

A Theoretical Study of the Electronic Spectra of Adenine and Guanine

Markus P. Fülcher, Luis Serrano-Andrés,* and Björn O. Roos

Contribution from the Department of Theoretical Chemistry, Chemical Centre, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden

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Abstract: The complete active space (CAS) SCF method and multiconfigurational second-order perturbation theory (CASPT2) have been used to study the electronic spectra of the nucleic acid base monomers guanine and two tautomers of adenine (the N(9)H and N(7)H forms). The calculations include vertical excitation energies, oscillator strengths, and transition moment directions in gas phase. For guanine solvent effects were included using a self-consistent reaction field model. The lowest $\pi \rightarrow \pi^*$ excited valence states of N(9)H-adenine are calculated at (experimental data in parentheses) 5.1, 5.2 (4.9), 6.2 (5.7–6.1), 6.7, 7.0 (6.8), and 7.6 (7.7) eV. The first two almost degenerate states are characterized by small and medium intensities, respectively. The third and fifth transitions have large oscillator strengths. Two less clear assignments can be performed to the transitions observed in experiment at 4.6 and 6.3–6.4 eV. Presently they can be assigned to the $2^1A'$ and $5^1A'$ states of the N(9)H tautomer of adenine, but possible contributions to the $3^1A'$ and $6^1A'$ states of the N(7)H tautomer of adenine cannot be ruled out. As both tautomers appear to be present in experiment, the measured and calculated polarization angles differ substantially. For guanine the following energies are obtained for the lowest $\pi \rightarrow \pi^*$ valence excited states: 4.7 (4.5–4.8), 5.1 (4.9–5.0), 6.0 (5.5–5.8), 6.5 (6.0–6.4), 6.6, 6.7 (6.6–6.7), and 6.7 eV. The polarization vectors of the first two transitions are almost perpendicular and point along the short and long axes, respectively. The fourth and sixth transitions are the most intense peaks of the spectrum. The experimental transition moment directions are reproduced with an accuracy better than 6°. The fourth transition is strongly shifted to lower energies in polar solvents.

1. Introduction

This contribution is the third paper in a row of publications aiming at the description of electronic spectra of nucleic acids in the gas phase.^{1,2} Even though theoretical studies on isolated molecules may be of limited value to bench chemists, detailed knowledge of the electronic structure of nucleic acids and related molecules is important for our understanding of static and dynamic properties of polynucleotides. In particular, transition moment directions in a molecule-fixed frame and oscillator strengths are needed—e.g., to calculate the GC content of DNA from CD spectra—but difficult to measure. Solvent effects and/or the influence of a crystalline environment on transition moments are sometimes large.³ Solvatochromic shifts for guanine are studied in the present work. A more thorough discussion of environmental effects will be presented in forthcoming publications.

A large number of theoretical and experimental studies have been performed on the photophysics of DNA bases. An extensive review of this work has been published by Callis.⁴ Theoretically, most of the *ab initio* work has focused on the geometry of DNA bases in the ground state, and calculations on excited states have in general applied semiempirical methods. To the authors' knowledge, only two *ab initio* investigations of excited states of adenine and guanine are available in the literature. Petke *et al.*⁵ applied the random phase approximation (RPA) and limited configuration interaction (CI) methods in

combination with basis sets of double- ζ quality which were extended with diffuse p_π functions. Fülcher *et al.*⁶ determined MCSCF wave functions using small basis sets and experimental average geometries.

In our previous studies of the electronic spectra of the pyrimidine bases cytosine, uracil, and thymine, tautomerism was not a problem. In contrast, experiments indicate that, even if the N(9)H form of adenine dominates in aqueous solutions, up to about 22% of the molecules may adopt the N(7)H tautomeric form.^{7,8} Here, we shall not enter this discussion in more detail, but refer the interested reader to ref 9 and citations therein. However, because a significant fraction of adenine may adopt the N(7)H form, we have calculated the electronic spectra for both forms. In the case of guanine experiments clearly give preference to the N(9)H form.¹⁰ For practical reasons, a planar form of the molecules was used in the calculations. According to what is known about the geometry, this is not a serious idealization.⁹

The UV/vis absorption spectra of aqueous solutions of adenine and guanine exhibit two complex band systems.⁴ The low-energy band (240–280 nm) is due to two electronic transitions with maxima at about 4.5 and 5.0 eV. The second band system begins at about 6.0 eV and extends into the far UV. In the energy range 6.0–7.0 eV at least two electronic

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transitions have been identified by experiment. In guanine, the electronic transition lowest in energy is polarized along the short axis of the molecule and the transition moment vector of the second electronic transition points along the long axis.¹¹ In adenine, the transition moments exhibit a similar polarization pattern, but the difficult assignment of the bands has obscured a detailed assignment.¹²

To describe ground and electronic excited states of adenine and guanine, we have applied the complete active space (CAS) SCF method supplemented by multireference, second-order perturbation theory, the CASPT2 method.^{13,14} In general, the method gives excitation energies with an accuracy of 0.2 eV or better. However, the interpretation of electronic spectra of the nucleotides is not easy due to intense, broad, and strongly overlapping bands and involves a number of experimental uncertainties. In addition, small structural differences, tautomerism, and solvation effects make the comparison between experiment and theory difficult.

The computed excitation energies exhibit a similar pattern for all three species: At low energies we predict a weak band followed by a second, stronger transition. In accord with experiment we predict that the second band system begins at about 6.0 eV. We also report excitation energies for the lowest $n \rightarrow \pi^*$ transitions, for which no experimental evidence is yet available.

2. Methods and Details of the Calculations

Geometries. Theoretically determined geometries of the N(9)H and N(7)H forms of adenine were taken from the literature.^{9,15} It was also assumed that the molecules are planar. For a discussion of the quality of these structures and comparison with experiment the interested reader is referred to ref 9. Identical methods were used in the present work to optimize the geometry of guanine: the MP2 approximation and 6-31G* basis sets. Figure 1 shows the chemical structure, the atom numbering, and the definition of the transition polarization angle.

