Tautomerism of the DNA Base Guanine and Its Methylated Derivatives as Studied by Gas-Phase Infrared and Ultraviolet Spectroscopy

Michel Mons,* Iliana Dimicoli, François Piuzzi, Benjamin Tardivel, and Mohamed Elhanine

Laboratoire Francis Perrin (FRE CNRS−CEA 2298), Service des Photons, Atomes et Molécules, Centre d'Etudes de Saclay, Bât. 522, 91191 Gif-sur-Yvette Cedex, France

Received: October 26, 2001; In Final Form: January 25, 2002

The present IR−UV depletion spectroscopic study extends the recent data of literature by providing evidence for the existence of a fourth form of guanine in the gas phase. The comparison of the UV and IR signatures of the four forms together with those of the 7- and 9-methylated derivatives allows us to build up a new assignment in terms of enol/keto and 7/9NH tautomerisms. From this complete picture, it turns out that the UV spectroscopy of free guanine is mainly controlled by the 7/9NH tautomerism: both 7NH tautomers observed are red-shifted compared to the 9NH *ones*, with the following origin transition order, from red to blue: 7NH enol (32864 ± 5 cm^{-1}), 7NH keto (+405 cm^{-1}), 9NH keto (+1046 cm^{-1}), and 9NH enol (+1891 cm^{-1}); 7-, 9- or 1-methylations are found to cause only moderate red shifts (less than 400 cm^{-1}). The opposite trend is observed for the IR spectroscopy, which appears to be essentially controlled by the enol/keto tautomerism. This study exemplifies the need for cross-checked experimental approaches, namely the IR/UV depletion spectroscopy or the study of relevant methylated species, to reach a global and consistent assignment, even in rather simple biological systems such as purine bases.

1. Introduction

Among the molecular bricks of the living world, DNA bases play a specific role with their ability to establish noncovalent hydrogen bonds responsible for the encoding and the expression of genetic information. Their very rich photophysics due to the numerous n and π electrons is very important since the ultraviolet (UV) spectrum and the excited-state lifetimes control the crucial questions of radiation-induced damages in DNA and the possible occurrence of undesired photochemical effects as well.[1,2]

Much is known on the photophysics of DNA purine bases, like guanine, in condensed phases.[3−5] However, several points remain open, especially those concerning the rich tautomerism of these species (Figure 1); in particular 9NH forms are though to be much less fluorescent that their 7NH counterparts.[4] Infrared (IR) studies in argon or nitrogen matrixes have helped a lot fifteen years ago for a better characterization of their vibrational spectroscopy, but no relationship between IR spectroscopy and UV absorption were carried out.[5−10] From a theoretical point of view, a wealth of ab initio studies is now available in the literature pertaining to both gas and condensed phase.[10−18]

The possibility to form and cool environment-free molecules of biological interest in a supersonic expansion enables us nowadays to study intrinsic effects that condensed phase studies cannot envisage. The field of photophysical studies of biological molecules in the gas-phase is encountering an impressive development.[19] Laser-based techniques combined to supersonic jets now allows experimentalists to distinguish the contribution of different tautomers as well as to measure their intrinsic photophysical features.[20] Very recently, guanine (Gua), known as a thermolabile and elusive molecule, was put in a supersonic expansion using laser desorption and its UV spectroscopy was published.[21] Spectroscopic evidence for the existence of three forms (referred to as **A**, **B**, and **C** in the present paper), with origin UV bands located at 32864, 33269, and 33910 cm^{-1} respectively, was provided.[22−24] Surprisingly, the fluorescence lifetime measurement[22] of **A** and **B** species revealed a nanosecond time scale resonant fluorescence,[25] in disagreement with the assumption that intrinsic short lifetimes (at least in the condensed phase) would be a necessary condition to ensure an efficient protection of DNA against UV-induced photochemical damages.

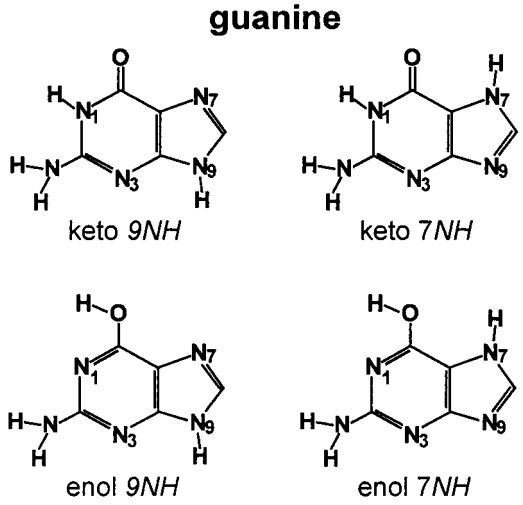

Figure 1. Schematic structures and atom numbering for the calculated most stable tautomeric forms of guanine in the gas phase. Only one rotamer of the enol forms is drawn: we will refer to it as the $enol_1$ rotamer (the OH moiety pointing toward the N_1 side). The second rotamer (not shown), with the OH on the N_7 side, will be referred to as the $enol_7$ form.

