Gas Phase Rotamers of the Nucleobase 9-Methylguanine Enol and Its Monohydrate: Optical Spectroscopy and Quantum Mechanical Calculations

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Received: April 6, 2004; In Final Form: July 1, 2004

The present work reports a combined experimental and theoretical study of the 9-methylguanine (9MG) nucleobase and its monohydrate in the gas phase. Laser spectroscopy provides optical evidence for the presence of only one tautomer of 9MG, among the three forms expected from an energetic basis. Comparison with the calculated vibrational frequencies enables us to assign with confidence this form to the anti rotamer of the 9MG enol tautomer. The same approach allows us to conclude that the monomer conformation is retained in the monohydrate, with the water molecule bridging the OH proton donor and the N7 acceptor sites of 9MG. A complete comparison between all IR absorption data known for the enol tautomers of guanine and related compounds in the gas phase and the present calculations enables us to propose a consistent assignment for all of these enol tautomers in terms of rotamers. Finally, the fact that one does not observe either the most stable (and biologically relevant) keto form or the other enol rotamer (very close in energy) raises the question of the efficiency of UV spectroscopy to properly detect these missing species.

1. Introduction

Knowledge of the fundamental properties of nucleic acid bases and their interaction with water is a key factor to understanding the chemical and physical processes at play in biological systems. Spectroscopic studies in the gas phase may address such questions by offering several unique features. First, the gas phase data, when available, are much easier to interpret, without perturbations from DNA macromolecular structure or from solvent molecules. Second, meaningful ab initio calculations are feasible on these medium-sized biological systems, allowing a fruitful interplay between experiment and theory. Finally, extrapolation of the gas phase results to a biological environment requires studying the effects of a solvent. Therefore, complexes of DNA bases with a selected number of water molecules, as produced in supersonic expansion, turn out to be reasonable models for a theoretical investigation of DNA hydration.[1−3]

The first gas phase spectroscopic data on purine and pyrimidine DNA nucleobases have been obtained only very recently[3] due to the huge progress in the development of relevant techniques for vaporization by laser desorption in combination with jet cooling and in double-resonance laser spectroscopy.[1,4,5] Phenomena as complex as tautomerisms and the nature and dynamics of the excited states have been experimentally addressed,[4,6−14] providing at last an experimental counterpart to the numerous theoretical studies.[15−29] Hydration of functional sites of nucleobases has been investigated so far only on model systems such as 2-hydroxypyridine or 2-pyridone,[30−33] essentially because these molecules are much easier to handle than the thermolabile nucleobases. No experimental data have been published on the structures of gas phase hydrates of DNA bases. In contrast, many computational studies have been carried out on the energetics of the isolated bases, on base pairing, as well as on their hydration. Calculations were mainly performed on the biologically relevant conformers, for example the keto form of guanine.[21,22] Only very recently more thorough studies of the relative energies of all types of tautomers of guanine and their hydrates have been reported.[6,23−25,29,34]

In addition to being the most complex system among the five nucleobases, the purine base guanine has been one of the most studied species in the gas phase. Our present understanding of this DNA base stems from a wide range of investigations, beginning with the pioneering work of de Vries et al.,[4] later completed by more thorough studies, including one-color resonant two-photon ionization (R2PI), laser induced fluorescence, as well as UV/UV and IR/UV double-resonance depopulation spectroscopies.[6,8,11,13] Four tautomers of guanine have been found to coexist in supersonic expansion and have been characterized by both their IR and UV (absorption and radiative) properties.[11,13]

For the sake of relevance to biology as well as of simplification, we have focused our efforts on the gas phase properties of the methylated derivative of the nucleic acid base guanine, namely 9-methylguanine (9MG) and its hydrate. Substitution of the 9N hydrogen atom by a methyl group provides a reasonable model for the base moiety in the guanine nucleotide and permits a more realistic study of DNA properties. The model nucleotide 9MG can adopt several tautomeric forms due to the mobility of its hydrogen atoms. The 7/9NH tautomerism existing in guanine is blocked by methylation, leaving only the enol/keto tautomerism. In Scheme 1 the structures and atom numbering of its most stable forms[25] are shown, namely the canonical amino-oxo (keto) 9MG form (9MGk) and the two

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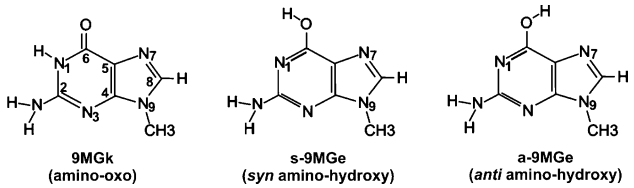
10.1021/jp048492f CCC: $27.50
Published on Web 09/09/2004

SCHEME 1: Conformers of 9-Methylguanine



rotamers of the amino-hydroxy (enol) 9MG form differing in the orientation of the hydroxy group toward either the N1 or the N7 atom, labeled s-9MGe and a-9MGe, respectively.

