Imino Tautomers of Gas-Phase Guanine from Mid-Infrared Laser Spectroscopy

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Received: February 23, 2007; In Final Form: May 8, 2007

We reinvestigated the assignment of the three major guanine conformers detected via resonance enhanced two-photon ionization (R2PI) in supersonic expansions and present IR/UV double resonance spectra in the spectral region between 1500 and 1800 cm^{-1}. Comparison with B3LYP/TZVPP and RI-MP2/cc-pVQZ calculations shows that both conformers B and C are 7H-keto tautomers with an imine group in the 2-position. They differ only in the local conformation of the imine group but are otherwise identical. Conformer A is an amino−enol form with the OH group in the trans position.

1. Introduction

Tautomers of guanine have been studied by several groups in the past.[1−11] Four conformers were observed in the vibronic spectra of laser-desorbed guanine in supersonic expansions detected by resonant two-photon ionization (R2PI) and with electronic origins at 32 864 cm^{-1} (A), +405 cm^{-1} (B), +1046 cm^{-1} (C)[4,5] and +1891 cm^{-1} (D).[5] Using IR/UV double-resonance spectra, two of them were assigned to amino−enol structures (A and D), and the other two were assigned to amino−keto structures (B and C).[4−7] There remained some doubt as to whether the observed conformers were 7H or 9H tautomers because the IR spectra of 7H- and 9H-guanine are very similar.

Very recently Choi and Miller presented IR spectra of guanine in helium droplets.[12] They identified the 9H-amino-keto, the 7H-amino-keto and two 9H-amino-enol tautomers by the orientation of their transition moment angles. Remarkably, the IR spectra of the amino−keto tautomers in helium droplets did not agree with the IR spectra of tautomers B and C, which were assigned to amino−keto structures in the gas-phase studies.[4−7] Because the keto assignment was certain (no OH stretch vibration of the enol form), imino−keto tautomers remained the most obvious assignment of tautomers B and C. This was pointed out in ref 8 but no new experimental results were presented there to prove that possibility. The NH stretch vibration of the imine group, which absorbs in the near-IR, could give clear evidence for imino tautomers, but its absorption intensity is weak and could not be detected in refs 4−8.

Imino−keto and amino−keto structures are clearly distinguishable in the mid-IR spectral region by the presence of the very strong C═N stretch absorption of the imine group next to the C═O stretch band of the keto group. In the following, we present the spectra of guanine tautomers between 1500 and 1800 cm^{-1} that unambiguously prove the imino−keto assignment of tautomers B and C. Recent calculations of Marian[11] predict fast internal conversion of the amino−keto tautomers. Therefore, we concluded that the most stable and abundant amino−keto tautomers could not be detected via R2PI because their electronically excited-state is too short-lived for efficient ionization. On the other hand, the rare imino−keto tautomers were observed because of their efficient ionization pathway in R2PI experiments. R2PI only allows the detection of conformers that have an efficient ionization channel.

2. Experimental Methods

The basic principles of our IR/UV experimental setup were described in detail elsewhere.[13−15] Solid samples of guanine are vaporized into an argon jet (5 bar) by laser desorption and were investigated by IR/UV hole burning spectroscopy (Figure 1). Guanine (Merck >98%) is mixed with graphite powder (Aldrich) in a 1:2 ratio and is applied to the edge of a 2 mm thick graphite wheel (diameter 60 mm), which is placed underneath the orifice of a 300 μm pulsed valve (General Valve). Slow rotation of the graphite wheel (1 turn per 45 min) constantly provides fresh material to be exposed to the desorption laser (Minilite, Spectra Physics, 1064 nm, ∼5 mJ/cm^2).

The skimmed molecular beam (skimmer diameter 1 mm) crosses the UV ionization laser (LAS, frequency doubled, attenuated to <0.1 mJ/pulse) at a right angle inside the ion extraction region of a linear time-of-flight (TOF) mass spectrometer in the Wiley−McLaren configuration. A pulsed IR laser beam (burn laser) is aligned collinear to the UV beam (probe laser) and is fired 100 ns before the latter. The burn laser (IR) frequency is scanned over the vibrational transitions, while the ionization laser (UV probe laser) is kept at a frequency resonant with a vibronic transition of a single guanine tautomer. By monitoring the ion mass signal as a function of IR frequency, mass and conformer selective IR spectra can be obtained.

