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Theoretical Study on the Excitation Energies of Six Tautomers of Guanine: Evidence for the Assignment of the Rare Tautomers

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The CASPT2//CASSCF method with the 6-31G* basis set and an active space up to (16,12) was used to calculate the excitation energies for six tautomers of guanine. Our calculations provide further support on the recent reassignment of the near-UV resonant two-photon ionization (R2PI) spectrum, in which two rare tautomers of the 7*H*-oxo-imino form were proposed to replace the previously assigned 7*H*/9*H*-oxo-amino tautomers. The adiabatic excitation energies of the 7*H*-oxo-imino tautomers are calculated to be 0.3−0.5 eV higher than those of the 7*H*/9*H*-oxo-amino tautomers. Our calculations also indicate that the missing most stable tautomers (7*H*/9*H*-oxo-amino tautomers) in the R2PI experiment is possibly due to the existence of an ultrafast nonradiative deactivation process in the excited-state of these two tautomers.

Since the first observation of the sharp transition in the jet-cooled guanine in 1999,[1] many works on the electronic spectroscopy of the isolated nucleic acid bases have appeared.[2−10] These experimental works have provided many intrinsic excited-state features of the bases.[11−14] Due to the existence of many tautomers, guanine has a complex near-UV spectrum. Through the laser resonant two-photon ionization (R2PI) technique, some researchers identified several tautomers of guanine in supersonic expansion. Nir et al. identified origin bands of three different tautomers, 9*H*-hydroxo-amino form (either **3** or its rotamer), 9*H*-oxo-amino form (**1**), and 7*H*-oxo-amino form (**2**) (as shown in Figure 1) based on the ground-state vibrational spectroscopy.[15] However, based on a comparison of the R2PI spectrum with the UV spectra of relevant methylated guanine species, Mons et al. identified four tautomers and gave a different assignment for the three tautomers observed by Nir et al.[10,16,17]

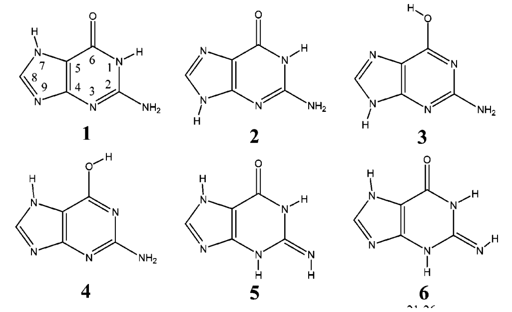

Figure 1. Six relative low-energy tautomers of guanine.[21−26]

Recently, Miller and his co-worker published their study on the IR spectra of guanine in He nanodroplets.[18] Based on the good agreement between the experimental and theoretical IR spectra, they convincingly assigned their IR spectrum to a mixture of the four most stable guanine tautomers, **1**−**3** and its rotamer, 9*H*-hydroxo-amino syn form. Inspired by this result, Mons et al. compared the IR/UV spectra with the IR spectrum obtained by Miller et al. and found that except for the 9*H*-hydroxo-amino anti form (**3**) observed simultaneously in both experiments, the other three tautomers observed in the R2PI experiment are not the same as those observed in He droplets.[19] That is to say, the most stable forms (**1** and **2**) of guanine tautomers were indeed not observed in the R2PI experiment. As a result, Mons et al. reassigned the two observed tautomers to the two rotamers **5** and **6** of the 7*H*-oxo-imino form. This reassignment has great influence on the study of the jet-cooled guanine. Very recently, we found that for biologically relevant 9*H*-oxo-amino guanine (**2**) the calculated adiabatic transition energy is much lower than the assigned experimental value if one tautomer in the R2PI experiment is assigned to this 9*H*-keto form.[20] This indicates that the previous assignment for this 9*H*-keto form may be incorrect. Stimulated by the new assignment of Mons et al.,[19] we have carried out theoretical calculations on the electronic transition energies of various guanine tautomers including the rare tautomers, such as **4**−**6**, using the CASPT2//CASSCF method. The results reported in this Letter are expected to provide further support for the new assignment of the guanine R2PI spectrum given by Mons et al.

Here we used the complete active-space self-consistent-field (CASSCF) method[27] to locate the minimum structure on the lowest singlet excited state. For the ground-state equilibrium geometry optimization, we employed the second-order Møller-Plesset (MP2) method based on the restricted Hartree−Fock reference. To correct the energetics by the dynamical electron correlation, we used the CASPT2 method[28,29] with the same active space and basis set as in the CASSCF calculation. The 6-31G* basis set is employed throughout this work. Unless stated otherwise, the active space is 14 electrons distributed in 11 π orbitals. The geometry optimizations for stationary points were performed with the Gaussian03 program.[30] Single-point