In the studies reported so far on 9-methylated guanine and parent compounds, only one main tautomer of the enol type was found,[11−13] in contrast to the case of 9*H*-guanine (9HG).

The aim of the present comprehensive experimental and computational study of 9-methylguanine and its monohydrate in the gas phase is to shed light on the several open issues related to these biologically relevant systems. First, a refined scan of the UV spectrum of 9MG was carried out, searching for eventual weakly populated tautomers. Second, the first UV and IR spectra of a nucleobase monohydrate have been recorded. A precise assignment in terms of rotamers of the enol conformers could be proposed based on the comparative analysis of the IR spectroscopy of the 9MG monomer and its hydrate. Third, a more complete picture has been obtained from quantum chemistry calculations, which confirm the experimental assignments on 9MG and in addition allow rotamer assignments on other 7- or 9-substituted guanine molecules.

2. Theoretical Calculations

A. Methodology. The DFT/B3LYP method was used for the study of the molecular structure, energetics, and interaction of guanine and methylguanine tautomers with water molecules. The choice of this level of theory and basis set was motivated by our need for reliable calculated IR frequencies which would help us to assign the experimental IR data. Excellent agreement between experimental IR data and this type of calculation was indeed achieved by Zwier and co-workers on the 1:1 hydrates of two nucleobase mimetic molecules, 2-pyridone and 2-hydroxypyridine.[32] The calculations were carried out with the Gaussian 98 program.[35] The standard 6-31+G(d) basis set was used. All geometries of local minima were optimized without symmetry restrictions (C_1 symmetry was assumed) by the gradient procedure. The local minima were verified by establishing that the matrices of the energy second derivatives (Hessians) have zero eigenvalues.

Single-point calculations at the MP2/aug-ccpvtz level have also been carried out for the most stable B3LYP/6-31+G(d) equilibrium geometries of 9MG and its monohydrate in order to improve the accuracy of the tautomer energetic ordering as well as to allow comparison with recent accurate RI-MP2/TZVPP calculations of guanine by Hobza and co-workers.[25]

B. Guanine and 9-Methylguanine. Three different conformers of the 9MG molecule displayed in Scheme 1 have been calculated at the B3LYP/6-31+G(d) level of theory. The values of zero point energy (ZPE) corrected MP2/aug-ccpvtz//B3LYP/6-31+G(d) relative energies, given in Table 1, indicate that 9MGk, s-9MGe, and a-9MGe are nearly isoenergetic. This finding is similar to the most accurate data of the three 9*H* tautomers of guanine (RI-MP2/TZVPP calculations of ref 25, Table 1), which suggests the small influence of 9-methylation upon the tautomer's energetics. This result together with the large energy reported for the syn ↔ anti barrier in guanine enol (10 kcal/mol [25,28]) suggests that these three species could be expected in a supersonic expansion.

TABLE 1: ZPE-Corrected Relative Energies (in kcal/mol) of the Enol and Keto Tautomers of 9-Methylguanine and Corresponding Hydrates of Figure 1 Calculated at the MP2/aug-ccpvtz//B3LYP/6-31+G(d) Level of Theory, with Similar Energetic Data on Tautomers of Guanine and Corresponding Hydrates, Adapted from Ref 25, Given for Comparison[a]

	MP2/aug-ccpvtz//B3LYP/6-31+G(d)[b]		RI-MP2/TZVPP	
	9MG	9MG−H_2O	Gua[c]	Gua−H_2O[d]
anti 9-enol	0.11	−0.88	0.24	−0.45
syn 9-enol	0.10	2.16	0.04	1.87
9-keto	0	0	0	0
syn 7-enol			2.85	
7-keto			−0.43	

[a] The canonical 9-keto tautomer has been taken arbitrarily as the energy reference for each type of calculation. [b] ZPE taken at the B3LYP/6-31+G(d) level. [c] RI-MP2/TZVPP level; ZPE at the MP2/6-31G(d,p) level, from ref 25. [d] RI-MP2/TZVPP energies from ref 25; ZPE at the B3LYP/6-31+G(d) level, calculated in the present work.

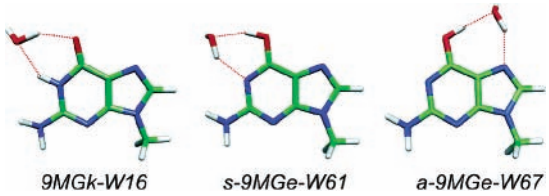


Figure 1. DFT minimum energy structures of the three most stable monohydrates of 9MG, obtained at the B3LYP/6-31+G(d) level of theory. The terminology used in the text is given, with the letters "k", "s", and "a" standing for keto, syn enol, and anti enol, respectively, and the numbers standing for the 9MG sites involved in the binding of the water molecule.