Excited State Energies and Transition Moments. To describe the ground and excited states of adenine and guanine, the complete active space (CAS)-SCF method¹⁶ was used and supplemented by a second-order perturbation approach for estimating effects of dynamic electron correlation, the CASPT2 method.^{17,18} The CAS state interaction method, CASSI, was used to compute transition moments.¹⁹ To avoid the effect of intruder states in the CASPT2 calculations, common to many calculations on excited states, a level shift technique was used, the so-called LS-CASPT2 approach^{14,20} (a level shift of 0.3 was used). The transition dipole moments were combined with excitation energies obtained by the CASPT2 method to calculate oscillator strengths. The combined use of CASSCF transition moments and CASPT2 excitation energies is, by now, a well-established tool to study electronic excitation spectra of aromatic and conjugated, organic molecules. For detailed

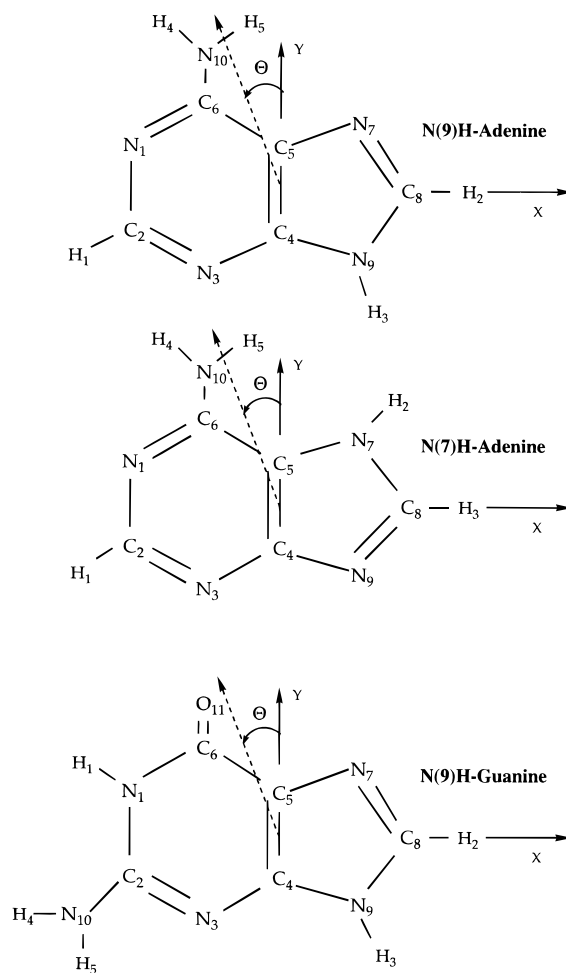


Figure 1. Molecular structures and atom numbering for N(7)H-adenine, N(9)H-adenine, and N(9)H-guanine. The angle Θ gives the orientation (positive values) of molecular and transition dipole moment vectors relative to the y axis, according to the DeVoe–Tinoco convention.

reviews of the methods and applications the interested reader is referred to refs 13 and 21.

It has been shown^{1,13,22} that calculations of electronic spectra require extended basis sets. Therefore, we used for this purpose large ANO-type basis sets,²³ which were contracted to 4s3p1d and 2s for the first-row atoms and hydrogen, respectively. They were supplemented with a 1s1p1d set of Rydberg-type functions (contracted from eight primitives for each angular momentum type), which were built closely following the recipe outlined elsewhere.¹³ A single set of Rydberg functions was constructed for both the N(9)H and N(7)H forms of adenine by determining wave functions for the $1^2A'$ and $1^2A''$ cations of both species. The ANO basis sets were contracted to double- ζ quality to represent the valence orbitals in these calculations.

Active Spaces. The crucial step in the construction of the CASSCF wave functions is the choice of the active space. In general, all orbitals with occupation numbers appreciably different from two or zero should be considered as active. Thereby all static correlation and near-degeneracy effects are included in the CASSCF reference function, and consequently there will be no large terms in the perturbation expansion. For aromatic or conjugated systems the CAS space, typically, includes the valence π -orbitals. In heteroatomic systems one may want to include also the lone pair orbitals and electrons to allow for $n \rightarrow \pi^*$ excitations.

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To describe the $\pi \rightarrow \pi^*$ excited states of adenine and guanine (numbers in parentheses), it was found from test studies that the π -orbital lowest in energy can be kept inactive throughout, leading to active spaces including 10 (12) electrons in 10 (11) orbitals, respectively. Moreover, we observed interferences with Rydberg states for some excited states. As we are primarily interested in predicting properties of valence excited states, state average calculations were first performed with one additional active π -orbital such that the Rydberg state was included. The associated Rydberg orbital was then identified and deleted from the MO basis. For guanine and the N(7)H form of adenine it was sufficient to repeat this step three times whereas two additional steps were needed for the N(9)H form of adenine. In contrast, the Rydberg orbitals of σ symmetry can be readily identified among the secondary orbitals and are also deleted from the MO basis. With all appropriate Rydberg-type orbitals deleted from the basis set, the final calculations will now only involve valence excited states with no valence Rydberg mixing. Finally, to calculate the $n \rightarrow \pi^*$ excitation lowest in energy, the lone pair orbital of σ symmetry and the corresponding electron pair are added to the active space.

Solvent Effects. To study solvation effects on the excitation energies and transition moments of guanine, we used a modified version of the self-consistent reaction field (SCRF) model.^{24–26} In this method the solvent is mimicked by a dielectric continuum with dielectric constant, ϵ , surrounding a spherical cavity which contains the molecule. The latter polarizes the solvent and induces an electric field, which interacts with the solute. In the SCRF model the solute–solvent interaction is added as a perturbation to the Hamiltonian of the free solute and the wave function is determined by appropriate iterative methods. Thus, equilibrium between the electronic state of the solute and the reaction field is assumed. However, in vertical electronic excitation processes the relaxation of the reaction field may be incomplete. Therefore, the time dependence of the absorption process has been accounted for by partitioning the reaction field factor into slow and fast components. The former is determined by the properties of the ground state. The fast component can be considered as the instantaneous electronic polarization that follows the absorption of a photon and is in a first approximation proportional to the dielectric constant at infinite frequency ϵ^{inf} where $\epsilon^{\text{inf}} \approx \eta^2$ and η is the refractive index of the solvent. The reaction field is computed at the CASSCF level and added as an external perturbation to the zero-order Hamiltonian of the CASPT2 method. For more details, the interested reader is referred to ref 27.

To estimate the solvent shifts in water ($\epsilon = 80.0$, $\eta = 1.33$), a cavity with a radius of 8.4 au was used. The basis sets were identical to those used for the isolated molecule. No repulsive potential representing the exchange interaction between the solute and the environment was included in the model.

CASSCF, CASPT2, and CASSI calculations were performed with the MOLCAS-3 software.²⁸ The MP2 geometry optimizations were performed with the MULLIKEN program.²⁹ All calculations were carried out on IBM RS/6000 workstations.

3. Results and Discussion

The presentation of our results is divided into four subsections. The first describes the electronic properties of N(7)H- and N(9)H-adenine and guanine in the ground state. The second

discusses the spectrum of adenine and the third the spectrum of guanine. Finally, the fourth subsection is devoted to the discussion of the general aspects of the spectra of both systems and the relation to other similar molecules.