IR laser light is generated by a three-stage difference frequency generation (DFG)/optical parametric amplification (OPA) setup.[14] Basically, a dye laser (Precision Scan, Sirah), with Styryl 8 or 9 in methanol or dimethylsulfoxide (DMSO), is pumped by the 532 nm output of a frequency doubled Nd:YAG laser (GCR 230 Pro, Spectra Physics). The dye laser output is mixed with a part of the fundamental (1064 nm) of the same Nd:YAG laser in a $LiNbO_3$ crystal for difference frequency generation, which produces IR radiation around 2.6 μm that is amplified in an OPA stage. In the third stage, after proper rotation of the polarizations, IR radiation between 5 and 8 μm (∼300 μJ) is produced by difference frequency mixing of the idler and the signal beams of the OPA stage in an antireflection coated $AgGaSe_2$ crystal.

Intensity noise due to shot-to-shot fluctuations can largely be compensated by creating a reference signal. We let the very

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10.1021/jp0715169 CCC: $37.00
Published on Web 06/23/2007

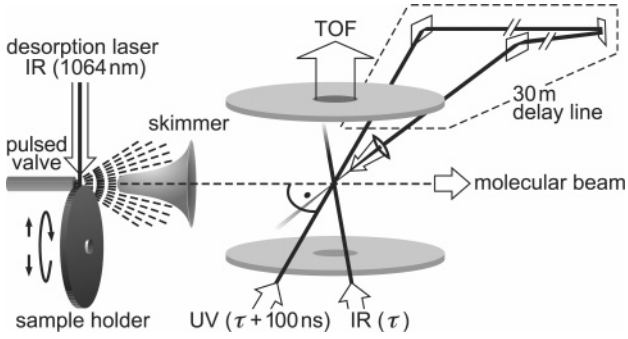

Figure 1. Experimental setup, showing the laser desorption and ionization region. The UV analysis laser travels more than 30 m after crossing the molecular beam, and then it is redirected and focused into the ionization region again to generate a reference signal of ions used to normalize the hole-burning signal.

same UV laser pulse that was used to ionize the molecules pass a 30 m delay line; then we re-direct and focus it into the sample region again, causing a second ion signal (reference signal) to appear ~100 ns after the first. Because the redirected beam is focused, its interaction volume with the IR beam is small and shows only a small IR dip, if at all. The reference signal is scaled to match the baseline of the first ion signal and is subtracted from the latter. In this case, the baseline corresponds to the constant ion signal without the IR laser. Because both ion signals originate from the very same laser pulse and sample the same gas pulse, common noise due to energy and number density fluctuations is mostly eliminated in the resulting signal.

The ro-vibrational transitions of the water bending (1595 cm^{-1}) and stretching vibrations (3657 and 3756 cm^{-1}) were used for frequency calibration. We did not normalize the IR spectra to the IR laser intensity, but the intensities in each measurement were the same to within 20%, so the intensity scales in the spectra are comparable for each conformer.

3. Calculations

We calculated all 20 tautomers and conformers of guanine, which are displayed in Figure 2 together with their relative energies. To get a more reliable estimate of the relative energies, geometry optimization at the B3LYP/TZVPP level of theory was followed by a RI-MP2/cc-pVQZ single point calculation at the optimized DFT geometries. Vibrational frequencies were calculated at the DFT level and were scaled by 0.9552 (O−H stretch), 0.9588 (N−H stretch), and 0.982 ($\tilde{\nu}$ < 2000 cm^{-1}). The different scaling factors for the OH and NH stretch vibrations were derived from calculations of 3- and 4-aminophenol. The relative energies in Figure 2 are corrected for the zero-point energy obtained by the above-mentioned DFT calculations. For the imino−keto tautomers we also calculated vibrational frequencies at the RI-MP2/cc-pVDZ, RI-MP2/cc-pVTZ, and RI-MP2/TZVPP levels of theory. All calculations were performed using the Turbomole program package.[16−24]

We use the following abbreviations to label the different tautomers: 7H/9H represents the H-atom in the 7N or 9N position, 3H represents the tautomer with the hydrogen atom moved from the 1- to the 3-position, *A* is the amino group in the 2-position, I_u/I_d is the imine group (up/down) in the 2-position, *O* is the keto form, and E_c/E_t is the enol form (cis/trans). Our nomenclature differs slightly from that introduced in ref 11. Except for the amino group, guanine is a planar molecule in the electronic ground state, with the hydrogen atom of the enol- and imino-groups lying in the molecular plane. The definitions for cis/trans refer to the position of the enol hydrogen. In the cis conformer the hydrogen is on the N7 side (e.g., 7H−*A*−E_c). Similarly, up/down refer to the position of the imino-hydrogen, with the hydrogen on the N1 side in the up conformer (e.g., 7H−I_u−*O*).

