around 40%. The TIP4P water model [24] was used for calculations for aqueous solution, and the standard optimized potential for liquid simulations (OPLS) parameters were used for DMSO [25]. The charges for the solutes were determined using the restrained electrostatic potential procedure [26] by fitting the electrostatic potential computed at the HF/6-31G(d) level. The van der Waals parameters were adopted from related atoms in the OPLS force field. Simulations were performed at constant pressure and temperature (1 atm, 298 K).

Differences in the free energy of solvation of the N-, C5- and C3-protonated species were determined using the windowing scheme. Mutation between tautomers was performed using 21 double-width sampling windows, each consisting of 5×10^6 configurations for equilibration and 7.5×10^6 configurations for averaging. The geometry of the solute was gradually mutated along the mutation, without sampling of the internal degrees of freedom. In all cases there was close agreement between the relative solvation free energy estimated from "forward" and "reverse" mutations (differences less than 0.4 kcal/mol), and thermodynamic cycles corresponding to the mutations C5 → N, C3 → N, and C5 → C3 were closed with an error less than 0.6 kcal/mol. All these results indicate good convergence in the calculations, and give confidence in the statistical quality of the results. MC-FEP simulations were performed using the BOSS computer program [27].

For 1-acetyl-2-amino-3-cyanopyrrole (IV) the effect of the cosolute (TFA anion) on the differential solvation of the tautomeric conjugate acids in DMSO was also investigated. To this end, additional systems containing each of the protonated species together with one TFA anion molecule were considered. The orientation of the TFA anion in the complex was determined on the basis of the inspection of the molecular interaction potential (MIP) [28] maps computed for the tautomeric conjugated acids. MIP calculations were performed by treating each conjugated acid quantum mechanically at the HF/6-31G(d) level and considering the interaction with a classical point particle having a negative unit charge and van der Waals parameters of an oxygen atom (O probe). On the basis of the MIP maps, mutations were performed by placing the TFA anion around the ammonium (immonium) group (in-plane position) and above the heterocyclic ring (out-of-plane position). MIP calculations were performed with the MOPETE program [29].

The systems containing TFA as cosolute were built up by replacing one DMSO molecule by the TFA anion in selected snapshots taken from the MC simulations performed for each of the protonated species. The systems were heated and equilibrated for 5×10^6 configurations (the former 3×10^6 configurations were performed by fixing the relative position of the TFA anion). The final structure was the starting point for the MC-FEP simulations, which were carried out using the same protocol as noted earlier. In all cases the relative orientation of the conjugated acid and the TFA anion was not significantly modified at the end of the mutation. Again, there was agreement between free-energy differences determined for "forward" and "reverse" mutations (deviation less than 0.7 kcal/mol), and the thermodynamic cycles for the mutations C5 → N, C3 → N, and C5 → C3 were closed with errors of 0.4 kcal/mol (in plane position) and 1.2 kcal/mol (out-of-plane).

Results and discussion

Gas-phase calculations

The prototropic tautomerism of the protonated 2-aminopyrrole in the gas phase was studied at different levels of ab initio quantum mechanical theory. In contrast to the tautomeric equilibrium of the neutral 2-aminopyrrole [5], the equilibrium between N-, C3- and C5-protonated forms is particularly sensitive to the level of theory used in calculations, as evident from the data given in Table 1. Compared to the N-protonated form, the greater stability of the ring-protonated species found at the HF level is reduced by 40–50% (around 11–14 kcal/mol) upon inclusion of electron correlation effects at the MP2 level. Extension of the basis set from 6-31G(d,p) to aug-cc-pVTZ leads to a smaller, but

Table 1. Free-energy differences (kcal/mol) between the species protonated at the exocyclic nitrogen and carbon atoms C3 and C5 of 2-aminopyrrole (I). Values are given relative to the N-protonated species

