Effect of Different Substituents on Uracil and its 2-Hydroxy-4-oxo Enol Tautomer – A Theoretical Study

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Z. Naturforsch. **63a,** 693 – 702 (2008); received March 10, 2008

The uracil/2-hydroxy-4-oxo uracil tautomeric system was studied by employing the MNDO semi-empirical method for the calculations. The uracil structure was found to be energetically favourable as indicated by the calculated heat of formation, the Gibbs free energy, the HOMO and LUMO energies, and charges. The substitution by F, OH, NH_2, CH_3, and BH_2 at the carbon-6 position was found to affect the geometrical parameters of the substituted molecules. All of the substituents were found to shift the equilibrium in forward direction compared to the unsubstituted tautomeric pair as indicated by the calculated values for the equilibrium constants.

Key words: Uracil; 2-Hydroxy-4-oxo Uracil; Tautomerization; MNDO.

1. Introduction

Uracil is a monocyclic base, belonging to a family called pyrimidines. Thymine and cytosine belong to the same family (Scheme 1). They are polyfunctional bases and may exist in tautomeric forms because they contain N–H and nearby carbonyl groups in their structure [1]. Uracil is one of the four bases in the ribonucleic acid (RNA); it replaces the thymine base in the deoxyribonucleic acid (DNA) [2, 3]. This replacement is very important because it protects the DNA from being easily attacked by some kinds of viruses or bacteria. Tautomerism is an important process in biological systems and it is believed to have a role in the mutagenesis of DNA [4 – 6]. This is probably because different tautomers have different electronic structures and consequently have different physical properties, which alter the base-pairing preferences during time of gene replication [7, 8].

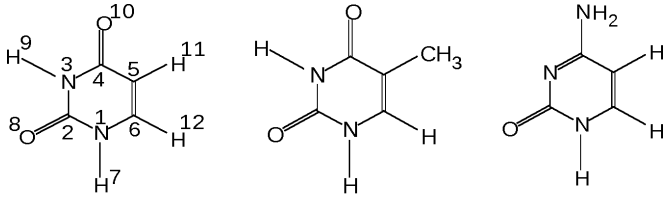
Tautomerism in the uracil molecule has been extensively studied by both theoretical [6, 9 – 13] and experimental methods [14 – 17]. The tautomerization equilibrium is largely affected by substituents [10]. Some studies have been made on the effect of methyl substitution at N1 of pyrimidine bases [18, 19]. It is well-known that 5- and 6-substituted uracils are biologically active and several of them have antitumour activity against experimental mouse tumour [20]. Also they have antibacterial and antiviral activity [21]. Preparation of some 5-substituted uracils by microwave irradiation is described in [22]. Specifically, 5-fluoro-uracil and its derivatives have been widely used, either alone or in combination with other drugs, for the treatment of advanced gastric cancer [23, 24]. In fact, this biological and pharmaceutical importance of uracil has motivated significant research.

2. Results and Discussion

2.1. Parent Uracil and its Enol (Hydroxy) Forms

Uracil

The bond lengths obtained in this work by using the MNDO semi-empirical method [25] for uracil are



Uracil Thymine Cytosine

Scheme 1.

Table 1. Bond lengths (Å) obtained by MNDO and by experimental and other computational methods.

Bond[a]	Experimental[b]	MP2/6-31G(d,p)	DFT B97-1/6-311G**	MNDO
N1C2	1.399	1.396	1.396	1.415
C2N3	1.399	1.385	1.385	1.404
N3C4	1.399	1.414	1.414	1.429
C4C5	1.462	1.460	1.463	1.477
C5C6	1.343	1.350	1.350	1.360
C2O8	1.212	1.217	1.210	1.227
C4O10	1.212	1.219	1.213	1.225
N1C6	1.399	1.376	1.374	1.397

[a] See Scheme 1 for atom numbering. [b] Obtained by NMR [23].

compared with those obtained experimentally by NMR studies [23] and computationally by MP2 at 6-31G(d,p) level of theory [26] and by density functional theory (DFT B97-1) at 6-311G** level of theory [8]. The results are shown in Table 1.

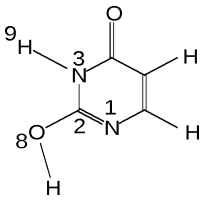
The bond lengths (l) obtained in this work (MNDO) are in good agreement with those obtained by NMR. Mostly they are larger than the experimental results; the largest difference was in the case of the N3C4 bond ($\Delta l = 0.030$ Å). The other differences were 0.016 Å or lower. Shishkin and co-workers [26] concluded in their extensive computational study that the application of a large basis set is not a necessary condition to obtain reliable geometrical parameters of a molecule.