4. Results

Figure 3 shows the IR/UV double resonance spectra of the three guanine conformers A, B, and C in the mid-IR region (top three traces). The UV probe laser was tuned to 32 864 (A), 32 864 + 405 (B), and 32 864 + 1046 cm^{-1} (C), respectively.[4,5,7] Although we tried different experimental conditions (laser energy, timings, etc.), we did not observe the vibronic or IR spectrum of conformer D[5,7] in our experiment. Contrary to non-methylated guanine, the vibronic and the mid-IR spectra of conformer D of 9-methylguanine could be easily obtained.[25] Also shown in Figure 3 are the calculated and scaled stick spectra of the eleven most stable tautomers of guanine. The corresponding structures are indicated to the left of each stick spectrum. For the spectral assignment, we only considered tautomers with relative energies lower than 6000 cm^{-1}. Tautomers with higher energies are assumed to not be present in the supersonic expansion, or their contribution is assumed to be negligible compared to that of the major conformers. Vertical colored lines originating from the experimentally observed absorption maxima help to compare experimental and calculated spectra (A = blue, B = red, C = green lines).

Conformers B and C. The mid-IR spectra of conformers B and C are very similar (Figure 3) and show only two absorption maxima around 1690 and 1738 cm^{-1}. From previous work we know that both conformers B and C are keto forms, because they do not show the OH stretch vibration of the enol tautomers (see Figure 4).[4,5] Consequently, the two bands at 1738 and 1739 cm^{-1} can be attributed to the C═O stretch vibrations of the keto group. This is supported by the FTIR gas-phase spectrum of thymine[26] and by mid-IR spectra of guanine−cytosine base pairs.[27] Now we compare the experimental spectra to the calculated spectra of keto tautomers in Figure 3. Only the imino−keto tautomers 7H−$I_{u/d}$−*O* and 9H−$I_{u/d}$−*O* reasonably agree with the experimental spectra. Therefore, the bands at 1689 and 1692 cm^{-1} must be the C═N stretch vibrations of the imine groups.

Figure 4 shows previous measurements in the region of the OH/NH stretch vibrations[4] together with the calculated and scaled stick spectra in that region. Conformers B and C show three absorptions between 3450 and 3510 cm^{-1}. According to the calculated spectra of the 7H−$I_{u/d}$−*O* and 9H−$I_{u/d}$−*O* tautomers, the red-most band belongs to the N_1H stretch vibration, whereas the other two are the N_3H and $N_{7/9}H$ stretch vibrations. The NH stretch vibration of the imino group (below 3400 cm^{-1}) is weak and is not discernible in the experimental spectra.

The mid-IR spectra show that conformers B and C are indeed imino−keto conformers, but the spectra differ only by small changes in the N−H stretch region, which makes the differentiation between 7H/9H and up/down tautomers difficult. Unfortunately, the calculated IR spectra of the imino tautomers are also very similar to each other. We did calculate the vibrational frequencies of the four imino−keto tautomers at different levels of theory to predict the reliability of the small frequency shifts between the different tautomers. If the shifts are systematic and consistent at each level of theory, then we could use them to distinguish between 7H/9H and up/down imino−keto tautomers and to provide tentative assignments for conformers B and C.