* To whom correspondence should be addressed. E-mail: mmons@cea.fr. Phone: 33 1 69 08 20 01. FAX: 33 69 08 87 07.

10.1021/jp0139742 CCC: $22.00
Published on Web 04/26/2002

With the help of ab initio calculations, tentative assignments were proposed. Calculations suggest that the keto (amino-oxo) and enol (amino-hydroxy) forms of Figure 1 are the most stable forms, with similar energies (within 1 kcal/mol) for the 9NH enol and keto and the 7NH keto, and a slightly higher energy for the 7NH enol form (2 kcal/mol): the two rotamers ($enol_1$ and $enol_7$) of the enol 9NH tautomer are found to have similar stabilities in contrast to the $enol_7$ rotamer of the 7NH tautomer that is much higher in energy for obvious steric reasons between OH and N7H groups). All the other forms (amino-oxo 3NH, imino-oxo, amino-enol, and imino-enol) are found much less stable.[13−18] On the basis of the assumption that only forms of Figure 1 should be observed in a supersonic expansion, contradictory assignments (in terms of enol/keto and 7/9NH tautomerism) have been derived for **A**, **B**, and **C**, depending upon the data considered, namely, ground-state vibrational spectroscopy[24] or lifetimes and electronic shifts.[22]

Beyond this disappointing disagreement, several questions still remain open and deserve further investigations. First, among the four forms (and even five, if one includes the two 9NH rotamers, $enol_1$ and $enol_7$) predicted to be significantly populated at a jet temperature, only three have been found so far. Second, only one of them, the 9NH keto form, is biologically relevant, and the absence of unambiguous assignment for guanine spectroscopy affects the relevance of the gas-phase studies to biology.

To shed light on these questions and to propose a convincing assignment for both the IR and UV spectroscopy of guanine, we have chosen a purely experimental approach, based on the comparison of guanine with methylated guanines. In a series of IR/UV experiments, we have measured the infrared gas-phase spectrum in the spectral region of the OH and NH stretches for the three guanine forms detected so far, using the resonant ion dip method.[20,26] A variant of the same technique was used to measure a "purified" UV spectrum, in which the spectral contribution of the most prominent species is eliminated. This powerful technique allowed us to provide experimental evidence for the presence of a fourth form in the jet, whose IR spectrum was also recorded. For assignment purposes, the IR and UV spectroscopy of related methylated compounds (7-methyl and 9-methyl guanine), in which the 7/9NH tautomerism is blocked have also been measured. The careful comparison of the IR and UV spectra of all these species allows us to build up a new and consistent assignment of the four forms of guanine observed in the gas phase in terms of tautomers. The reason for the disagreement with earlier studies is discussed and explained by the failure of a Boltzmann description of the tautomer population for a laser-desorbed species. Consequences in terms of biological relevance of gas-phase studies of life machinery building blocks are also discussed.

2. Experimental Section

The pulsed jet of guanine molecules was generated with a pulsed valve system (General Valve, 300 μm nozzle), fitted with a laser desorption module described elsewhere.[27] Argon was used as carrier gas (4 bar baking pressure). The IR/UV depletion experiment was carried out on a classical pulsed molecular beam setup.[22] The infrared radiation (2.5−3.0 μm, several mJ) was generated as the idler of a Nd:YAG pumped $LiNbO_3$ OPO (Euroscan/BM Industries). A line width of 1 cm^{-1} was achieved by an intracavity IR Pérot-Fabry plate. The spectral scan of the OPO, which is controlled by a personal computer (PC), was carried out by tilting the plate, simultaneously with the $LiNbO_3$ crystal. A typical energy of 3 mJ per pulse could be obtained, except in the 3470−3500 cm^{-1} energy range where the OPO emission vanishes because of an intense IR absorption in the crystal. The IR light was mildly focused (f = 2000 mm) onto the supersonic jet in the source of a time-of-flight mass spectrometer. The guanine molecules were photoionized by resonant two-photon process using the output of an excimer-pumped (Lambda-Physik EMG 200) dye laser (Lambda-Physik FL 2002E) after a time delay of 100 ns. The mass-selected Gua^+ signal was then acquired and averaged over 50 laser shots in a LeCroy digital oscilloscope as function of the frequency of the laser scanned and then transferred and processed in a PC.

Several types of spectra were measured with this setup:

•*UV spectra*, from one-color resonant two-photon ionization (R2PI), which mimic the absorption spectrum, provided that the photoionization efficiency and the lifetime of the excited species do not evolve too much in the spectral range considered.

•*Ground-state IR spectra*, detected by resonant ion dip (RIDIR spectroscopy[20]), using an IR−UV two-color scheme. The IR laser is scanned while the IR-induced depletion of one of the tautomers is probed by the UV laser tuned on a transition of this species.