The dihedral angles obtained from the optimized geometry of uracil in this work are all 0° or 180°, which indicates that the molecule is completely planar with C_s symmetry. Millefiori and Alparone [8] obtained dihedral angles less than 0.1° when they applied ab initio (CCSD/6-31G**) and DFT methods (B97-1). This is also in good agreement with the experimental gas-phase electron diffraction results [27].

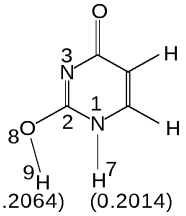
The heat of formation of uracil obtained in this work is found to be −271.74 kJ/mol, whereas the experimental value for the gas-phase uracil was found to be (−303.05 ± 2.26) kJ/mol [28]. So the value obtained by this method (MNDO) is in good agreement with the experimental value where the error is about 10% taking into account that the value obtained experimentally is the true value for the heat of formation of uracil.

Enol Counter Parts of Uracil

The number of possible enol tautomers is determined by the number of possible combinations of the proton sites on the oxygen and nitrogen atoms. This gives 12 possible combinations as illustrated by many workers. The relative energies of these tautomers together with those of rotamers were presented in [6, 8, 9], the most stable enol tautomer is shown in Scheme 2.



Scheme 2.



Scheme 3.

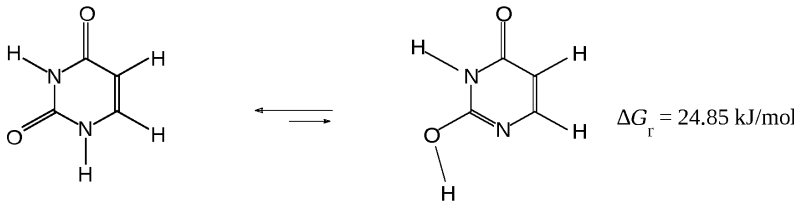
Its heat of formation is found to be −253.58 kJ/mol, i. e., 18.16 kJ/mol higher than that of the diketo form (uracil). This difference was found to be 39.75 kJ/mol by other workers [29, 30].

On the other hand the least stable enol tautomer was the one which appears in Scheme 3.

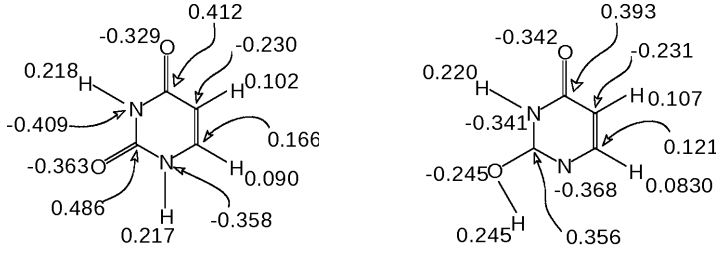
The reason for this low stability apparently is the large repulsion between the hydrogen atoms H7 and H9, where the charges are 0.2014 and 0.2064, respectively. This repulsion most probably is the reason for the increase in the C2O8H9 bond angle, which is found to be 115.05°, where this angle is 110.27° in the other (more stable) tautomer in Scheme 2. The role of the repulsion and attraction effect on the stability of different enol tautomers of uracil is also studied by Jalout and co-workers [9].

The calculated heat of formation for this tautomer (Scheme 3) is found to be −205.85 kJ/mol, i. e., it is 65.90 kJ/mol less stable than the diketo form (uracil). This difference was experimentally found to be (79.50 ± 25.10) kJ/mol by other workers [31].

As calculated in this work, the entropies of uracil and the most stable tautomer (Scheme 2) are found to be 233.61 J/(K mol) and 211.18 J/(K mol), respectively, i. e., the entropy change is −22.43 J/(K mol). Therefore, provided that the enthalpy change (ΔH_r) is 18.18 kJ/mol, the calculated Gibbs free energy change (ΔG_r) for the tautomerization equilibrium process (Scheme 4) at 25 °C is 24.85 kJ/mol. This means that the equilibrium is shifted towards the diketo form **1a**, where both energy and entropy factors are in favor of forming the diketo form.



1a **1b** Scheme 4.



1a **1b** Scheme 5.

The calculated value of the equilibrium constant (K) by the use of the relation $\Delta G = -RT \ln K$ [32] is $4.428 \cdot 10^{-5}$. This value is very low, which means that the concentration of the enol form in a uracil sample is very low and its detection by absorption spectroscopy techniques is extremely difficult. The successful detection of very small amounts of enol tautomers by Tsuchiya and co-workers [30] by the fluorescence spectroscopy technique seems to be due to their high fluorescence yields.

