Assignment of Electronic Transition Moment Directions of Adenine from Linear Dichroism Measurements

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Abstract: The electronic spectra of a series of adenine derivatives have been investigated with respect to the number of electronic transitions, their intensities, and transition moments. The experimental work includes linear dichroism (LD) measurements on samples partially oriented in stretched polymer poly(vinyl alcohol) films, fluorescence anisotropy (FA), and magnetic circular dichroism (MCD). The UV spectra of both 7-methyladenine (7MA) and 9-methyladenine (9MA) are resolved into contributions from five $\pi \rightarrow \pi^*$ transitions (I−V). Their polarizations relative to the C_4−C_5 axis are for 7MA +45° (I, 36 600 cm^{-1}), −16° (II, 39 500 cm^{-1}), −28° (III, 42 600 cm^{-1}), +76° (IV, 45 800 cm^{-1}) and ∼−29° (V, ∼47 700 cm^{-1}); and for 9MA +66° (I, 36 700 cm^{-1}), +19° (II, 38 820 cm^{-1}), −15° (III, 43 400 cm^{-1}), −21° (IV, 46 800 cm^{-1}), and ∼−64° (V, ∼48 320 cm^{-1}). The experimental results are correlated with results from semiempirical INDO/S and *ab initio* CIS/6-31G(d) and CASPT2 molecular orbital calculations. The transition moments are found to be conserved when the adenine amino group has been substituted with either one or two methyl groups. In addition, LD and MCD spectra for 6-(dimethylamino)-9-ethylpurine, which is soluble in nonpolar solvents, have been measured in stretched polyethylene film and cyclohexane, respectively. The results indicate that the electronic transition moments of the 9-substituted adenine chromophore are essentially the same in a polar and a nonpolar solvent. On the basis of the results for 7MA and 9MA, the reduced LD and absorption spectra of adenine are analyzed in terms of contributions from the 7-H and 9-H tautomers of adenine. By comparison with theoretical and experimental results for purine and 2-aminopurine regarding the position of the lowest $n \rightarrow \pi^*$ transition, we are able to confidently position the lowest $n \rightarrow \pi^*$ transition in 9MA very close to the lowest $\pi \rightarrow \pi^*$ transition. The proximity of the first $^1n\pi^*$ and $^1\pi\pi^*$ states in adenine might be related to the effecient nonradiative deactivation of the lowest excited $^1\pi\pi^*$ state.

Introduction

The interpretation of various optical spectroscopic characteristics of the nucleic acids relies heavily on data for the excited-state properties of the constituent chromophores, the nucleic acid bases. One important experimental method for obtaining such data is polarized reflection or absorption spectroscopy on single crystals of the bases. All of the common bases have been studied using this technique.[1−5] However, one potential disadvantage of the crystal mesurements is that they only represent the molecules in the solid state, whereas most spectroscopic studies of nucleic acids refer to dilute aqueous solutions. Calculations by Callis and co-workers[6,7] for several guanine and adenine derivatives indicate that substantial electrostatic perturbations have to be assumed in order to get a reasonable agreement between INDO/S gas-phase calculations and experimental crystal data. However, recent results in our laboratory indicate that the INDO/S results for adenine are not very sensitive to the field from a polar solvent and it is therefore

possible that INDO/S calculations still may provide reasonable spectral properties for nucleic acid bases in the gas phase as well as in liquid solutions.[8]

Linear dichroism (LD) measurements on solutes partially oriented in stretched polymer films is an alternative to the crystal measurements as both techniques yield absolute transition dipole moment directions within the molecular coordinate system. In the case of the nucleic acid bases, several film LD studies[9,10] have been performed, which have helped to resolve some of the uncertainties regarding the transition moment directions and corresponding intensity distributions. In the case of adenine, however, the film measurements have not been in agreement with the polarized-reflection studies by Clark on 9-methyl-adenine (9MA), 6-(methylamino)purine (6MAP), and 2′-deoxy-adenosine (Ado).[5] Clark determined the polarization for the lowest lying $\pi \rightarrow \pi^*$ transition to be +83° to +67° and the second $\pi \rightarrow \pi^*$ transition to +35 to +25°. The film study on adenine reported by Matsouka and Nordén gave no polarization for the lowest transition, and an angle of +9° or −70° for the second transition.[9] However, their data was obtained for adenine which is known to exist as a mixture of the 7-H (∼23%) and the 9-H tautomer (∼77%) in PVA film.[11] The 7-H and 9-H tautomers are expected to have different transition moment directions and, thus, precludes quantitative interpretation of these earlier measurements.

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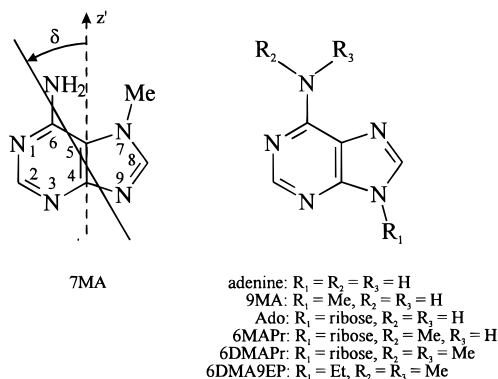(11) Holmén, A. *J. Phys. Chem.* **1997,** *101,* 4361.

Figure 1. Molecular structures of 7-methyladenine (7MA) and the 9-substituted adenine derivatives with definitions of the atomic numbering scheme, and an in-plane angle (δ). z' is a molecule-fixed reference axis parallel to the C_4-C_5 bond.

Circular dichroism (CD)[12,13] and magnetic circular dichroism (MCD)[12-15] spectra of adenine derivatives have presented evidence for a weak, possibly in-plane polarized transition around 230 nm. The 230 nm transition was not observed by Clark.[5] Clark has tentatively assigned n $\rightarrow$ π^* transitions in adenine at 244 nm and around 204 nm.[5c] Recent film dichroism and emission anisotropy studies of the purine[16] and 2-amino-purine[17] chromophores have enabled the identification of n $\rightarrow$ π^* transitions below (purine) and above (2-aminopurine) the lowest $\pi \rightarrow \pi^*$ transition. The lowest n $\rightarrow$ π^* transition in adenine has yet not been assigned.

In an attempt to resolve the ambiguity that still remains regarding the number of as well as the transition moment directions of the electronic transitions of the adenine chromphore, we have investigated the UV linear dichroism (LD) of 7-methyladenine (7MA), 9MA, Ado, 6-(methylamino)purine riboside (N^6-methyladenosine, 6MAPr), and 6-(dimethylamino)-purine riboside (N^6,N^6-dimethyladenosine, 6DMAPr) (Figure 1) in stretched poly(vinyl alcohol) (PVA) films. The concentration of the molecules in the film is low, which guarantees monomeric behavior, in contrast to solid-state samples. The effect of the polar PVA matrix or aqueous solution on the electronic transitions has been assessed by studying the LD of the adenine derivative, 6-(dimethylamino)-9-ethylpurine (6DMA9EP), oriented in a nonpolar polyethylene matrix, and the MCD in cyclohexane. In the case of 7MA and 9MA we have performed MCD and also fluorescence excitation anisotropy (FA) measurements to aid the assignments of the electronic transitions. The experimental results are compared with results from INDO/S and *ab initio* molecular orbital calculations.

Experimental Methods

Chemicals. Adenine, adenosine (Ado), 6-(methylamino)purine riboside (6MAPr, N^6-methyladenosine), and 6-(dimethylamino)purine riboside (6DMAPr, N^6,N^6-dimethyladenosine) were purchased from Sigma Chemical Co. and used without further purification. The synthesis and purification of 7MA[18,19] and 9MA[20] has been described previously.[11] 6-(Dimethylamino)-9-ethylpurine (6DMA9EP) was syn-

thesized from 6-(dimethylamino)purine, by ethylation with ethyl iodide according to the methods described by Hedayatullah.[20] 6DMA9EP was purified by chromatography on silica gel with chloroform/methanol/ammonia (90/10/1) as eluant. After evaporation of the solvent, an oily substance was obtained. The purity was checked by ^{1}H NMR and ^{13}C NMR spectroscopy and mass spectrometry. Poly(vinyl alcohol) (PVA) was obtained as powder from E. I. du Pont de Nemours Co. (Elvanol). All aqueous solutions were prepared from deionized water (Millipore). All organic solvents were of spectrophotometric grade.