Once the IR spectroscopy of at least one species is known, one can record population labeling[20,26] spectra by carrying out two kinds of IR−UV two-color experiments:

•*IR-purified UV spectra*, in which the contribution of a given tautomer, identified by one of its IR transitions, is suppressed. The UV laser is scanned while the IR laser is tuned on an intense IR transition of one of the tautomers causing its disappearance. This method, when applicable (i.e., when IR transition and/or laser intensities are high enough to provide intense depopulation), enables us to get rid of the contribution of the IR-excited tautomer on the UV spectroscopy. This technique turns out to be very useful to find modest spectroscopic features hidden in a congested part of the spectrum due to a prominent species. In the present work, IR-purified spectra have been carried out on two tautomers of Gua (the **A** and **D** forms, see below) by tuning the IR laser on two bands that lead to depletions as large as 90%: the OH and $NH_{2\ anti}$ bands. These high depletion efficiencies can be assigned to an intense pumping up to high-lying vibrational levels of the molecule (IR multiphoton excitation), due for instance to quasi-resonant excitation steps. To our knowledge, such large depopulations were reported only for the 5-hydroxytropolone monomer, by Zwier and co-workers.[28]

•*IR-labeled UV spectra* of a species selected by a given IR frequency ω_{IR}. When scanning the UV frequency, while the IR laser is tuned on ω_{IR}, the signals (IR-on) and (IR-off) are measured using a PC-controlled shutter and their difference is recorded.

3. Results

3.1. The Three Forms of Guanine Observed Previously. The near UV spectrum of gas-phase guanine, as obtained with our R2PI setup, is shown in Figure 2c. The spectrum, which shows apparently an intense Franck−Condon activity, is similar in shape to the spectrum published by de Vries and co-workers.[21] In Figure 2c are also indicated the origin bands of the three forms **A**, **B**, and **C**, previously identified by UV−UV hole-burning spectroscopy[24] or by their lifetime.[22]

The RIDIR spectra recorded when interrogating the IR spectroscopy of these species in the spectral region of the OH and NH (amino NH_2 antisymmetric and symmetric, 1NH, 7/9NH) chromophores are shown on Figure 3. The **A** species exhibits four bands, two of them looking as a doublet (close in

TABLE 1: Experimental UV Origin Transitions and IR Frequencies Obtained in the Present Experiment on the Four Tautomers of Guanine Observed in the Gas Phase (A−D), as Well as the Corresponding Species in 7- and 9-Methyl Guanine: Precision on UV and IR Frequencies = 2 cm^{-1}, the Assignment in Terms of Enol vs Keto and 7 vs 9NH Tautomerisms Is Discussed in the Text

	guanine				7MeGua		9MeGua
	A	**B**	**C**	**D**	**A**	**B**	**D**
assignment	enol 7NH	keto 7NH	keto 9NH	enol 9NH	enol	keto	enol
UV frequency[a]	0	405	1046	1891	−303	198	1748[b]
IR frequencies							
OH	3587			3590	3582		3589
$NH_{2\ anti}$	3577	3490[c,d]	3503[e]	3583	3575	not observed	3582
7/9 NH	3515	3504	3497[c,d,e]	3508	*f*	*f*	*f*
1 NH		3456[c]	3449			3456	
$NH_{2\ sym}$	3462	not observed	not observed	not observed	3461	not observed	not observed

[a] Relative to the origin of tautomer **A**: 32864 ± 5 cm^{-1}. [b] Center of a doublet (splitting: 2.4 cm^{-1}). [c] From ref 24. [d] Not observable in our experiment; see Experimental Section. [e] Tentative assignment; see discussion section. [f] Methylation site.

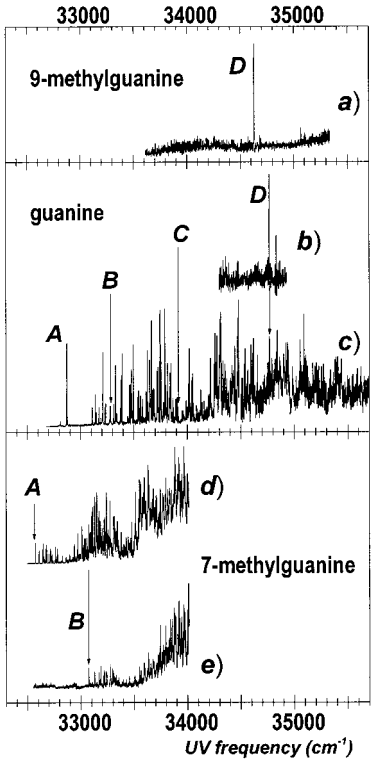

Figure 2. Mass-selected, one-color R2PI UV spectra of guanine and related methylated compounds: (a) 9-methyl guanine, the origin band, labeled D, is located at 34612 cm^{-1}; (b) IR-labeled UV spectrum of the tautomer **D** of guanine, origin band labeled D located at 34755 cm^{-1}, IR tuned to the OH mode frequency of Gua **D** (3590 cm^{-1}, see Figure 3); (c) guanine, the origin bands of the four tautomers are labeled A (32864 cm^{-1}), B (33269 cm^{-1}), C (33910 cm^{-1}), and D (34755 cm^{-1}); (d) 7-methyl guanine, the origin band of the most red tautomer, labeled A, is located at 32561 cm^{-1}; (e) IR-purified UV spectrum of the tautomer B of 7-methyl guanine, the origin band, labeled B, is located at 32060 cm^{-1}, IR tuned to the OH mode frequency of tautomer **A** of 7-methyl guanine (3582 cm^{-1}).