A look at the charges (Scheme 5) of different atoms in both the keto **1a** and enol **1b** structures (see structures **1a** and **1b** in Table 2 for the atom numbering) shows that the former is more stable than the latter. For example, the charges at H7 are 0.217 and 0.245 for the keto **1a** and the enol form, **1b**, respectively. This means that H7 in the enol form is more acidic than that in the keto form, so this hydrogen (H7) can easily move from O8 in the enol to N1 in the keto form, which suggests that the keto form **1a** is more stable than the enol form **1b**. An inspection of the charges at N1, C2, O8, N3, and C4 shows that the attraction forces in the keto form **1a** are greater than those in the enol form **1b** which adds stability to the keto form and shifts the reaction towards the reactants **1a**. This result is consistent with the result predicted from the free energy change calculations.

2.2. Effect of Substituents

Now the effect of F, OH, NH_2, BH_2, and CH_3 on the tautomerization of uracil (**1a**) and its most stable enol tautomer **1b** is considered. It is found that all of the substituents affect the geometrical parameters (bond lengths and bond angles) when the substituted compounds are compared with their parent unsubstituted keto and enol forms. The results are summarized in Table 2. These effects on the bond lengths and bond angles are noticeable at the position of the substitution. The rest of the molecule is affected to a smaller extent.

The effects of these substituents on the position of equilibrium is also studied. The position of equilibrium is measured by the magnitude of the free energy change of the reaction (ΔG_r) which is determined by an energy factor (ΔH_r) and entropy factor ($T\Delta S_r$) according to the equations $\Delta G_r = \Delta H_r - T\Delta S_r$, $\Delta H_r = \Delta H_f(\text{products}) - \Delta H_f(\text{reactants})$ [32]. Enthalpy changes (ΔH_r) for the tautomerization reactions, entropy changes (ΔS_r), Gibbs free energy changes (ΔG_r), and the equilibrium constants (K) obtained in this work for different substituents calculated at 25 °C are listed in Table 3.

The entropy of a molecule is associated with the number of atoms and their physical arrangement in that molecule. Since in a tautomerization reaction the number of atoms and the number of bonds is the same, and the arrangement of the atoms in space is similar, it is expected that the entropy change of such reactions is small. An entropy change of 10.0 J/(K mol) decreases the free energy (ΔG_r) by about 2.98 kJ/mol at 25 °C. Therefore, the free energy change for the tautomerization reactions is expected to be largely determined by the energy factor, i. e., the reaction's enthalpy change (ΔH_r).

Table 2. Optimized geometrical parameters (bond lengths in Å, bond angles and dihedral angles in °) calculated by MNDO for the unsubstituted and substituted uracil and its 2-hydroxy tautomer.