3.1. Ground State Properties. The computed dipole moments of N(7)H-adenine and guanine are remarkably similar and large, 6.83 and 6.70 D, respectively. No experimental values seem to be available. The dipole moment of N(9)H-adenine is, however, much lower, 2.50 D, and is related to the partial charge of the protonated/deprotonated N-atom. The computed dipole moment of N(9)H-adenine is in agreement with the experimental estimate of the dipole moment of crystalline 9-methyladenine, 2.4 D,³⁰ and is also comparable to the dipole moment of 9-butyladenine in solution, 3.0 ± 0.2 D.³¹ Earlier calculations on gas-phase dipole moments are close to our results. Petke *et al.*⁵ obtained MRCI values of 7.89 D for guanine and 2.68 D for N(9)H-adenine.

3.2. Electronic Spectrum of Adenine. The UV/vis absorption spectrum of adenine exhibits a low-energy band with a peak measured at 252 nm (4.92 eV) in the gas phase,³² which is red-shifted to 260 nm (4.77 eV) in aqueous solution.³² In 1963 Stewart and Davidson³³ studied crystals of 1:1 complexes of 9-methyladenine and 1-methylthymine and concluded that this band contains at least two electronic transitions. Since then, the observation has been confirmed multiple times using various spectroscopic techniques. Somewhat surprising is that the splitting is not observed in circular dichroism (CD) spectra^{34–36} but is clearly seen in magnetic CD (MCD)^{37,38} and linear dichroism (LD)^{39,40} spectra. In aqueous solutions and crystal environments the transitions are separated by approximately 10 nm, and typically, the first transition is weaker and centered at approximately 270 nm (4.6 eV).⁴¹ The high-energy branch of the UV/vis spectrum also consists of a superposition of several Gaussian bands. In particular, experiment reveals two strong oscillators centered at approximately 210 and 182 nm (5.9 and 6.8 eV)^{12,42} and in addition a high and intense band at 160 nm.^{41,42} Recently Clark⁴¹ suggested that three distinct bands should be present in the 5.9–6.8 eV band envelope of the crystal spectrum of 9-methyladenine. Additionally, a band at 6.36 eV has not been reported in CD^{34,35} and MCD³⁸ spectra, which has not been described by any of the other experimental techniques.

$n \rightarrow \pi^*$ transitions are also expected to be present in the electronic spectrum at intermediate energies. They are, however, about 2 orders of magnitude less intense than $\pi \rightarrow \pi^*$ transitions. Therefore, they are difficult to observe in absorption spectra, but may be observed in CD or MCD spectra. It has been speculated that a band appearing at 230 nm (5.4 eV) in CD spectra^{34,35} of adenosine may be due to $n \rightarrow \pi^*$ transitions. Its position is however rather insensitive to solvent, pH, and temperature, and it has not been described in any other spectra. Table 1 summarizes the experimental and theoretical information about the adenine spectrum.

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Table 1. Summary of Experimental and Present Theoretical Values for the Excitation Energies (ΔE , eV), Oscillator Strengths (f), and Transition Moment Directions (Θ , deg) for Adenine and Adenine Derivatives

| | band I | band II | band III | band IV | band V | band VI | authors |
|-----------------------------|------------|-----------------|------------|------------|--------|---------|---|
| Absorption in Solution | | | | | | | |
| ΔE | 4.63 | 4.77 | 6.05 | | | | Voelter <i>et al.</i> ³⁷ |
| ΔE | 4.63 | 4.77 | 5.99 | | | | Sutherland and Griffin ³⁸ |
| ΔE | | 4.77 | 5.99 | | | | Voet ⁴⁸ |
| ΔE | | 4.61 | 5.90 | | 6.81 | 7.75 | Yamada and Fukutome ⁴² |
| ΔE | | 4.92 | 5.99 | | | | Clark <i>et al.</i> (vapor) ³² |
| ΔE | | 4.77 | 5.96 | | | | Clark <i>et al.</i> (TMP) ³² |
| ΔE | | 4.77 | 5.99 | | | | Clark <i>et al.</i> (water) ³² |
| Linear Dichorism | | | | | | | |
| ΔE | 4.59 | 4.71 (5.27) | | | | | Matsuoka and Nordén ⁴⁰ |
| Θ | | +9/-70 (-75/16) | | | | | |
| Θ | | -3 | +82/-88 | | | | Fucaloro and Forster ³⁹ |
| Circular dichorism | | | | | | | |
| ΔE | | 4.68 | 5.77 | 6.36 | | | Sprecher and Johnson ³⁴ |
| ΔE | | 4.63 | 5.93 | 6.36 | | | Brunner and Maestre ³⁵ |
| ΔE | | 4.92 | 6.08 | | | | Miles <i>et al.</i> ³⁶ |
| Magnetic Circular Dichorism | | | | | | | |
| ΔE | 4.59 | 4.92 | 5.90 | | | | Voelter <i>et al.</i> ³⁷ |
| ΔE | 4.63 | 4.92 | 5.84 | 6.33 | | | Sutherland and Griffin ³⁸ |
| Electron Scattering | | | | | | | |
| ΔE | 4.53 | | 5.84 | | 6.50 | 7.70 | Isaacson ⁴⁵ |
| Polarized Absorption | | | | | | | |
| ΔE | 4.54 | 4.82 | 6.02 | 6.33 | | | Chen and Clark ⁴⁴ |
| f | 0.08 | 0.20 | 0.16 | 0.51 | | | adenine hydrochloride |
| Θ | -28 | -80 | +15 | -60 | | | (crystal) |
| ΔE | 4.51 | 4.68 | 5.82 | 6.08 | 6.81 | 7.75 | Clark (crystal) ⁴¹ |
| f | 0.1 | 0.2 | 0.25 | 0.11 | 0.30 | 0.23 | 9-methyladenine |
| Θ | +83 | +25 | -45 | +15 | +72 | +6 | |
| ΔE | 4.6 | 4.8 | 5.9 | 6.3 | 6.8 | 7.7 | average experiment |
| ΔE | 5.1 | 5.2 | 6.2 | 6.7 | 7.0 | 7.6 | Ad9H calculated |
| f | 0.07 | 0.37 | 0.85 | 0.16 | 0.56 | 0.41 | |
| Θ | +23 | +37 | -57 | +40 | +27 | +89 | |
| ΔE | 4.6, 5.0 | | 6.0, 6.2 | 6.3, 6.5 | | | Ad7H calculated |
| f | 0.05, 0.19 | | 0.36, 0.12 | 0.07, 0.58 | | | |
| Θ | +23, -10 | | +3, -49 | +52, +33 | | | |