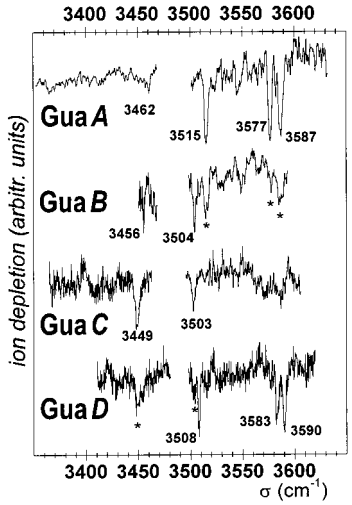

Figure 3. RIDIR spectroscopy in the spectral region of the OH/NH stretches for the four tautomers **A**, **B**, **C**, and **D** of guanine identified by R2PI UV spectroscopy. The UV frequency is tuned on the origin bands for A (32864 cm^{-1}), B (+ 405 cm^{-1} above A origin), C (+1044 cm^{-1}), and D (+1891 cm^{-1}). The bands marked by a star in the Gua **B** RIDIR spectrum are IR transitions of the **A** species; their observation is due to accidental spectral overlaps of the UV bands of **B** and A, combined with the low population of B in our expansion. For the same reasons, the bands marked by a star in the RIDIR spectrum of Gua **D** are due to IR transitions of the Gua **C**. The spectra are not corrected for the IR laser intensity, which drops when going to low frequencies, except in the 3470−3500 cm^{-1} energy range, where an intense IR absorption occurs in the $LiNbO_3$ crystal.

energy and of similar intensity) located in the blue region of the spectrum (around 3580 cm^{-1}) and two isolated bands, one in the intermediate part (ca. 3510 cm^{-1}) and the other in the red part (ca. 3460 cm^{-1}). The IR spectra of the **B** and **C** species are very similar in shape: they present two bands in the domain investigated, one in the intermediate part and the other in the red part. The spectra of the **B** and **C** are very similar to those published by de Vries and co-workers,[24] apart from a third band (located at 3490 and 3497 cm^{-1}, respectively), which is missing in our spectra because of the absorption in our OPO crystal.

The values of the IR frequencies and the energy of the UV origin transitions of the studied species are given in Table 1.

At this stage, a preliminary assignment in terms of enol/keto tautomerism can already be proposed. Indeed, the compilation of the several matrix IR data on guanine, 9-methyl guanine, or dimethylated guanine on the amino group (2-dimethylamino, 6-hydroxy purine)[6−9,29] together with the IR supersonic jet data on the keto and enol model compounds, 2-pyridone[30,31] and 2-hydroxypyridine,[31,32] shows that

(i) the two bands in the blue of spectrum (>3550 cm^{-1}) are essentially due to the OH and amino antisymmetric ($NH_{2\ anti}$) stretch modes of enol forms,

(ii) the intense band in the red (<3460 cm^{-1}) is ascribed to the 1NH stretch of keto forms.

Such general features allows us to conclude that the **A** form is an enol tautomer and **B** and **C** forms are keto tautomers. This first assignment is confirmed by the very recent study by de Vries and co-workers,[24] whose approach consists of comparing

experimental gas-phase IR data with ab initio calculated frequencies. It should be noticed that this clear IR signature invalids our tentative assignment,[22] which was based on the red shifts and lifetime considerations alone.

On the basis of their calculations de Vries and co-workers went one step further, since they assigned **B** and **C** to the keto 9NH and 7NH tautomers respectively, and the apparently most populated form **A** to the enol 9NH species theoretically found to be the most stable enol form.[11−18,24] We will see below that this assignment in terms of 7/9NH tautomerism is not confirmed by additional IR data on methylated species.

3.2. Experimental Evidence for a Fourth Form. One of the striking points of the previous results concerns the UV spectroscopy of guanine. There is a large difference between the location of the origin bands of species **A**, **B**, and **C** (all below 34000 cm^{-1}) and that of 9-methyl guanine (9MeGua) located at 34612 cm^{-1}.[23] This apparent blue shift for 9MeGua is in contrast to what is expected for methylation on a ring system, which generally causes a moderate red-shift [33,34](a few hundreds of cm^{-1}). This observation suggested to us to look for the spectral signature of a fourth guanine species, hidden in the blue UV spectral region, that would be a counterpart to 9MeGua. However, as indicated in Figure 2c, the density of bands is very large in this region and renders difficult an unambiguous identification by UV/UV depletion spectroscopy. Our strategy is based on the fact that, surprisingly enough, it was rather easy to obtain large (>90%) IR depletion of the **A** species. Taking advantage of this we have carried out an **A**-free UV spectrum of this region, using IR-purified UV spectroscopy (see section 2). The resulting spectra (not shown) were composed of a noisy background corresponding to the large density of A bands partially depopulated, on which were superimposed a few isolated bands belonging to other species. Besides bands originating from **B** and **C**, was also found a band, at 34755 cm^{-1}, that did not exhibit any of the intense IR depletions of **A**, **B**, or **C** forms. This allowed us to conclude that this band originates from a new form, which we have labeled Gua **D**. The RIDIR spectrum (Figure 3) of **D** was performed in the same way as for the three other species. Three bands were strong enough to be detected. The resemblance of this spectrum to the spectrum of **A**, in particular the presence of two bands in the blue range, slightly blue-shifted relative to the band of **A**, enables us immediately to assign this new **D** form to a second enol tautomer.