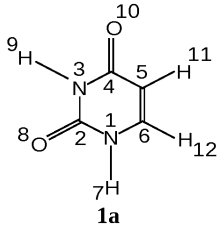
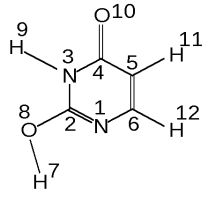
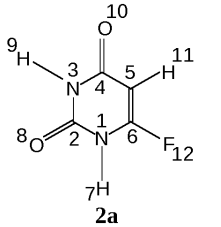
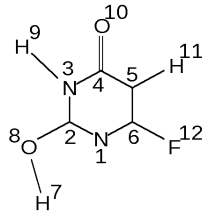
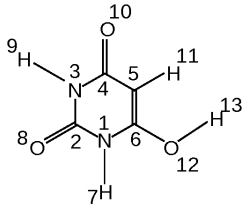
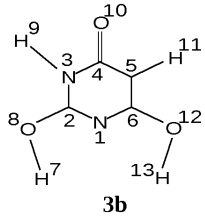
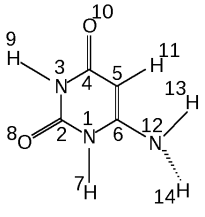
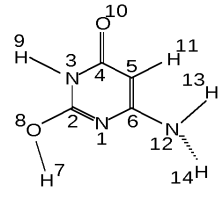
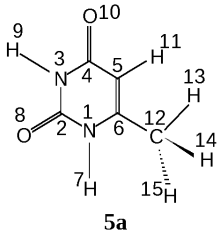
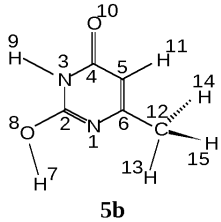
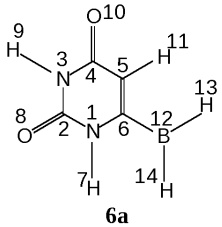
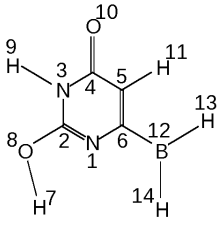
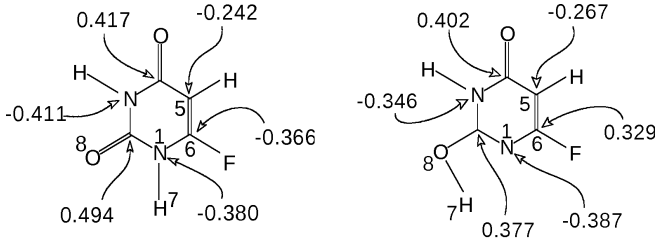
 **1a**	H12C6: 1.0944; C6C5: 1.3599; N1C6: 1.3966; H7N1: 1.0021; H11C5: 1.0893; C4C5: 1.4767; N1C2: 1.4153; H12C6C5: 123.78; C6C5C4: 121.30; C5C4C3: 114.68; H11C5C4: 117.44; all dihedral angles are 180.00° or 0.00°.	 **1b**	H12C6: 1.0949; C6C5: 1.3728; C4C5: 1.4624; N1C6: 1.3923; H11C5: 1.0866; N1C2: 1.3155; H12C6C5: 121.29; C6C5C4: 117.01; C5C4C3: 114.95; H11C5C4: 119.37; all dihedral angles are in the range of $(0.00 \pm 0.2)°$ and $(180 \pm 0.2)°$.
 **2a**	F12C6: 1.3162; C6C5: 1.3712; N1C6: 1.4047; H7N1: 1.0050; H11C5: 1.0894; C4C5: 1.4742; N1C2: 1.4187; F12C6C5: 124.85; C6C6C4: 120.22; C5C4C3: 115.13; H11C5C4: 117.77; all dihedral angles are $(0.00 \pm 0.00)°$ and $(180 \pm 0.00)°$.	 **2b**	F12C6: 1.3147; C6C5: 1.3851; N1C6: 1.3893; H11C5: 1.0869; C5C4: 1.4583; N1C2: 1.3260; F12C6C5: 122.58; C6C5C4: 116.12; C5C4C3: 115.15; H11C5C4: 119.32; all dihedral angles are in the range of $(180 \pm 0.1)°$ or $(0 \pm 0.1)°$.
 **3a**	O12C6: 1.3497; C6C5: 1.3756; N1C6: 1.3992; H7N1: 1.0053; H11C5: 1.0885; O12H13: 0.9494; C4C5: 1.4671; N1C2: 1.4194; O12C6C5: 127.14; C6C5C4: 120.68; C5C4C3: 115.01; H11C5C4: 117.10; H13O12C6: 113.41; all dihedral angles are in the range of $(0.00 \pm 0.00)°$ and $(180 \pm 0.00)°$.	 **3b**	O12C6: 1.3596; C6C5: 1.3796; N1C6: 1.4042; H11C5: 1.0867; C4C5: 1.4603; H13O12: 0.9523; N1C2: 1.3276; O12C6C5: 121.37; C6C5C4: 117.33; C5C4C3: 117.20; H11C5C4: 119.31; H13O12C6: 110.28; H7O8C2: 110.06; all dihedral angles are in the range of $(180.00 \pm 0.10)°$ or $(0.00 \pm 0.20)°$.
 **4a**	C6N12: 1.4180; C6C5: 1.3714; N1C6: 1.4005; H7N1: 1.0048; H11C5: 1.0888; C4C5: 1.4711; H13N12: 1.0074; H14N12: 1.0081; N1C2: 1.4180; N12C6C5: 125.42; C6C5C4: 121.37; C5C4C3: 114.92; H11C5C4: 116.47; H13N12C6: 111.83; H14N12C6: 112.86; O8C2N41: 123.46; N12C6C5C4: 173.51; H7N1C6C5: 173.44; H14N12C6N1: 302.16; all the other dihedral angles are $(180 \pm 1)°$ or $(0 \pm 1)°$.	 **4b**	N12C6: 1.4277; C6C5: 1.3920; N1C6: 1.4036; H11C5: 1.0862; N1C2: 1.3239; C4C5: 1.4672; N12C6C5: 122.78; C6C5C4: 117.60; C5C4C3: 115.80; H11C5C4: 118.99; H13N12C6: 110.25; H14N12C6: 120.21; N12C6C5C4: 180.90; H7O8C2N3: 180.04; H13N12C6N1: 179.48; H14N12C6N1: 59.85.
 **5a**	C12C6: 1.5101; C6C5: 1.3665; N1C6: 1.4041; H7N1: 1.0029; H11C5: 1.0903; C4C5: 1.4760; H13C12: 1.1085; H14C12: 1.1101; H15C12: 1.1101; N1C2: 1.4143; H12C6C5: 124.65; C6C5C4: 122.23; C5C4C3: 114.88; H11C5C4: 116.24; H13C12C6: 111.23; H14C12C6: 111.16; H15C12C6: 111.16; H14C12C6N1: 299.64; H15C12C6N1: 60.36; all other dihedral angles are $(180.00 \pm 0.00)°$.	 **5b**	C12C6: 1.5081; C6C5: 1.3777; C4C5: 1.4657; N1C6: 1.4266; H11C5: 1.0912; N1C2: 1.3158; C12C6C5: 122.97; C6C5C4: 122.58; C5C4C3: 113.68; H11C5C4: 117.62; H13C12C6: 112.62; H14C12C6: 110.50; H15C12C6: 110.50; H14C12C6N1: 120.10; H15C12C6N: 239.90; all other dihedral angles are $(180 \pm 1)°$ or $(0 \pm 1)°$.

