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Solvent effects on tautomerism equilibria in heterocycles

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Abstract. High-level *ab initio* quantum mechanical methods have been used to analyze the tautomeric preferences in the gas phase and in aqueous solution of three important five-member heterocycles: 4-(5-)methylimidazole, 5-hydroxyisoxazole, and 3-hydroxypyrazole. Solvent effects have been introduced by means of self-consistent reaction field (SCRF) calculations at the *ab initio* level using our parametrized version of the polarizable continuum model developed by Miertus, Scrocco and Tomasi (MST), including geometry relaxation upon solvation. The extent to which the MST model, and SCRF methods in general, are suitable for the study of processes of this type is discussed.

Keywords: Tautomeric equilibria – Hetro-cycles – Solvent effects – Self-consistent reaction field – Continuum electro statics

1 Introduction

The determination of the tautomeric preferences of heterocycles is a challenging task with direct implications for the interpretation and prediction of chemical reactivity and identification of molecular recognition patterns [1, 2]. Knowledge of the most abundant tautomers is particularly important for understanding the molecular basis of biochemical and pharmacological processes, such as the maintenance of the genetic code [3], substrate binding to enzymes [4], or the specific reading of DNA along the major and minor grooves by drugs and proteins [5]. Indeed, the origin of spontaneous mutagenic processes has been related to the appearance of the nucleic bases in minor tautomeric forms [6]. All these considerations emphasize the relevance of tautomerism equilibria in biomolecular structure and function and explain the experimental and theoretical effort devoted to the study of tautomerism and of the factors modulating the relative stability between tautomers. The influence of the solvent is especially interesting.

High level *ab initio* quantum mechanical calculations allow us to estimate with reasonable accuracy the difference in stability between tautomers in the gas phase for small or even medium-sized heterocycles. The development of efficient computational algorithms and the increase in computer power have facilitated the application of large-scale electronic structure calculations, which can be performed with extended basis sets and with the inclusion of electron correlation effects. The theoretical study of tautomerism in solution is, however, more difficult, since it involves the explicit treatment of a large number of solvent molecules, which is not affordable by current quantum mechanical methods, and the description of subtle effects arising from the interaction of the solute with the bulk solvent molecules, which are not always easy to represent at the classical level.

Current theoretical approaches to tautomerism in solution rely on the calculation of the free energy of solvation, which can be used, in conjunction with the free energy difference between tautomers in the gas phase, to discuss the tautomeric equilibria in solution. The free energy of solvation can be determined from the ensemble of statistically sampled configurations of the solute/solvent system by using force-field-derived methods or mixed quantum mechanical/molecular mechanical techniques. Alternatively, it can be estimated at a low computational cost using self-consistent reaction field (SCRF) continuum models, which replace the microscopic description of the solvent by a polarizable dielectric medium [7].

In this paper we examine the tautomerism equilibria in aqueous solution of three heterocyclic molecules: 4-(5-)methylimidazole, 5-hydroxyisoxazole, and 3-hydroxypyrazole. The calculation combines high level *ab initio* quantum mechanical procedures for the solute in the gas phase with SCRF calculations of the free energy of hydration performed with the *ab initio* parametrized version of the continuum model developed by Miertus, Scrocco and Tomasi (MST [8], also called polarizable continuum model, PCM [9]). Comparison with previous

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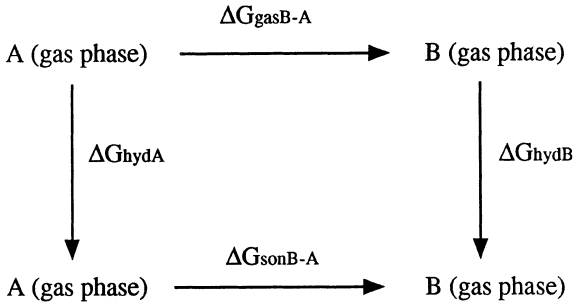
results reported by other authors using these systems allows us to discuss the strengths and shortcomings of the theoretical methods for computation of solvent effects on tautomerism in heterocycles.

2 Methods

The equilibrium geometries for the tautomers were optimized at the gas-phase self-consistent-field restricted Hartree-Fock (SCF-RHF) level using the 6–31G(d,p) basis [10]. Force-constant analyses were carried out to verify the minimum-energy state of the stationary points. Single-point energy calculations were performed at the RHF, MP2, MP3 and MP4(SDTQ) levels of theory using the 6–311+G(d,p) basis set [11]. The frozen-core approximation was used in Møller-Plesset calculations. Differences in gas-phase free energy between tautomers were determined by the addition of zero-point vibrational energies, and thermal and entropic effects [12], which were determined using the standard statistical thermodynamic formulas for harmonic oscillator-rigid rotor [13].