C. 9-Methylguanine−Water Complex. The geometries of monohydrates of the three tautomers of 9MG have been optimized starting from the structure of the lowest energy hydrates of 9*H*-guanine (compatible with a 9-methylation) found by Hobza and co-workers[25] at the MP2/6-31G(d,p) level. All of these structures correspond to a water molecule linking a donor and an acceptor site of guanine: the O6 site of guanine with either the N1 or N7 nitrogen atom (Figure 1). The donor or acceptor character of these sites depends on the tautomer. The keto form gives rise to a six-membered ring bridging the N1H and the O6 sites (structure labeled as 9MGk-W16). The s-9MGe form leads to a similar six-atom bridging pattern (s-9MGe-W61), however with a reversed direction for H-bonding since it actually corresponds to the final state of an H atom transfer from the N1H site in the previous structure. The a-9MGe form is involved in a seven-membered ring (a-9MGe-W67) pointing toward the N7 site of guanine.

The most stable form (Table 1) is found to be the hydrate of the anti enol tautomer (a-9MGe-W67). The hydrated keto (9MGk-W16) and syn enol (s-9MGe-W61) tautomers are less stable by 0.9 and 3.0 kcal/mol, respectively, yielding a stability pattern very similar to that of guanine hydrates. One should notice that the energy difference between both enol hydrates is much larger compared to the monomers. Obviously, the seven-membered ring leads to a relative stabilization of ∼3 kcal/mol for the a-9MGe rotamer compared to the six-membered ring on s-9MGe, presumably because it offers the best geometric arrangement for H-bonding. The distances are indeed shorter for the a-9MGe-W67 bridge (1.73 and 1.85 Å for the O_6H-O_w and O_wH-N_7 distances, respectively) compared to the s-9MGe-

W61 bridge (1.80 and 1.94 Å for the O_6H-O_w and O_wH-N_1 distances, respectively).

3. Experimental Methods

The spectroscopic measurements with laser-desorbed nucleobases were performed with an apparatus described in detail elsewhere.[5,11,13] Briefly, the setup combines a supersonic expansion of laser-desorbed species, tunable UV and IR lasers, as well as fluorescence and mass spectrometry detection.

The production of intact vapor phase molecules was achieved by desorption from a compressed mixture of the molecule with graphite using a Nd:YAG laser light (532 nm, 0.5 J/cm^2) transported by an optical fiber in front of the pulsed nozzle (General valve).[5] Argon (or a mixture with water vapor) at a backing pressure of ~5 bar was employed as the carrier gas. The seeded molecular beam crosses at a right angle the UV and/or IR laser beams at a distance of 1 cm for the fluorescence measurements and inside the source region of a time-of-flight mass spectrometer (~20 cm) for the ionization measurements. Two tunable dye lasers (Lambda Physika 3002) pumped by excimer lasers (Lambda Physika EMG 102 and LPX) operating in the region of 33 000−36 000 cm^{-1} (pulse energy on the order of 400 μJ) were employed. The infrared radiation (2.5−3.7μm, several millijoules) was generated as the idler of a Nd:YAG pumped $LiNbO_3$ OPO (Euroscan /BM Industries). The computer controlled spectral scan was achieved by tilting an intracavity IR Pérot−Fabry plate (line width 1 cm^{-1}) simultaneously with the crystal.

The spectroscopic methods used were based on fluorescence and resonant two-photon ionization (R2PI) detection. Fluorescence excitation spectra and lifetimes were measured after filtering of the emitted light by a wide band monochromator. The ionization excitation spectra were obtained by monitoring specific selected mass peaks while varying the UV laser wavelength.

Double-resonance experiments (UV/UV or IR/UV depopulation spectroscopy[1,2]) were used to record conformer-specific vibronic or ground-state infrared spectra. IR spectroscopy in the 3000−4000 cm^{-1} region (NH and OH stretches) is an excellent technique for the detection of enol/keto tautomerism and the presence of hydrogen bonding.[6,11]

4. Results and Discussion

A. Refined Identification of the 9-Methylguanine Rotamers. *UV Spectroscopy.* The mass-selected R2PI vibronic spectrum of jet-cooled 9MG is shown in Figure 2. The spectrum is composed of only a few bands, located over 150 cm^{-1} to the blue of one intense origin band at 34 612 cm^{-1}. Although the experimental sensitivity has been increased, no additional spectral features of 9MG have been detected in the large spectral range covered (~2000 cm^{-1}) in both the R2PI and fluorescence measurements. The weak bands located at 439, 479, and 524 cm^{-1} higher in energy have been shown to exhibit the spectral signature of the 9MG−H_2O complex (see Figure 2).