Method	C3	C5
HF/6-31G(d,p)[a]	−27.8 (−29.2)	−35.0 (−36.3)
HF/6-31 + G(d,p)	−26.5	−33.8
HF/6-311 + + G(d,p)	−26.3	−33.6
HF/aug-cc-pVTZ	−26.4	−33.8
MP2/6-31G(d,p)[a]	−16.6 (−17.7)	−21.5 (−22.9)
MP2/6-31 + G(d,p)	−15.4	−20.4
MP2/aug-cc-pVDZ	−14.8	−19.9
MP2/6-311 + + G(d,p)	−14.4	−19.5
MP2/aug-cc-pVTZ	−13.5	−18.8
MP4SDQ/6-31 + G(d,p)	−20.9	−25.8
MP4SDQ/6-311 + + G(d,p)	−20.4	−25.2
QCISD/6-31 + G(d,p)	−21.3	−26.3
QCISD/6-311 + + G(d,p)	−20.8	−25.7
QCISD(T)/6-31 + G(d,p)	−20.0	−24.8
QCISD(T)/6-311 + + G(d,p)	−19.4	−24.2
Composite[b]	−18.5	−23.5

[a]Results determined for the MP2/6-31G(d,p) optimized geometries. The relative stability determined using the HF/6-31G(d,p) optimized geometries is given in parentheses

[b]The composite value was determined by adding the energy difference between QCISD(T) and MP2 levels of theory with the 6-311 + + G(d,p) basis to the relative free-energy difference calculatthe MP2/aug-cc-pVTZ level

nonnegligible reduction (around 3 kcal/mol) of the relative stability of the ring-protonated forms at this level of theory. Higher-order correlation effects reverse partially this effect, as the relative stability of the ring-protonated forms is enhanced by around 5 kcal/mol. Triple excitations have only a moderate effect on the results. Finally, the MP4 and QCI calculations seem reasonably stable towards the extension of the basis set.

Following standard procedures on the study of tautomeric equilibria in the gas phase [22, 30], the free-energy differences between the tautomeric conjugated acids were estimated by adding the energy difference computed at the QCISD(T) and MP2 levels of theory with the 6-311 + + G(d,p) [6-31 + G(d,p) for the C3-protonated species of compound IV] to the relative free energy computed at the MP2/aug-cc-pVTZ(pVDZ) level. From the results in Table 1, our best estimates indicate that the C3- and C5-protonated forms of 2-aminopyrrole are more stable than the N-protonated species in the gas phase by around 18 and 23 kcal/mol, respectively. Since both the extension of the basis set and the inclusion of higher-order correlation effects tend to reduce the stability of the ring-protonated forms, these estimates should be regarded more properly as upper-bound limits of the relative stability between tautomeric conjugated acids. However, despite numerical uncertainties in the calculations, the energy difference is so large that the existence of the N-protonated tautomer can be precluded in the gas phase and the C5-protonated form must be (with a large difference) the dominant tautomer of 2-aminopyrrole in the gas phase.

The preference of the ring-protonated forms compared to the N-protonated tautomer can be rationalized

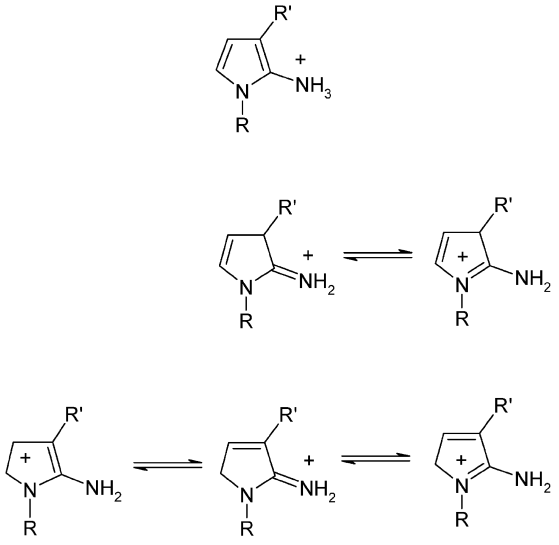


Fig. 2. Schematic representation of the charge delocalization in the N-, C3- and C5-protonated forms of 2-aminopyrrole

Table 2. Free-energy differences (kcal/mol) between the species protonated at the exocyclic nitrogen and carbon atoms C3 and C5 of 2-aminopyrrole derivatives II–V. Values are given relative to the N-protonated species

Method	II		III		IV	
	C3	C5	C3	C5	C3	C5
MP2/6-31G(d,p)	−5.7	−16.3	−9.2	−14.8	−0.2	−11.2
MP2/6-311 + + G(d,p)	−4.6	−14.7	−7.8	−13.6	0.7[b]	−9.8
MP2/aug-cc-pVTZ	−2.5	−13.8	−7.2	−13.1	1.6	−9.4
QCISD(T)/ 6-311 + + G(d,p)	−10.7	−20.5	−12.3	−17.8	−5.1[b]	−15.1
Composite[a]	−8.6	−19.6	−11.7	−17.3	−4.2	−14.7