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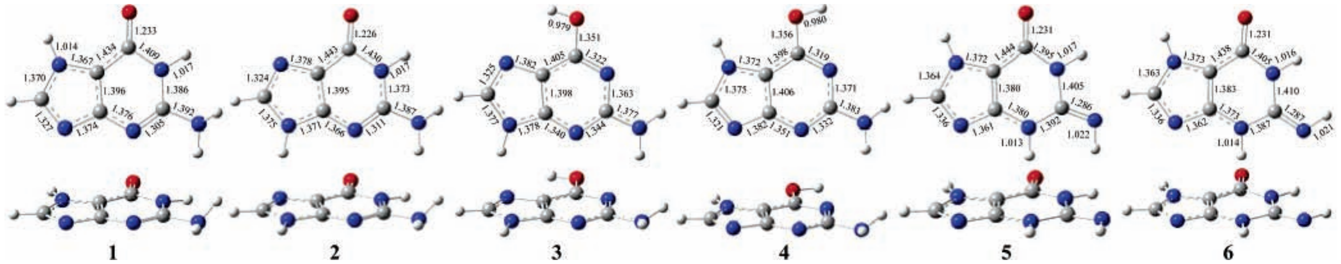

Figure 2. Structures of the MP2-optimized ground-state equilibrium geometries of **1**−**6** (top and side view).

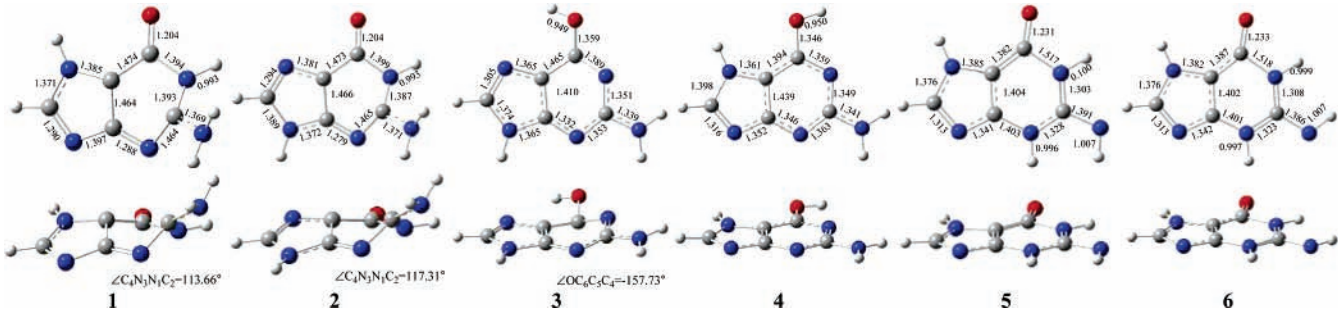

Figure 3. Structures of the CASSCF-optimized equilibrium geometries of **1**−**6** on the S_1 surface (top and side view).

TABLE 1: Calculated Vertical and Adiabatic Excitation Energies for the Lowest Excited-State of Guanine Tautomers 1−6[a]

	S_1 ($\pi\pi^*$) excitation energy (eV)				
	vertical		adiabatic		
tautomers	CASSCF	CASPT2[b]	CASSCF	CASPT2	exp[c]
1	5.986	4.618 (0.184)	4.298[d]	3.968[d]	
2	5.811	4.780 (0.200)	4.250[d]	3.887[d]	
3	5.311	4.597 (0.041)	4.982	4.276	4.309
4	5.112	4.313 (0.070)	4.807	4.010	4.075
5	5.790	4.488 (0.071)	4.952	4.365	4.204
6	5.578	4.340 (0.091)	4.874	4.286	4.125

[a] Oscillator strengths are in parentheses. [b] The ground-state geometry is optimized by the MP2/6-31G* method. [c] New assignment in ref 19. [d] An active space (16,12) is used for the S_1 ($\pi\pi^*$) state.

CASPT2 energy calculations were performed with the MOLPRO software.[31]

The optimized ground-state and lowest exited-state equilibrium geometries of the six guanine tautomers **1**−**6** are shown in Figures 2 and 3, respectively. One can see that for all six tautomers, their ground-state equilibrium geometries are nearly planar except the slight pyramidalization of the nitrogen atom in the amino group of **1**−**4**. As for the S_1 excited state, the CASSCF-optimized geometries of **1**−**3** exhibit large out-of-plane distortions. For the 7*H*/9*H*-oxo-amino forms **1** and **2**, the distortion is mainly of the bending of the amino group, where the C_2 atom is pyramidalized. For the 9*H*-hydroxo-amino anti form **3**, the distortion is mainly of the bending of the hydroxy group, where the C_6 atom is pyramidalized. The other tautomers **4**−**6** show no out-of-plane distortions at the minimum geometries of their S_1 states.