Linear dichroism (LD) is defined as

$$LD(\tilde{\nu}) = A(\tilde{\nu})_{\parallel} - A_{\perp}(\tilde{\nu}) \tag{1}$$

where $A_{\parallel}(\tilde{\nu})$ and $A_{\perp}(\tilde{\nu})$ are the absorbances measured with plane-polarized light respectively parallel and perpendicular to the macroscopical sample axis (the stretching direction). The reduced linear dichroism, $LD^r(\tilde{\nu})$, is defined:

$$LD^r(\tilde{\nu}) = (A_{\parallel}(\tilde{\nu}) - A_{\perp}(\tilde{\nu}))/A_{iso}(\tilde{\nu}) \tag{2}$$

where $A_{iso}(\tilde{\nu})$ is the absorbance of a corresponding isotropic sample. For a uniaxial orientation of the sample molecules, such as in a polymer matrix, $A_{iso}(\tilde{\nu})$ can be calculated from the polarized components as[21]

$$A_{iso}(\tilde{\nu}) = {}^1/_3 (A_{\parallel}(\tilde{\nu}) + 2A_{\perp}(\tilde{\nu})) \tag{3}$$

For a planar molecule, the LD^r for a transition i can be written[22]

$$LD^r_i = 3(S_{yy} \sin^2 \theta_i + S_{zz} \cos^2 \theta_i) \quad \text{(in-plane transition)} \tag{4}$$

$$LD^r_i = 3S_{xx} \quad \text{(out-of-plane transition)} \tag{5}$$

where S_{zz}, S_{yy}, and S_{xx} are the Saupe orientation parameters[23] for the diagonal in-plane axes z and y, and the out-of-plane axis x, characterizing the orientation of the solute molecules and θ_i is the angle between the transition dipole moment and the preferred molecular orientation axis z. For an in-plane polarized transition the LD^r can have values between $3S_{zz}$ and $3S_{yy}$ and the orientation parameters are interrelated according to

$$S_{xx} + S_{yy} + S_{zz} = 0 \tag{6}$$

For overlapping transitions the observed LD^r is a weighted average of the LD^r_i values of the contributing transitions[22]

$$LD^r(\tilde{\nu}) = \frac{\sum_i \epsilon_i(\tilde{\nu}) LD^r_i}{\sum_i \epsilon_i(\tilde{\nu})} \tag{7}$$

where $\epsilon_i(\lambda)$ is the molar absorptivity associated with transition i at wavelength λ. The pure reduced linear dichroism LD^r_i is determined by the TEM method.[24] This method is based on forming linear combinations of the type $A_{\parallel} - dA_{\perp}$ with varying values of the subtraction coefficient, d. The subtraction coefficient, d_i, for which a specific spectral feature, i, disappears is related to the LD^r_i of the transition containing that feature by[25]

$$LD^r_i = 3\frac{d_i - 1}{d_i + 2} \tag{8}$$

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When the reduction coefficient is known it can be used to obtain the reduced absorption spectrum for a specific transition as outlined by Michl and Thulstrup.[21] This approach has been used by us to obtain approximate band shapes for the observed transitions. It should be noted that the resolution of band shapes is made under the assumption that there is only pairwise overlap between neighboring (in energy) transitions and that the resolved band shapes correspond to the purely polarized transitions.

The experimental details of polarized UV measurements on solutes in stretched PVA films have been reported before.[26] In the case of 6DMA9EP, low-density polyethylene (PE) that had been stretched five times its original length was used as the anisotropic host. The PE film was exposed to the sample dissolved in cyclohexane and the film surface was subsequently rinsed with ethanol. A reference PE film was prepared similarly and used for correction of the sample spectra with regards to scattering and absorption of the PE film.

Emission measurements were performed on a Spex fluorolog $\tau 2$ spectrofluorometer. The quantum yield of 7MA in a room temperature methanol/ethanol (1/9) solution was determined using an argon purged solution of 2,5-diphenyloxazole (PPO) in cyclohexane ($\phi_f = 0.85$)[27] as a reference. The fluorescence excitation anisotropy (FA) spectra were measured with the samples in either in propylene glycol glass at 190 K, or in EPA glass (a 5/5/2 mixture of diethyl ether, 2-methyl-butane, and ethanol) at 80 K, with Glan polarizers both in the excitation beam and in the emission beam. The fluorescence intensities were measured with polarizers set either vertically (v) or horizontally (h) in the excitation and emission beams. The emission was measured at 330 nm with a bandpass of 7 nm. The bandpass of the excitation monochromator was 2 nm and five data points per nanometer were collected. The degree of anisotropy, r, was calculated as

$$r = \frac{I_{vv} - I_{vh}G}{I_{vv} + 2I_{vh}G} \qquad (9)$$

where G is the ratio I_{hv}/I_{hh} used for instrumental correction. The first index refers to the excitation polarizer and the second to the emission polarizer. The theoretically limiting value of the anisotropy for any pure electronic transition, i, is reached when no molecular reorientation occurs during the excited state lifetime. This limiting value, r_{oi}, is given by[28]

$$r_{oi} = r_{01}(3\cos^2\beta, - 1)/2 \qquad (10)$$

where β_i is the angle between the ith transition moment of absorption and the emitting moment. The r_{01} is the observed limiting anisotropy for the lowest lying transition. Fluorescence lifetimes were determined using a time-correlated single-photon-counting (SPC) instrument described previously.[29] A scattering solution was used as a reference.

Magnetic Circular Dichroism (MCD). The MCD measurements were made on a JASCO J-720 spectropolarimeter equipped with a permanent horseshoe magnet.[30] The magnetic field was calibrated to be 0.58 T by using the MCD signal at 510 nm of a 1 M solution of $CoSO_4$ ($\Delta\epsilon_{510} = -1.88 \times 10^{-2}$ M^{-1} cm^{-1} T^{-1}).[31] The MCD was measured for 7MA and 9MA in neutral aqueous solution and for 6DMA9EP in cyclohexane.

Quantum Chemical Calculations. Electronic spectra were calculated using the semiempirical intermediate neglect of differential overlap (INDO/S)[32−34] model Hamiltonian. The two-center electron repulsion

integrals were calculated using the Mataga−Nishimoto scheme.[35] Generally, all singly substituted configurations involving n → π* and π → π* transitions were included in the configuration interaction (CI) calculations. Larger CI spaces were tried (up to 260 singly substituted configurations), but this had no significant effect on the results. Inclusion of doubly excited configurations had the general effect to lower the oscillator strengths but not to change transition moment directions to any significant extent.

In the INDO/S calculations, the effect of solvent (water) on the calculated spectra was incorporated using the self-consistent reaction field (SCRF) model B described by Karelson and Zerner.[36] A spherical cavity was used in the SCRF calculations. The cavity radius was calculated using the mass-density approach. This gave a radius of 3.77 Å for 7-H-adenine (7HA) and 9-H-adenine (9HA) and a radius of 3.89 Å for the two methylated adenines. The solvent (water) is modeled as a dielectric continuum outside the cavity, characterized by its refractive index (1.33) and low-frequency dielectric constant (78.5). To test the supermolecule treatment proposed by Karelson and Zerner,[36] a solute/ water complex was constructed by placing six or seven water molecules around the solute molecule at plausible hydrogen bonding sites,[37] and keeping the lengths of the hydrogen bonds constant and equal to 2.0 Å. The supermolecule was then used in the SCRF calculation. The use of the supermolecule instead of the bare solute had very little effect on the calculated spectroscopic properties of the adenine derivatives.