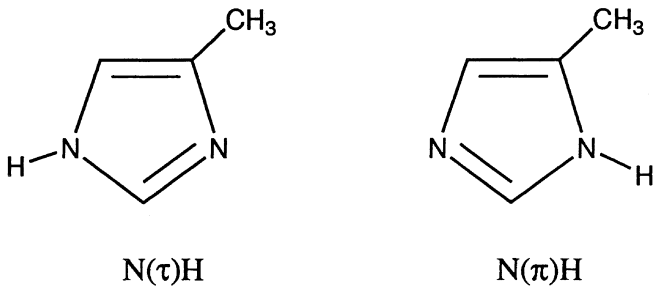
The relative stability in aqueous solution was determined by following the thermodynamic cycle shown in Fig. 1. The contribution to the free energy difference between tautomers due to the change in the solute-solvent interaction ($\Delta\Delta G_{hyd^{B-A}}$) was estimated from SCRF calculations, which were performed using the *ab initio* 6–31G(d) optimized version [8] of the MST continuum model [9]. The method computes the free energy of solvation as the addition of the electrostatic, cavitation and van der Waals components. According to the linear response approximation [1d], the electrostatic term is determined as half the solute-solvent interaction energy. In the MST method the solvent reaction field is described by a set of imaginary charges spread over the solute-solvent interface, which are determined by solving the Laplace equation at the interface with the appropriate boundary conditions (see Ref. [9] for details). Standard charge compensation corrections are used to account for the tails in electron distribution [9a] placed outside the solute cavity. The electrostatic potential originated from the solute charge distribution is determined rigorously at the SCF level [14]. The cavitation component is evaluated following Pierotti's scaled particle theory [15], while the van der Waals term is estimated using a linear relationship with the solvent-excluded surface [8b,c] that represents the solute-solvent interface, which was defined as a set of solvent-excluded surfaces, determined from a set of overlapping spheres centered at the nuclei [16]. Other energy contributions not explicitly considered are accounted for through parameterization of the MST method to experimental free energies of solvation [8].

MST calculations were performed using both the geometry optimized in the gas phase and that obtained after full geometrical relaxation in aqueous solution. Geometry optimizations in solution were performed using the analytical expressions reported recently by the Pisa group [17].



$$\Delta G_{sonB\text{-}A} = \Delta G_{gasB\text{-}A} + \Delta G_{hydB} - \Delta G_{hydA} = \Delta G_{gasB\text{-}A} + \Delta\Delta G_{hydB\text{-}A}$$

Fig. 1. Thermodynamic cycle used to compute free energy differences between tautomers in solution



N(τ)H N(π)H

Fig. 2. Representation of the N(π)H or proximal and N(τ)H or distal tautomers of 4-(5-)methylimidazole

Gas phase calculations were carried out using the Gaussian-94 computer program [18]. MST calculations were performed with locally modified versions of the MonsterGauss [19] and Hondo-8 [20] computer programs. All the calculations were carried out using the SP2 computer at the Centre de Supercomputació de Catalunya (CESCA) and on SGI and HP workstations in our laboratory.

3 Results and discussion

3.1 4-(5-)Methylimidazole

Tautomerism of 4-(5-)methylimidazole (see Fig. 2) is important because of its biological implications. Not only is the imidazole ring the side chain constituent of the amino acid histidine, but it is also involved in various biochemical and pharmacological response mechanisms [21]. Indeed, tautomerism at the imidazole ring has been implicated in the activation mechanism at histaminergic receptors [22].

Table 1 reports the relative energy, enthalpy and free energy between the N(π)H (4-methylimidazole) and N(τ)H (5-methylimidazole) tautomers in the gas phase. At the highest level of theory the N(τ)H tautomer is favored by around 0.7 kcal/mol, and the results do not change significantly when lower-level calculations are considered. Thus, inspection of the values in the table shows that electron correlation effects on the relative stability of the tautomers is only around 0.2 kcal/mol. This is as expected, since the tautomerism of imidazole involves the formation and breaking of N—H bonds in very similar chemical environments, as shown by com-

Table 1. Relative energies, enthalpies and free energies (kcal/mol) in the gas phase for the tautomeric shift N(π)H→N(τ)H of 4-(5-)methylimidazole

Level[a]	ΔE	ΔH	ΔG_{gas}
HF/6-31G(d,p)	−0.2	−0.3	−0.3
HF/6-311+G(d,p)	−0.4	−0.5	−0.5
MP2/6-311+G(d,p)	−0.6	−0.7	−0.7
MP3/6-311+G(d,p)	−0.4	−0.5	−0.5
MP4/6-311+G(d,p)	−0.6	−0.7	−0.7

[a] Single-point energy calculations at the HF/6-31G(d,p) optimized geometries. Zero-point energy, thermal and entropic corrections (298 K) estimated from HF/6-31G(d,p) calculations

parison of the chemical structures of the two tautomers (Fig. 2). Accordingly, correlation effects are expected to almost cancel and the HF level of theory estimates the relative energy accurately enough. Furthermore, comparison of relative energies and free energies shows the negligible effect of zero point, thermal energetic, and entropic effects. Finally, the influence of the basis set seems to be only moderate. In fact, even an RHF calculation performed with the 6-31G(d,p) basis satisfactorily reproduces within 0.4 kcal/mol the best results reported here. Furthermore, these calculations match the MP2/6-31G(d,p)//HF-6-31G estimate of the free energy difference between tautomers reported by Worth and Richards [23].

The free energy difference in aqueous solution (ΔG_{sol}) was determined by the addition of the gas phase free energy difference (ΔG_{gas}) at the MP4/6-311+G(d,p) level to the relative free energy of hydration ($\Delta\Delta G_{hyd}$), which was estimated from MST calculations using the gas phase optimized geometry or upon geometry relaxation in solution (see Methods). The solvent effect on the geometrical parameters is very small, since bond lengths and angles change by less than 0.006 Å and 0.4°. Results in Table 2 show that the effect of the geometry relaxation on the free energy of hydration is rather modest, since it only changes by 0.6 kcal/mol. Indeed, the magnitude of real interest, i.e. $\Delta\Delta G_{hyd}$, is insensitive to the geometry used in the calculation, the tautomer N(π)H being better solvated than the N(τ)H, which agrees with the larger dipole moment of the former tautomer [N(π)H: 4.02 D; N(τ)H: 3.54 D at the HF/6-31G(d,p) level]. The preferential hydration of the N(π)H species is large enough to revert the tautomeric equilibrium in the gas phase, since the free energy difference in aqueous solution ranges from 0.3 to 0.1 kcal/mol when the ΔG_{sol} determined at the HF and MP4/6-311+G(d,p) levels are used.