The UV spectrum reveals a striking similarity to the spectrum of the 9*H* enol conformer of guanine, namely a rather blue origin band and very weak Franck−Condon activity. However, the origin transition of 9MG centered at 34 612 cm^{-1} is composed of a doublet with two narrow bands split by 2.5 cm^{-1} and FWHM of 1.65 and 1.61 cm^{-1}, respectively (see insert in Figure 2). The rotational contour of each component of the doublet shows a dip on the top. A group of four lower intensity bands, blue-shifted from the origin by ~55 cm^{-1}, is presumably linked to the vibrational activity of the methyl group, eventually

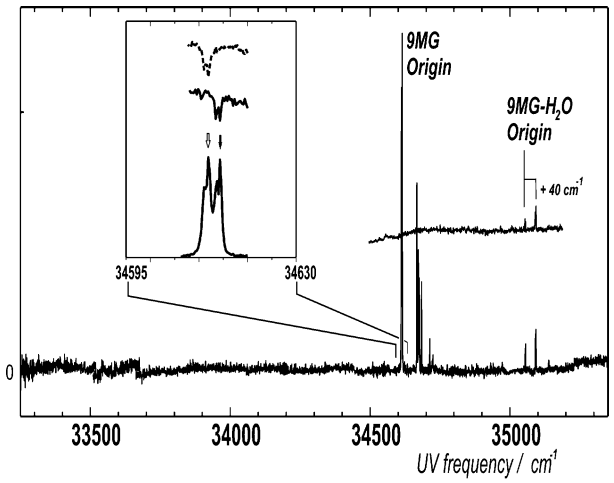


Figure 2. Mass-selected one-color R2PI spectrum of 9MG in the presence of traces of water in the expansion. The lower and upper traces correspond to the $9MG^+$ and $9MG^+-H_2O$ mass channels, respectively. The scan has been extended far to the red of the 9MG origin of the main tautomer in order to check for the existence of a minor 9MG species. The insert displays a close-up of the 9MG origin doublet (lower trace) together with the UV/UV double resonance depopulation spectra (upper and middle trace) carried out with the probe laser tuned on the band of each component (left arrow and right arrow, respectively).

perturbed by a coupling to the torsional motion of the amino group since the frequency observed differs slightly from the nearly free methyl rotation frequency (50 cm^{-1}).[36]

The fluorescence excitation spectrum (not shown here) is very similar to the R2PI spectrum. The excited-state decay time measurements on each doublet component gave a value of 21 ± 2 ns. This value is comparable to that of the four guanine tautomers measured at their origins, namely 12 (7*H* enol), 22 (7*H* keto), 25 (9*H* keto), and 17 ns (9*H* enol), respectively.

Finally, it turns out that the UV spectrum of 9MG looks fairly simple, like the 9*H* enol guanine conformer spectrum, in contrast to those of other guanine conformers which exhibit extended and complex progressions. At this stage of the study it was necessary to identify the doublet structure of the origin band.

Double-Resonance UV/UV and IR/UV Spectroscopy. UV/UV depopulation experiments on 9MG (see insert in Figure 2) show that the two components of the origin doublet (split by 2.5 cm^{-1}) belong to either different ground-state species (conformers) or different quantum states (ground state and hot band). Moreover, the four bands at ~55 cm^{-1} are associated, two by two, to each component, with a splitting increasing to 5.4 and 8.5 cm^{-1}. However, one can notice that the rotational contour is the same for both doublet components, with similar widths and intensities. This pleads in favor of two ground states of the same conformer.

For elucidating the nature of the two species, the IR spectra have been measured for all bands by IR/UV depopulation spectroscopy. All spectra are identical, mainly composed of two narrow bands in the 3600 cm^{-1} region, the red most being slightly less intense (Figure 3a). Comparison with the experimental gas phase and matrix IR spectra of the tautomers of guanine and 7-methylguanine shows that such an IR feature in the 3600 cm^{-1} region is characteristic of the enol conformers.[11]

IR intensities as well as measurements of the splitting between symmetric and antisymmetric NH stretches of the amino group were used to assign these two bands to the antisymmetric amino NH stretch (3582 cm^{-1}) and the OH stretch (3589 cm^{-1}) of an enol conformer. The symmetric NH_2 stretch, which is expected in comparison to guanine[6] to be red-shifted by ca. 120 cm^{-1} compared to the antisymmetric NH_2 stretch, was presumably