Table 2. CASSCF and CASPT2 Excitation Energies (eV), Oscillator Strengths (f), Dipole Moments (μ (D), θ (deg)), and Transition Moment Directions (Θ (deg)) of Singlet Valence Excited States of Adenine^a

| state | N(7)H-adenine | | | | | | N(9)H-adenine | | | | | |
|-------------------------|---------------|------|-------|----------|-------|----------|---------------|------|-------|----------|-------|----------|
| | CAS | PT2 | f | Θ | μ | θ | CAS | PT2 | f | Θ | μ | θ |
| ground state | | | | | 6.83 | -22 | | | | | 2.50 | -96 |
| $\pi-\pi^*$ Transitions | | | | | | | | | | | | |
| 2 ¹ A' | 5.12 | 4.61 | 0.050 | +23 | 5.95 | -18 | 5.73 | 5.13 | 0.070 | +23 | 2.37 | -106 |
| 3 ¹ A' | 6.63 | 4.97 | 0.187 | -10 | 9.64 | -7 | 6.48 | 5.20 | 0.370 | +37 | 2.30 | -39 |
| 4 ¹ A' | 7.81 | 6.02 | 0.363 | +3 | 8.68 | -11 | 7.80 | 6.24 | 0.851 | -57 | 2.13 | -53 |
| 5 ¹ A' | 7.22 | 6.15 | 0.123 | -49 | 6.70 | -29 | 8.30 | 6.72 | 0.159 | +40 | 4.60 | -83 |
| 6 ¹ A' | 8.12 | 6.32 | 0.077 | +52 | 6.72 | -15 | 8.77 | 6.99 | 0.565 | +27 | 3.42 | -98 |
| 7 ¹ A' | 8.41 | 6.49 | 0.581 | +33 | 7.36 | -13 | 9.29 | 7.57 | 0.406 | +89 | 6.41 | -72 |
| $n-\pi^*$ Transitions | | | | | | | | | | | | |
| 1 ¹ A'' | | | | | | | 6.43 | 6.15 | 0.001 | | 2.14 | +97 |
| 2 ¹ A'' | | | | | | | 7.16 | 6.86 | 0.001 | | 1.93 | +3 |

^a Angles defined in Figure 1.

Information concerning transition moment directions stems, on the one hand, from linear dichroism spectra of 6-(methylamino)-9-methyladenine and 9-methyladenine dissolved in stretched films of poly(vinyl alcohol).^{39,40} On the other hand, polarized absorption spectra have been taken from adenine hydrochloride, 6-(methylamino)purine, and 9-methyladenine crystals to calculate polarization angles.^{12,41} There is no evident agreement between the different experiments. It seems clear that at low energies the most intense transition around 4.7 eV is polarized along the short axis of the molecule.^{40,41} In contrast, the transition moment vector of the 182 nm (6.8 eV) band points along the long axis while the intermediate band at 210 nm (5.9 eV) has an intermediate polarization (cf. Table 1). Table 2 gives more details about the computed excitation energies, oscillator

strengths, and transition dipole moment directions computed for N(9)H-adenine and N(7)H-adenine in the gas phase.

The interpretation of the adenine spectrum is difficult and remains unclear. The first aspect to consider is the presence of both N(7)H and N(9)H tautomers in vapor and solvated spectra. As the N(9)H tautomer has been established as the predominant form both in the gas phase and in solution⁹ and also because the most recent and extensive measurements are available for 9-methyladenine crystals,⁴¹ we will try to interpret the adenine spectrum preferentially considering the N(9)H-adenine transitions.

For the N(7)H form of adenine we predict the lowest $\pi \rightarrow \pi^*$ excited valence state at 4.61 eV with a low oscillator strength of 0.05. The second lowest $\pi \rightarrow \pi^*$ excited valence state is

computed at 4.97 eV with a larger oscillator strength, 0.19. The energy splitting with respect to the $2^1A'$ state is 0.36 eV; however, the dipole moment of the $3^1A'$ is 41% larger than that of the ground state, and therefore the band is expected to strongly shift to lower energies in polar solvents. For the N(9)H-adenine tautomer the lowest singlet $\pi \rightarrow \pi^*$ transition is computed at 5.13 eV with an oscillator strength of 0.07. The second transition is computed at 5.20 eV with a much larger oscillator strength, 0.37. Both the $2^1A'$ and $3^1A'$ states in N(9)H-adenine have dipole moments close to that of the ground state; therefore, no larger solvent shifts are predicted for these transitions.

The N(9)H forms has been established as the predominant form both in the gas phase and in solution,⁹ but the presence of the N(7)H tautomer cannot be ruled out, especially in polar solvents, where the higher polarity of the N(7)H-adenine stabilizes this tautomer with respect to the N(9)H form. In the gas phase only one broad band is detected, peaking at 4.92 eV.^{32,43} As is observed in Table 1 the band splits in solution into two components, approximately at 4.6 and 4.8–4.9 eV. The splitting seems clear in the optical absorption spectra in solution,^{37,38} linear dichroism (LD),⁴⁰ and magnetic circular dichroism (MCD).^{37,38} Two bands also appear clearly in the crystal spectra of adenine hydrochloride⁴⁴ and 9-methyladenine.⁴¹ In this last case only the N(9)-methyl tautomer is present, and therefore the measured excitations at 4.51 eV ($f = 0.1$) and 4.68 eV ($f = 0.2$) should better match those of the N(9)H tautomer. To estimate how the crystal environment affects the excitation energies, we can use the example of 9-ethylguanine.¹¹ In that case both transitions were red-shifted by more than 0.1 eV with respect to the values in solution. Added to the small effects of the methylation (usually also decreasing the excitation energies), the crystal values would compare well to the values obtained in solution.

The previous discussion leads to an interpretation of the spectrum basically based on the N(9)H-adenine tautomer. We have computed a band at 5.13 eV with low intensity and another band at 5.20 eV with medium intensity. Considering the bathochromic effect of the solvent, which can be as large as 0.2 eV, the match of the medium-intensity 4.8–4.9 eV band with the 5.20 eV computed transition for the isolated molecule seems clear. A larger deviation is observed for the low-lying transition: 4.63 eV observed in solution and 5.13 eV computed in isolation. Although the deviation falls still within the limits of accuracy expected for the CASPT2 method, we also have to consider the possibility that the contribution of the N(7)H-adenine tautomer shifts the band maximum to lower energies. First we have to take into account that the $2^1A'$ state of the N(9)H-adenine has a low oscillator strength (computed $f = 0.07$). The $2^1A'$ of N(7)H-adenine, computed at 4.61 eV, has too low intensity, but the intensity is higher for the $3^1A'$ state computed at 4.97 eV with an oscillator strength of 0.19. The transition should be expected at much lower energies due to the high dipole moment of the $3^1A'$ state. Therefore, although limited by the relatively small presence of the corresponding tautomer, the contribution to the intensity of the transition of the N(7)H-adenine $3^1A'$ state cannot be ruled out completely for the spectra of adenine in solution. This does not apply for the transition observed in the crystal spectrum of 9-methyladenine.

Matsouka and Nordén⁴⁰ using linear dichroism (LD) spectroscopy propose the presence of three low-lying transitions at

4.59, 4.71, and 5.27 eV. Considering the other experimental information and our own computed values, we infer that, unless the two adenine isomers participate in the observed spectrum, only two electronic transitions are involved. Although the value at 5.27 eV matches better with our computed 5.20 eV transition for N(9)H-adenine, the absence of such a transition in all other experimental spectra indicates that the 5.27 eV is simply a higher component of the transition to the $3^1A'$ state of N(9)H-adenine.