Table 2 (continued).

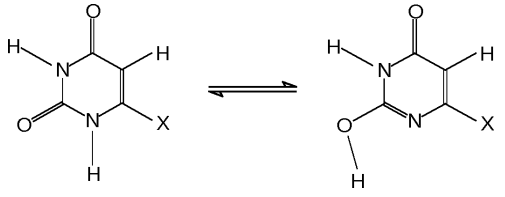
 **6a**	B12C6: 1.5386; C6C5: 1.3711; N1C6: 1.4083; H7N1: 1.0034; H11C5: 1.0914; C4C5: 1.4831; H13B12: 1.1579; H14B12: 1.1584; N1C2: 1.4109; B12C6C5: 122.95; C6C5C4: 122.51; C5C4C3: 114.87; H11C5C4: 116.05; H13B12C6: 119.50; H14B12C6: 121.34; all dihedral angles are 180.00° or 0.00°.	 **6b**	B12C6: 1.5429; C6C5: 1.3787; C4C5: 1.4702; N1C6: 1.3507; H11C5: 1.0923; N1C2: 1.3338; H13B12: 1.1607; H14B12: 1.1592; B12C6C5: 122.75357; C6C5C4: 122.61; C5C4C3: 113.20; H11C5C4: 117.04; H13B12C6: 119.76; H14B12C6: 121.40; B12C6C5C4: 180.00; H7O8C2N3: 180.00; all dihedral angles are (180.0 ± 1)° or (0 ± 1)°.



2a **2b**

Scheme 6.

Table 3. The calculated enthalpy changes (ΔH_r), entropy changes (ΔS_r), Gibbs free energy changes (ΔG_r) and the equilibrium constants (K) obtained for different substituents X at the uracil/2-hydroxy-4-oxo enol tautomeric system.



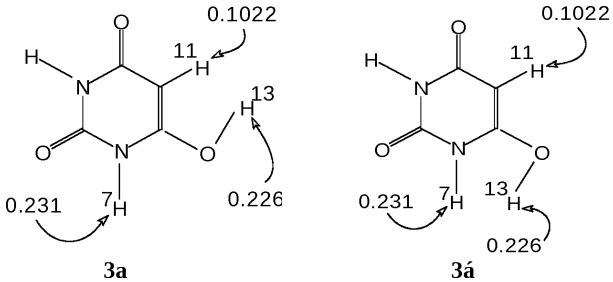
X	ΔH_r (kJ/mol)	ΔS_r [J/(K mol)]	ΔG_r (kJ/mol)	10^3 K
F	9.09	6.32	7.210	54.64
OH	1.09	−6.58	3.052	291.9
NH_2	6.60	−2.58	7.369	51.16
CH_3	2.90	−6.41	4.811	143.6
BH_2	6.37	5.73	4.662	152.5

Effect of F

The calculated heat of formation of the F-substituted uracil compound **2a** is found to be −467.27 kJ/mol and that of its substituted enol parent **2b** is −458.18 kJ/mol. This means that the substituted keto form is slightly more stable than the substituted enol form. The energy difference (ΔH_r) is 9.09 kJ/mol. The calculated entropies for the F-substituted keto and enol forms are 246.80 J/(K mol) and 253.12 J/(K mol), respectively, i. e., the entropy change (ΔS_r) is 6.32 J/(K mol). Therefore, the calculated free energy change (ΔG_r) at 25 °C is 7.21 kJ/mol. The positive value of ΔG_r means that the equilibrium prefers the reactants' side, i. e., it is shifted in the backward direction. In this case, because the entropy change (ΔS_r) is positive, the entropy factor prefers the enol **2b** formation but its magnitude is not sufficient to force the equilibrium in the forward direction.