Electronic absorption spectra were also calculated *ab initio* by using configuration interaction with single substitutions (CIS) and the 6-31G(d) basis set. The active space consisted of all occupied valence orbitals and all virtual orbitals. The Gaussian94 program was used for these calculations.[38]

The molecular geometries used in the INDO/S and the CIS/6-31G(d) calculations were obtained from *ab initio* calculations at the SCRF DFT(B3LYP)/6-31G(d,p) level of theory with no constraint regarding the planarity of the molecules.[11]

Results

In this section we present results from linear dichroism measurements on 7MA and 9MA partially aligned in stretched PVA film. The magnetic circular dichroism (MCD) spectra of these molecules in water solutions have also been examined, as well as the fluorescence excitation anisotropy (FA) in low-temperature glasses. On the basis of the experimental results, the electronic absorption spectra of both 7MA and 9MA are resolved into intensity distributions of five electronic transitions and the corresponding possible polarizations of the transitions are deduced. The experimental data is then compared to the results from INDO/S and *ab initio* calculations of the excited states of 9-H-adenine and 7-H-adenine. The changes in the isotropic and reduced linear dichroism spectra when methylating the adenine amino group is examined by comparing results for Ado, 6MAPr, and 6DMAPr in stretched PVA films, and 6DMA9EP in stretched PE film. Finally, the absorption and reduced linear dichroism spectra of adenine in stretched PVA film are analyzed in terms of contributions from the 7-H and 9-H tautomers.

The Electronic Spectra of 7MA and 9MA. The isotropic absorption (A_{iso}) spectra and reduced linear dichroism (LD^r)

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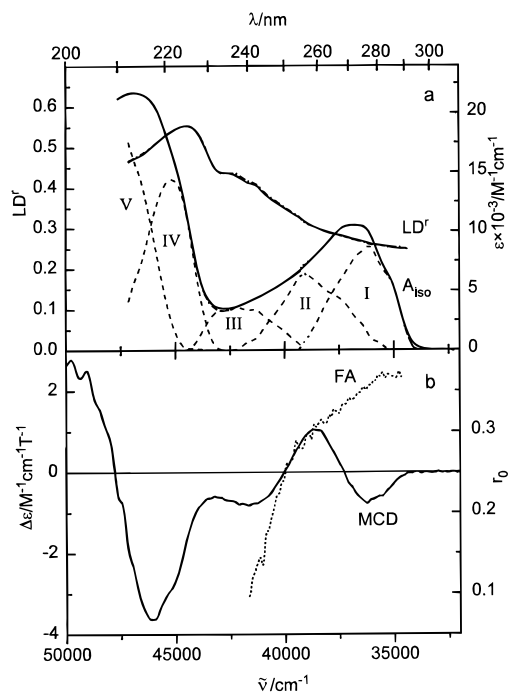(33) Bacon, A. D.; Zerner, M. C. *Theor. Chim. Acta* **1979**, *53*, 21.

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Figure 2. (a) Isotropic absorption (A_{iso}, —) and reduced linear dichroism (LD^r, —) spectra of 7MA in stretched PVA film. Also presented are the resolved band shapes of transitions I–V (- - -) and the fitted A_{iso} (- · -) and LD^r spectra (- · ·). (b) The magnetic circular dichroism (MCD, —) and fluorescence excitation anisotropy (FA, - - -) spectra of 7MA. MCD measured in neutral aqueous solution and FA in propylene glycol glass at 190 K.
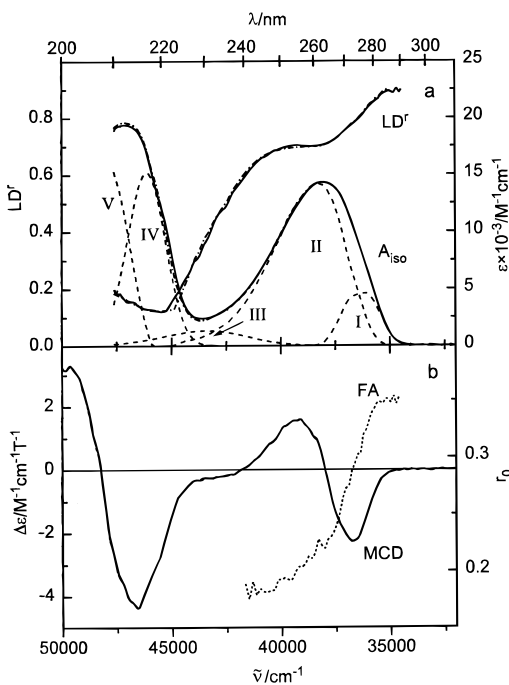

Figure 3. (a) Isotropic absorption (A_{iso}, —) and reduced linear dichroism (LD^r, —) spectra of 9MA in stretched PVA film. Also presented are the resolved band shapes of transitions I–V (- - -), and the fitted A_{iso} (- · -) and LD^r spectra (- · ·). (b) The magnetic circular dichroism (MCD, —) and fluorescence excitation anisotropy (FA, - - -) spectra of 9MA. MCD measured in neutral aqueous solution and FA in EPA glass at 80 K.

spectra of 7MA and 9MA in stretched PVA film are presented in Figures 2a and 3a. For 7MA, the LD^r increases slightly toward shorter wavelengths over the first band centered around 270 nm and reaches a plateau value of 0.43 at 238 nm. This

indicates that at least two differently polarized in-plane transitions are located in the region between 230 and 300 nm. At 226 nm, a maximum in the LD^r is found ($LD^r = 0.55$), indicating a transition at the red edge of the relatively strong band centered at 215 nm. The large LD^r indicates that this transition is polarized relatively close to the molecular orientation axis. At shorter wavelengths the LD^r decreases, suggesting at least one strong transition at the high energy side of the 215 nm band. In the case of 9MA, the LD^r equals 0.90 at 290–285 nm and then drops to 0.70 between 260 and 250 nm. This is a clear indication of the presence of two transitions within the 260 nm band, the low-energy transition being the weakest one. Both these transitions make relatively small angles with the orientation axis. A decrease in the LD^r starts at 250 nm and a minimum ($LD^r = 0.12$) is reached at 220 nm. This is clear evidence for a relatively strong transition at the red edge of the strong band, polarized with a large angle to the orientation axis. At higher energies, the LD^r increases which indicates the presence of another strong transition.

The FA spectra of 7MA in propylene glycol glass at 190 K, and of 9MA in EPA glass at 80 K, are presented in Figures 2b and 3b. For 7MA, the FA decreases slowly over the lowest band reaching a value of 0.25 at 250 nm which corresponds to an angle between the absorbing and emitting moments of 27°. At wavelengths shorter than 250 nm the FA decreases rapidly. The FA of 9MA varies strongly over the band lowest in energy in agreement with the pronounced variation in LD^r. The angle between the two lowest transitions is estimated to be 35°.

The MCD spectra of 7MA and 9MA in aqueous solutions have been reported before,[14] but for comparison reasons they have been remeasured and are presented in Figures 2b and 3b. The MCD changes sign from negative to positive within the first absorption band for both 7MA and 9MA, indicating the presence of two nonparallel $\pi \rightarrow \pi^*$ transitions in this region.[39] This was not clear from either the LD^r or FA spectra of 7MA. At about 240 nm there is a negative feature in the MCD for 7MA and 9MA. This is more pronounced for 7MA than for 9MA, indicating that the transition at about 240 nm in 7MA is stronger than the putative transition of 9MA at about the same wavelength. At shorter wavelengths, the MCD becomes first more negative with minima at 217 and 215 nm for 7MA and 9MA, respectively, and then changes sign at about 208 nm. The two strong lobes observed both for 7MA and 9MA are in agreement with the LD results which indicated two relatively strong $\pi \rightarrow \pi^*$ transitions within the 215 nm bands of 7MA and 9MA.