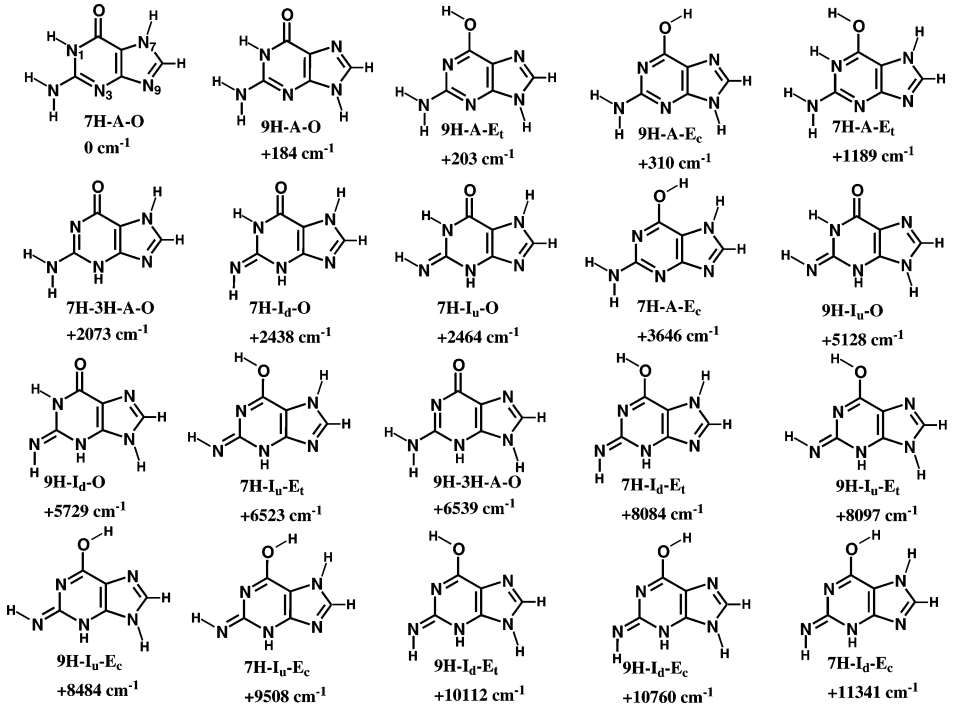

Figure 2. Structures, labels, and relative energies (B3LYP/TZVPP//RIMP2/cc-pVQZ, ZPE corrected) of all 20 tautomers of guanine and their relative energies in ascending order. The numbering of the atoms is indicated in the top−left structure. Abbreviations are as follows: 7H/9H = H-atom in 7N or 9N position, 3H = tautomer with the hydrogen atom moved from the 1- to the 3-position, *A* = amino group in the 2-position, I_u/I_d = imine group (up/down) in the 2-position, *O* = keto form, E_c/E_t = enol form (cis/trans).

Table 1 lists the unscaled C═N, C═O, and N−H stretch frequencies obtained at the B3LYP/TZVPP, RI-MP2/cc-pVDZ, RI-MP2/cc-pVTZ, and RI-MP2/TZVPP levels of theory. δ is the spacing between the C═N and C═O stretch vibrations. From Table 1 it is clear that the spacing between and the energetic order of the N_3H and $N_{7/9}H$ vibrations strongly depend on the theoretical method and the basis set. The δ value also depends on the theoretical level, but it is consistently larger for the 9H-tautomers as compared to the 7H-tautomers. In the experimental spectra, the spacing is the same for conformers B and C, indicating that both conformers are probably either 7H- or 9H-tautomers. If one conformer belongs to 7H and the other to 9H, then the experimental spacing between C═N and C═O vibrations would most likely differ by at least 10 cm^{-1}. Finally, there is a systematic, albeit small, blue shift of the N_1H vibration when going from an I_d to an I_u conformer. If the small shift is reliable, then we can assign conformer B to an up (I_u) and conformer C to a down (I_d) conformer.

Considering the uncertainties in the calculated spectra, we cannot derive conclusive assignments of the experimental spectra to specific tautomers of guanine (e.g., a 7H/9H assignment) exclusively based on the IR spectra. Further information is provided by energetic arguments and by comparison with previous calculations.[11] Experimentally, conformers B and C have ion signals of comparable intensities at their electronic origins. If we assume an equal detection probability for both conformers, then their number densities in the supersonic jet are comparable as well. Then, using the zero-order picture of a Boltzmann distribution, both conformers should have about the same energy relative to the most stable conformer. Upon inspection of Figure 2, the energies of tautomers 7H−I_d−*O* and 7H−I_u−*O* differ only by about 30 cm^{-1}, whereas 9H−I_u−*O* and 9H−I_d−*O* are 600 cm^{-1} apart and are about 3000 cm^{-1} higher in energy than the corresponding 7H tautomers. On the basis of an energetic argument, we would assign conformers B and C to 7H. If we now take into account the small shift of the N_1H stretch vibration, then conformers B and C can be assigned to tautomers 7H−I_u−*O* and 7H−I_d−*O*, respectively. A similar energetic argument could be derived from the IR spectra, because of the similar IR intensities. However, one has to be careful when comparing IR intensities in double-resonance experiments because saturation effects and dissociation (mainly for clusters) can alter relative intensities significantly.