[a]The composite value was determined by adding the energy difference computed at the QCISD(T) and MP2 levels of theory with the 6-311 + + G(d,p) [6-31 + G(d,p) for C3 of compound IV] to the relative free-energy difference calculated at the MP2/aug-cc-pVTZ level. Geometries optimized at the MP2/6-31G(d) level

[b]Determined with the 6-31 + G(d,p) basis

from the different degrees of charge delocalization in the three species. Thus, whereas the positive charge is highly localized on the exocyclic nitrogen for the N-protonated form, the charge delocalization is greater upon protonation at C3 and particularly C5, as noted in the resonance structures shown in Fig. 2.

The predicted free-energy differences in the gas phase between the protonated forms for derivatives II–IV of 2-aminopyrrole are reported in Table 2. In all cases the species protonated at the exocyclic nitrogen and at the C5 carbon of the ring are the least stable and the stablest species. Compared to the 2-aminopyrrole, nevertheless, there are remarkable differences in the relative stability of the protonated forms, depending on the nature of the substituent. Thus, attachment of the acetyl group (at the heterocyclic N) reduces the stability of the C5- and C3-protonated forms by around 4 and 10 kcal/mol relative to that of the N-protonated species. Similarly, inclusion

of the cyano group (at position 3) reduces the stability of the C5- and C3-protonated forms by around 6 and 7 kcal/mol. Comparison of the results reported for compound IV (1-acetyl-3-cyano) with those determined for II (3-cyano) and III (1-acetyl) indicates that their effects are quite additive, and that introduction of several substituents into the aromatic ring can induce large changes in the tautomeric preference of aminopyrroles.

The destabilization of the ring-protonated species relative to the exocyclic N-protonated tautomer can be explained by the electron-withdrawing nature of both cyano and acetyl groups, which decreases the stabilization arising from delocalization of the positive charge (Fig. 2). Despite the dramatic influence exerted by these substituents on the relative stability of tautomeric conjugate acids, our best estimates indicate that the C5-protonated form of 1-acetyl-2-amino-3-cyanopyrrole (compound IV) is preferred by nearly 15 kcal/mol with regard to the N-protonated tautomer.

Solvation in pure solvents

The differences in the free energy of solvation of N-, C5, and C3-protonated forms of 2-aminopyrrole derivatives were used to explore the influence of solvation on the equilibrium between the tautomeric conjugated acids. To this end, both MST and MC-FEP calculations were carried out. The HF/6-31G(d) parameterization of the MST model was used to determine the free energy of solvation in water, DMSO, octanol, chloroform and carbon tetrachloride. Owing to the large computational cost of MC-FEP simulations, these calculations were limited to water and DMSO, which are the solvents that have the greatest influence on the relative stability between tautomers (see later).

The differences in the solvation free energy between tautomeric conjugated acids are given in Table 3. Keeping in mind the different formalism of the two computational procedures and the ionized state of the conjugated acids, there is reasonable agreement between the MST and the MC-FEP results, especially for DMSO. Larger differences are found for the relative free energy of solvation in water, especially concerning the relative hydration of the C5-protonated form, which presumably stems from the different treatment of polarization effects in MST and MC-FEP calculations. As expected from the usual gas-phase/solvation compensation effect, the results point out a marked stabilization of the species protonated at the exocyclic nitrogen compared to the ring-protonated tautomers (Table 3), this effect being clearly more important in water or DMSO than in the rest of the solvents. The preferential solvent-induced stabilization of the exocyclic N-protonated form can be explained by the greater delocalization of the positive charge in the ring-protonated conjugated acids (see Fig. 2); however, this effect is largely modulated by the electronic and steric properties of the substituents attached to the heterocyclic ring. Thus, inclusion of the cyano group at position 3 of the ring (compound II) tends to slightly increase the solvent-induced stabilization of the N-protonated form compared to the parent 2-aminopyrrole (compound I). In contrast, acylation of the ring nitrogen (compound III) reduces the preferential stabilization of the N-protonated form, which is around 50% compared to the solvent-induced stabilization of this latter species in 2-aminopyrrole. This effect reflects the decrease in the solvent exposure of the ammonium group upon acylation of the pyrrole nitrogen.