Resolution of Absorption Components. On the basis of the variations in the LD^r, FA, and MCD spectra for 7MA and 9MA, the electronic absorption spectra of these compunds in the region 210–300 nm have been resolved into contributions from five individual electronic transitions (I–V, in Figure 2a,b). This has been accomplished by using the stepwise-reduction method devised by Thulstrup, Eggers, and Michl (TEM) in which linear combinations of the polarized absorption components ($A_{\parallel}$ and $A_{\perp}$) are used to construct the band shapes of the corresponding purely polarized transitions. The resolved band shapes are deduced under the assumption of pairwise overlap which of course is not always the case in reality. Thus, some of the resolved bands look somewhat "unphysical" probably due to the restriction to only pairwise overlap. For band III of

(39) Numerous examples are found in the literature where two isolated overlapping electronic transitions give rise to a bisignate MCD spectrum. See for example: Voelter, W.; Records, R.; Bunnenberg, E.; Djerassi, C. *J. Am. Chem. Soc.* **1968**, *90*, 6163. Nordén, B.; Håkansson, R.; Pedersen, P. B.; Thulstrup, E. W. *Chem. Phys.* **1978**, *33*, 355. Albinsson, B.; Eriksson, S.; Lyng, R.; Kubista, M. *Chem. Phys.* **1991**, *151*, 149.

Table 1. Observed Electronic Transitions of 7MA, 9MA, and Ado in PVA Film[a]

	trans	$\tilde{\nu}$,[b] cm^{-1}	ϵ,[c] M^{-1} cm^{-1}	LD^r	θ,[d] deg	FA	β,[e] deg
7MA	I	36580	8750	0.25 ± 0.01	63 ± 2	0.37	
$S_{xx} = [-0.28, -0.29]$	II	39550	6450	0.32 ± 0.01	56 ± 2	0.25	>27
$S_{yy} = [0.04, 0.05]$	III	42630	3514	0.43 ± 0.01	44 ± 1	0.10	>45
$S_{zz} = [0.24, 0.25]$	IV	45760	15200	0.56 ± 0.01	32 ± 2		
$\alpha = -72 \pm 5°$	V	≈ 47682	17442	0.45 ± 0.05	43 ± 5		
9MA	I	36710	4550	0.90 ± 0.01	15 ± 2	0.35	
$S_{xx} = -0.33$	II	38820	14090	0.70 ± 0.01	32 ± 1	0.19	34
$S_{yy} = 0.01$	III	43370	1320	0.18 ± 0.01	66 ± 1		
$S_{zz} = 0.32$	IV	46840	16910	0.12 ± 0.01	72 ± 2	-0.08[f]	70
$\alpha = +51 \pm 5°$	V	≈ 48320	≈ 16420	0.20 ± 0.05	65 ± 5		
Ado	I	36710	4550	0.69 ± 0.01	20 ± 2		
$S_{xx} = -0.27$	II	38820	14650	0.71 ± 0.01	18 ± 2		
$S_{yy} = 0.01$	III	43370	1320	0.22 ± 0.01	60 ± 1		
$S_{zz} = 0.26$	IV	46840	16410	0.13 ± 0.01	68 ± 2		
$\alpha \approx +45°$	V	≈ 48320	≈ 15940	0.23 ± 0.05	59 ± 5		

[a] Orientation parameters and direction of orientation axis from Holmén.[11] [b] Wavenumber of absorption maximum in water solution. Estimated from resolution of band shapes in PVA film. [c] Molar absorptivities in water solution. [d] Angle between transition moment and the orientation axis. Error bars estimated from the uncertainties in orientation parameters and the LD^r values (see text). [e] Angle between the ith transition moment and the transition moment of the lowest lying transition. [f] Data for 6DMAPr in ethanol glass at 77 K (ref 42).

9MA, another approach was made on the basis of a Gaussian fit to evaluate the residiual intensity when the bands II and IV had been subtracted from the measured isotropic absorption spectrum. Also shown in Figures 2a and 3a are the sums of the resolved absorption bands, and the LD^r spectra simulated using the resolved band shapes and the intrinsic LD^r values (eq 7) obtained by the TEM method. The agreement between the measured and simulated spectra is almost perfect. The spectral parameters obtained in the resolution of band shapes are collected in Table 1. The LD^r spectrum of adenosine (Ado) in stretched PVA film (Figure 4a) has also been evaluated quantitatively and the results are given in Table 1.

Molecular Orientation in Film and Transition Moment Directions. In order to obtain absolute polarizations within the molecular framework, the orientation axis and the orientation parameters must be known (eqs 4 and 5). We have recently characterized the orientational properties of 7MA, 9MA, and Ado in stretched PVA films using polarized IR spectroscopy,[11] and the orientation parameters and directions of the orientation axis determined in that study are cited in Table 1.

Uncertainties in the orientation parameters will of course introduce uncertainties in the final transition moment directions. In the case of 9MA and Ado, the estimated uncertainties were smaller than the experimental accuracy of the polarized IR measurements. However, for 7MA the orientation parameters were found to have a small degree of uncertainty associated with them. Furthermore, the determined LD^r values are estimated to be correct within ± 0.01 for transition I to IV and within ± 0.05 for transition V. On the basis of this knowledge, the angles between the transition moments and the orientation axis (θ) can be estimated (Table 1).

The transition moment direction within the molecular framework of transition i, δ_i, is related to the angle θ_i by

$$\delta_i = \alpha \pm \theta_i \qquad (11)$$

where α is the angle between the orientation axis and the z' axis. Any uncertainty in the direction of the orientation axis will, thus, be reflected in the final transition moment directions. For 7MA and 9MA we estimate that the direction of the orientation axis is correct within $\pm 5°$.

In order to choose between the two alternative transition moment directions (δ) for each transition, complementary information is needed. In the case of 7MA, we have indications

from FA that the angles between the transitions I and II, and I and III are at least 27° and 45°, respectively. By comparing these angles with the possible relative angles (Table 1) obtained from the LD measurement we conclude that both transitions II and III are on the opposite side of the orientation axis compared to transition I. However, in order to obtain absolute polarizations for 7MA we have to rely, partly, on results from quantum chemical calculations (*vide infra*).

For 9MA, the situation is more favorable since we have also investigated in detail another 9-substituted adenine derivative, adenosine (Ado). It is then possible to exploit the changes in the angles θ between the transition moments and the orientation axis when changing the methyl group in 9MA to a ribose sugar in adenosine. The use of such substitutional perturbation has been applied successfully in several stretched film experiments in order to solve the sign ambiguities regarding the polarization angles.[11,40,41] From the IR study of 9MA and Ado it was found that the orientation axis of Ado was realigned on average $-6°$ compared to that of 9MA. This agrees with the expectation that changing the methyl group to a ribose will enhance the orientation in the direction of the short molecular axis. The angle θ for transition I in 9MA equals 15° while for Ado the angle has increased to 20°. For transition II in 9MA, $\theta = 32°$, and for Ado it equals 18°. These relative changes strongly indicate that transition I is polarized at $+66°$ (and not $+36°$) and that transition II is polarized at $+19°$ (and not $+83°$) in 9MA. The same line of reasoning has been applied to transitions III−IV. The concluded polarizations for transitions I and II are also consistent with the angle $\beta = 34°$ obtained from the FA analysis (Table 1). Furthermore, the polarization of transition IV is consistent with the angle of about 70° between transitions I and IV obtained from the fluorescence polarization measurements by Tohara and Hirakawa on 6-(dimethylamino)-purine riboside (6DMAPr) in ethanol glass at 77 K.[42] However, this conclusion is only justified if there is no appreciable perturbation of the electronic transitions in 6DMAPr compared to 9MA and Ado (*vide infra*). For transition V, the experimental data do not extend sufficiently high in energy to allow a confident assignment of the polarization of this transition. The LD^r value obtained at the blue edge of the 215 nm band

(40) Albinsson, B.; Kubista, M.; Nordén, B.; Thulstrup, E. W. *J. Phys. Chem.* **1989**, *93*, 6646. Albinsson, B.; Nordén, B. *J. Phys. Chem.* **1992**, *96*, 6204.