Our estimate of $\Delta\Delta G_{hyd}$ agrees with previous results determined from Molecular Dynamics–Free Energy Perturbation (MD–FEP) simulations, which indicate a difference in free energy of hydration of the two tautomers around 0.2 kcal/mol [23]. Indeed, the relative stability predicted in aqueous solution is in agreement with the range of experimental values for the equilibrium constant, which varies from 0.5 to 1.2 for the tautomeric shift N(π)H→N(τ)H, corresponding to −0.1 to 0.4 kcal/mol for ΔG_{sol} (values taken from Ref. [23]).

Table 2. Free energy of hydration (ΔG_{hyd}; kcal/mol) of tautomers N(π)H and N(τ)H of 4-(5-)methylimidazole, relative free energy of hydration ($\Delta\Delta G_{hyd}$) and free energy of tautomerization (ΔG_{sol}) in aqueous solution for the tautomeric shift N(π)H→N(τ)H

Geometry[a]	ΔG_{hyd}(N(π)H)	ΔG_{hyd}(N(τ)H)	$\Delta\Delta G_{hyd}$	ΔG^{b}_{sol}
Gas	−7.4	−6.6	0.8	0.1
Solution	−8.1	−7.3	0.8	0.1

[a] Optimized in the gas phase or in aqueous solution at the HF/6-31G(d,p) level
[b] Determined using the free energy difference in the gas phase estimated at the MP4/6-311+G(d,p) level (see Table 1)

3.2 5-Hydroxyisoxazole

The tautomeric equilibria of this heterocycle (Fig. 3) are difficult to assess experimentally because of the instability of this compound. They have, however, been the subject of recent stimulating theoretical studies in the gas phase and solution [24].

The gas phase tautomeric equilibria of compounds **1**–**4** have recently been examined by Cramer and Truhlar, who used both HF and correlated methods [Møller-Plesset (MP) up to fourth order, and coupled-cluster single-and-double (CCSD) excitations with perturbative triple excitations] with large, extended basis sets, and geometry optimization at the HF and MP2 levels [24c]. For the purposes of this study we simply adopt their best estimated free energy differences in the gas phase (kcal/mol): (**1**) 6.2; (**2**) 0.0; (**3**) 5.6; (**4**) 5.8. These values were determined by adding the difference between CCSD excitations and MP2 levels of correlation using the correlation-consistent polarized valence double-ζ basis set of Dunning, augmented with diffuse and higher angular momentum functions to the MP2 results obtained using the analogous triple-ζ basis.

Table 3 reports the electrostatic and non-electrostatic components of the free energy of hydration determined with several geometries and various solvation models. All the MST results suggest that tautomer **1** is the best hydrated, followed by tautomer **2**, **4** and **3**, which are about 1, 2 and 4 kcal/mol less well stabilized by the solvent than **1**.

The first row in Table 3 reports the results determined from the MST model using the gas phase optimized

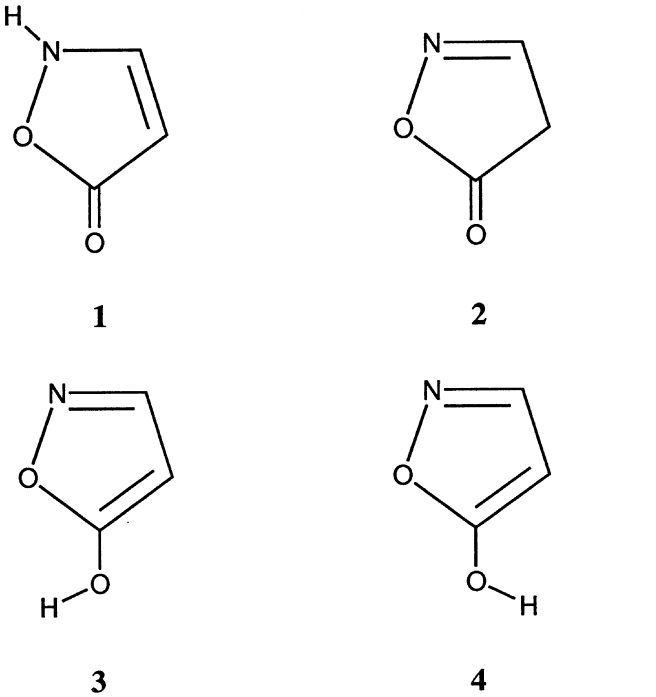


Fig. 3. Representation of the four tautomers of 5-hydroxyisoxazole: (**1**) 5(*2H*)-isoxazolone; (**2**) 5(*4H*)-isoxazolone; (**3**) *syn*-5-hydroxyisoxazole; (**4**) *anti*-5-hydroxyisoxazole

Table 3. Electrostatic (ΔG_{el}) and non-electrostatic ($\Delta G_{non\text{-}el}$) components (kcal/mol) of the free energy of hydration (ΔG_{hyd}), and relative free energies of hydration ($\Delta\Delta G_{hyd}$) for tautomers of 5-hydroxyisoxazole