The free energies of tautomerization in solution determined by adding our best estimates of the free-energy difference in the gas phase to the relative free energies of solvation (averaged MST and MC-FEP results for water and DMSO) are reported in Table 4. For 2-aminopyrrole (I), the ring-protonated tautomers are predicted to be stabler than the N-protonated form by more than 4 kcal/mol (C3-protonation) and 7 kcal/mol (C5-protonated) in all solvents. With regard to the

Table 3. Relative free energies of solvation (kcal/mol) in chloroform, dimethyl sulfoxide (*DMSO*) and water for the N-, C3- and C5-protonated species of 2-aminopyrrole derivatives. Values are given relative to the N-protonated species

Method	I		II		III		IV	
	C3	C5	C3	C5	C3	C5	C3	C5
Carbon tetrachloride								
MST[a]	4.6	5.0	4.8	5.3	2.5	2.5	2.8	2.7
Chloroform								
MST	8.3	9.1	8.5	9.5	4.5	4.4	5.5	5.4
Octanol								
MST	11.3	12.9	12.2	13.7	6.7	7.0	7.9	7.9
DMSO								
MST	14.2	16.4	15.7	17.4	8.3	8.9	10.2	10.1
MC-FEP	12.7	14.1	15.5	16.5	6.4	5.9	8.0	8.9
Average	13.5	15.3	15.6	16.9	7.4	7.4	9.1	9.5
Water								
MST	16.8	18.9	16.5	18.4	8.0	8.7	11.7	11.4
MC-FEP	12.0	12.8	14.7	15.6	5.8	4.2	8.1	6.3
Average	14.4	15.9	15.6	17.0	6.9	6.5	9.9	8.9

[a]Calculations performed by using the HF/6-31G(d) parameterization of the MST continuum method

Table 4. Relative free energies of tautomerization (kcal/mol) in chloroform, DMSO and water for the N-, C3- and C5-protonated species of 2-aminopyrrole derivatives. Values are given relative to the N-protonated species

Solvent	I		II		III		IV	
	C3	C5	C3	C5	C3	C5	C3	C5
Carbontetrachloride	−13.9	−18.5	−6.1	−17.1	−9.5	−14.8	−1.4	−12.1
Chloroform	−10.2	−14.4	−0.1	−10.1	−7.2	−12.9	1.3	−9.4
Octanol	−7.2	−10.6	3.6	−5.9	−5.0	−10.3	3.7	−6.9
DMSO[a]	−5.0	−8.2	7.0	−2.7	−4.3	−9.9	4.9	−5.3
Water[a]	−4.1	−7.6	7.0	−2.6	−4.8	−10.8	5.7	−5.9

[a]Average values of MST and MC-FEP relative free energies of solvation used to determine the relative free energies of tautomerization between conjugated acids

N-protonated form of the 3-cyano derivative (II), the C3-protonated form is predicted to be disfavored by around 7 kcal/mol in both DMSO and water and to be nearly equivalent in chloroform. Moreover, though the C5-protonated form is predicted to be the most populated tautomer of compound II in all the solvents, the difference in stability with the N-protonated species is reduced to 2–3 kcal/mol in water and DMSO, despite the fact that the difference in stability between C5- and N-protonated species was nearly 20 kcal/mol in the gas phase (Table 2). Despite the solvent effect, the 1-acetyl derivative (III) exhibits a marked preference for the C5-protonated form, and even the C3-protonated form is stabler than the N-protonated one. The large influence played by the cyano group is also noted when tautomerization free energies of compounds III and IV are compared (Table 4). Thus, it is found that the introduction of a cyano group into the 1-acetyl derivative (III) leads to a dramatic increase in the stability of the N-protonated form with respect to both the C3- and C5-protonated species.

Effect of cosolute

The preceding results point out that choice of appropriate subtituents and solvent might lead to a dramatic reduction in the gas-phase preference of the ring-protonated forms of aminopyrrole compounds. For the 1-acetyl-3-cyano derivative (compound IV) such a reduction amounts to around 10 kcal/mol upon solvation in DMSO; however, solvation alone does not suffice to revert the equilibrium between immonium and ammonium species, since calculations still predict the C5-protonated form to be stabler than the N-protonated conjugated acid by around 5 kcal/mol. This disagrees with the experimental evidence, which points out that the ammonium species of 1-acetyl-2-amino-3-cyanopyrrole derivatives are formed in DMSO upon addition of 2 Eq TFA. On the basis of our previous experience in related systems [31], we might suggest that the TFA anion can act as a cosolute modifying the tautomeric equilibrium between immonium and ammoniun tautomers.