(41) Holmén, A.; Albinsson, B.; Nordén, B. *J. Phys. Chem.* **1994**, *98*, 13460.

Table 2. Calculated Transitions for the 7-H Adenine Chromophore

	SCRF INDO/S[a]				CIS/6-31G(d)[b]				CASPT2[c]			
trans	character	$\tilde{\nu}$, cm^{-1}	f[d]	δ,[e] deg	character	$\tilde{\nu}$, cm^{-1}	f[d]	δ,[e] deg	character	$\tilde{\nu}$, cm^{-1}	f[d]	δ,[e] deg
$S_0 \to S_1$	$(\pi \to \pi^*)$	34107	0.19	+33	$(\pi \to \pi^*)$	35923	0.16	+28	$(\pi \to \pi^*)$	37180	0.050	+23
$S_0 \to S_2$	$(n \to \pi^*)$	35989	0.003	oop	$(\pi \to \pi^*)$	38816	0.082	−6	$(\pi \to \pi^*)$	40080	0.19	−10
$S_0 \to S_3$	$(n \to \pi^*)$	37381	0.004	oop	$(n \to \pi^*)$	40383	0.005	oop	$(\pi \to \pi^*)$	48550	0.36	+3
$S_0 \to S_4$	$(\pi \to \pi^*)$	39626	0.11	+36	$(n \to \pi^*)$	42776	0.001	oop	$(\pi \to \pi^*)$	49600	0.12	−49
$S_0 \to S_5$	$(n \to \pi^*)$	42123	0.023	oop	$(n \to \pi^*)$	44827	0.002	oop	$(\pi \to \pi^*)$	50970	0.077	+52
$S_0 \to S_6$	$(\pi \to \pi^*)$	43543	0.032	+79	$(n \to \pi^*)$	44961	0.016	oop	$(\pi \to \pi^*)$	52340	0.58	+33
$S_0 \to S_7$	$(\pi \to \pi^*)$	45891	0.69	+83	$(\pi \to \pi^*)$	45835	0.82	+77				
$S_0 \to S_8$	$(n \to \pi^*)$	46580	0.003	oop	$(\pi \to \pi^*)$	48473	0.47	−24				
$S_0 \to S_9$	$(\pi \to \pi^*)$	47565	0.63	−26	$(\pi \to \pi^*)$	49161	0.23	−89				

[a] CI including 61 singly excited configurations. SCRF calculation using the SCRF model B of Karelson and Zerner.[36] [b] Transition energies scaled with a factor of 0.72.[8] [c] Results from ref 44. No n → π* transitions were calculated. [d] Oscillator strength. [e] In-plane angle defined in Figure 1.

Table 3. Calculated Transitions for the 9-H-Adenine Chromophore

	SCRF INDO/S[a]				CIS/6-31G(d)[b]				CASPT2[c]			
trans	character	$\tilde{\nu}$, cm^{-1}	f[d]	δ,[e] deg	character	$\tilde{\nu}$, cm^{-1}	f[d]	δ,[e] deg	character	$\tilde{\nu}$, cm^{-1}	f[d]	δ,[e] deg
$S_0 \to S_1$	$(\pi \to \pi^*)$	34 950	0.12	+34	$(\pi \to \pi^*)$	37 206	0.35	+52	$(\pi \to \pi^*)$	38100	0.24	+43
$S_0 \to S_2$	$(n \to \pi^*)$	36 471	0.005	oop	$(\pi \to \pi^*)$	37 678	0.065	+90	$(\pi \to \pi^*)$	38200	0.001	−45
$S_0 \to S_3$	$(\pi \to \pi^*)$	38 039	0.32	+73	$(n \to \pi^*)$	41 269	0.001	oop	$(\pi \to \pi^*)$	47000	0.307	−45
$S_0 \to S_4$	$(n \to \pi^*)$	41 963	0.023	oop	$(n \to \pi^*)$	43 696	0.003	oop	$(\pi \to \pi^*)$	47600	0.167	−40
$S_0 \to S_5$	$(\pi \to \pi^*)$	44 113	0.073	−29	$(n \to \pi^*)$	45 741	0.020	oop	$(\pi \to \pi^*)$	53500	0.055	+89
$S_0 \to S_6$	$(n \to \pi^*)$	46 566	0.003	oop	$(\pi \to \pi^*)$	46 575	0.39	−40	$(\pi \to \pi^*)$	61052	0.406	+89
$S_0 \to S_7$	$(\pi \to \pi^*)$	48 080	0.72	−29	$(\pi \to \pi^*)$	48 971	0.43	+8				
$S_0 \to S_8$	$(\pi \to \pi^*)$	50 104	0.52	−67	$(\pi \to \pi^*)$	49 671	0.57	+89				

[a] CI including 61 singly excited configurations. SCRF calculation using the SCRF model B of Karelson and Zerner.[36] [b] Transition energies scaled with a factor of 0.72.[8] [c] From Holmén et al.[8] No n → π* transitions were reported. [d] Oscillator strength. [e] In-plane angle defined in Figure 1.

corresponds to a transition moment direction of −11° or −61°. The −61° alternative is in agreement with the strong increase in the fluorescence polarization toward shorter wavelengths.[42]

Molecular Orbital Calculations−Transition Assignments. To aid the assignment of electronic transitions of 7MA and 9MA and in particular the choice of the correct polarizations in 7MA, we have performed SCRF INDO/S and CIS/6-31G(d) calculations of the excited states. The results from calculations on 7-H-adenine (7-H-A) and 9-H-adenine (9-H-A) are presented in Tables 2 and 3, respectively. It should be noted that the SCRF INDO/S data (not shown) for 7MA and 9MA are nearly identical to the data of 7-H and 9-H tautomers of adenine, respectively. The CIS/6-31G(d) transition energies have been scaled with a linear scaling factor of 0.72 which has been found to give the best overall agreement between experiment and CIS/6-31G(d) theory for the transition energies of the DNA bases.[8] The sensitivity to the molecular geometry of INDO/S results for adenine has been demonstrated recently.[8] As a basis for the presented calculations we have used molecular geometries calculated by SCRF DFT(B3LYP)/6-31G(d,p) theory which is known to provide good quality geometries for this type of molecules.[11,43] Also included in Tables 2 and 3 are results from CASPT2 calculations of the lowest π → π* transitions in 7-H-A[44] and in 9-H-A.[8]

(a) 7MA. The INDO/S, CIS/6-31G(d) and CASPT2 methods predict as the lowest transition ($S_0 \to S_1$) a medium intensity π → π* transition polarized at an angle of +32° to +28°. This transition is assigned to the experimentally resolved transition I. One of the two experimentally possible polarizations, +45°, agrees reasonably well with the theoretically predicted polarizations. The second and third INDO/S transitions are very weak out-of-plane-polarized n → π* transitions which should be

hidden below the strong π → π* transitions of the 260 nm band. The $S_0 \to S_4$ (π → π*) transition in the INDO/S calculation is assigned to transition II. This transition is shifted 1000 cm^{-1} to the red in the SCRF calculation compared to the calculated gas-phase transition energy (data not shown). This is the largest calculated solvent shift noted for any of the studied adenines.

The same transition, in terms of dominating configurations, is found in the CIS calculation as the $S_0 \to S_2$ transition. However, the polarization for this transition differs between the INDO/S and the CIS calculation. If the choice of polarization for transition I is correct, then the choice for transition II must be −15° on the basis of the FA results. Thus, there is good agreement with the CIS (−6°) and CASPT2 (−10°) polarizations. In the region of transition III (237 nm, 42 200 cm^{-1}) there is one weak n → π* transition and one weak π → π* transition according to the INDO/S calculation. The CIS calculation predicts two very weak n → π* transitions and one weak n → π* transition ($S_0 \to S_6$). The high LD^r value and the observed intensity of transition III ($f = 0.052$), indicates that this is probably a weak π → π* transition. The experimentally determined polarization is either +64° or −28° of which the first option agrees with the calculated polarization of +73°. However, this choice is inconsistent with the angle (β) between the transition I and III of being at least 45° (Table 2), suggesting instead the −28° alternative. At higher energies, the INDO/S calculation predicts two strong π → π* transitions at about 45 900 cm^{-1} ($S_0 \to S_6$) and 47 600 cm^{-1} ($S_0 \to S_8$). They correspond to the strong $S_0 \to S_6$ and $S_0 \to S_7$ transitions, respectively, in the CIS calculation. The calculated polarizations of the INDO/S and the CIS calculations are very similar and agree with one of the experimentally determined polarizations for each of these transitions. We have therefore chosen the alternatives +76° for transition IV and −29° for transition V.

