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Effect of Water on the Tautomeric Equilibrium of Uracil Monoanions. A PCILO Study*

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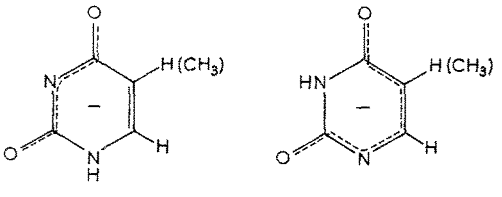
The PCILO method is used to calculate the stabilization energies of the anionic forms of uracil by water molecules forming the first hydration shell of the tautomers. The results show that the 1-HU⁻ tautomer is more stabilized by the solvent effect than the 3-HU⁻ tautomer by about 8 kcal/mole. The effect brings closer together the energies of the two tautomers which differ intrinsically by about 13 kcal/mole in favour of the 3-HU⁻ form. It operates in the direction inferred from experimental studies.

Key words: Tautomeric equilibrium of uracil monoanions - Uracil monoanions, tautomeric equilibrium of ~

1. Introduction

Uracil (or thymine) in an alkaline solution deprotonates at N_1 or N_3 to give two monoanionic forms 3-HU$^-$ (3-HT$^-$) or 1-HU$^-$ (1-HT$^-$), respectively. Both tautomers exist in solution as a mixture of comparable amounts (see Ref. [1] for the collection of tautomeric ratios of anionic forms of uracil, thymine and their derivatives). A more detailed comparison indicates that the proportion of 1-HU$^-$ (1-HT$^-$) and 3-HU$^-$ (3-HT$^-$) tautomers varies with the nature and concentration of the buffer employed [2, 3].



1-HU⁻(1-HT⁻) 3-HU⁻(3-HT⁻)

Unfortunately, there are no direct experimental data on the proportion of the anions in the gas phase. Starting from the observation that the anions 3-HU$^-$ (3-HT$^-$) are favoured by concentrated phosphate buffers of low dielectric constant [1-3] and assuming that the solvation energy of the anions is directly proportional to the sum of the calculated squares

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of atomic charges and inversely proportional to the dielectric constant and extrapolating then to a dielectric constant of unity, Snyder *et al.* [4] have estimated that in the gas phase the anion 3-HT$^-$ is more stable than 1-HT$^-$ by about 13 kcal/mole. A practically identical value is obtained by theoretical computations carried out by the PCILO method, while *ab initio* and CNDO/2 methods yield a somewhat larger value [4, 5]. Both the "experimental" and theoretical values can only be considered as very rough approximations (probably by excess [6]) to the true situation, the theoretical values if only because of uncertainties in the input geometries.

The purpose of this note is an attempt to interpret the influence of the solvation on the change of the tautomeric equilibrium observed for the monoanions of uracil when going from the vapour phase to water solution.

2. The Method

In order to interpret the effect of the water molecules on the shift of the tautomeric equilibrium 1-HU$^-$ $\rightleftarrows$ 3-HU$^-$, we have used the microscopic supermolecule approach, which has been satisfactorily applied to predict the hydration sites of a large number of biomolecules as well as to explain the change of the conformational properties of such molecules upon solvation [7] and very recently to interpret the shift of the tautomeric lactam-lactim equilibrium of oxopyridines upon solvation [8, 9]. The approach was used within the PCILO methodology [10, 11]. Concerning the input data, the individual geometries of the monoanions and water molecules have been kept constant in all calculations. The experimental geometry ($r_{\text{O-H}}$ = 0.957 Å, HOH = 104.52°) was used for water. For the monoanions of uracil, the positions of the heavy atoms were assumed to be the same as those in 1-methylthymine as shown in a neutron diffraction study [12]. The values of 1.047 Å for the bond N_1-H and 1.096 Å for the bond C_6-H were assumed to be the same as those for the bonds N_3-H and C_5-H, respectively [12].

3. Results and Discussion

The calculations have been performed in two steps. First, preferred hydration sites have been computed for the two uracil monoanions using the supermolecule approach involving one water molecule at a time. The oxygen atom of the water was moved around in the plane of the tautomers, and for a fixed oxygen position the most probable geometrical structure of the supermolecule has been calculated changing the distance, the configuration of the water relative to the monoanions and rotating it about the O-H bonds. In this way we have been able to determine the hydration sites as the loci of the surface revealing the energetically preferred water-monoanion configurations. As we are essentially interested in the difference between the solvation energies of the two tautomers, we did not investigate the hydration sites surrounding the C_5-C_6 region of the monoanions, previous tentative CNDO/2 calculations [8] showing no significant differences in this respect. Calculations for which the oxygen atom of the water molecule would be located out of the plane of the tautomers were not carried out either, because non-empirical computations indicate that this type of interaction between water and the nucleic acid bases is not very important [7] compared to the in-plane interactions.

The preferred hydration sites computed in the manner indicated above are shown for both tautomeric monoanions of uracil in Figs. 1a and b which contain also the corresponding stabilization energies.