TABLE 2: Experimental and Calculated (B3LYP/6-31+G(d) Level of Theory) Harmonic Vibrational Stretch Frequencies (in cm^{-1}) of the N7/9H, NH_2, and OH Groups of Several Tautomers of Guanine, 9MG, and 7MG[a]

molecule	N9H	N7H	NH_2 sym	NH_2 anti	OH	N1H
9MG exptl			b	3582	3589	
syn 9MG enol			3590	3709	3687	
anti 9MG enol			3594	3715	3702	
9MG keto			3561	3666		3580
7MG exptl			3461	3575	3582	
syn 7MG enol			3586	3704	3687	
anti 7MG enol			3591	3711	3772	
9HG exptl (Gua D)	3508		b	3583	3590	
syn 9HG enol	3551		3592	3712	3688	
anti 9HG enol	3645		3596	3718	3700	
7HG exptl (Gua A)		3515	3460	3577	3587	
syn 7HG enol		3655	3588	3707	3689	
anti 7HG enol		3650	3592	3712	3762	

[a] Calculated frequencies (in italics) are not scaled. [b] Not observed.

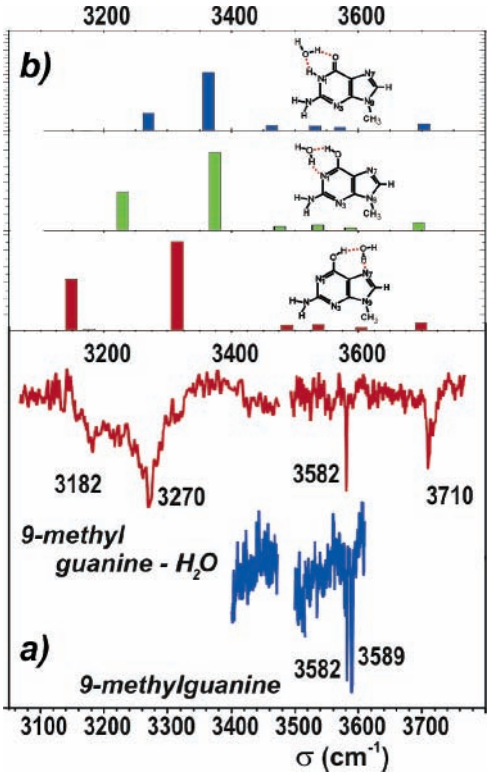


Figure 3. (a) IR/UV double resonance depopulation spectrum of the 9MG and 9MG−H_2O species in the 2.65−3.25 μm region, carried out with the UV probe laser tuned on the origins. (b) IR frequencies and relative intensities of the three most stable conformers of the 9MG−H_2O system (Figure 1) as obtained by DFT calculations at the B3LYP/6-31+G(d) level of theory. A scaling factor of 0.974 has been applied to the frequencies (see text).

too weak to be observed in our experiment. It is worth noting that the frequencies of the antisymmetric NH_2 and OH stretch modes in 9*H* enol guanine are very similar, 3583 and 3590 cm^{-1}, respectively. This assignment was not a straightforward task, especially because of the modest precision of the calculated absolute frequencies for such modes of different nature in the same spectral range.

The issue of the very similar IR spectra observed for the two UV doublet components could be accounted for by (i) either two enol rotamers (with very similar spectra) or (ii) by two quantum states of the *same* enol rotamer. To clarify this point, the IR spectra of the a-9MGe and s-9MGe forms have been calculated (Table 2). The agreement with the experimental frequencies is quite poor, as emphasized above. Even when one introduces a scaling factor, the calculated frequencies are found with a precision of only 30 cm^{-1}, leading in particular to an inversion order between the OH and antisymmetric NH_2 stretches. However, frequency comparisons *for the same mode between similar species* (both enols) are expected to be much more reliable. Calculations indicate that the OH stretch frequency is significantly red-shifted (by 15 cm^{-1}) in the syn rotamer compared to the anti rotamer, and the antisymmetric NH_2 stretch is red-shifted by 6 cm^{-1} as well. The same trend is found for both rotamers of 9HG enol. This shows unambiguously that the IR spectroscopy is sensitive enough to distinguish changes in the OH environment between the two enol rotamers.

From this important result, it turns out that only one enol rotamer is responsible for the gas phase UV spectrum of 9MG. No spectral signature of the keto form could be provided despite its similar stability. Both components of the UV doublet, evidenced by UV/UV depopulation spectroscopy, should be assigned to different states of the same rotamer, for instance arising from the existence of an eventual double potential well. The nonplanarity of the NH_2 group in the purine bases,[17,23,37] as well as its possible coupling with the methyl group in 9MG, could account for such an observation.