We collected the calculated vertical and adiabatic excitation energies of **1**−**6** in Table 1. One can see that the calculated vertical excitation energies of **1** and **2** are apparently higher than those of **3**−**6**. The calculated adiabatic excitation energies for **3** and **4** at the CASPT2 level agree quite well with the experimental values, indicating that the previous assignment of two tautomers A and D in the R2PI spectrum to **4** and **3** given by Mons et al.[10,16,17] should be right. If tautomers B and C[10,16,17] were assigned to **1** and **2**, the calculated adiabatic transition energies of **1** and **2** at the CASPT2 level would be much lower than the corresponding experimental values. Thus, the assignment of the tautomers B and C may be wrong. In comparison with the adiabatic excitation energies of **1** and **2**, those of **5** and **6** are higher by 0.3−0.5 eV and are much closer to the experimental values previously assigned to **1** and **2**. Thus, tautomers B and C should be assigned to **6** and **5**, respectively. In addition, the calculated adiabatic excitation energy order of **5** and **6** is consistent with that observed experimentally. This agreement between theoretical calculations and experiments gives further support for Mons et al.'s new assignment of the tautomers B and C. It should be mentioned that our results here are different from those calculated with the TD-B3LYP//CIS approach, where the adiabatic transition energies of **1** and **2** were predicted to be larger than that of **4**.[32]

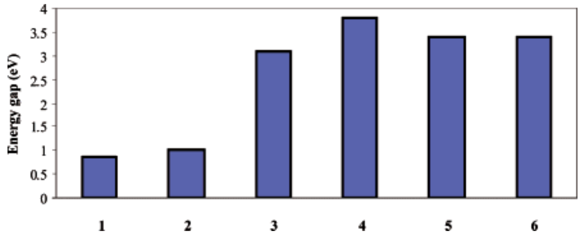

Figure 4. Energy gap between the S_1 and S_0 states at the geometries of the S_1 minima of **1**−**6**.

According to Mons et al.'s new assignment, the guanine tautomers **1** and **2** were not observed in the R2PI experiment. To probe the possible reasons for the absence of **1** and **2**, we calculated the energy gaps between the S_1 and S_0 states at the geometries of the S_1 minimum for **1**−**6**. The results at the CASPT2 level are shown in Figure 4. One can see that in species **1** and **2** the energy gap is less than 1.1 eV, being much smaller than those in the other tautomers. The small energy gaps in **1** and **2** indicate that a conical intersection (CI) near the geometry of the S_1 minimum may exist, which can help the system to decay to the ground state via an ultrafast nonradiative relaxation. In a recent work,[20] we have located this CI for **2** and found that the access from the S_1 minimum to this CI is energetically favorable. From the geometrical similarity between the S_1 minimum of **1** and that of **2**, and their similar S_1−S_0 energy gaps, we can speculate that the excited-state of **1** may also decay in an ultrafast nonradiative pathway to the ground state. So the ultrafast nonradiative deactivation in **1** and **2** may prevent the observation of these two tautomers in the R2PI experiment. On the contrary, for **3**−**6**, their relatively large energy gaps between the S_1 and S_0 states (no less than 3 eV) imply that an ultrafast nonradiative deactivation to the ground state through the CI is unlikely to occur in their S_1 states. So our results suggest that the excited-state dynamics of guanine is tautomer-dependent. We also notice that the calculated vertical emission energy for **4** (3.8 eV) is in good agreement with the experimental fluorescence maximum (3.78−3.81 eV) of guanine in the neutral ethylene glycol−water glasses[33] at low temperatures and in aqueous solution at room temperature.[34] The vertical emission energies of all the other species calculated in this work are less than 3.4 eV. This result, along with the previous experimental assessment that the fluorescence of guanine may be dominated by the 7*H* tautomers,[33] suggests that the highly fluorescent tautomer of guanine may be the 7*H*-hydroxo-amino form **4**. However, it is necessary to point out that since in our calculations the condensed phase effect (such as the solvent effect) was not taken into account, our results could not give quantitative descriptions on the experimental results in the condensed phase. It is well-known that the lifetime of the nucleic base in condensed phase at low temperature is much longer than that in gas phase of supersonic jets.[11] As a result, the fluorescence quantum yield of the nucleic base in condensed phase is much higher than that in gas phase.

On the basis of the results described above, we can draw the following conclusions. First, the recently proposed assignment for the four tautomers of guanine identified in the R2PI spectrum is supported by our calculated adiabatic excitation energies. Two tautomers, observed in the R2PI spectrum, may correspond to 7*H*-oxo-imino rotamers (**5** and **6**), rather than 7*H*/9*H*-oxo-amino (**1** and **2**) assigned previously. Second, the adiabatic excitation energies of **1** and **2** are calculated to be about 0.3−0.5 eV lower than those of **5** and **6**. This information may be helpful for spectroscopists to find the tautomers **1** and **2** in the future if the more advanced experimental techniques can allow the short-lived species **1** and **2** to be detected. Third, our calculations suggest that the “missing” tautomers **1** and **2** in the R2PI experiment may be caused by an ultrafast nonradiative decay pathway, which forbids these two tautomers to be detected in the R2PI experiment.

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Supporting Information Available: Computational details, calculated energies, Cartesian coordinates of all stationary points, and active orbitals for excited-state calculations of **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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