The knowledge of the RIDIR spectrum of the new form Gua **D** enabled us to perform an IR-labeled UV spectrum of this species (Figure 2b), which exhibits one additional band at 66 cm^{-1} to the blue of the main intense band (assigned to the origin). The RIDIR spectrum carried out on this satellite band showed the same three frequencies as on the origin, which suggests that these bands belong to the same species.

3.3. Comparison with 9-Methyl Guanine. To assign more precisely the four forms observed to guanine tautomers, in particular in terms of 7NH vs 9NH tautomerism, we have chosen a strategy based on an experimental comparison of guanine with its 7- and 9-methylated derivatives, in which this tautomerism is blocked.

The R2PI UV spectrum of 9MeGua, similar to that of de Vries' group,[23] is shown in Figure 2a. Its origin at 34612 cm^{-1} is composed of a doublet, the center of which is located 143 cm^{-1} to the red of Gua **D**. The Franck−Condon activity is rather weak compared to Gua **A**. One can notice another doublet at +60 cm^{-1}, similarly to Gua **D**, as well as another weak feature at +438 cm^{-1} to the blue. The RIDIR spectrum carried out from the red-most band of the doublet (Figure 4, Table 1) is nearly identical to that of Gua **D**, with the same pair of bands in the blue part (same location within 1 cm^{-1}) and without any line in the 3510 cm^{-1} region. This observation shows unambiguously that this species is a 9MeGua enol tautomer, that we will label **D**, owing to the strong similarities with the Gua **D** species.

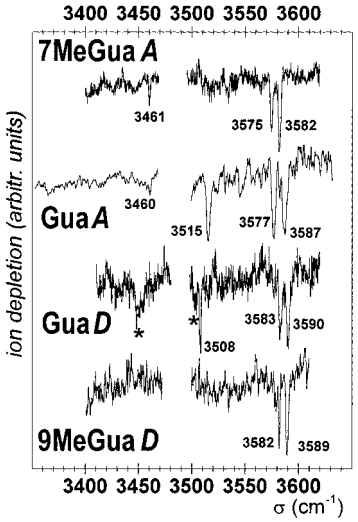

Figure 4. Comparison between the RIDIR spectra in the spectral region of the OH/NH stretches for 7-methyl guanine **A**, the tautomers **A** and **D** of guanine and 9-methyl guanine **D** identified by R2PI UV spectroscopy. The UV frequency is tuned on the origin bands for 7MeGua **A** (32561 cm^{-1}), Gua **A** (32864 cm^{-1}), Gua **D** (34755 cm^{-1}), and 9MeGua **D** (34612 cm^{-1}). The bands marked by star in the RIDIR spectrum of Gua **D** are due to IR transitions of Gua **C** (see Figure 3 caption).

All the 9MeGua vibronic bands mentioned above exhibit the same IR depletions as the origin. Evidence for the presence of additional UV bands originating from other tautomers, i.e., a keto form, has been looked for systematically. No significant signal could be found so far even at frequencies as low as 1000 cm^{-1} to the red of 9MeGua **D** origin.

3.4. Comparison with 7-Methyl Guanine. The R2PI UV spectrum of 7MeGua, shown on Figure 2d, resembles that of Gua **A**, with its large Franck−Condon activity. The red-most band at 32561 cm^{-1} is red-shifted by 303 cm^{-1} relative to the origin of Gua **A**. Intense vibronic bands can also be found at 367, 503 and 667 cm^{-1}. A careful examination shows that similar vibrational pattern, consisting of about 10 features, is present in combination with each of these bands. Being observed neither in any guanine form nor in 9MeGua, one should assign these patterns to vibrational excitations of the methyl group hindered rotation in 7MeGua excited state. To characterize the species responsible for the red-most bands, its RIDIR spectrum was carried out. This spectrum (Figure 4, Table 1) is very similar to that of Gua **A** or **D**, with, however, one missing band, i.e., that lying in the intermediate 3510 cm^{-1} region. The observation of a blue doublet enables us to assign it to an enol form, which we will label **A**. This labeling will be justified below.