B. Structure of the 9-Methylguanine−H_2O Complex. *UV Spectroscopy.* The UV spectrum of the water complex of 9MG (Figure 2) measured when monitoring the ion signal corresponding to the 9MG-W mass is composed of two main bands separated by 40 cm^{-1}, the origin band being located at 35 051 cm^{-1}. The fluorescence excitation spectrum presents a similar pattern. The fluorescence intensity measured, similar to what is observed on the monomer, suggests a decay time of the same order (~20 ns). The same two bands are also observed in the UV spectrum of 9MG and are respectively blue-shifted by 439 and 478 cm^{-1} from the 9MG origin (doublet center).

The observation of the same band pattern in both the 9MG-W and 9MG mass channels is the signature of a fragmentation of the water complex either in the excited or in the ionic state. Ion signals from these bands measured in both $9MG^+$ and $9MG^+$-W mass channels exhibit the same appearance time in the jet, which shows that they originate from the same species.[38] Moreover, the typical delay of 15 μs relative to the arrival time of the bare molecule indicates that the spectral features observed in both mass channels are that of a 1:1 hydrate.

The spectral blue-shift observed indicates a strong weakening of the H-bonding in the excited state, if one assumes the same

TABLE 3: Experimental and Calculated (B3LYP/6-31+G(d) Level of Theory) Harmonic Vibrational Stretch Frequencies (in cm^{-1}) of the NH_2 and OH/N1H Groups for the Three Low Energy Conformers of the Monohydrate of 9MG, with the NH_2 Antisymmetric Frequencies of the Respective Monomers Given in the Last Column for Comparison[a]

	9MG monohydrate					9MG monomer	
	bridge1, mainly OH/N1H (9MG)	bridge2, mainly OH(water)	NH_2 sym	NH_2 anti	water, "free OH"		NH_2 anti
expt	3182	3270	b	3582	3710		3582
s-9MGe-W16	3245	3379	3488	3599	3709	s-9MGe	3612
a-9MGe-W67	3159	3314	3499	3617	3713	a-9MGe	3618
9MGk-W61	3284	3368	3474	3583	3718	9MGk	3671

[a] Calculated frequencies are scaled by the same factor as ref 32 (0.974), successfully applied to account for the H-bonding of the monohydrate of 2-pyridone and 2-hydroxypyridine. [b] Not observed.

form in both the bare molecule and the hydrate. This issue, however, must still be addressed.

IR Spectroscopy. The infrared data in the 3000−3800 cm^{-1} region enable us to provide a complementary picture of the hydrate. Figure 3a shows that the IR spectrum of 9MG-W is composed of two broad overlapping bands centered respectively at 3182 and 3270 cm^{-1}, accompanied by two narrow bands at 3582 and 3710 cm^{-1}. Interestingly, one of the latter corresponds exactly within experimental error to the red component of the IR doublet (3582 cm^{-1}) of (anti or syn) 9MG enol.

The spectral features of Figure 3a, namely the persistence of one of the components of the enol IR doublet as well as the presence of two broad and intense bands in the red part of the spectrum obviously suggesting strong H-bonding, can readily be rationalized in the following way:

The persistence of the 3582 cm^{-1} component of the IR doublet of the 9MG enol monomer in the hydrate spectrum suggests that 9MG exhibits the same enol conformer in the hydrate. The unchanged frequency of the 3582 cm^{-1} band, assigned to the antisymmetric stretch of the amino group in the enol monomer, shows that this group is *not involved* in the bonding. In contrast, the disappearance of the other component, assigned to the OH stretch, is explained by the involvement of the 9MG OH group in a hydrogen bond with water. The frequency of the proton donor OH group is expected to be red-shifted, therefore accounting for one of the two intense broad bands in the 3200 cm^{-1} region.

Concerning water, the ν_1 and ν_3 OH stretch doublet (3657 and 3757 cm^{-1}) due to the vibrational coupling existing in free water is not observed. Instead, one observes the presence of an IR band at 3710 cm^{-1} close to midway between both ν_1 and ν_3 OH stretches of free water. This indicates a water molecule acting as a proton donor, one of its OH bonds being involved in a H-bond to 9MG and the other being free.[30,32] The initial vibrational coupling in the free molecule is now negligible compared to the H-bonding, leaving therefore two uncoupled OH stretch modes. The band at 3710 cm^{-1} corresponds to a free OH stretch local mode, the other OH being donor to a proton-accepting site on 9MG.

The present analysis therefore suggests that the water molecule actually acts as both a proton donor and proton acceptor, bridging the OH site of an enol conformer of 9MG and an acceptor site, which according to the hydrate stable forms calculated (Table 1) can be either the N1 or the N7 nitrogen atom of 9MG.