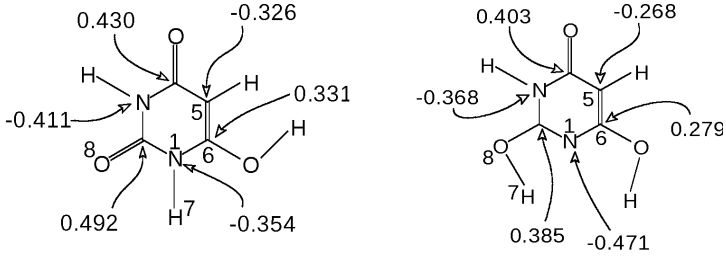
From the charge distribution on the atoms of the F-substituted keto and enol forms (Scheme 6) it can be seen that compound **2a** has larger charge and oppositely charged atoms compared with compound **2b**. This suggests that the substituted keto form **2a** is more stabilized in comparison to **2b** by stronger attraction forces, which means that the equilibrium prefers the backward direction, i. e., the formation of compound **2a**. This agrees with the non-spontaneity, predicted from the calculated positive free energy change (ΔG_r).

Also, H7 in compound **2b** is more acidic as compared to H7 in compound **2a**, because it carries more positive charge. The charges are 0.257 in compound **2b** and 0.232 in compound **2a**. This suggests that compound **2b** is more easily deprotonated than compound **2a**, and the equilibrium prefers the backward direction as predicted from the ΔG_r calculations. It is also noticed that the N1H7 distance (2.236 Å) in compound **2b** is shorter than the H7O8 distance (2.525) in compound **2a**, which encourages the proton H7 being transferred to N1 obtaining structure **2a**.



3a **3á**

Scheme 7.



3a **3b**

Scheme 8.

The F substituent decreases the electron density at the position of substitution (C6) and increases it at the nearby atoms (C5 and N1). At C6 the electron density is 3.834 and becomes 3.634 after substitution. The electron densities at C5 and N1 are 4.230 and 5.358, respectively. After substitution the electron density becomes 4.242 at C5 and 5.380 at N1. These electron density changes, the decrease at the atom of substitution (C6) and the increase at the adjacent atoms (C5 and N1), suggest that the F substituent behaves as electron-releasing substitutent [33, 34].

Effect of OH

It is found that **3a** is more stable than **3á** (Scheme 7) by 1.09 kJ/mol. This is believed to be due to the charges on the hydrogen atoms H11, H13 and H7. These charges are found to be 0.102, 0.226, and 0.231, respectively. Therefore **3a** is preferred because of the lower repulsion between H11 and H13 as compared to that between H7 and H13 in **3á**.

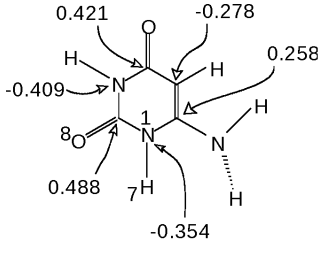
The calculations show that the energy of the OH-substituted keto form **3a** is slightly lower than that of the OH-substituted enol form **3b**. The heat of formation of the former **3a** is −482.60 kJ/mol and that of the latter is **3b** −481.50 kJ/mol. The calculated entropy of the product **3b** is 245.12 J/(K mol) and that of the reactant **3a** is 251.70 J/(K mol), i. e., there is an increase in the energy by 1.09 kJ/mol and a decrease in the entropy by 6.58 J/(K mol). This means that both energy and entropy factors favor the reactant **3a**. The calculated Gibbs free energy (ΔG_r) at 25 °C is found to be 3.062 kJ/mol.

The charge distributions in the **3a** and **3b** (Scheme 8) show that the total attraction forces in the substituted keto form **3a** are higher than those in the substituted enol form **3b**, which suggests that the keto form is more stable than the enol form and the reaction favors the formation of the reactant **3a**, i. e., it is non-spontaneous in the forward direction as suggested by the calculated positive value of the free energy change (ΔG_r). This direction of equilibrium shift is encouraged by the more positively charged H7 in **3b** (0.291) as compared to that in **3a** (0.231), which makes H7 in the enol **3b** more acidic. The extra acidity of H7 in compound **3b** together with the smaller distance between N1 and H7 (2.365 Å) can be compared with the H7O8 distance (2.301 Å) in the keto **3a**. The shorter distance makes the proton transfer easier and the keto form formation is encouraged, i. e., the equilibrium is shifted in the reverse direction.

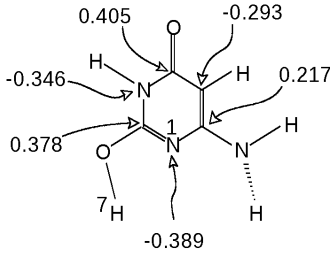
The OH group behaves like a weak electron releasing group because it decreases the electron density at C6, increases it at C5, and slightly increases it at N1. The electron density after substitution becomes 3.669 at C6, 4.326 at C5, and 5.354 at N1.