The next step, in the investigation of 7MeGua, was to seek for the presence of a keto form. For this purpose, taking advantage of the large IR-induced depletion measured, we recorded IR-purified UV spectra. Figure 2e shows the **A**-free UV spectrum obtained, when the IR is tuned on the IR transition of 7MeGua **A** at 3582 cm^{-1}. This spectrum resembles that of 7MeGua **A**, but its origin is blue-shifted by 501 cm^{-1} relative to 7MeGua **A**. A vibronic pattern of similar shape is also present, although the frequencies involved are slightly different. The RIDIR spectrum carried out from the origin of this species

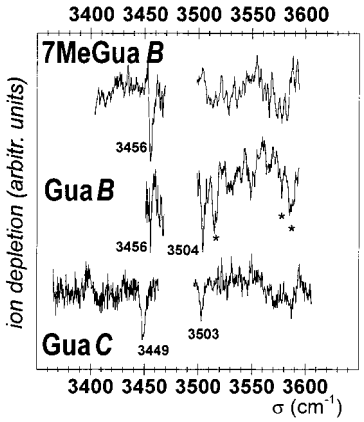

Figure 5. Comparison between the RIDIR spectra in the spectral region of the OH/NH stretches for 7-methyl guanine **B** and the tautomers **B** and **C** of guanine identified by R2PI UV spectroscopy. The UV frequency is tuned on the origin bands for 7MeGua **B** (33062 cm^{-1}), Gua **B** (33269 cm^{-1}), and Gua **C** (33910 cm^{-1}). The bands marked by star in the RIDIR spectrum of Gua **B** are due to IR transitions of the Gua **A** (see Figure 3 caption).

(Figure 5, Table 1) is very similar to that of Gua **B** or **C**, with again one missing band, i.e., that lying in the intermediate 3510 cm^{-1} region. The observation of an intense red line together with the absence of a blue doublet suggest to assign this second species of 7MeGua to a keto form that we will label **B**.

3.5. Summary of the Experimental Evidence. Four tautomers, distinguished from their IR spectroscopy, are identified in the UV spectrum of guanine (Figure 2c). From red to blue, one finds Gua **A** (enol), **B** (keto), **C** (keto), and **D** (enol).

One main tautomer (enol) of 9MeGua is found; its UV transition is close to that of Gua **D**.

Two tautomers are found for 7MeGua: from red to blue: first an enol form and then a keto form.

4. Discussion

4.1. Assignment of the Four Forms of Guanine Observed. The experimental evidences gathered above enables us now to propose an assignment of these species in terms of 7/9NH tautomerism. Methylation is here the safest argument. Indeed, methylation blocks the 7/9NH tautomerism and is not expected to strongly perturb neither the vibrational (IR spectra), nor the electronic properties (UV spectra) of the molecule. Hence, one expects the enol 9NH tautomer of Gua to have the same properties as the enol tautomer of 9MeGua, and so on. The present assignment of the four forms of guanine observed is done on the basis of a consistent correspondence with the three species identified in the 7- and 9-MeGua, for both the UV and IR properties.

One should notice here that the comparison between the four Gua tautomers alone, as an alternative assignment strategy, would probably fail because these four forms are actually distinct molecules, with a loose parenthood between them, much looser than the parenthood due to methylation. Consequently their IR and/or UV properties can be very different, without any intuitive connection between them. This point will be illustrated later on.

The examination of Figure 2, 4, and 5 shows that each form of 7- or 9-MeGua observed has a counterpart among the guanine species.

•*9MeGua **D***: UV transition slightly red-shifted (143 cm^{-1}) to Gua **D**, and has the same IR blue frequencies as those of Gua **D**, within 1 cm^{-1}.

•*7MeGua **A** and **B***: both UV transitions are also red-shifted compared to Gua **A** and **B**, by 303 and 207 cm^{-1}, respectively. The only IR band observed for 7MeGua **B** does correspond exactly to that of Gua **B**. The blue IR bands of 7MeGua **A** are shifted relative to those of Gua **A** and Gua **D**, but are closer to those of Gua **A**.

These correspondences justify the labeling of sections 3.3 and 3.4.

The present experimental results build up a self-consistent set of IR and UV data, which enables us to propose the following assignment for guanine: **A** and **B** are the *enol* and *keto 7NH tautomers*, respectively, and **C** and **D** are the *keto* and *enol 9NH tautomers*, respectively.

4.2. Assignment of the IR Spectra of the Guanine Tautomers. *a. Enol Tautomers.* The present enol IR data (Gua **A** and Gua **D**, 9MeGua **D**, and 7MeGua **A**) all exhibit the blue doublet due to the OH and antisymmetric amino ($NH_{2\ anti}$) stretch modes, confirming the matrix IR data, the OH band being the most blue and the most intense. The band at ca. 3460 cm^{-1} matches well a symmetric amino ($NH_{2\ sym}$) stretch mode, with a splitting of the two amino modes of 115 cm^{-1}. The band in the intermediate region (ca. 3510 cm^{-1}) in Gua disappears in the methylated species, indicating these bands' assignment to the 7NH or 9NH stretch modes (Table 1).

Interestingly one can note that the effect of the 7/9NH tautomerism on the OH and NH_2 vibrations remains rather modest, and has the same order of magnitude than the frequency difference between the 7NH and 9NH stretch modes.