(b) 9MA. For the two lowest π → π* transitions ($S_0 \to S_1$ and $S_0 \to S_3$) of 9MA, the INDO/S calculation yields energies

(42) Tohara, A; Hirakawa, A. Y. *Chem. Phys. Lett.* **1980**, *75*, 145.
(43) Broo, A.; Holmén, A. *Chem. Phys.* **1996**, *211*, 147.
(44) Fülscher, M. P.; Serrano-Andrés, L.; Roos, B. O. *J. Am. Chem. Soc.* **1997**, *119*, 6168.

Table 4. Concluded Spectral Parameters of 7MA and 9MA

trans	$\tilde{\nu},^a$ cm^{-1}	f^b	$\delta,^c$ deg
	7MA		
I($\pi \rightarrow \pi^*$)	36580	0.11	$+45 \pm 7$
II($\pi \rightarrow \pi^*$)	39550	0.094	-16 ± 7
III($\pi \rightarrow \pi$)	42630	0.052	-28 ± 6
IV($\pi \rightarrow \pi$)	45760	0.16	$+76 \pm 7$
V($\pi \rightarrow \pi^*$)	≈ 47682	≈ 0.19	-29 ± 10
	9MA		
I($\pi \rightarrow \pi^*$)	36710	0.047	$+66 \pm 7$
II($\pi \rightarrow \pi^*$)	38820	0.24	$+19 \pm 7$
III($\pi \rightarrow \pi$)	43370	0.027	-15 ± 6
IV($\pi \rightarrow \pi$)	46840	0.14	-21 ± 7
V($\pi \rightarrow \pi^*$)	≈ 48320	≈ 0.12	-64 ± 10

^{a} Wavenumber of absorption maximum in water solution. ^{b} Oscillator strength. ^{c} In-plane angle defined in Figure 1. Error bars estimated from the uncertainties in orientation parameters, in LD^r values, and in direction of the orientation axis (see text).

and oscillator strengths that are in good agreement with experiment. However, the calculated polarizations differ from the experimentally determined ones which in turn are quite similar to the polarizations determined in the polarized reflection studies of Clark.[5] Between the two $\pi \rightarrow \pi^*$ transitions a very weak n $\rightarrow \pi^*$ transition is predicted from the calculation. Due to the overlap from the $\pi \rightarrow \pi^*$ transitions in this region, such a transition would be very difficult to observe. However, the presence of an n $\rightarrow \pi^*$ transition at this position could be of significant importance for the photophysical properties of the 9-substituted adenine chromophore as will be discussed later.

The CIS and CASPT2 calculations both yield two $\pi \rightarrow \pi^*$ transitions that are essentially degenerate as the lowest transitions, with a medium-intensity transition as the $S_0 \rightarrow S_1$ transition and a weak (CIS/6-31G(d)), or a very weak (CASPT2), $S_0 \rightarrow S_2$ transition. This order is reversed when compared to experiments and the splitting in energy equals about 2000 cm^{-1}. The CIS/6-31G(d) polarizations are $+52°$ ($S_0 \rightarrow S_1$) and $+90°$ ($S_0 \rightarrow S_2$). If the order of these transitions is allowed to be reversed there is an acceptable agreement with the experimentally determined polarizations. The transition moment direction of the very weak CASPT2 $S_0 \rightarrow S_2$ transition is not in agreement with experiment. However, the transition moment for the medium intensity transition, $+43°$, is in fair agreement with our experimentally determined polarization of $+19°$ for transition II (*vide supra*).

In the region of transition III, the INDO/S method predicts one weak n $\rightarrow \pi^*$ transition ($S_0 \rightarrow S_4$) and one weak $\pi \rightarrow \pi^*$ transition ($S_0 \rightarrow S_5$). The CIS/6-31G(d) method predicts one weak n $\rightarrow \pi^*$ transition ($S_0 \rightarrow S_5$) in this region. The CASPT2 calculation does not predict any $\pi \rightarrow \pi^*$ transitions in the region of transition III. The effective polarization obtained from the spectral resolution was $-15°$ which is not far from the $-29°$ obtained in the INDO/S calculation for the $\pi \rightarrow \pi^*$ transition ($S_0 \rightarrow S_5$).

Two relatively strong transitions are obtained in both the INDO/S ($S_0 \rightarrow S_7$ and $S_0 \rightarrow S_8$) and the CIS calculations ($S_0 \rightarrow S_6$ and $S_0 \rightarrow S_7$) that should correspond to the two strong $\pi \rightarrow \pi^*$ transitions IV and V, respectively. The CASPT2 calculation gave two strong transitions around 47 000 cm^{-1}. The first of these have a polarization of $-38°$. The second one has a low weight ($\omega = 0.29$) of the zeroth-order wave function and thus must be regarded as unreliable both with respect to the transition energy and oscillator strength.[8] The experimental polarization of transition IV, $-21°$, is in quite good agreement with the corresponding calculated polarizations. For transition V, there is fair agreement with the results from both the INDO/S and
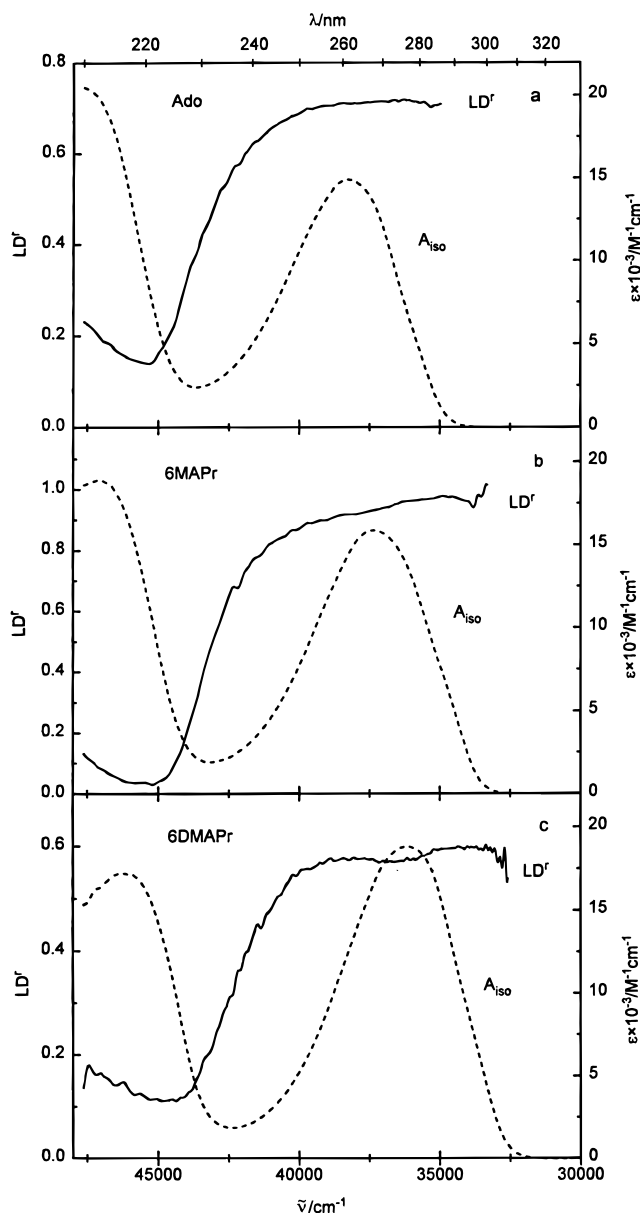

Figure 4. Isotropic absorption (A$_{iso}$, - - -) and reduced linear dichroism (LD^r, —) spectra in stretched PVA film of (a) Ado, (b) 6MAPr, and (c) 6DMAPr.