The two most prominent bands of adenine (bands III and V in Table 1) have been measured at 5.9 eV (6.0 eV in vapor³²) and 6.8 eV. Isaacson⁴⁵ found an excitation energy of 6.5 eV for the most intense band using electron scattering. Calculated energies and oscillator strengths makes it possible to correlate these bands with the $4^1A'$ and $6^1A'$ states in N(9)H-adenine, computed at 6.24 and 6.99 eV. Equally clear seems the assignment of the band VI of Table 1 located at 7.7 eV with an oscillator strength of 0.23.⁴¹ We have computed the $7^1A'$ state of N(9)H-adenine at 7.6 eV and the transition to this state with an oscillator strength of 0.41 and a transition moment direction of 89°. The transition of 6.24 eV also carries the maximum intensity of the computed spectrum, which is not in agreement with the observed profiles of the bands in solution. Apart from that the assignments of bands III, V, and VI seem clear.

The situation is less clear for band IV in Table 1. The measured energies range from 6.1 eV in Clark's⁴¹ crystal spectrum to 6.4 eV in the CD spectrum.^{34,36} Our computed excitation energy for the $5^1A'$ state of N(9)H-adenine is 6.72 eV, with an oscillator strength of 0.16. We observed that the computed dipole moment of the $5^1A'$ state (4.6 D) almost doubles the ground state value. Therefore, a strong red shift can be expected for this band in solvated environments. This could be an explanation for the assignment of the 6.3–6.4 eV transition observed in the CD^{34,36} and MCD³⁸ spectra. The deviation from the measured value in the crystal phase is however too large. One possibility is to consider that the two bands proposed by Clark⁴¹ in the crystal model spectrum at 5.82 and 6.08 eV were simply one. Otherwise the crystal effects in the 6.08 eV transition seem too large (compare to the solution values near 6.4 eV). There is in addition a final possibility that cannot be discarded: If the somewhat weak transition computed at 6.72 eV is not shifted toward lower energies by the solvent, it could be easily obscured by the presence of the more intense fifth band at 6.8 eV. In that case there are no prominent features in the spectrum of N(9)H-adenine from 6.0 to 6.6–6.7 eV. Therefore, it is possible that the N(7)H-adenine contributes with some intensity to this region. We have computed the $6^1A'$ state of N(7)H-adenine at 6.49 eV and an oscillator strength of 0.58. This is the most intense transition computed for the N(7)H-adenine, and it could explain the observation of a weak band at 6.3–6.4 eV in the CD and MCD spectra in solution, where the N(7)H-adenine tautomer is expected to contribute 20% to the composition of the sample.

Finally, we have computed the low-lying $n \rightarrow \pi^*$ transitions in adenine. The lowest transition is calculated at 6.15 eV with a very low oscillator strength. It is therefore not likely that such a weak transition can be observed in an absorption spectrum since it is buried under the envelope of the more intense $4^1A'$ transition.

Much attention has been devoted to the polarization of the transition bands in adenine. Unfortunately the experimental situation is unclear, and we have not obtained a good agreement with our theoretical values. Only two angles have been reported for the low-lying transition at 4.5 eV: -28° ⁴⁴ for adenine hydrochloride crystals and $+83^\circ$ ⁴¹ for 9-methyladenine crystals.

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Table 3. Summary of Experimental and Present Theoretical Values for the Excitation Energies (ΔE , eV), Oscillator Strengths (f), and Transition Moment Directions (Θ , deg) for Guanine and Guanine Derivatives

| | band I | band II | band III | band IV | band V | band VI | band VII | authors |
|-----------------------------|--------|---------|----------|---------|--------|---------|----------|--|
| Absorption in Liquids | | | | | | | | |
| ΔE | 4.51 | 4.96 | 5.51 | 6.33 | | | | Voet <i>et al.</i> ⁴⁸ |
| ΔE | 4.51 | 4.96 | | | | | | Voelter <i>et al.</i> ³⁷ |
| ΔE | 4.59 | 4.96 | | 5.99 | | 6.59 | | Clark (9-ethylguanine, pH 7) ¹¹ |
| f | 0.14 | 0.21 | | 0.38 | | 0.42 | | |
| ΔE | 4.51 | 4.96 | 5.79 | 6.39 | | | | Clark (guanine, pH 2) ¹¹ |
| f | 0.13 | 0.22 | | 0.7 | | | | |
| ΔE | 4.31 | 4.90 | | 6.20 | | 6.70 | | Yamada and Fukutome ⁴² |
| Linear Dichorism | | | | | | | | |
| ΔE | 4.40 | 5.00 | | | | | | Matsuoka and Nordén ⁴⁰ |
| Θ | +4/-61 | -88/31 | | | | | | |
| Circular Dichorism | | | | | | | | |
| ΔE | 4.59 | 4.90 | 5.51 | 6.20 | | 6.59 | | Sprecher and Johnson ³⁴ |
| ΔE | 4.81 | 5.00 | 5.71 | 6.29 | | | | Miles <i>et al.</i> ³⁶ |
| Magnetic Circular Dichorism | | | | | | | | |
| ΔE | 4.51 | 4.96 | | | | | | Voelter <i>et al.</i> ³⁷ |
| ΔE | 4.51 | 4.96 | | 6.11 | | 6.59 | | Sutherland and Griffin ³⁸ |
| Polarized Absorption | | | | | | | | |
| ΔE | 4.55 | 4.98 | | 6.03 | | 6.63 | | Clark (solution) ¹¹ |
| f | 0.16 | 0.25 | | 0.41 | | 0.48 | | 9-ethylguanine |
| Θ | -4/+35 | -75 | | -71/-79 | | -9/+41 | | |
| ΔE | 4.5 | 5.0 | 5.7 | 6.2 | | 6.6 | | average experiment |
| ΔE | 4.73 | 5.11 | 5.98 | 6.49 | 6.59 | 6.72 | 6.74 | guanine computed ^a |
| f | 0.15 | 0.24 | 0.02 | 0.29 | 0.18 | 0.41 | 0.15 | |
| Θ | -4 | +75 | +6 | -85 | -42 | +36 | +58 | |

^a This work. Includes an estimation of the solvation effects in water.

For the second transition the same compounds and authors give -80° and $+25^\circ$, respectively. LD spectroscopy, on the other hand, reported $+9/-70^\circ$.⁴⁰ Our computed transition dipole moment directions for $2^1A'$ and $3^1A'$ of both the N(7)H and N(9)H tautomers of adenine give short-axis polarization. Selecting the more reliable $+25^\circ$ and $+9^\circ$ values for the more intense transition at 4.7–4.8 eV and comparing them with our computed value for the $3^1A'$ state of N(9)H-adenine, $+37^\circ$, we can agree with the experiment that the most intense band of this region of the spectrum is short-axis-polarized, in contrast to the situation for the guanine molecule. The situation for the low-lying band remains unclear, perhaps due to the low intensity of the band.