Effect of NH_2

The calculated heat of formation of the NH_2-substituted keto form **4a** is −272.94 kJ/mol and that

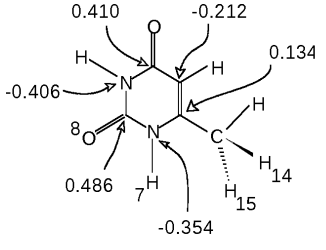


4a

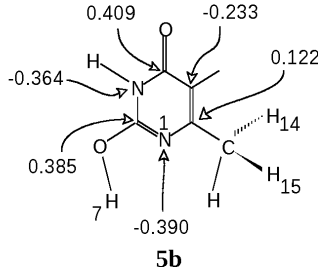


4b

Scheme 9.



5a



5b

Scheme 10.

of the parent enol form **4b** is −266.34 kJ/mol. The calculated absolute entropies of the compounds **4a** and **4b** are 257.65 and 255.07 J/(K mol), respectively. This means that, energetically, the NH_2-substituted keto form **4a** is more stable than the NH_2-substituted enol **4b** by 6.60 kJ/mol. Also, the entropy change for the reaction (ΔS_r) is −2.58 J/K mol, so both energy and entropy factors favor the formation of the keto form. The calculated free energy change (ΔG_r) at 25 °C is 7.369 kJ/mol and the equilibrium is shifted towards the reactant **4a**.

The charge distribution of **4a** and **4b** is shown in Scheme 9. The charges of **4a** are larger and opposite in sign, so a large stability of **4a** as compared to **4b** is created due to the larger attraction forces, which make **4a** more stable than **4b**. Because the charge at H7 in the enol form **4b** (0.248) is more positive as compared to that at H7 in the keto form **4a** (0.221), and the H7N1 distance (2.254 Å) in **4b** is smaller as compared to the H7O8 distance (2.511 Å) in **4a**, the proton H7 leaves **4b** easily towards the formation of the keto form **4a**. This means that the equilibrium tends to be shifted in the reverse direction. This is consistent with the thermodynamic calculations of positive free energy change.

The electron density change here indicates that this substituent behaves as a weak electron-releasing group, because there is a decrease in the electron density at C6, an increase at C5 and a slight decrease at N1, these charges being 3.742, 4.278, and 5.354, respectively.

Effect of CH_3

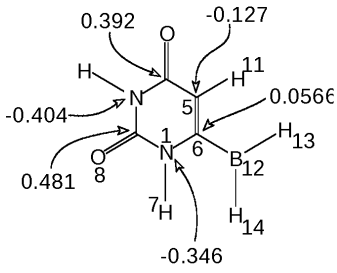
For this substituent, the calculations show that the substituted keto form **5a** is more stable than the substituted enol form **5b**, by 2.90 kJ/mol. The heats of formation are −305.46 kJ/mol and −302.56 kJ/mol for **5a** and **5b**, respectively. A decrease in entropy by 6.41 J/(K mol) is also observed, where the entropy of compound **5a** is 258.57 J/(K mol) and that of compound **5b** is 252.16 J/(K mol), respectively. Both energy and entropy factors favor the reactant's side. The calculated free energy change (ΔG_r) for the reaction at 25 °C is 4.811 kJ/mol and the reaction is shifted towards the formation of the substituted keto form **5a**.

The charge distribution in **5a** and **5b** (Scheme 10), and the distances H7O8 (2.515 Å) in **5a** and H7N1 (2.345 Å) in **5b** suggest more stability of **5a** over **5b** due to greater net attraction forces. This is consistent with the calculation of ΔG_r, where the equilibrium prefers the formation of the substituted keto form.

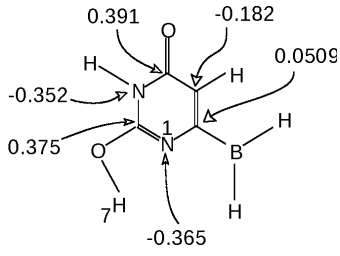
The charges at H14 (0.00934) and H15 (0.00935) in **5b** suggest that these two hydrogen atoms are equivalent; also these two hydrogen atoms are equivalent in the keto form **5a**, where the charges are 0.0172 on both of these two hydrogen atoms.

Effect of BH_2

The calculated heats of formation of the BH_2-substituted keto form **6a** and that of the BH_2-



6a



6b

Scheme 11.

substituted enol form **6b** are −248.84 kJ/mol and −242.47 kJ/mol, respectively. The absolute entropy values are 259.91 J/(K mol) for compound **6a** and 265.64 J/(K mol) for compound **6b**. These results indicate that the energy factor favors the keto form formation and the entropy factor favors the enol form formation. But since the entropy factor at 25 °C is less effective than the energy factor, the equilibrium is shifted backwards towards the keto form. The calculated free energy change is 4.662 kJ/mol.