To obtain a rough estimate of the influence of the TFA anion in the relative stability of the conjugated acids, the free-energy differences determined for the mutations between tautomers were recomputed from MC-FEP simulations by introducing one TFA anion in the simulated system. MC or molecular dynamics simulations for systems containing cosolutes may have problems owing to the convergence in the configurational sampling, and accordingly the starting configurations must be carefully selected. For this purpose, MIP calculations for the N-, C5- and C3-protonated acids interacting with a negatively charged oxygen (O^-) probe were computed. Inspection of the isocontour potential profiles (Fig. 3) showed the existence of favorable contacts in a wide area around the region proximal to the exocyclic amino group as well as in the space above and below the ring. For the C3-protonated compound, the O^- probe interacts preferentially through the face of the ring opposite the cyano group. On the basis of the MIP maps, two locations of the TFA anion around the protonated 1-acetyl-2-amino-3-cyanopyrrole were considered. In one case (in-plane orientation) the TFA anion was located in the plane of the ring and proximal to the ammonium/immonium group. In the other case (out-of-plane orientation) it was located above the heterocyclic ring (in the face opposite the cyano group for the C3-protonated tautomer). As noted in Methods, the relative orientation of the TFA anion in the complex was initially fixed in the setup of the system, but was allowed to move after equilibration. At the end of the MC-FEP simulations, the TFA anion retained its relative orientation with regard to the conjugated acid.

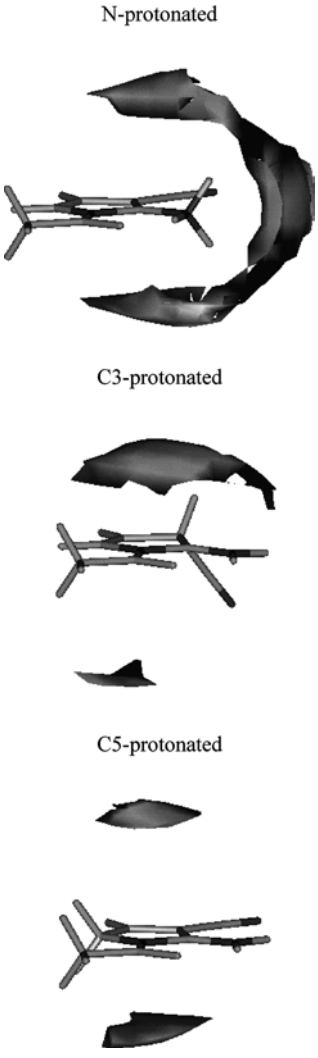


Fig. 3. Representation of the most favorable regions for the interaction between N-protonated (*top*), C3-protonated (*middle*) and C5-protonated (*bottom*) forms of 1-acetyl-2-amino-3-cyanopyrrole with a negatively charged oxygen probe determined from molecular interaction potential calculations. The plotted contour corresponds to an interaction (electrostatic plus van der Waals) energy of −40 kcal/mol

The corresponding changes in the free energy for the mutations between N-, C5- and C3-protonated species in presence of the TFA anion are reported in Table 5. Similar results are obtained for the two orientations of the TFA anion, leading to a preferential solvation of the N-protonated tautomer by 7.1 and 11.8 kcal/mol with regard to the C3- and C5-protonated forms. Comparison with the MC-FEP free energy differences for the solvation of the conjugated acids of compound IV in pure DMSO indicates that the single TFA anion makes an additional contribution of nearly 3 kcal/mol to the stability of the ammonium species. On the basis of the preceding results, compound IV is predicted to exist mainly as the C5-protonated form, though the species protonated at the exocyclic nitrogen is less favored by only 2.4 kcal/mol.

Comparison with experimental data

Analysis of the NMR data demonstrates that 1-acetyl-2-amino-3-cyano-4-*R*-5-*R*′-pyrroles in DMSO undergo protonation at the exocyclic nitrogen after addition of 2 Eq TFA (Scheme 1). Within the uncertainty of the calculated relative stabilities in the gas phase and the differences in solvation free energies, as well as the simplified description of the cosolute effects, the results clearly indicate that the concerted effect of suitable substituents, solvent and cosolute can lead to a dramatic decrease (around 22 kcal/mol) in the gas-phase preference of the ring-protonated species. Comparison of the results obtained for the parent 2-aminopyrrole (I) and its 1-acetyl-3-cyano derivative (IV) points out that such a decrease can be further attributed to the gas-phase destabilization of the ring-protonated forms upon attachment of cyano and acetyl groups (around 10 kcal/mol), solvation in DMSO (around 9 kcal/mol) and the presence of the TFA anion as cosolute (around 3 kcal/mol).