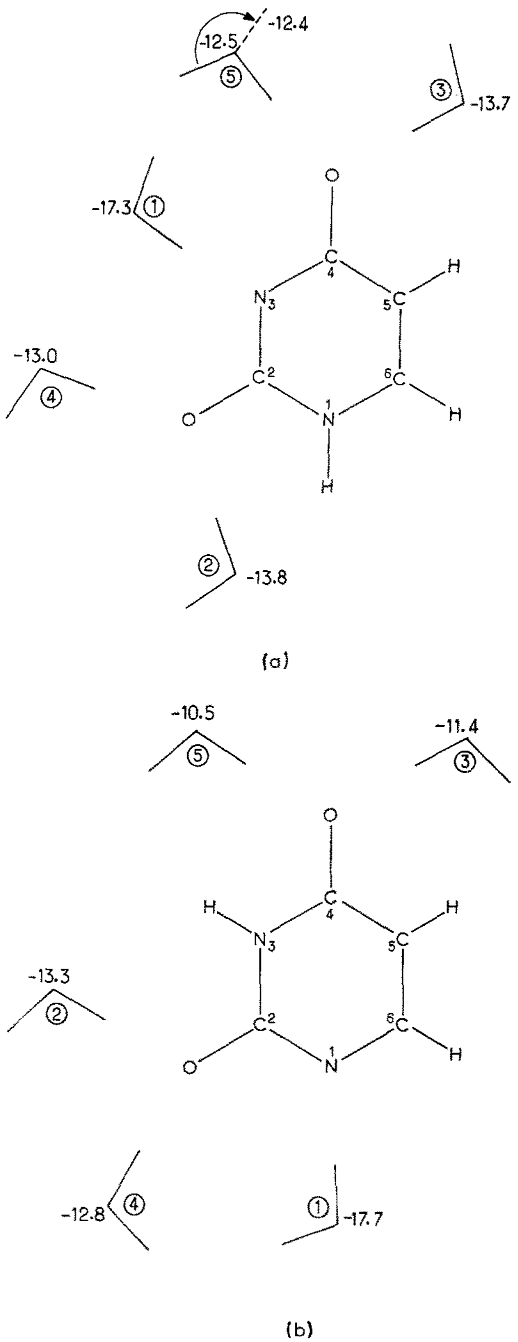

Fig. 1. Stabilization energies (in kcal/mole), i.e. the differences between the total energies of the complexes (monoanion + H_2O) and the energies of their components (monoanion, H_2O). (a) The 1-HU^- anion, (b) the 3-HU^- anion

Applying a simple additivity rule to the results of Figs. 1a and b would lead to a preferential stabilization by the solvent of 1-HU^- by about 4.6 kcal/mole.

In the second step of the computation the *simultaneous* presence of the five water molecules in the first hydration shell of the anions was taken into account by a reoptimization of their respective positions and a recomputation of the stabilization energy of the supermolecule formed by the tautomers + five H_2O. The results indicate that there are no significant differences between the configurations of the single water molecules placed at the sites ①-⑤ of the first hydration shell and the configurations of the five water molecules fixed simultaneously around the tautomers. The only significant difference concerns the position of the water ⑤ in the anion 1-HU$^-$ where it is changed as indicated in Fig. 1a by an arrow. On the other hand, the differential stabilization of the two tautomeric anions amounts, in this refined approximation, to 7.7 kcal/mole in favour of the anion 1-HU$^-$.

It may be interesting to remark that a similar result is obtained when the above indicated result obtained by simple additivity rule based on Fig. 1a, b is completed by a rough evaluation of the effect of water-water interactions. Thus for hydrated 1-HU$^-$ (Fig. 1a) the distances between water molecules are about 3.8, 3.0, 3.0 and 4.5 Å, while for 3-HU$^-$ (Fig. 1b) they are about 3.6, 3.5, 4.9 and 4.5 Å. Now, the stabilizing interaction (dimerization) energy of water molecules calculated by the PCILO method for the distance of $\simeq$3.0 Å is about 2 kcal/mole [13], while the interaction energies between water dimers having $r_{O \ldots O} > 3.5$ Å are much smaller. Taking into account only the two water-water interactions in the 1-HU$^-$ anion which correspond to $r_{O \ldots O} \simeq 3$ Å and neglecting the others would add 4 kcal/mole to the stabilization of this monoanion, giving a rough value of 4.6 + 4 = 8.6 kcal/mole as the contribution of the solvent effect to its overall stabilization with respect to the 3-HU$^-$ anion. The value obtained in this way is of similar order of magnitude as the one computed exactly. The theoretical value of the differential water effect is somewhat too small to compensate entirely Snyder's [4] estimation of the intrinsic excess of stability of the 3-HU$^-$ tautomer. It shows, however, the correct trend towards the description of the influence of the solvent on the tautomeric equilibrium. Because of the numerous uncertainties related to the problem both from the experimental and the theoretical point of view (the energy difference between the tautomers in the vapour phase, the geometries of the uracil anions, the limitation of the solvent effect to the influence of the first hydration shell etc.), the results cannot and should not be used in a quantitative way. Nevertheless qualitatively they appear to account correctly for the general nature of the solvent effect.

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