Geometry[a]	Method		**1**	**2**	**3**	**4**
Gas, HF/6-31G(d,p)	MST, 6-31G(d)	ΔG_{el}	−13.4	−11.1	−9.3	−11.5
		$\Delta G_{non\text{-}el}$	3.0	3.5	2.9	2.9
		ΔG_{hyd}	−10.4	−7.6	−6.4	−8.6
		$\Delta\Delta G_{hyd}$	−2.8	0.0	1.1	−1.1
Gas, MP2/cc-pVDZ[b]	MST, 6-31G(d)	ΔG_{el}	−14.1	−11.8	−9.6	−12.1
		$\Delta G_{non\text{-}el}$	3.0	3.6	2.2	2.9
		ΔG_{hyd}	−11.1	−8.2	−7.4	−9.2
		$\Delta\Delta G_{hyd}$	−2.9	0.0	1.5	−1.0
Solution, HF/6-31G(d,p)	MST, 6-31G(d)	ΔG_{el}	−13.7	−11.2	−9.5	−11.6
		$\Delta G_{non\text{-}el}$	3.0	3.5	3.2	3.2
		ΔG_{hyd}	−10.7	−7.7	−6.3	−8.4
		$\Delta\Delta G_{hyd}$	−3.0	0.0	1.5	−0.7
Gas, HF/3-21G[c]	PCM, 6-31G(d,p)	ΔG_{el}	−17.2	−16.3	−12.3	–
Gas, HF/3-21G[d]	Onsager, 6-31G(d,p)	ΔG_{el}	−6.2	−5.0	−0.7	−4.3
Gas, AM1[d]	AM1-SM2	ΔG_{el}	−5.8	−4.4	−3.1	−3.5
		$\Delta G_{non\text{-}el}$	−4.7	−3.8	−6.4	−6.3
		ΔG_{hyd}	−10.5	−8.2	−9.5	−9.8
		$\Delta\Delta G_{hyd}$	−2.4	0.0	−1.4	−1.7
Solution, AM1[d]	AM1-SM2	ΔG_{el}	−6.2	−4.5	−3.2	−3.6
		$\Delta G_{non\text{-}el}$	−4.7	−3.7	−6.4	−6.3
		ΔG_{hyd}	−10.9	−8.2	−9.5	−9.9
		$\Delta\Delta G_{hyd}$	−2.7	0.0	−1.3	−1.7
Gas, HF/3-21G [c]	MD-FEP	$\Delta\Delta G_{hyd}$	−2.1	0.0	1.8	–

[a] Determined in the gas phase or in aqueous solution at the corresponding level of theory
[b] cc-pVDZ: correlation-consistent polarized valence double-ζ basis set of Dunning
[c] Ref. 25b
[d] Ref. 25c

geometry at the HF/6-31G(d,p) level. The differences in the free energy of hydration between tautomers arise mainly from the electrostatic contribution, which is the largest component of ΔG_{hyd}, while the destabilizing effect of the non-electrostatic component is nearly the same for all the tautomers. The differences in the electrostatic free energy roughly reflect the differences in the dipole moments of the tautomers. Thus, tautomers **1** and **3** have the largest and lowest dipole moments [**1**: 5.72 D; **2**: 5.08 D; **3**: 1.81 D; **4**: 4.74 D at the HF/6-31G(d,p) level], which agrees with the relative electrostatic stabilization induced by the solvent upon solvation. In contrast, tautomer **4** is better hydrated than **2** by around 1 kcal/mol, in spite of its lower dipole moment, partially because of the effect of non-electrostatic factors.

Comparison of results in the first row with the values in the second and third rows allows us to gain insight into the effect of molecular geometry on hydration. Inspection of the geometries optimized at the MP2/cc-pVDZ and HF/6-31G(d,p) levels reveals differences in bond lengths and angles of around 0.05 Å and 4°, which have little influence on the total free energy of hydration, since the electrostatic term changes by around 0.3–0.7 kcal/mol, while the non-electrostatic term remains largely unaffected. Geometry relaxation in water leads to even smaller changes in geometry (around 0.02 Å and 2° in bond lengths and angles), which are reflected in negligible variations of the hydration free energy. Therefore, these results indicate that the choice of geometry is not expected to have a great influence on the free energies of hydration for this tautomeric equilibrium.

Comparison of MST results with the PCM values reported by Woodcock et al. [24b] is especially interesting, since both are based on the same physical model. Such comparison reveals that the solvent-induced electrostatic stabilization exhibits qualitatively similar trends, but there are clear quantitative differences in the results. Thus, the preferential stabilization of tautomer **1** with regard to **2** is reduced by around 60%, while the destabilization of tautomer **3** is greatly enhanced up to 4.0 kcal/mol. Indeed, the PCM results are larger, in absolute values, by 3.0–5.2 kcal/mol than the MST ones.

These differences may stem from the difference in the basis set or from the definition of the solute/solvent interface, since the preceding discussion rules out a significant influence of the geometry. To test the effect of the basis set, we perfomed MST calculations with the 6-31G(d,p) basis. The results (data not shown) indicate that the electrostatic contribution to ΔG_{hyd} reproduces the results at the 6-31G(d) level within 0.2 kcal/mol. Therefore, the origin of such quantitative discrepancies can be attributed to the definition of the solute cavity. In the MST method the cavity is built up using a universal empirical scaling factor of 1.25, i.e., van der Waals radii of 1.4 Å(O), 1.5 Å(C), 1.5 Å(N), 0.9 Å (H bound to polar atom), and 1.2 Å (H bound to carbon) are multiplied by a factor of 1.25 to give the effective radii used to build up the cavity. In contrast, the cavity in the PCM calculations was defined following Aguilar and Olivares del Valle [25], who suggested determining the atomic radii in terms of the Mulliken partial charge, which allows to include the influence of the local environment. In particular, they used a model involving a linear dependence between the radius and the atomic charge, which leads to important differences between the two sets of atomic radii. Interestingly, there is overall compensation, since the volume of the cavity ($\sim$96 Å^3) is similar to that found in MST calculations (90–95 Å^3), but there are notable differences in the atomic contributions to the volume. Thus, the contribution of hydrogens and carbonyl carbon to the cavity is negligible using the atomic radii as determined following Aguilar and Olivares del