CIS calculations. The concluded spectral parameters of 7MA and 9MA are given in Table 4.

Substituent and Environment Effects on the Adenine Chromophore. In Figure 4, the absorption and LD^r spectra of Ado, 6MAPr, and 6DMAPr in stretched PVA films are presented. The absorption spectra of the adenosine derivatives Ado, 6MAPr, and 6DMAPr differ in that the they are gradually shifted to the red upon methylation of the amino group, followed by an increase of the intensity of the 260 nm band compared to the 220 nm band as was noted early by Mason.[45] All these derivatives are expected to orient roughly the same way in the stretched film as they differ only marginally in shape. The LD^r spectra of 6MAPr and 6DMAPr have not been analyzed quantitatively but as judged from the LD^r spectra, the transition moment directions of Ado are to a large extent preserved in the methylated molecules. This means that the results from the fluorescence polarization study of 6DMAPr[42] are valid also for the 9-substituted adenine chromophore.
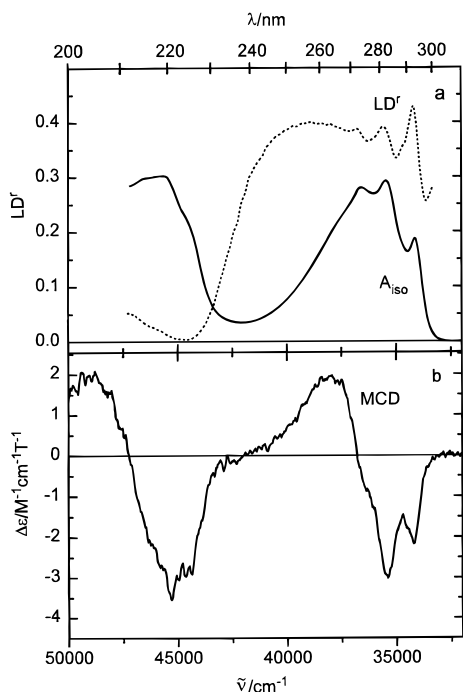
(45) Mason, S. F. *J. Chem. Soc.* **1954**, 2071.

Figure 5. (a) Isotropic absorption (A_{iso}, - - -) and reduced linear dichroism (LD^r, —) spectra of 6DM9EAP in stretched PE film and (b) MCD of 6DM9EAP in cyclohexane.

In order to investigate how transitions of the adenine chromophore behave in a nonpolar environment we examined 6-(dimethylamino)-9-ethylpurine (6DMA9EP) which is soluble in the nonpolar polymer polyethylene (PE). In Figure 5, the isotropic absorption and LD^r spectra of 6DMA9EP in stretched PE film are presented together with the MCD spectrum of 6DMA9EP in cyclohexane. It interesting to note the pronounced vibrational structure of the first band which is also present in the first lobe of the MCD spectrum. The LD^r spectrum is quite similar in overall shape to the LD^r spectra of all the 9-substituted adenine derivatives investigated by us. This applies also to the MCD spectrum which has the same features as the MCD spectrum of 9MA in water (Figure 3b). Since it is expected that the orientation axis of 6DMA9EP in stretched PE film should be very nearly the same as for example that of 6DMAPr in stretched PVA film, we have by the similar LD^r features a strong indication that the UV transition moment directions of the adenine chromophore are not very sensitive to the polarity of the environment.

Tautomerism of Adenine. Adenine is known to exist as a mixture of the 7-H and 9-H tautomers in polar solvents.[46] In PVA film we have previously estimated the relative concentration of the 7-H tautomer to be about 23% from IR spectroscopy.[11] The LD^r spectrum of adenine (Figure 6) differs drastically from that of 9MA and Ado and exhibits at least one prominent feature that can be directly attributed to the 7-H tautomer, namely the maximum in LD^r at 224 nm (44 720 cm^{-1}) which almost coincides with the maximum in the LD^r of 7MA at 226 nm (Figure 2a). Using the resolved band shapes of 7MA and 9MA (Table 1) and the corresponding absolute transition moment directions (Table 4), we have simulated the isotropic absorption and LD^r spectra of a mixture of 23% 7MA and 77%
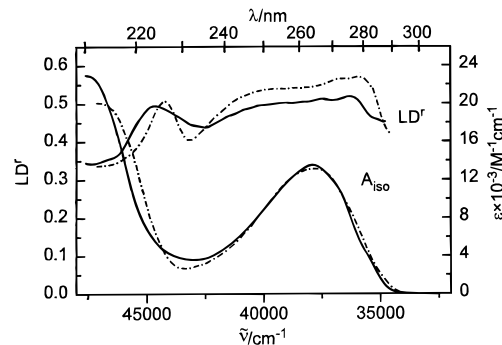

Figure 6. Measured (—) and simulated[47] (- · -) isotropic absorption (A_{iso}) and reduced linear dichroism (LD^r) spectra of adenine in stretched PVA film.

9MA using eq 7.[47] The resulting spectra are shown in Figure 6. The gross features of the adenine LD^r spectrum are reproduced in the simulated spectrum. The isotropic absorption spectrum of adenine is reasonably well described as the weighted sum for the first absorption band at about 260 nm but not for the next stronger band at 215 nm.

Discussion

Assignment of Electronic Transitions. Apart from the position of the double bond in the imidazole ring, the geometries of the 7MA and 9MA chromophores are quite similar.[48] Thus, the electronic spectra of the two molecules are expected to be quite similar, as they have approximately the same orbital energy levels. The dominant features of the UV spectra are indeed found to be common to 7MA and 9MA, and the spectra of both molecules can be described by a model of five electronic transitions in the 200–300 nm region. Transitions I and II are located within the first absorption band centered at about 270 nm in 7MA and 261 nm in 9MA. Transition III is in the valley between the first and second band, and the transitions IV and V are located in the second band centered at about 215 nm. It should be noted that these are the minimum number of transitions needed to account for the experimental observations (A_{iso}, LD, FA, and MCD). In addition, from theoretical calculations, several weak out-of-plane polarized n $\rightarrow$ $\pi*$ transitions are expected to occur in the 200–300 nm region, but we have not observed any spectral features that could be assigned to any such transitions. In the following discussion, the results for 7MA and 9MA will be compared.

The first absorption band is clearly due to two overlapping $\pi \rightarrow \pi*$ transitions. This is concluded from the bisignate MCD features, the varying absorption and fluorescence excitation polarizations and the results from the theoretical calculations. By using the Strickler–Berg equation[49] and the resolution of the lowest bands in 7MA and 9MA (Figures 2a and 3a), the natural lifetimes, $\tau_0 = \tau_f/\phi_f$, are estimated to be 9 and 20 ns for 7MA and 9MA, respectively. For 7MA, we have determined the fluorescence lifetime and quantum yield to be 2.0 ns and 0.26, respectively, at 100 K in ethanol/methanol (9/1) glass. This corresponds to $\tau_0 = 7.7$ ns, in good agreement with the

(46) (a) Dreyfus, M., Dodin, G.; Bensaude, O.; Dubois. J. E. *J. Am. Chem. Soc.* **1975**, *97*, 2369. (b) Gonnella, N. C.; Nakanishi, H.; Holtwick, J. B.; Horowitz, D. S.; Kanamori, K.; Leonard, N. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1983**, *105*, 2050.