At this point of the discussion, one should say that the experimental data do not allow us to decide which rotamer ($enol_1$ or $enol_7$) we do observe for the enol forms. In the case of 7NH, a strong steric effect between the OH and 7NH hydrogen atoms suggests that the $enol_7$ form should be energetically disfavored, which is confirmed by ab initio calculations.[12,14,16,24] In the case of 9NH, this effect is not present and our only guidance is the *ab initio* calculations. The $enol_1$ rotamer is predicted to be more stable than the $enol_7$ by less than one kcal/mol,[12,14,16,24] which precludes any firm assignment.

b. Keto Tautomers. The assignment of the keto IR spectra (Gua **B** and Gua **C** and 7MeGua **B**) is less obvious, apart from the red-most band obviously due to the 1NH stretch. Indeed this latter point has been nicely confirmed by the IR spectra of the 1-methyl guanine[24] in which this band disappears.

In the intermediate range, however, it is more difficult to assign unambiguously the two bands observed to the $NH_{2\ anti}$ and the 7NH or 9NH stretches without relying on methylated species. Figure 5 shows no band in the IR spectrum of 7MeGua **B** in the region of the 3504 cm^{-1} feature of Gua **B**. This supports the assignment of this latter band as the 7NH stretch of the 7NH keto tautomer. Consequently, the other band of Gua **B** in this region (3490 cm^{-1} in de Vries' spectrum[24]) should be the $NH_{2\ anti}$ stretch, which seems to be more intense than the 7NH stretch in the spectra of ref 24.

For the corresponding 9NH tautomer Gua **C**, the absence of 9MeGua analogue precludes such an assignment. Relying on the intensities in Gua **C** IR spectra of ref 24, one can tentatively assign the most intense band (3503 cm^{-1}) to the $NH_{2\ anti}$ stretch and the 3490 cm^{-1} band to the 9NH stretch.

This new tentative assignment for the keto forms allows us to compare the IR shifts between 7NH and 9NH forms. For the keto forms, the 7NH stretch (3504 cm^{-1}) seems to be blue-shifted relative to the 9NH stretch (3497 cm^{-1}) and the $NH_{2\ anti}$ stretch seems to be more red-shifted in the 7NH tautomer (3490 cm^{-1}) than in the 9NH species (3503 cm^{-1}). This trend is

consistent with the IR shifts observed on the enol forms: the 7NH stretch (3515 cm^{-1}) is blue-shifted relative to the 9NH stretch (3508 cm^{-1}), and the $NH_{2\ anti}$ stretch is more red-shifted in the 7NH tautomer (3577 cm^{-1}) than that in the 9NH species (3583 cm^{-1}).

One can notice that our assignments for these two bands, based on the effects of methylation, differ from that proposed by the de Vries' group, who relied on the ab initio calculations alone. This is probably due to the difficulty of the ab initio harmonic mode calculations to accurately reproduce the relative position of vibrations of different nature (here *different* stretch oscillators in *different* tautomers). The present case, in which three bands are expected within a 20 cm^{-1} frequency range in two distinct molecules (the 9NH and 7NH tautomers are different molecules even despite their similar shape) is indeed very severe test for the *ab initio* frequency mode calculations in a rather modest basis set.

One can finally note that the striking features used for the basic enol-keto assignment of section 3.2, namely, the disappearance of the blue doublet in the 3580 cm^{-1} region for the keto forms, is due to the difference between the OH and 1NH stretches together with the drastic red shift of the NH_2 modes when going from enol to keto (again the tautomers are different molecules, although their similar shape).

4.3. Considerations on the Tautomer Abundances in the Supersonic Expansion. The four lowest energy tautomers (2 enol and 2 keto) of guanine have been observed experimentally with populations having the same order of magnitude (within a factor of 3, assuming similar detection efficiencies), despite the relatively large difference of stability between the two enol forms for example. In our experiment we have had much difficulties to observe the **B** species (one of the two lowest energy forms) in the R2PI experiment. By comparison with the spectra of de Vries' group,[24] this species seems to be much less populated in our desorption device. In the same line, the assignment of the most intense feature (**A**) to the most stable enol form according to calculations (9NH) leads to a misassignment. All these facts tend to indicate that, although being indicative, the calculated stabilities should not be used as unquestionable arguments. These findings are in contrast to a previous gas-phase photoelectron study that suggests that the keto N7H form is the most abundant species.[35]

When the 7/9NH tautomerism is blocked by 7-methylation, the enol and keto forms (7MeGua) are both observed with similar intensities in our experiment. In 9MeGua only the enol form is observed: the keto form, which is biologically relevant, is unfortunately not detected. A similar observation, although less marked, was reported in gas-phase photoelectron studies: the enol form is found to be prominent.[36] This seems to indicate that results on one methylated molecule cannot be extrapolated straightforwardly to another. The comparison with theory is even more disappointing: the keto form is expected to be nearly as stable as the enol in 9MeGua and to be more stable by ca. 4 kcal/mol in 7MeGua![10]

The observed discrepancy concerning the tautomer population suggests that either (i) the abundances of the desorbed species is far from an equilibrium distribution and can be setup dependent, (ii) the precision of the calculated relative energies is not better than a few kcal/mol, or (iii) the UV oscillator strengths are strongly dependent upon the species. This latter point seems to be quite likely, according to calculations of Broo and Holmen,[13] which indicate a more intense $\pi\pi^*$ transition for 9NH enol than for 9NH keto by a factor of ca. 2. In any case, one has to be careful when using the relative abundances as assignment arguments.