Valle, whereas the contribution of the rest of atoms is larger than in the MST model. The different atomic contributions to the cavity surface have a large impact on ΔG_{hyd}. Thus, test calculations performed with the MST model and the 6–31G(d,p) basis indicated that the electrostatic term is increased by nearly 3 kcal/mol, which explains most of the difference between the values reported by Woodcock et al. [24b] and present results.

MST results can also be compared with the values computed from the Onsager model [26]. Differences in the electrostatic free energy range between 6 and 9 kcal/mol, which represents a reduction of more than 50% (in absolute terms) with regard to the MST values. Such a great discrepancy can mainly be attributed to the treatment of (a) the solvent reaction field, and (b) the solute/solvent interface. Onsager's calculation uses a simple dipole representation of the solute charge distribution, neglecting the influence of higher order multipolar contributions, which can be important. Furthermore, these calculations were performed using a single sphere of radius 3.6 Å determined following Wong et al. [27]. In the MST method the solute cavity is defined in terms of the molecular-shaped solvent-excluded surface, which for tautomers of 5-hydroxyisoxazole encloses a volume of 90–95 $Å^3$, contrasting with the volume of $\sim$195 $Å^3$ in the Onsager calculations. Since in this latter model the electrostatic free energy is inversely proportional to the volume of the spherical cavity, the Onsager values are expected to be lower than the MST ones by 53%, which explains semiquantitatively the reduction in the electrostatic component for tautomers **1**, **2** and **4**. The large error in the Onsager estimate of ΔG_{el} of tautomer **3** is probably also due to the intrinsic shortcomings of the dipolar representation of the solute.

The MST results can also be compared with the AM1-SM2 values determined with and without geometry relaxation in aqueous solution. Inspection of results in the last two rows of Table 3 confirms the small influence (less than 0.5 kcal/mol) of geometry relaxation in the determination of the hydration free energy, which agrees with our findings derived from MST calculations. Comparing MST and SM2 calculation, there is clear qualitative agreement in the electrostatic stabilization of the tautomers, but the absolute value of the electrostatic free energy is underestimated by 60% in AM1-SM2 calculations with respect to the MST values in all cases. These differences probably stems from the lower charge separation of the tautomers in the context of AM1 calculations, and from the use of slightly different cavities in SM2 calculations. The non-electrostatic term has a net stabilizing influence in the AM1-SM2 model, which contrasts with the MST results. However, despite the differences in the various contributions, it is worth noting the agreement between AM1-SM2 and MST absolute free energies of hydration, particularly for tautomers **1**, **2** and **4**. This is not surprising, since the two models were parametrized to reproduce experimental free energies of solvation and, accordingly, overall agreement in ΔG_{hyd} is expected in spite of the differences in the separate components. In fact, Cramer and Truhlar have pointed out that "non-electrostatic" terms in the AM1-SM2 method include not only cavity effects, but also make up for the systematic underestimation of the electrostatic contribution due to the use of AM1 Hamiltonians [28]. The only serious discrepancy between AM1-SM2 and MST calculations is found for tautomer **3**, which is around 1 kcal/mol more or less stabilized than **2** according to the AM1-SM2 and MST results respectively. This difference is due to the non-electrostatic term, since the two methods indicate that the electrostatic contribution destabilizes **3** around 1.3 kcal/mol. However, the non-electrostatic component favors **3** by 2.6 kcal/mol at the AM1-SM2 level, while it stabilizes **3** by only 0.6 kcal/mol in the MST model.

Finally, MST results compare well with the relative free energies of hydration determined from MD-FEP simulations [24b]. MD-FEP calculations give the following ordering of stability (kcal/mol): (**1**) −2.1; (**2**) 0.0; (**3**) 1.8, in almost quantitative agreement with MST estimates. A planar geometry was used for tautomer **1** in the MD-FEP studies, but this is not expected to introduce significant changes in the relative free energy of hydration, which indicates this tautomer to be better hydrated than **2**, in agreement with SCRF results. Indeed, MD–FEP simulations reveal that tautomer **3** is destabilized with regard to **2** upon solvation, which agrees with the finding derived from MST calculations.

Table 4 reports the relative free energies of tautomerization in aqueous solution determined according to the thermodynamic cycle in Fig. 1. The small influence of the geometrical parameters used in SCRF calculations is reflected in the similarity of the results obtained with either MST or AM1-SM2 calculations. Indeed, as expected, the agreement between the values determined with AM1-SM2 and MST SCRF for tautomers **1**, **2** and **4** is good. The results suggest that the most abundant species of 5-hydroxyisoxazole is tautomer **2**, since the difference in relative stability of tautomer **1** is at least 3.2 kcal/mol, and the hydroxy forms **3** and **4** are even less stable. Unfortunately, there is no

Table 4. Relative free energy of tautomerization in solution (kcal/mol) for tautomers of 5-hydroxyisoxazole

