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Tautomerism

Rotational Spectral Signatures of Four Tautomers of Guanine**

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Many efforts have been devoted to the identification of the preferred tautomeric forms of the nucleobase guanine. Theoretical calculations[1–6] predict the existence of four low-energy tautomers with stabilities in the range 0–400 cm^{-1} (see Table 1), whereby the amino–oxo (keto) tautomers with a hydrogen atom at N7 or N9 are the most stable. The analysis of gas-phase UV and IR spectra on the basis of ab initio calculations has led to discordant identification of the tautomers. By using two-photon ionization (R2PI) and IR–UV double-resonance spectroscopy, de Vries and co-workers found spectral features that they assigned to the N9H keto, N7H keto, and N9H enol (*cis* or *trans*) forms.[1,2] However, the most intense band assigned to the N9H enol was later attributed by Mons and co-workers[7,8] to a higher-energy form of the N7H enol tautomer. Furthermore, they observed a fourth band, which they assigned to the N9H enol *cis* form (see Table 1). Choi and Miller studied guanine molecules embedded in He droplets[9] and assigned the IR spectroscopic data to a mixture of the four more stable tautomeric forms: keto N7H, keto N9H, and enol N9H *cis* and *trans*.

Mons et al.[10] later reported a new interpretation of the R2PI spectra. They suggested the occurrence of a fast nonradiative relaxation of the excited states of the N7H keto, N9H keto, and N9H enol *trans* tautomeric forms that prevents the observation of these species in the R2PI spectra. They proposed that the R2PI spectrum is actually dominated by three less-stable "rare" tautomers, the N7H enol and the two N7 keto imine tautomers, whose stabilities lie in the 1000–2700 cm^{-1} range (see Table 1). This hypothesis was confirmed in a recent investigation by Seefeld et al.[11] In a very recent ultrafast electron diffraction study of guanine,[12] the electron diffraction patterns were interpreted in terms of the existence of the N9H keto form, since the patterns of the N9H and N7H keto forms are indistinguishable. The enol forms were not considered in this analysis, although they exist in tautomeric equilibrium.

The difficulties encountered in attempts to conclusively detect the most stable tautomers of guanine showed the need for the use of a gas-phase structural probe that is insensitive to excited-state dynamics. Microwave spectroscopy appeared particularly suitable. Because of the high melting point (>300 °C) and thermal instability of guanine, its rotational spectra have not yet been reported. Despite the extensive use of laser ablation as an alternative method for the vaporization of solid organic compounds, few research groups have explored the possibility of combining laser ablation with Fourier transform microwave spectroscopy in supersonic jets. Laser ablation devices developed independently by Suenram et al.[13] and Walker and Gerry[14] were used successfully to investigate the rotational spectra of metal oxides and halides. The device developed by Suenram et al. was applied to a structural study of glycine in the gas phase;[15] however, it was reported that the classical heating method was more reliable. Similar results were reported by Kretschmer et al. for a study on urea.[16] Experiments on solid organic molecules were discontinued until we introduced laser ablation molecular beam Fourier transform microwave (LA MB FTMW) spectroscopy as an approach for the investigation of thermally fragile biomolecules.[17,18] This technique has been applied successfully to the study of natural amino acids[19] and the pyrimidine bases uracil[20] and thymine.[21] Herein we describe its use in the search for rotational spectral probes of the tautomers of guanine in the gas phase. Previously, we also extended this experimental method to observe the microsolvation of glycine.[22]

Our spectroscopic searches were guided by theoretical predictions. The structural and electronic properties of the tautomers were determined by ab initio calculations.[23] The derived theoretical rotational constants and dipole-moment components (Table 1) were used to predict the spectral position and intensity of rotational transitions. On this basis, the rotational spectra of four different rotamers were recognized. All observed transitions were split into many components. Guanine contains five ^{14}N nuclei with spin $I = 1$ and with a nuclear quadrupole moment that couples to the molecular-electric-field gradient at the site of the nuclei and causes the coupling of the nuclear spin to the overall rotational momentum. The coupling of the five N nuclei of guanine results in very complex hyperfine splitting patterns for all observed rotational lines (see the Supporting Information). No attempt was made to assign the quadrupole hyperfine components, and the rotational frequencies were measured as the intensity-weighted mean of the line clusters. The measured rotational spectra, which consist of R-branch rotational transitions allowed by μ_a- and μ_b-type selection rules (see Tables S1–S4 in the Supporting Information), were fitted to a rigid rotor Hamiltonian to give the sets of rotational constants labeled I, II, III, and IV in Table 1.