As predicted from the thermodynamic calculations, the charge distribution on the atoms of **6a** and **6b** (Scheme 11) also predicts that the reverse direction of the reaction is preferred. For example, the charge at H7 (0.235) in **6b** is higher than that at H7 (0.216) in **6a** indicating that H7 in **6b** is more acidic. Therefore **6b** is less stable as compared to **6a** because H7 leaves compound **6b** more easily to form compound **6a**, i. e., the keto form is preferred.

The geometry of the BH_2-substituted molecule **6a** is planar; all of the dihedral angles are either 180.0° or 0.0°. This molecule belongs to the C_s symmetry group. Even though the rotation around the C6B12 bond is free, still H13 and H14 are in the plane of the molecule. This is due to the negative charges of these hydrogen atoms (H14: −0.0478 and H13: −0.0396), while the attraction by positive H11 (0.100) from one side and by H7 (0.216) from the other side of the BH_2 group keeps the planarity of the molecule.

The electron density at C6 is increased (3.943) but it is decreased at C5 (4.127) and N1 (5.346). This suggests that BH_2 behaves as electron-withdrawing group.

2.3. Equilibrium Constants

The values of the equilibrium constants for the substituted keto/enol systems in this work are calculated at 25 °C according to the relationship $\Delta G = -RT \ln K$. The results are shown in Table 3.

In order to compare the extent of the tautomerization reactions in the forward direction, it is better to compare their equilibrium constants (K) rather than just comparing their values of the free energy change (ΔG_r). The calculations indicate that there is a significant increase in these equilibrium constants for the tautomerization reactions after substituting all of the substituents as compared to the equilibrium constants before substitution. An increase in the equilibrium constant means that the reaction goes further in the forward direction. Therefore, it can be concluded that all of the substituents have increased the extent of the reaction in the forward direction to different extents according to the order of the increase in the K values, implying the ordering $OH > BH_2 > CH_3 > F > NH_2$.

Therefore, in a substituted uracil sample it is expected to have the highest substituted enol concentration, if the substituent is NH_2 as compared to the other substituents. But at the same time, none of the substituents was able to make the reaction spontaneous in the forward direction, since none of the equilibrium constants is greater than or equal to unity.

2.4. Orbital Energies (HOMO and LUMO)

An introduction of substituents to the uracil/2-hydroxy-4-oxo uracil tautomeric system is found to affect the electronic properties of both the keto and enol form. This effect shows up as a change in the energies of the HOMO and LUMO and in the energy gaps of the substituted keto and enol forms as compared to those of the parent tautomeric pair **1a** and **1b** before substitution. The results are shown in Table 4.

From these results it is seen that the energy gaps (E_g) for all of the substituted keto and enol forms are decreased as compared to those of the unsubstituted keto **1a** and enol **1b** parent compounds. The energy gaps for the unsubstituted keto and enol compounds are 9.604 eV and 9.113 eV, respectively. The electronic

Table 4. Calculated HOMO and LUMO energies and energy gaps for the unsubstituted and substituted uracil and its 2-hydroxy-4-oxo tautomers.

	Substituted uracil			Substituted 2-hydroxy-4-oxo uracil		
Substituent X	HOMO (eV)	LUMO (eV)	Energy gap E_g (eV)	HOMO (eV)	LUMO (eV)	Energy gap E_g (eV)
H	−9.938	−0.334	9.604	−9.580	−0.467	9.113
F	−10.258	−0.830	9.428	−9.842	−0.899	8.943
OH	−9.792	−0.360	9.432	−9.304	−0.399	8.905
NH_2	−9.706	−0.380	9.326	−9.300	−0.440	8.860
CH_3	−9.861	−0.438	9.423	−9.546	−0.475	9.071
BH_2	−10.042	−1.354	8.688	−9.460	−1.068	8.392

stability is measured by the magnitude of the energy gaps, where the stability is higher when the energy gap is larger [35 – 37]. So, it can be concluded that all of the substituents have destabilizing effects on their corresponding keto and enol counterparts. The BH_2 substituent has the largest destabilization effect on both keto ($E_g = 8.688$ eV) and enol ($E_g = 8.392$ eV) substituted forms since the energy gaps were smallest as compared to those of the other substituents.

3. Conclusion

It is found that uracil is more stable than its most stable enol (2-hydroxy-4-oxo uracil) counter part. The keto/enol equilibrium prefers the keto form with a smaller equilibrium constant. All of the substituents push the equilibrium towards the enol formation as compared to the unsubstituted tautomerization system but still with preference of the keto form as found from the calculated ΔG_r values. The charge distribution criterion can also be used to explain the results.

A similar study with cytosine and thymine would be of great importance since they are constituents of the DNA, where the tautomerization role in mutation is of paramount importance.

Acknowledgement

The author would like to thank Prof. K. Al-Tarawneh from the Department of Biology at Mutah University for his fruitful discussion about the role of tautomerization in mutation. Thanks are also due to Prof. S. M. Khalil for providing the computer program to produce this work.

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