The assumption of an equal detection probability is supported by recent calculations of Marian.[11] There, the corresponding 7H−$I_{u/d}$ tautomers have the same oscillator strength and similar Franck−Condon factors for vertical excitations, reflecting the fact that both structures differ only by the orientation of the imine group. Furthermore, the electronic origin of the up tautomer is predicted to be to the red of the corresponding down tautomer, which supports our assignment of conformers B and C to 7H−I_u−*O* and 7H−I_d−*O*, respectively. According to Shukla and Leszczynski[9] the 9H−I−*O* tautomers absorb about 4000 cm^{-1} to the blue of the corresponding 7H tautomers.

Additional support for the 7H assignment comes from measurements of 7-methylguanine.[25] There, the C═O stretch vibration of the corresponding 7-methylguanine conformer is red-shifted by 10 cm^{-1} as compared to non-methylated guanine, which is in good agreement with theory. However, the C═O stretch vibration of 9-methylguanine remains unchanged in the calculations. Also, 9-methylguanine has only one prominent peak in the REMPI spectrum, which correlates with conformer D of non-methylated guanine (a 9H−*A*−E tautomer).[5,7,8] There is no vibronic transition of 9-methylguanine in the spectral range of conformers A, B, and C of guanine. However, 7-methylguanine has two conformers with electronic origins close to conformers A and B of guanine and with IR spectra that are similar to those of the non-methylated conformers.[5,25] Either the number densities of the 9H−I−*O* tautomers are too small for detection or, if they are formed in sufficient amounts, they do not absorb in the same spectral region as the 7H−I−*O* tautomers (see above), or we simply cannot detect them because