The situation for the remaining transitions of adenine is not much better. Experimental⁴¹ and our CASSCF theoretical results agree in obtaining an intermediate polarization for the 5.9–6.0 eV transition. We observe, however, that for the 6.8 and 7.7 eV transitions the results are reversed. Clark⁴¹ reports long- and short-axis polarizations, respectively, for the two bands, while the theoretical values clearly point to short- and long-axis polarizations, respectively. We do not have an explanation for such discrepancies, but we also do not have any reason for suspicion of the theoretical results. In systems where the experimental situation is clear such as guanine (see below), indole,⁴⁶ and different amides,⁴⁷ we have never found discrepancies larger than $\pm 20^\circ$ in the polarization angles from crystal-phase measurements. Also here the crystal effects are not expected to be so important. It is clear that more experiments are required to clarify the situation.

3.3. Electronic Spectrum of Guanine. The electronic spectrum of guanine is rather similar to that of adenine, but the agreement among different experiments is better. Table 3 summarizes the experimental excitation energies, oscillator strengths, and transition moment directions for guanine and some

derivatives. Two excited states are observed at low energies. Unlike adenine, they are clearly separated by about 25 nm and positioned at 275 (4.51 eV) and 250 (4.95 eV) nm, respectively. Both have similar oscillator strengths, 0.16 and 0.25, respectively (cf. Table 3). At energies higher than 6.0 eV the spectra show two strong bands centered at about 203 (6.11 eV) and 188 (6.59 eV) nm. In the energy range from 5.0 to 6.0 eV an additional weak state has also been observed.^{11,34,36,48} As for adenine, $n \rightarrow \pi^*$ transitions have not been assigned.

Table 4 compiles the calculated excited state properties of guanine, both in the gas phase and in water. Table 5 compiles the final interpreted results for adenine and guanine spectra. The two lowest excited singlet valence states of guanine have been found at 4.76 and 5.09 eV, respectively. The calculations also showed that transitions to the $2^1A'$ and $3^1A'$ states are characterized by rather strong oscillator strengths, 0.13 and 0.23, respectively, and are polarized along the short (-15°) and the long ($+73^\circ$) axes of the molecule. For both states the dipole moment is similar to that of the ground state. Since the dipole moments of the different excited states are similar, only small changes were found in the excited state properties when solvent effects were included in the calculations. The largest influence concerns the transition moment direction of the $2^1A'$ state, which changes from -15° to -4° (the $5^1A'$ state is an exception, as discussed below).

The third $\pi \rightarrow \pi^*$ transition of guanine is computed to be a weak band at 5.96 eV (5.98 eV in water) with an oscillator strength of 0.023 and short-axis polarization. Evidence of such a band has been found in CD spectra^{34,36} and also in the crystal spectrum of guanine and 9-ethylguanine and in the solution spectrum of protonated guanine¹¹ close to 5.8 eV. It has been suggested that this band is due to a $n \rightarrow \pi^*$ transition. In fact, our calculations predict the lowest $n \rightarrow \pi^*$ state at 5.79 eV (see below), which may explain the experimental uncertainties.

Four transitions were found in the energy range 6.0–7.0 eV.

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Table 4. CASSCF and CASPT2 Excitation Energies (eV), Oscillator Strengths (f), Dipole Moments (μ (D), θ (deg)), and Transition Moment Directions (Θ (deg)) of Singlet Valence Excited States of Guanine^a

| state | gas phase | | | | | | water | | | experiment ^b | | |
|--------------------------------------|-----------|------|------------------|----------|-------------------------|----------|-------|-------|----------|-------------------------|--------------------|------------|
| | CAS | PT2 | f | Θ | μ | θ | PT2 | f | Θ | ΔE | f | Θ |
| ground state | | | | | 6.89 | +157 | | | | | | |
| | | | | | $\pi-\pi^*$ Transitions | | | | | | | |
| 2 ¹ A' | 6.08 | 4.76 | 0.133 | -15 | 7.72 | +170 | 4.73 | 0.154 | -4 | 4.4-4.5 | 0.16 | (-4, +35) |
| 3 ¹ A' | 6.99 | 5.09 | 0.231 | +73 | 6.03 | +168 | 5.11 | 0.242 | +75 | 4.9-5.0 | 0.25 | (-75) |
| 4 ¹ A' | 7.89 | 5.96 | 0.023 | +7 | 5.54 | +161 | 5.98 | 0.021 | +6 | 5.7-5.8 | <0.05 ^c | |
| 5 ¹ A' | 8.60 | 6.65 | 0.161 | -80 | 10.17 | -172 | 6.49 | 0.287 | -85 | 6.1-6.3 | 0.41 | (-71, -79) |
| 6 ¹ A' | 9.76 | 6.55 | 0.225 | -41 | 6.11 | +160 | 6.59 | 0.183 | -42 | } 6.6-6.7 | 0.48 | (-9, +41) |
| 7 ¹ A' | 8.69 | 6.66 | 0.479 | +43 | 6.57 | +179 | 6.72 | 0.412 | +36 | | | |
| 8 ¹ A' | 9.43 | 6.77 | 0.098 | +52 | 7.17 | -178 | 6.74 | 0.154 | +58 | | | |
| | | | | | $n-\pi^*$ Transitions | | | | | | | |
| 1 ¹ A'' (n _O) | 6.22 | 5.79 | 10 ⁻⁴ | | 4.31 | +141 | | | | | | |
| 2 ¹ A'' (n _N) | 8.05 | 6.60 | 0.013 | | 4.63 | +167 | | | | | | |
| 3 ¹ A'' (n _O) | 7.97 | 6.63 | 0.002 | | 2.64 | +116 | | | | | | |
| 4 ¹ A'' (n _N) | 8.99 | 7.16 | 0.002 | | 6.10 | -137 | | | | | | |

^a Angles defined in Figure 1. ^b The most reliable experimental data have been included. See Table 3 for a survey of results and references. ^c Weak band in the spectra of 9-ethylguanine¹¹ and guanine.⁵⁴ See also ref 34.