Assignments. DFT calculations of the vibrational frequencies confirm the qualitative arguments presented above and provide us with a firm assignment of the structure of 9MG observed in the hydrate based on the three following arguments.

The calculated IR spectra of the three minimum energy structures found in section 2 are compared to the experimental one in Figure 3. As discussed above, whereas a detailed match is not expected for the frequencies of free OH and NH stretches, fair agreement should be found for the H-bonded groups. On the basis of the OH bridge band positions, the calculated spectrum of a-9MGe-W67, exhibiting the red-most bands, gives the best match with experiment.

The calculated relative energies of the most stable hydrates of the three conformers of 9MG (Table 1) show that a-9MGe-W67 is the most stable, followed by the canonical 9MGk-W16 conformer at +0.9 kcal/mol. The s-9MGe-W61 conformer is still less stable by another 2.2 kcal/mol. As mentioned above, the first hydration step increases the energy difference between rotamers because of an increased stabilization of the a-9MGe conformer compared to the s-9MGe conformer.

The effect of hydration on both rotamers of 9MG enol, also investigated theoretically (Table 3), shows that the NH_2 antisymmetric stretch frequency of s-9MGe is changed by −13 cm^{-1} due to the sensitivity of the NH_2 group to the neighboring water molecule in this complex, whereas that of a-9MGe is nearly unchanged upon hydration ($\Delta\nu = -1$ cm^{-1}) as observed experimentally. Interestingly, the change of the NH_2 antisymmetric stretch frequency upon hydration in the 9MG keto tautomer is also predicted to be significant (by +12 cm^{-1}), which forbids this form to account for the observations.

On the basis of these three items, we can assign confidently the 9MG conformer in both the hydrate and free monomer to the a-9MG enol rotamer. Vibrational calculations show that the two OH stretches involved in H-bonding (OH enol and one OH water) are strongly coupled by the closed H-bridge, giving rise to two bridge modes based on mixings of local OH stretch motions. This coupling together with the vicinity of the two frequencies explains the two overlapping broad bands in the 3150−3350 cm^{-1} domain of the spectrum.

5. Rotamerism of Enol Guanine Molecules in the Gas Phase

A. Assignments from IR Spectroscopy. The present conclusion, resulting from the convergence of three independent arguments combining experimental and theoretical considerations, is the first firm assignment in terms of rotamers for an enol conformer of a guanine compound. This encouraging result suggests that the present ab initio calculations carried out on a series of enol guanine molecules should be compared with the experimental IR data available for guanine and related molecules. Experimental IR spectra of several enol guanine conformers are indeed now available. The spectra of 9MG and 9HG enol are very similar as are those of 7MG and 7HG enol, which also appear as an IR doublet, slightly red-shifted compared to the 9-substituted ones.[11] The gas phase spectroscopy of guanosine, the 9-substituted guanine with a ribose sugar, and related molecules was also reported recently by Nir et al.[12] An analysis of the UV data provides evidence for an enol tautomeric form.[11] This was nicely confirmed by the IR experimental spectra, which exhibit a typical IR enol doublet that is interestingly very close to that of the 7H enol guanine.[12]

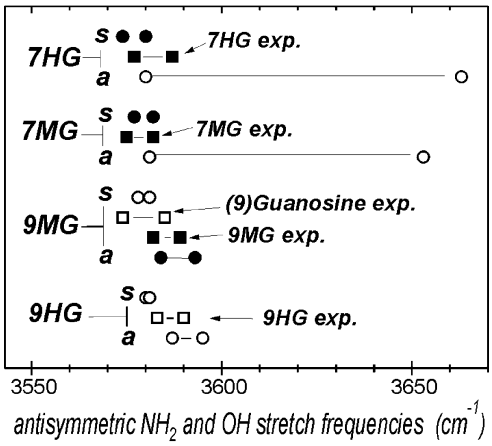


Figure 4. Comparison between calculated and relevant experimental frequencies of the antisymmetric NH_2 (red-most frequency) and OH stretch (blue-most frequency) vibrations for enol conformers of guanine and related compounds. Experimental data (squares) are taken from the present work as well as from refs 11 and 12. The calculated frequencies (circles) of each vibration (Table 2) have been scaled independently with the same scaling factor for all the species considered. The scaling factors (0.9648 for the antisymmetric NH_2 stretch and 0.971 for the OH stretch) have been adjusted to fit those experimental data, for which a secure assignment was available (see text); the corresponding reference data (both experimental and calculated) are indicated by filled symbols.

As mentioned earlier, the close comparison between experiment and DFT calculations is difficult because of the different nature of the OH and NH stretches. To circumvent this problem, different scaling factors can be applied to these two series of vibrations, provided these factors can be adjusted on the experimental spectra of species for which a secure assignment could be obtained.