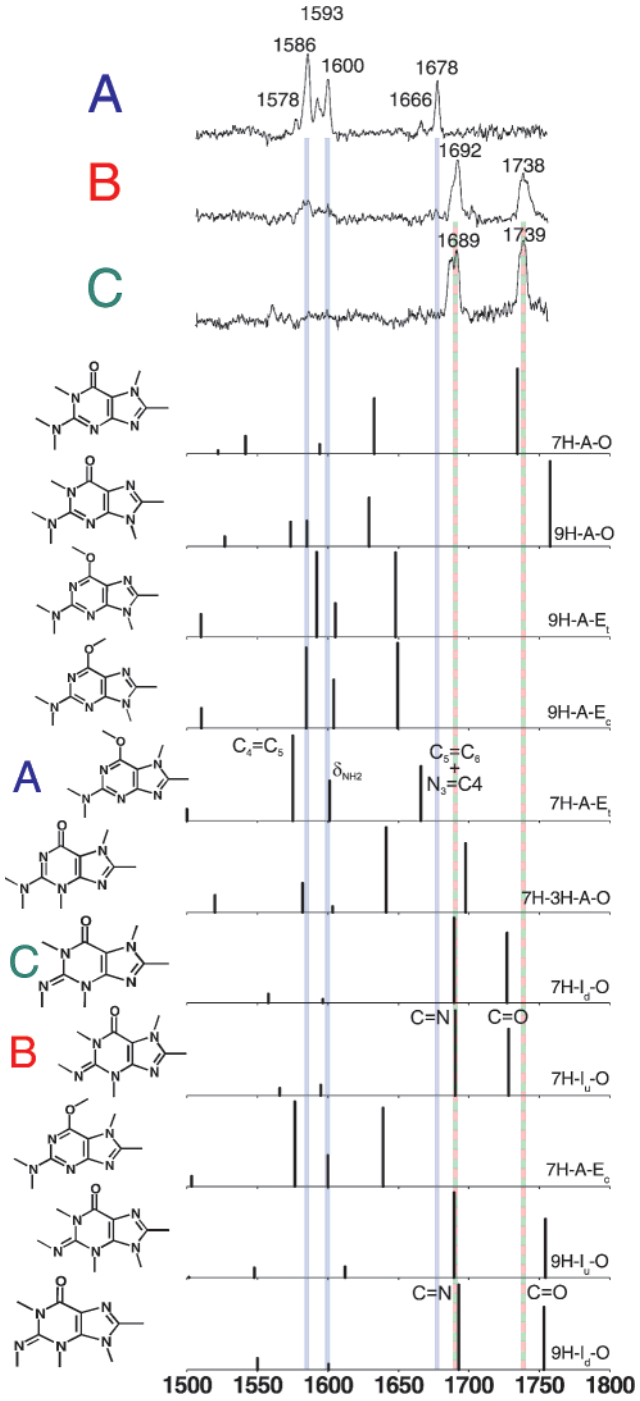

Figure 3. IR/UV double resonance spectra of the three guanine conformers A, B, and C between 1500 and 1800 cm^{-1} (top three traces). Also shown are the calculated and scaled stick spectra of the 11 most stable tautomers with relative energies less than 6000 cm^{-1} (cf. Figure 2). Colored lines indicate the positions of experimentally determined absorption maxima (A = blue, B = red, C = green). Conformers B and C are imino−keto structures, whereas conformer A is an amino−enol structure.

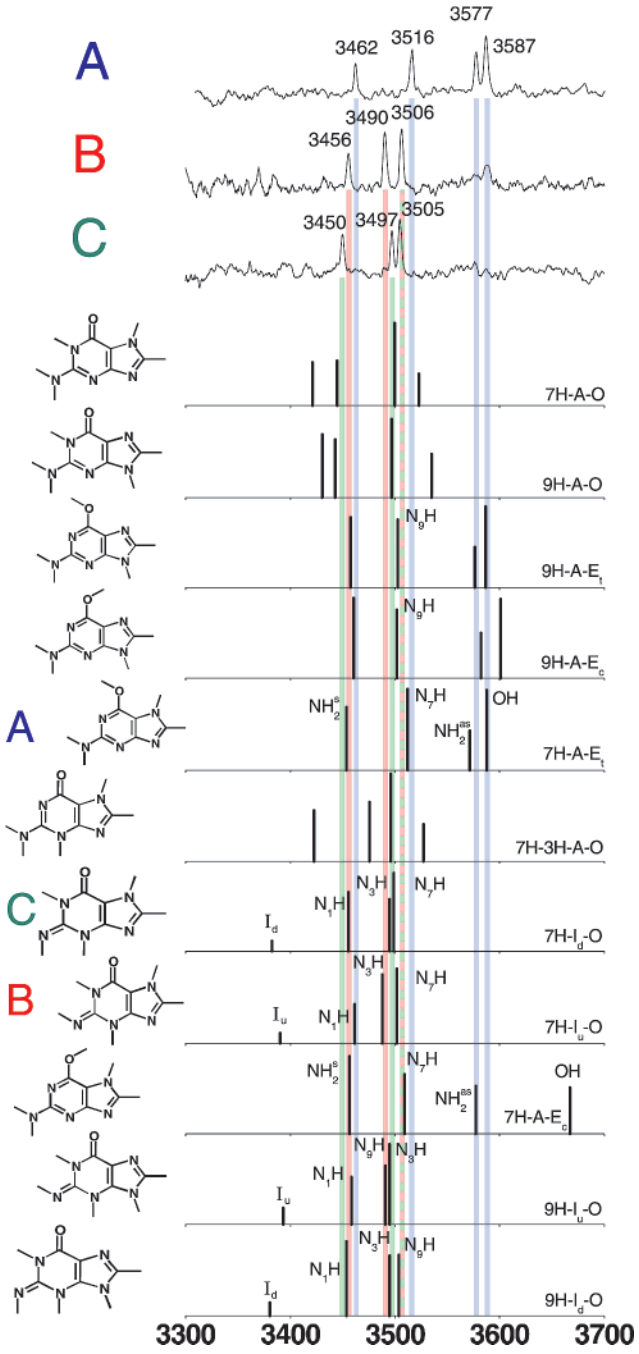

Figure 4. IR/UV double resonance spectra of the major guanine conformers A, B, and C between 3300 and 3700 cm^{-1} (top three traces). Also shown are the calculated and scaled stick spectra of the 11 most stable tautomers with relative energies less than 6000 cm^{-1} (cf. Figure 2). Colored lines indicate the positions of experimentally determined absorption maxima (A = blue, B = red, C = green). Conformers B and C are imino−keto structures, whereas conformer A is an amino−enol structure.

of unfavorable Franck−Condon factors or fast internal conversion. In either case, the non-observation of 9-methylguanine tautomers close to conformers B and C supports our 7H assignment of the those conformers. Therefore, together with the IR spectra and predictions of the electronic origins of conformers B and C, we can safely assign conformers B and C to 7H−I$_u$−*O* and 7H−I$_d$−*O*, respectively.