Table 5. Interpretation of the Adenine and Guanine Spectra Based on the Experimental^a and Presently Computed Theoretical Excitation Energies (ΔE , eV), Oscillator Strengths (f), and Transition Moment Directions (Θ , deg)^b

| state | N(9)H-adenine | | | | | | N(9)H-guanine | | | | | |
|-------------------|--------------------|------|----------|--------------|------|----------|--------------------------|------|----------|--------------|-------|----------|
| | theoretical | | | experimental | | | theoretical ^c | | | experimental | | |
| | ΔE | f | Θ | ΔE | f | Θ | ΔE | f | Θ | ΔE | f | Θ |
| 2 ¹ A' | 5.1 ^d | 0.07 | -23 | 4.6 | 0.10 | +83 | 4.7 | 0.15 | -4 | 4.4-4.5 | 0.16 | -4 |
| 3 ¹ A' | 5.2 | 0.37 | +37 | 4.8-4.9 | 0.20 | +25 | 5.1 | 0.24 | +75 | 4.9-5.0 | 0.25 | -75 |
| 4 ¹ A' | 6.2 | 0.85 | -57 | 5.9-6.0 | 0.30 | -45 | 6.0 | 0.02 | +6 | 5.7-5.8 | <0.05 | |
| 5 ¹ A' | 6.7 ^{e,f} | 0.16 | +40 | 6.3-6.4 | | | 6.5 | 0.29 | -85 | 6.1-6.3 | 0.41 | -79 |
| 6 ¹ A' | 7.0 | 0.56 | +27 | 6.8 | 0.30 | +72 | 6.6 | 0.18 | -42 | } 6.6-6.7 | 0.48 | +41 |
| 7 ¹ A' | 7.6 | 0.41 | +89 | 7.7 | 0.23 | +6 | 6.7 | 0.41 | +36 | | | |
| 8 ¹ A' | | | | | | | 6.8 | 0.15 | +58 | | | |

^a The most reliable experimental data in different media have been included. See Tables 1 and 3 for a survey of results and references. ^b Angles defined in Figure 1. ^c Includes an estimation of solvent effects in water by a SCRf model. ^d The 2¹A' and 3¹A' states of N(7)H-adenine at 4.6 and 5.0 eV could also contribute to the band. ^e The transition is expected to shift strongly to lower energies in polar solvents. ^f The 6¹A' state of N(7)H-adenine at 6.5 eV could also contribute to the band.

The dipole moment of the 5¹A' state is almost 50% larger than that of the ground state. Taking solvation effects into account, the excitation energy shifts 0.16 eV to the red. As the SCRf model tends to underestimate solvatochromic shifts, one may assign the 5¹A' state as the origin of the band observed at 6.1-6.3 eV. With such an assignment, the calculated polarization, -80°, is in agreement with the experimental value, -71/-79°. ¹¹

The 6¹A' and 7¹A' states are predicted 6.55 and 6.66 eV above the ground state. The polarization directions are almost perpendicular and close to $\pm 40^\circ$ with calculated oscillator strengths of 0.23 and 0.48, respectively. Finally, the 8¹A' state is computed at 6.77 eV with a low oscillator strength, 0.10. As no indication of a splitting of the most intense band at 6.6-6.8 eV can be found in any of the experiments, presumably, all intensity is assigned to the 7¹A' state. The agreement with the observed value of the oscillator strength, 0.48, and transition dipole moment direction, 41° (preferred to -9°), is excellent for the 7¹A' state.

We have in addition computed the low-lying $n \rightarrow \pi^*$ transitions of guanine. The 1¹A'' state is calculated at 5.79 eV with an oscillator strength of 10⁻⁴. Clark¹¹ detected a weak bump in the perpendicularly polarized crystal spectrum of 9-ethylguanine around 4.1 eV with no correspondence in the parallel spectrum. This feature was assigned to a possible $n \rightarrow \pi^*$ transition or the shifted parallel component of the band origin of the first $\pi \rightarrow \pi^*$ transition. Our calculations strongly support the second possibility. Hydrogen bonding of lone pair electrons,

in general, leads to large blue shifts of $n \rightarrow \pi^*$ transitions, which will make the discrepancy between theory and experiment even larger.

3.4. General Aspects. Our calculations predict, in accord with experiment, that the electronic spectra of adenine and guanine can be separated into two band systems. The low-energy band system is due to transitions to at least two excited states with small to modest intensity. The second band system starts out at ~ 6.0 eV and includes the bulk of intensity. In the energy range 6.0-7.0 eV we find at least three states.

The general structure of the spectra resembles that of systems like purine,⁴⁹ indole,⁴⁶ and naphthalene⁵⁰ (references are to CASSCF/CASPT2 studies). In these systems too, a low-intensity region separates the first and second band systems. The general pattern of oscillator strengths is also remarkably similar: Two weak oscillators are followed by two strong ones. However, because of the extra π -electrons and centers contributed by the substituents, deviations are expected.

In contrast to naphthalene and indole, however, where double excitations contributed up to 10-20% to the CASSCF wave functions (even for some of the intense states), these excitations play in general a minor role in adenine and guanine, especially for the L_a, L_b, B_b, and B_a states.

The intense B_b state in naphthalene is best described by the symmetric combination of the nearly degenerate HOMO - 1

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Table 6. Configurations and Weights of Excited Configurations (weight >5%) for the Most Representative Valence Singlet Excited States of N(7)H-Adenine, N(9)H-Adenine, and Guanine

| state | CSF ^a | N(7)H-adenine (%) ^b | N(9)H-adenine (%) ^b | guanine (%) ^b |
|-----------------------------|-----------------------|--------------------------------|--------------------------------|--------------------------|
| ¹ L _a | H - 1 → L | | | |
| | H → L + 1 | 6 | 14 | 7 |
| | H → L | 42 | 63 | 68 |
| | H - 1 → L + 1 | 17 | | 5 |
| | N (W, %) ^c | 4 (70) | 2 (77) | 3 (81) |
| ¹ L _b | | 3 ¹ A' | 2 ¹ A' | 3 ¹ A' |
| | H → L | | 13 | 7 |
| | H - 1 → L + 1 | 14 | | |
| | H → L + 1 | | 41 | 69 |
| | H → L + 2 | 10 | | |
| ¹ B _b | H - 1 → L | 25 | 31 | |
| | N (W, %) ^c | 4 (68) | 3 (85) | 3 (80) |
| | | 4 ¹ A' | 4 ¹ A' | 5 ¹ A' |
| | H - 1 → L | 30 | 46 | 51 |
| | H → L + 1 | 10 | 26 | 2 |
| ¹ B _a | H - 2 → L + 1 | | | 3 |
| | H - 1 → L + 1 | | | 14 |
| | N (W, %) ^c | 3 (58) | 2 (72) | 6 (77) |
| | | 7 ¹ A' | 6 ¹ A' | 7 ¹ A' |
| | H - 1 → L + 1 | 16 | 60 | 30 |
| ¹ B _a | H → L | 28 | | 5 |
| | H, H → L, L | | | 1 |
| | N (W, %) ^c | 4 (53) | 1 (60) | 9 (74) |

^a Main configurations. H = HOMO, L = LUMO. ^b Weight of the configuration. ^c Number of configurations with weight >5% and accumulated weight (in parentheses).