In addition to the present work on 9MG, one can mention that the assignment in terms of rotamers can readily be given for 7HG and 7MG. In this case, indeed, the steric hindrance between the 7-substituent (H or methyl group) and the OH group of the anti rotamer leads to a significant destabilization (10 kcal/mol) of this rotamer compared to the syn rotamer, together with a large *calculated* blue-shift of the OH stretch (typically 70 cm^{-1}; see Table 3) compared to the syn rotamer. The presence of the usual IR enol doublet (split typically by $\sim$10 cm^{-1} and not 70 cm^{-1}) combined with the energetic argument allows us to assign to a syn rotamer the 7HG and 7MG enol conformers in the gas phase.

Using the three 9MG, 7MG, and 7HG species as benchmarks, one can use their experimental frequencies to scale independently the calculated OH and NH stretch frequencies. Figure 4 presents the comparison between experimental and scaled calculated IR frequencies in the 3580 cm^{-1} region for all rotamers of the enol conformers of guanine and methylguanine. A fair agreement, although not perfect, is obtained between the benchmark data and their calculated counterpart, showing the validity of the DFT calculation to correctly predict the IR spectroscopy of rotamers.

Taking advantage of this assessment, general trends can be derived. All syn doublets appear in the same range. The same trend can also be reported for the anti rotamers (with the exception of the OH stretches of the 7-substituted species, for the steric hindrance discussed above). In addition, whatever the 7- or 9-substitution considered, the anti rotamer exhibits the blue-most doublet compared to the syn.

Therefore, it turns out that the IR spectrum of the enol conformers in this region is not especially controlled by the 7/9 tautomerism but is rather controlled by the enol rotamerism. Such a global picture can then be used to infer the nature of the rotamers of 9HG and guanosine observed in jet experiments. For 9HG, the best fit is obtained for the anti rotamer, which seems quite consistent with the small effects of the methyl group. More surprising is the case of guanosine. The resemblance of its IR doublet with that of 7HG enol was already noted by Nir et al.[12] despite the difference in substitution (guanosine is a 9-substituted guanine). Figure 4 shows that it fits better the calculated IR frequencies of a syn rotamer than those of an anti rotamer. As for 7MG, we tentatively assign it to a syn enol rotamer. The difference in rotamer preferences in going from 9HGe and 9MGe to guanosine appears quite puzzling. Gas phase guanosine exhibits a strong intramolecular H-bond between one of the OH groups of the ribose sugar and the N3 nitrogen.[12] Its original rotamer preference might be induced by a significant charge modification of the purine ring upon H-bonding.

B. Abundances in the Jet Experiments. The apparent absence in the gas phase of a 9MGk conformer as well as of the syn enol tautomer, despite their similar calculated stability compared to the anti enol tautomer, is quite intriguing and unsatisfactorily explained so far.

A keto tautomer has indeed been observed in similar systems (9*H*-guanine, 7*H*-guanine, and 7MG) in the gas phase. A tentative explanation would invoke either a drastically reduced excited-state lifetime or a very different ionization energy or ionization cross section in 9MG, but neither of these explanations appears satisfactory since methylation is not thought to induce dramatic spectroscopic or dynamical effects, even if the possibility for strong changes of the excited-state dynamics upon methylation has been suggested by a recent theoretical work.[27] An alternative explanation would be that the calculated relative energies, which are found to be very close, would not be accurate.

The fact that the two rotamers of the enol conformers have never been observed simultaneously so far is difficult to explain. The relative energies of these rotamers are indeed very close, with a difference in stability being less than 1 kcal/mol. Even assuming an energy difference of 1 kcal/mol, a simple counting of harmonic vibrational levels leads to a ratio of 3:1 between the densities of states at an energy corresponding to the barrier height (10 kcal/mol[28]) which does not seem to be large enough to account for an efficient relaxation process during the expansion.

6. Conclusion

By associating computational and spectroscopic gas phase studies, it was possible to infer that only one conformer of the monohydrate of the 9MG molecule was present and to determine its structure. It was found that the tautomer involved in the 9MG−water complex is an enol tautomer, and we could also determine that it is the anti rotamer. Since the computational studies were also performed on the four guanine tautomers and the 7MG enol rotamers and 9MG enol rotamers, it was possible to identify the rotamer populated for the 9*H* enol tautomer of guanine and guanosine. In this respect, calculations have been proven to be a powerful assignment tool, whose precision enables us to tackle problems as difficult as the identification of rotamers.

The question of the absence, in the UV gas phase spectra of the 9MG molecule, of a keto tautomer, the biologically relevant species, remains open. Alternative probes of cooled biological species, not stemming from UV spectroscopy, such as IR

spectroscopy in helium droplets[39] would be an elegant way to solve the issue.

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