Conformer A. Conformer A shows four absorptions between 3450 and 3600 cm^{-1} (Figure 4), which were previously assigned to the enolic OH (3587 cm^{-1}), NH$_2^a$ (3577 cm^{-1}), N$_7$H or N$_9$H (3516 cm^{-1}), and NH$_2^s$ (3462 cm^{-1}) stretch vibrations.[4,5] Comparison with our calculations shows that its spectrum agrees best with 9H−*A*−E$_t$, 9H−*A*−E$_c$, and 7H−*A*−E$_t$. We can rule out 7H−*A*−E$_c$ because the OH stretch frequency is blue-shifted to about 3680 cm^{-1}, due to steric interactions between the OH and the N$_7$H group. Between 1500 and 1800 cm^{-1}, conformer A shows three strong bands at 1586, 1600, and 1678 cm^{-1} (Figure 3). For the three tautomers in question, these vibrations have predominantly C$_4$=C$_5$ stretch, NH$_2$ bending, and C$_5$=C$_6$ + N$_3$=C$_4$ stretch character, respectively. Although the assign-

TABLE 1: Unscaled C═N, C═O, and N−H Stretch Frequencies (in cm^{-1}) of the Four Imino Tautomers at Various Levels of Theory[a]

conformer	C═N	C═O	δ	N1−H	N3−H	N7/9−H
			B3LYP/TZVPP			
7H−I_d−*O*	1721	1759	(38)	3604	3646	3649
7H−I_u−*O*	1721	1760	(38)	3610	3638	3652
9H−I_d−*O*	1724	1785	(61)	3602	3654	3645
9H−I_u−*O*	1721	1786	(66)	3607	3641	3645
			RI-MP2/cc-pVDZ			
7H−I_d−*O*	1765	1830	(64)	3622	3673	3666
7H−I_u−*O*	1767	1833	(66)	3625	3669	3670
9H−I_d−*O*	1768	1837	(69)	3622	3680	3668
9H−I_u−*O*	1768	1840	(72)	3622	3672	3669
			RI-MP2/cc-pVTZ			
7H−I_d−*O*	1744	1801	(57)	3617	3671	3667
7H−I_u−*O*	1746	1804	(58)	3623	3664	3669
9H−I_d−*O*	1747	1812	(65)	3617	3680	3673
9H−I_u−*O*	1746	1815	(68)	3621	3669	3672
			RI-MP2/TZVPP			
7H−I_d−*O*	1738	1790	(53)	3616	3673	3669
7H−I_u−*O*	1740	1794	(53)	3624	3664	3671
9H−I_d−*O*	1741	1802	(61)	3617	3683	3675
9H−I_u−*O*	1741	1805	(65)	3623	3669	3674

[a] δ is the spacing between the C═N and C═O stretch vibrations.

ment is not as convincing as for conformers B and C, the mid-IR spectrum of conformer A agrees best with 7H−*A*−E_t, with the enolic OH group in the trans conformation. Our assignment confirms previous studies of Mons and co-workers.[5,7] This is in line with calculations of the vertical excitation energies.[11] Only 7H−*A*−E_t has an electronic origin at lower energies, whereas 9H−*A*−E_t and 9H−*A*−E_c have vertical transition energies higher than the 7H−I−*O* tautomers.

5. Summary

We presented the mid-IR spectra of the three guanine conformers A, B, and C. In combination with previous measurements in the OH/NH stretch region[4] and with DFT and ab initio calculations, we demonstrated the existence of imino−keto structures in supersonic expansions. Specifically, conformers B and C are assigned to 7H−I_u−*O* and 7H−I_d−*O*, respectively, which differ only in the orientation of the imino group. Our assignment is supported by comparison with UV and IR/UV spectra of 7- and 9-methylguanine,[5,7,8,25] as well as with recent predictions of electronic origins, oscillator strength, and relaxation pathways of different guanine tautomers.[11] In contrast, conformer A is an amino−enol form and is assigned to 7H−*A*−E_t, with the OH group in the trans position, in agreement with previous work.[5,7,11] Considering theoretical calculations[11] and our re-assignment, none of the three major conformers A, B, and C detected by R2PI are the biologically relevant 9H−*A*−*O* tautomer, which has not been observed via R2PI so far.

Acknowledgment. This work has been supported by the Deutsche Forschungsgemeinschaft (SFB 663/A4). The authors thank C. Marian for fruitful discussions. We gratefully acknowledge the referees' comments.

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