Geometry[a]	Method	**1**	**2**	**3**	**4**
Gas, HF/6-31G(d,p)	MST, 6-31G(d)	3.4	0.0	6.7	4.7
Gas, MP2/cc-pVDZ[b]	MST, 6-31G(d)	3.3	0.0	7.1	4.8
Solution, HF/6-31G(d,p)	MST, 6-31G(d)	3.2	0.0	7.1	5.1
Gas, AM1[c]	AM1-SM2	3.8	0.0	4.2	4.1
Solution, AM1[c]	AM1-MS2	3.5	0.0	4.3	4.1

[a] Determined in the gas phase or in aqueous solution at the corresponding level of theory
[b] cc-pVDZ: correlation-consistent polarized valence double-ζ basis set of Dunning
[c] Ref. 25c

definitive experimental evidence on the tautomeric equilibria of this compound, but the results clearly show that the keto species is favored in water. In the absence of direct experimental evidence, the agreement of MST with other high level estimates gives confidence in our calculations for this molecule.

3.3 3-Hydroxypyrazole

The tautomerism of 3-hydroxypyrazole is of particular relevance since it involves equilibria between chemical species with two vicinal nitrogen atoms. Various studies, most of them performed by Elguero and coworkers [1b, 29], led to the assumption that those tautomers with contiguous heteroatoms of the same hybridization type are greatly destabilized. This assumption has been used to discuss the tautomeric preference of 1,2,4- and 1,2,3-triazole, which mainly take N1H and N2H forms respectively. The preference of these tautomers is explained by the lone pair repulsion between vicinal pyridine-like nitrogens or by bond dipole repulsion between two pyrrol-like nitrogens. Recently, Elguero and coworkers have extended this assumption to the study of tautomerism in benzotriazole [30].

Previous studies [31] on the tautomerism of 3-hydroxypyrazole in the gas phase and in solution have limited the number of relevant tautomers to only five species, which are shown in Fig. 4: 3-hydroxypyrazole (**1** and **2**), 4-pyrazolin-3-one (**3**), 2-pyrazolin-5-one (**4**) and 5-hydroxypyrazole (**5**). The tautomeric equilibria between hydroxy and keto structures is modulated by the combined effect of three factors. First, the keto species have intrinsicically higher stability. Second, the presence of two vicinal pirrol-like nitrogens in tautomer **3** tends to decrease the stability of the keto tautomer. Third, the solvent affects the keto-enol tautomerism. Therefore, the population of the different tautomers in the gas phase or in solution is expected to be modulated by the subtle balance between these factors. To our knowledge, no gas phase data are available for the relative stability of these tautomers. However, experimental estimates [31b] in aqueous solution indicate the stability ordering to be **3** > (**1**, **2**) > **5** > **4**. Accordingly, for the purpose of this study we have limited our attention to tautomers **1**, **2** and **3**.

Cao et al. [31c] reported the hydroxy tautomer **1** to be the preferred species in the gas phase, it being favored by 2.9 and 7.7 kcal/mol with regard to tautomers **2** and **3**. In fact, the second most stable species in the gas phase was found to be tautomer **4** (1.2 kcal/mol less stable than **1**). In that study the geometry was optimized at the MP2/6-31G(d,p) level and single-point calculations were subsequently performed with larger basis and inclusion of higher order correlation effects. In particular, the preceding values were determined by adding the difference in energy between CCSD excitations and MP2 calculations performed with the 6-31G(d,p) basis to the MP2 energies determined with the 6-311 + G(3df,2p) basis (see Table 5). The difference in stability between tautomers **1** and **3** is close to the value of 6.6 kcal/mol reported by Parchment et al., which was determined from MP4/6-31G(d,p) calculations using the geometry optimized at the HF/3–21G level [31b].

The results reported by Cao et al. indicate that the difference in stability between hydroxy and keto forms is particularly sensitive to electron correlation effects [32]. This is illustrated by our MPx calculations with the 6-311 + G(d,p) basis (see Table 5), according to which the free energy difference between tautomers **1** and **3** changes from 9.7 (MP2) to 8.1 (MP3) and to 6.2 (MP4) kcal/

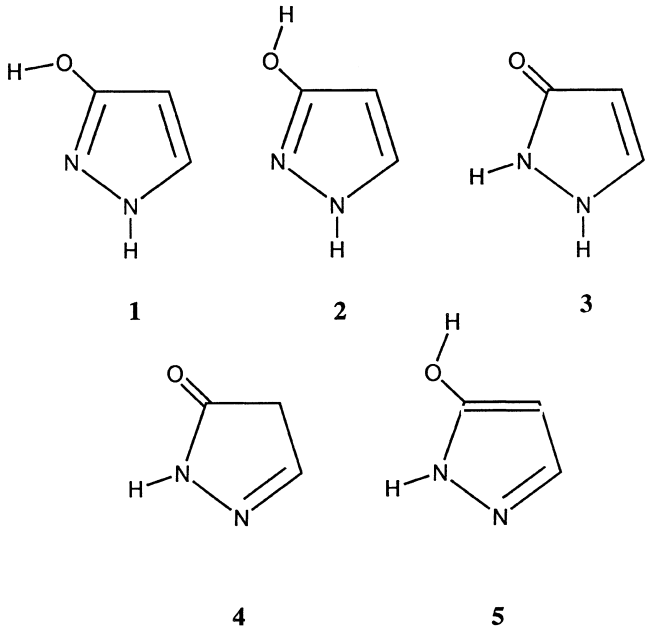


Fig. 4. Representation of the three tautomers of 3-hydroxypyrazole: (**1**) *syn*-3-hydroxypyrazole; (**2**) *anti*-3-hydroxypyrazole (**3**) 4-pyrazolin-3-one; (**4**) 2-pyrazolin-5-one; (**5**) 5-hydroxypyrazole