→ LUMO and HOMO → LUMO + 1 excited states, whereas the B_a state is well characterized by the HOMO - 1 → LUMO + 1 excited configuration. Thus, focusing on the oscillator strengths and disregarding states with large contributions from the HOMO - 2 → LUMO + 2 excited configuration, we can readily identify the states that can be related to the L and B states in indole and naphthalene. Table 6 summarizes the structure of the CASSCF wave functions for these states. Earlier,⁵¹ we observed that the L and B states in indole are blue-shifted as compared to the band position in naphthalene, and due to the loss of symmetry, the near degeneracy of the HOMO - 1 → LUMO and HOMO → LUMO + 1 excitations is lifted. Here, these trends are even more pronounced. In guanine, for example, the ratio between the two dominant CI coefficients in the ¹L_b and ¹B_b states is reduced to ~1:4. Moreover, the ¹L_b and ¹L_a states have switched order in both guanine and N(9)H-adenine.

As noted above, a number of additional electronic transitions are present in the spectra, which include configuration state functions involving the HOMO - 2 and LUMO + 2 orbitals. N(7)H-adenine exposes two such states, 5¹A' and 6¹A'. They have strong multiconfigurational character. In contrast, the 5¹A' and 7¹A' states of N(9)H-adenine are well described by the singly excited configurations HOMO - 2 → LUMO (weight 70%) and HOMO - 2 → LUMO + 1 (weight 77%), respectively. Finally, the 4¹A' state of guanine can be described as a mixture of three singly excited configurations: HOMO → LUMO + 2 (weight 49%), HOMO - 2 → LUMO + 1 (weight 10%), and HOMO, HOMO → LUMO + 1, LUMO + 1 (weight 7%). Likewise the 6¹A' state involves the configurations HOMO - 1 → LUMO + 1 (weight 22%), HOMO - 2 → LUMO (weight 37%), and HOMO, HOMO → LUMO, LUMO (weight 9%). Finally we note that the dipole-forbidden 2¹A_g and 1¹B_{1g} states in naphthalene also include a large contribution

from the HOMO - 2 → LUMO + 1 configuration and, due to the loss of symmetry, are expected to become visible in the nucleic acids.

Experiment and theory agree that the polarization of the two lowest transitions in indole and guanine are mutually perpendicular. For guanine the polarization vectors point along the short and long axes of the molecule, whereas they are rotated by ~20° in indole.⁴⁶ The computed transition dipole moment directions of guanine are in excellent agreement with the experimental data obtained by Clark⁴¹ for crystalline 9-ethylguanine. The comparison between theory and experiment for adenine is more difficult partially due to the existence of two tautomeric forms and an uncertain experimental scenery.

4. Summary and Conclusions

The CASSCF/CASPT2 method has been applied to predict a large number of singlet excited valence states of N(7)H-adenine, N(9)H-adenine, and N(9)H-guanine (cf. Figure 1). Table 5 contains the interpretation of adenine and guanine spectra based on the available experimental data and the present theoretical results.

For adenine the experimental situation is complex partially due to the presence of at least two tautomeric forms which are believed to contribute to the observed spectra in solution.⁸ Therefore, we computed the electronic spectra of the two forms N(7)H-adenine and N(9)H-adenine. We found that it is in general possible to interpret the observed spectra as only resulting from transitions belonging to the N(9)H-adenine tautomer, although contributions from the N(7)H-adenine tautomers cannot be discarded completely. We have computed the excited state energies of N(9)H-adenine blue-shifted as compared to N(7)H-adenine. The lowest weak transition observed in the spectrum at 4.6 eV can be assigned to the 2¹A' state in N(9)H-adenine, but both 2¹A' and, especially, 3¹A' states of N(7)H-adenine can contribute to the intensity in solution. The second component of the first band system (4.9 eV in the gas phase) is clearly assigned to excitations to the 3¹A' state in N(9)H-adenine. The second and intense band in adenine extends from 5.7 to 7.0 eV. Two clear peaks observed at 5.9 and 6.8 eV are assigned to the 4¹A' (¹B_b) and 6¹A' (¹B_a) states of N(9)H-adenine, whereas the intermediate transition observed between 6.3–6.4 eV could be assigned to the 5¹A' state of N(9)H-adenine, which is computed at 6.7 eV. This state is expected to be strongly shifted to lower energies in polar solvents. The absence of intense bands of the N(9)H species in this energy range should make it possible to observe the intense 6¹A' N(7)H-adenine transition, which is computed at 6.5 eV. Therefore, also here the participation of the N(7)H tautomer cannot be ruled out. Finally, the intense band observed at 7.7 eV is assigned to the 7¹A' state of N(9)H-adenine computed at 7.5 eV.

The polarization of the transitions in adenine is still an unsolved experimental problem, in part due to the simultaneous presence of both tautomers in the medium. The calculations confirm the short-axis polarization⁴¹ of the transition at 4.9 eV and the intermediate polarization of the 5.9 eV band, but do not match the measured transition dipole moment directions of transitions higher in energy than 6.2 eV.

For guanine the agreement with experiment is excellent. The excitation energies are reproduced with an accuracy better than 0.3 eV, and the transition moment directions agree within ±6°. As in adenine the lowest band is composed of two transitions, measured at 4.5 and 5.0 eV and computed at 4.73 and 5.11 eV. The first transition (¹L_a state) has a short-axis polarization, and

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the second (1L_b) is perpendicular to the first, in agreement with experiment. A weak band found at 5.7 eV has been assigned to the $4^1A'$ state of guanine. The envelope of the most intense band ranging from 6.1 to 6.8 eV includes four different transitions. Two peaks are observed in the spectrum near 6.2 and 6.6 eV. We assign the 6.2 eV transition to the $5^1A'$ state of guanine located at 6.65 eV in the gas phase. The state has shown larger sensitivity to the solvent effects than the other states. Calculations carried out by means of a reaction field model shifted the excitation energy to 6.49 eV and increased the intensity of the $5^1A'$ state. The transition to the $7^1A'$ state of guanine (1B_a) is the most intense one. The computed oscillator strength and polarization for this state agree with the experimental observations.

The agreement between experiment and theory for guanine suggests that the crystal field effects on the polarization directions of the electronic transitions are not as important as may be assumed on the basis of semiempirical calculations.^{3,52} The discrepancies between theory and experiment rather appear to be due to the limitations of the theoretical method employed (CNDO, INDO, or PPP³) than a physical effect. We also recall that similar observations have been reported earlier for such

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molecules as indole,⁴⁶ propanamide,⁴⁷ and *N*-acetyl glycine.⁵³ To this end we conclude that the somewhat larger discrepancies found in adenine are a consequence of the complex experimental spectrum and the presence of the two tautomers.

Finally, we showed that the lowest excited valence states of adenine and guanine can be related to the classical four-state model used to discuss simple annulenes such as naphthalene. A thorough analysis and comparison of wave functions and excited state properties of the series of molecules naphthalene, indole, purine, adenine, and guanine will be reported, together with a study on the excited states of the purine molecule, in a forthcoming publication.⁴⁹

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