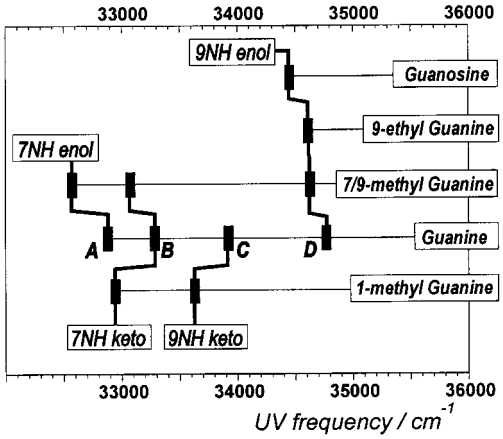

Figure 6. UV spectroscopy of guanine related species in the range between 280 and 320 nm. Spectral positions of the UV origin bands of guanosine, 9-ethyl guanine,[37] 7-methyl guanine, 9-methyl guanine, guanine (present work), and 1-methyl guanine.[24] The assignments in terms of enol/keto and 7H/9H tautomerisms are proposed on the basis of the present investigation. Vertical lines link corresponding tautomers in the different molecules. Note that guanosine is a 9-substituted guanine.

4.4. Influence of Tautomerism on the UV Spectroscopy. The present IR/UV data allow us to provide consistent tautomer-selective information on the UV spectroscopy of guanine derivatives (Figure 6). The UV spectroscopy of Gua is essentially controlled by the 7/9NH tautomerism: the two 7NH tautomers (**A** and **B**) have their origin band red-shifted compared to the 9NH ones (**C** and **D**). Within each of these tautomeric classes, the order of the enol/keto transition appears to be dependent upon the 7/9NH tautomer considered (Figure 6).

The assignment we propose is consistent with the result of the 7/9 substituted species. Methylation induces a spectral red shift of 150−350 cm^{-1} depending upon the tautomer considered as well as the methylation site. Other 9-substituted guanines, 9-ethyl guanine and guanosine,[37] exhibit origin transitions very close to that of Gua **D** and 9MeGua, which suggest that these species appear as enol forms in the gas phase.

The main message of the present paper, namely the large electronic splitting due to the 7/9NH tautomerism, holds also probably for other purine systems, e.g., adenine. For example, the small blue shift (+26 cm^{-1}) of the 9-methyl adenine origin[38] relative to that of adenine[23,38,39] should be considered as an evidence for the occurrence of adenine as a 9NH tautomer in the gas phase.

Finally, we would like to point out that our assignment is also consistent with energetic data on 1-methyl guanine.[24] The two keto tautomers observed, which correspond to the **B** and **C** keto forms of Gua, have their UV transition red-shifted compared to that of **B** and **C** by 384 and 286 cm^{-1}, respectively.[24]

This dependence of the UV spectrum upon the 7/9NH tautomerism is in line with the trends observed in the scarce absorption studies reported for guanine or 7-methylated species in rare gas or nitrogen matrixes.[5]

5. Conclusion

The present work provides evidence for the simultaneous existence of four tautomers of guanine in the gas phase. A new assignment of these forms (in terms of enol/keto and 7/9NH tautomerisms) is given, based on both their UV and IR

properties, as determined by double resonance IR/UV experiments. This assignment is consistent with the UV and IR data also collected on the 7- and 9-methylated guanine. A complete picture of the dependence of the UV absorption upon the enol/keto and 7/9NH tautomerisms is provided for isolated guanine and its derivatives and can be used as firm reference data for the assessment of UV spectra calculations.

From this study, it turns out that the IR and UV diagnostics are obviously complementary to assign the contribution of the several tautomers. Whereas IR spectroscopy is very efficient to sort out the enol/keto tautomerism, its use is much less appropriate for the 7/9NH tautomerism, even with a comparison with *ab initio* calculated frequencies. On the other hand, the UV spectroscopy, because of its sensitivity to this 7/9NH tautomerism, provides efficiently the missing information.

The relative abundances observed in the present gas-phase experiment, in particular the fact that the enol forms are quite well observed despite the lesser stability calculated for these forms, suggests that experimentalists should be careful when using calculated energies as assignment tools.

Finally, the present study shows that the 9-substituted guanine derivatives that experimentalists are using as model systems for studying bases interactions in DNA[37] might not be in the biologically relevant keto form in the supersonic expansion, unless intermolecular interactions in clusters are able to reverse the tautomer equilibrium.

Acknowledgment. The authors thank Dr. A. Peremans for helpful discussions and for the setting up of the infrared optical parametric oscillator as well as Miss W. Chen for her help during data collection.

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