Table 5. Relative free energies (kcal/mol) in the gas phase for tautomers of 3-hydroxypyrazole

Level[a]	1	2	3
HF/6-31G(d,p)	0.0	3.7	3.9
HF/6-311 + G(d,p)	0.0	3.7	3.5
HF/6-311 + G(3df,2p)[b]	0.0	3.4	2.9
MP2/6-311 + G(d,p)	0.0	3.1	9.7
MP3/6-311 + G(d,p)	0.0	3.0	8.1
MP4/6-311 + G(d,p)	0.0	3.1	6.2
MP4/6-31G(d,p)[c]	0.0	–	6.6
CCSD/6-31G(d,p)[b,d]	0.0	2.9	7.7
Composite[e]	0.0	2.8	5.7

[a] Single-point energy calculations at the HF/6-31G(d,p) optimized geometries. Zero-point energy, thermal and entropic corrections (298 K) estimated from HF/6-31G(d,p) calculations

[b] Ref. 31c

[c] Ref. 31b. The HF/3-21G optimized geometry was used in single-point calculations

[d] Estimated from addition of the difference between CCSD/6-31G(d,p) and MP2/6-31G(d,p) energies to the MP2/6-311 + G(3df,2p) value

[e] Estimated from addition of the difference between MP4/6-311 + G(d,p) and HF/6-311 + G(d,p) energies to the HF/6-311 + G(3df,2p) value

mol. The sensitivity to the basis set is smaller. This is shown by the values determined at the HF level, which show reasonable convergence, since the relative stability of **1** and **3** computed with the 6-31G(d,p), 6-311 + G(d,p) and 6-311 + G(3df,2p) basis decreases from 3.9 to 3.5 and to 2.9 kcal/mol. Furthermore, the smaller sensitivity to basis set is also shown by comparison of the correlation energy up to MP4 theory determined with the 6-31G(d,p) and 6-311 + G(d,p) basis, which amounts to 3.2 and 2.7 kcal/mol respectively. Accordingly, we have determined our best estimate for the relative stability between tautomers from the addition of the correlation energy determined up to MP4/6-311 + G(d,p) level to the difference in energy estimated from HF/6-311 + G(3df,2pd) calculations. These values are denoted as "composite" in Table 5.

Table 6 reports the electrostatic and non-electrostatic components of the free energy of hydration determined with different geometries and different solvation models. The first row reports the results determined from the MST model using the gas phase HF/6-31G(d,p) optimized geometry. As noted before for 5-hydroxyisoxazole, the differences in the free energy of hydration between tautomers are due mainly to the electrostatic term, while the non-electrostatic component, which has a destabilizing effect, ranges between 2.5 and 2.7 kcal/mol. Again, the differences in the electrostatic free energy follow approximately the variations in dipole moment of the tautomers (**1**: 2.49 D; **2**: 3.83 D; **3**: 4.97 D), tautomers **2** and **3** being better stabilized upon solvation than **1** by 3.1 and 3.6 kcal/mol respectively.

Comparison with the values in the second row provides insight into the effect of geometry relaxation in solution. Inspection of the geometries in the gas phase and in solution shows small changes in bond lengths and angles (around 0.01 Å and 1°) for **1** and **2**, but they are larger for **3** (around 0.03 Å and 2.5°), even though the most marked change is observed in the dihedral angle HN—NH, which decreases around 10°. The change in the free energy of hydration is around 1 kcal/mol, which is small (less than 10% the total value), but notably larger than the effect observed for 4-(5-)methylimidazole and 5-hydroxyisoxazole. This change arises from the electrostatic component, while the non-electrostatic term remains unaffected.

The present MST results can be compared with the PCM values reported by Parchment et al. [31b], which were determined using the atomic radius estimated following Aguilar and Olivares del Valle [26]. The PCM values of ΔG_{el} are around 3 kcal/mol larger in absolute values than the MST ones. As noted before for 5-hydroxyisoxazole, the effect of the basis set analyzed from MST calculations performed with the 6-31G(d,p) basis is estimated to be small (0.7 kcal/mol), and the discrepancy between MST and PCM results can mainly be attributed to the definition of the solute cavity. Thus, the radii for hydrogens are clearly smaller, while heteroatoms are notably larger than those used in the MST model. The electrostatic component increases in absolute value by around 2.0 kcal/mol when the atomic radii determined following Aguilar and Olivares del Valle's approach are used in MST calculations performed with the 6-31G(d,p) basis.

Finally, MST results can be compared with the relative free energies of hydration determined from FEP simulations [31b, c]. Surprisingly, there is large difference in the values determined from the two FEP simulations, since the Monte Carlo (MC)-FEP [31c] estimate is nearly twice the value determined from MD-FEP [31b] simulations. This large difference likely stems from the origin of the atomic partial charges. Thus, HF/6-31G(d) electrostatic charges were used in MD-FEP, whereas in MC-FEP simulations electrostatic charges were determined from HF/6-31G(d,p) SCRF calculations, which are expected to include a part of the solvent-induced polarization of the solute charge distribution [33].