The agreement between the ab initio and experimental rotational constants in Table 1 provided the first piece of

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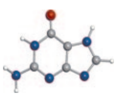
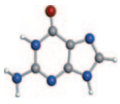
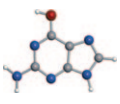
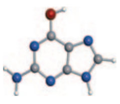
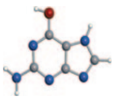
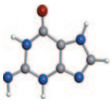
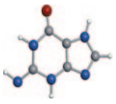
Table 1: Experimental and predicted spectroscopic constants for the tautomers of guanine.[a,b]

Experimental	I	II	III	IV			
A [MHz]	1922.155(12)	1922.2780(81)	1916.080(91)	1923.460(98)			
B [MHz]	1121.6840(97)	1116.6710(38)	1132.360(38)	1136.040(41)			
C [MHz]	709.0079(16)	706.8580(12)	712.1950(58)	714.7000(58)			
Δ [uÅ^2]	−0.6795(66)	−0.5174(37)	−0.455(24)	−0.484(25)			
σ [kHz]	49.2	44.7	41.0	48.6			
N	21	26	11	15			
Predicted[c]	Keto N7H	Keto N9H	Enol N9H *trans*	Enol N9H *cis*	Enol N7H *trans*	Imine 7H-I$_d$-O	Imine 7H-I$_u$-O
A [MHz]	1909.0	1909.7	1908.6	1915.6	1896.7	1912.1	1897.4
B [MHz]	1119.2	1113.5	1128.2	1131.9	1130.9	1120.0	1125.0
C [MHz]	706.6	704.2	709.5	712.0	709.1	709.0	709.1
Δ [uÅ^2]	−1.1	−0.8	−0.4	−0.4	−0.6	−2.8	−2.9
μ_a [D]	0.9	2.8	0.6	−2.1	−0.9	−3.4	−3.4
μ_b [D]	−1.3	5.5	2.9	3.3	−4.1	1.4	1.5
μ_c [D]	−1.0	−0.8	−1.0	−0.9	−0.8	−0.3	−0.3
ΔE_{MP2} [cm^{-1}]	0	100	141	347	1218	2682	2723
							

[a] A, B, and C are the rotational constants; $\Delta = I_c - I_a - I_b$ is the inertial defect; conversion factor: 505 379.1 MHz uÅ^2; σ is the root-mean-square deviation of the fit; N is the number of measured transitions; μ_a, μ_b, and μ_c are the electric-dipole-moment components. [b] Standard errors are shown in parentheses in units of the last digit. [c] Spectroscopic constants and relative energies (ΔE_{MP2}) from MP2/6-311++G(d,p) calculations.

evidence for the identity of the tautomers of guanine. A scale factor of 1.004 brings the ab initio values of the rotational constants for the N7H keto, N9H keto, N9H enol *trans*, and N9H enol *cis* forms nearly into coincidence with the experimental values for rotamers I, II, III, and IV, respectively. The values of the dipole-moment components served as a second piece of evidence to confirm this assignment. The applied microwave power for optimal polarization of the rotational transitions was consistent with the predicted values of the electric-dipole-moment components for each tautomer in Table 1. We evaluated the postexpansion abundances of the detected tautomers by relative-intensity measurements. The estimated ratios point to a higher stability of the N9H and N7H keto forms, which were more abundant than the N9H enol *cis* and *trans* forms, in agreement with the relative energies predicted ab initio. Our observations match the IR spectroscopic results with He droplets completely.[9] We did not detect the high-energy "rare" tautomers detected in the R2PI spectrum owing to low populations under our experimental conditions.

The negative values of the inertial defect, $\Delta = I_c - I_a - I_b$ (Table 1), show that the tautomers of guanine are nonplanar. The values are very similar to those of aniline[24] and related aminopyridines[25] (between −0.4 and −0.9 uÅ^2), for which a nonplanar configuration undergoing inversion of the amino group has been proposed.

This first rotational study of guanine confirms the power of LA MB FTMW spectroscopy for investigating the structure of solid biomolecules in the gas phase. The consistency between the experimental data and theoretical calculations enabled us to unequivocally identify the four most stable tautomers of guanine in the gas phase.

Experimental Section

The rotational spectrum of guanine was investigated by using an LA MB FTMW spectrometer described elsewhere.[17] A solid rod of guanine (m.p. > 300 °C) was ablated by using the third harmonic of a picosecond Nd:YAG laser (100–150 ps, 25 mJ/pulse; YAG = yttrium aluminum garnet). The vaporized molecules were entrained in several atmospheres of Ne (ca. 18 bar) and pulsed through a 1.0 mm nozzle into a vacuum chamber (where a Fabry–Pérot resonator was located) to create a supersonic expansion. A short microwave pulse in the 4–12 GHz frequency range polarized the species in the jet. After the polarizing microwave pulse had dissipated, the molecular emission signal containing the rotational transitions was captured in the time domain and Fourier transformed into the frequency domain.

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