It is also worth noting the differential effect of the two substituents in modulating the relative stability of tautomers in the gas phase and in solution. On the basis of the results given in Tables 1 and 2, acylation of the heterocyclic nitrogen has a larger destabilizing effect (around 6 kcal/mol) of the C5-protonated species than attachment of the cyano group at position 3 (around 4 kcal/mol). Besides the destabilization of the ring-protonated forms caused by the electron-withdrawing influence of the two substituents, the larger effect of the acetyl group also reflects the stronger intramolecular interaction of the carbonyl oxygen with the ammonium group in the N-protonated form compared to the interaction with the immonium group in the ring-protonated tautomer. However, the reverse trend is found in their modulation of the solvent-induced stabilization of the N-protonated conjugated acid. Thus, compared to the parent 2-aminopyrrole (I), inclusion of the cyano group further stabilizes the N-protonated species by around 1 kcal/mol (Table 3), but the acetyl group largely reduces (by around 9 kcal/mol) the solvent-induced destabilization of the ring-protonated forms. This effect can be related to the steric occlusion exerted by the acetyl group, which makes the solvation of the ammonium group difficult. As a result, the cyano group plays a more relevant role than acylation in displacing the equilibrum towards the ammonium species.

Table 5. Differences in free energies of solvation (kcal/mol) between N-, C3- and C5-protonated species of 1-acetyl-2-amino-3-cyanopyrrole (IV) in the presence of a trifluoro acetic acidanion as cosolute in DMSO. Values are given relative to the N-protonated species

Complex	C3	C5
In plane	7.3	12.0
Out of plane	7.1	11.6
Average	7.2	11.8

We hypothesize that cosolute effects play a significant role in modulating the tautomeric equilibrium between ammonium and immonium species of protonated aminopyrrole derivatives. Our estimates of the cosolute effect indicates that this factor exerts a modest, but sizeable, contribution (around 3 kcal/mol) in displacing the equilibrium towards the species protonated at the exocyclic amino nitrogen. At this point, it is worth noting that the model system used in our simulations is possibly a simplistic representation of the real microenvironment of the protonated aminopyrrole in solution, since the experimental procedure indicates that 2 Eq TFA was added to the DMSO solution [8]; therefore, the involvement of different ion aggregates cannot be ruled out. Finally, the potential role played by traces of other species, such as water molecules, which have been found to play a relevant contribution in other systems [31], cannot be completely excluded.

It is also worth noting that the cosolute effect should be solvent-specific. For instance, when 1-acetyl-2-amino-3-cyano-4-*R*-5-*R*′-pyrroles are dissolved in pure TFA, the differential stabilization of the ammonium tautomer owing to the formation of an ion pair with the TFA anion is precluded by the protic nature of this solvent, which should destabilize the ion pair with regard to the solvation of the separated cation and anion. Similar behavior is expected to occur in polar protic solvents such as water, which is able to act as a hydrogen-bond donor and acceptor and solvate efficiently both cations and anions [32]. As a solvent, DMSO is at the top of the solvent polarity/polarizability scale, at the medium-to-high solvent basicity scale and at the low end of the solvent acidity scale [33]; therefore, DMSO should be relatively poor at stabilizing anions where the negative charge is highly localized [34], and the ion pair is expected to be stabler than in TFA or in water, thus further contributing to the stabilization of the N-protonated tautomer.

In summary, the theoretical analysis presented here has allowed us to dissect the differential effects due to substituents, solvent and cosolute in modulating the ammonium/immonium equilibrium between the tauto-

meric conjugate acids of aminopyrrole derivatives. None of these factors alone suffice to change the tautomeric preference for the ring-protonated forms; however, the concerted occurrence of the three factors provides a basis to rationalize the shift in the tautomeric preference towards the conjugated species protonated at the exocyclic nitrogen observed under certain experimental conditions. Overall, our theoretical results pointed out the difficulties existing in the interpretation of experimental data when obtained in conditions far from the gas phase or ideal dilute solutions.

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