The MC–FEP solvent-induced stabilization for **3** is notably larger than the MST value. This suggests that first-solvation shell effects (hydrogen bonding), which cannot be properly reflected within the framework of current SCRF methods, play a decisive role in the preferential hydration of tautomer **3**. At this point, it is worth noting the partial zwitterionic nature of this tautomer due to the canonical resonance structure with net charge separation (see Fig. 5), whose contribution is expected to be enhanced upon transfer from the gas phase to aqueous solution. The increased negative charge on the oxygen atom reinforces the strength of (hydrogen bonding) interactions with the surrounding water molecules, which in turn leads to better hydration. This effect is not easy to represent at the SCRF level, since the same cavity definition is used for neutral molecules irrespective of the weight due to ionic resonance structures.

Table 6. Electrostatic (ΔG_{el}) and non-electrostatic ($\Delta G_{non\text{-}el}$) components (kcal/mol) of the free energy of hydration (ΔG_{hyd}) for tautomers of 3-hydroxypyrazole

Geometry[a]	Method		**1**	**2**	**3**
Gas, HF/6-31G(d,p)	MST, 6-31G(d)	ΔG_{el}	−10.0	−13.0	−13.7
		$\Delta G_{non\text{-}el}$	2.6	2.6	2.7
		ΔG_{hyd}	−7.4	−10.4	−11.0
		$\Delta\Delta G_{hyd}$	0.0	−3.1	−3.6
Solution, HF/6-31G(d,p)	MST, 6-31G(d)	ΔG_{el}	−10.8	−14.4	−14.9
		$\Delta G_{non\text{-}el}$	2.6	2.5	2.7
		ΔG_{hyd}	−8.3	−11.8	−12.2
		$\Delta\Delta G_{hyd}$	0.0	−3.6	−3.9
Gas, HF/3-21G[b]	PCM, 6-31G(d,p)	ΔG_{el}	−13.1	–	−16.3
Gas, HF/3-21G[b]	MD-FEP	$\Delta\Delta G_{hyd}$	0.0	–	−4.6
Gas, HF/3-21G[c]	MC-FEP	$\Delta\Delta G_{hyd}$	0.0	−2.9	−10.9

[a] Determined in the gas phase or in aqueous solution at the corresponding level of theory
[b] Ref. 31b
[c] Ref. 31c

Table 7. Relative free energy of tautomerization in solution (kcal/mol) for tautomers of 3-hydroxypyrazole

Geometry[a]	Method	1	2	3
Gas, HF/6-31G(d,p)	MST, 6-31G(d)	0.0	−0.3	2.1
Solution, HF/6-31G(d,p)	MST, 6-31G(d)	0.0	−0.8	1.8
Gas, HF/3-21G[b]	MD-FEP	0.0	–	1.2
Gas, HF/3-21G[c]	MC-FEP	0.0	−0.1	−5.2

[a] Determined in the gas phase or in aqueous solution at the corresponding level of theory
[b] Ref. 31b
[c] Ref. 31c

Table 7 reports the relative free energies of tautomerization in aqueous solution determined upon addition of the relative free energies of hydration to the gas phase tautomerization free energy. The results indicate that the stability of species **1** and **2** is very similar, the difference being less than 1 kcal/mol, in agreement with the experimental estimates [31b]. Both MST and MD-FEP results indicate that tautomer **3** is less stable than **1** in solution, even though the difference in stability is greatly reduced with respect to the situation in the gas phase. Experimental studies [31b] indicate, nevertheless, that tautomer **3** is favored by around 1.4 kcal/mol over **1** and **2**. It is worth noting that with our best gas phase estimate of the tautomerization free energy only the MC-FEP calculations qualitatively reproduce the experimental preference of **3** over species **1** and **2**. However, the stability seems to be overestimated in these calculations, since the relative free energy of hydration of tautomer **3** is nearly 3 kcal/mol larger than the experimental value.

Comparison of the different *ab initio* estimates of the gas phase relative stability for **1**, **2** and **3** raises doubts about the convergence of results even at our highest level (composite in Table 5). It is difficult, however, to believe that the uncertainty in the gas phase free energy differences would be large enough to justify the MST estimate of the free energy in solution. Rather, it seems that MST calculations are unable to reproduce properly the differential hydration of these tautomers, which is probably due to the inability of SCRF methods to account for changes in the solvent-solute interface occurring as a result of a drastic reorganization of the solute charge distribution upon solvation. In this case, it is suggested that the solvent can increase the weight of zwitterionic resonance structures (see Fig. 5), which interact with water molecules more firmly than would be the case for a typical neutral solute. This effect should be reflected at the MST level by a reduction in the solute cavity. In fact, our previous studies for solvation of ions in aqueous solution suggested that the scaling factor used to define the cavity should be reduced from 1.25 to 1.15 [34]. If the present MST calculations are repeated using a scaling factor of 1.2 for tautomer **3**, the tautomers **2** and **3** have the same stability in aqueous solution, while for an ionic cavity the free energy difference is slightly larger than experimental value.

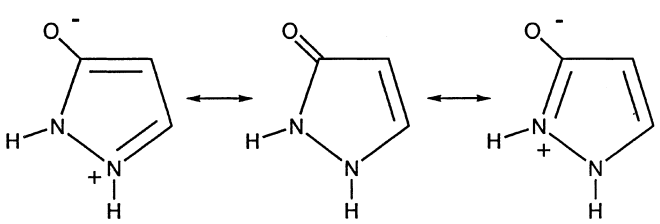


Fig. 5. Zwitterionic resonance structures of 3-hydroxypyrazole

The preceding discussion stresses the great dependence of the SCRF results on the cavity definition. In particular, the results point out the need to introduce further refinements in future versions of the MST method, and in general of SCRF methods, which should include more flexible cavity definitions, able to account for large changes in the solvent response to the solute charge distribution.

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