(47) The simulated LD^r spectrum has been made under several assumptions. The orientation axes of 7-H-adenine and 9-H-adenine are assumed to be the same as those of 7MA and 9MA, respectively. The same set of orientation parameters ($S_{zz} = 0.29$, $S_{yy} = 0.01$, $S_{xx} = -0.30$) have been used for both 7-H-adenine and 9-H-adenine. These parameters are obtained from polarized IR measurements on adenine.[11] No attempt was made to optimize the fit between simulation and experiments since the individual orientation parameters of 7-H-adenine and 9-H-adenine probably deviate from those determined for the mixture of the 7-H and 9-H tautomers.
(48) Holmén, A.; Broo, A. *Int. J. Quantum Chem.* **1995**, *QBS 22*, 113.
(49) Strickler, S. J.; Berg, R. A. *J. Chem. Phys.* **1962**, *37*, 814.

value from the resolved band shape of the lowest transition. In the case of 9-substituted adenines, the fluorescence lifetime has been estimated to be shorter than a few picoseconds in room-temperature aqueous solution. A recently determined value for Ado is 1.9 ± 0.3 ps.[50] The fluorescence quantum yield under the same conditions has been determined to be 0.6×10^{-4}.[51] Combining these values yields a value of $\tau_0 = 32 \pm 6$ ns. The lower boundary value is in reasonable agreement with the value estimated from the resolved band shape (Figure 2a). Transition I dominates the first absorption band of 7MA, while in 9MA, the oscillator strength of transition I is about 20% of that of transition II.

Evidence for the next transition (III) are features clearly visible in the LD^r and MCD spectra of 7MA. The relatively high LD^r value at about 240 nm indicates that transition III is in-plane polarized. Thus, it is reasonable to assume that it is a $\pi \rightarrow \pi^*$ transition. In order to account for the change in LD^r for 9MA when going from the first band toward the second band, we had to assume that a weak in-plane polarized transition is positioned between the two bands. Transition III might in principle be an $n \rightarrow \pi^*$ transition, having in-plane polarization due to vibronic borrowing, but on the basis of the above-mentioned arguments the same type of transitions are expected to contribute in both 7MA and 9MA although with different oscillator strengths. Thus, it is therefore more probable that the weak transition III of 9MA is also a $\pi \rightarrow \delta^*$ transition. For both 7MA and 9MA, the INDO/S calculations predict a third weak $\pi \rightarrow \pi^*$ transition which we assign as transition III. It should be noticed though that the calculated strength of this transition is quite sensitive to the details in geometry of the adenine base.[8]

The assignments of transitions IV and V which are both relatively strong $\pi \rightarrow \pi^*$ transitions are supported by the variations in LD^r and MCD, and the results from the theoretical calculations.

In Figure 7, the concluded transition moment directions of 7MA and 9MA are presented. The polarizations of transitions I and II in 9MA are rotated $+21°$ and $+35°$, respectively, compared with 7MA. These relatively small changes are surprising since the two molecules must be regarded as being two distinctly different chromophores due the change of the double bond in the imidazole ring. However, the same similarity between the polarizations of the two lowest $\pi \rightarrow \pi^*$ transitions have been found for the parent chromophores of 7MA and 9MA, *viz.* 7-methylpurine and 9-methylpurine.[16] Compared with the purines, the amino substitution in the sixth position in adenine has a drastic effect on the polarization of the second $\pi \rightarrow \pi^*$ transition which is found to be rotated toward the amino group. Such a substitution effect on the lowest $\pi \rightarrow \pi^*$ transition transitions in purine has been proposed by Mason.[45] Transition III has about the same polarizations in both 7MA and 9MA. For transitions IV and V, the transition moment directions differ considerably between 7MA and 9MA, indicating a sensitivity for these transitions to the shift of the double bond in the imidazole ring.

Comparison with Results from Other Studies. One of the goals of this work was to find out whether the transition polarizations for a series of 9-substituted adenine derivatives in the solid state determined by Clark[5] are also representative for the adenine chromophore in solution. The polymer matrix used as the orienting medium in this study behaves like an ordinary solvent in the respect that the sample molecules are
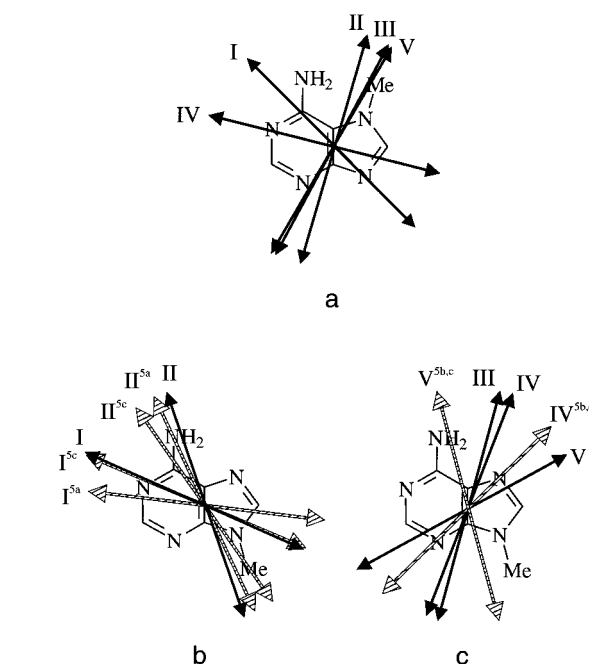
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Figure 7. Concluded transition moment directions of 7MA (a) and 9MA (b, 250−300 nm region, and c, 200−250 nm region). For 9MA, these are compared with transition moment directions (hatched) from previous determinations of Clark.[5a−c] Our transition III was not observed by Clark.

dispersed far apart from each other, thereby minimizing the interchromophoric interactions. Therefore, our results are expected to apply also for adenines in an aqueous environment. In Figure 7, the transition moment directions obtained in this work for the 9-substituted adenine chromophore are compared with those determined by Clark.[5] For transitions I and II our transition moment directions are obviously very similar to the ones obtained by Clark. Thus, both crystal measurements and stretched film measurements indicate that the lowest $\pi \rightarrow \pi^*$ transition is polarized at an angle of about $+83$ to $+66°$ from the short molecular axis and that the second transition is polarized at an angle of about $+19°$ to $+35°$, depending on the surrounding environment. However, the oscillator strengths of I and II show significant variations between the two different sets of experiments. The oscillator strength determined in this work for transition I is only 0.05 whereas Clark estimated it to be 0.09. For transition II, our estimate of the oscillator strength is 0.24 and Clark's is 0.18. It can be noted that the sum of the oscillator strengths for the lowest $\pi \rightarrow \pi^*$ transitions are almost the same regardless of methodology.

Transition III in our experiments was not observed by Clark, although he did observe some out-of-plane intensity at about 244 nm which was tentatively assigned to an $n \rightarrow \pi^*$ transition. The circular dichroism (CD) spectrum of adenosine measured by Brunner and Maestre[13] and by us as well (data not shown), shows a positive band between 222 and 237 nm. We believe this band originates from transition III.

For the strong $\pi \rightarrow \pi^*$ transition IV, the polarization is determined to be $-21°$. Clark concluded the polarization for this transition to be $-45°$ which is close to our rejected alternative $-57°$. However, we shall argue, on the basis of the changes in the relative angles, with respect to the orientation axis for this transition when comparing 9MA and Ado, together with the fluorescence polarization data of Tohara and Hiraka-wa,[42] that the most probable choice is the $-21°$ alternative. Also for transition V, we rely on the data of Tohara and Hirakawa which justifies the $-64°$ choice. However, our assignment of

the transition moment direction must be regarded relatively uncertain since we could not follow the complete change in LD^r over the 215 nm band. Clark has determined the transition moment direction of transition V to be $+15°$.

It should be noted that Clark's assignments of polarizations were based on the overlap of data for the crystals of 9MA and 6MAP. For the individual chromophores the uncertainties were relatively large.[5b] However, the recent crystal study of 2′-deoxyadenosine seems to confirm the earlier results and, thus, Clark concluded that the effects of the crystal environment on the deduced transition moments were negligable.[5c] This conclusion is in contrast to what have been proposed by Callis and co-workers who have evaluated crystal field effects for both guanine[6] and adenine[7] using INDO/S calculations. In the case of adenine, the 9MA and 6MAP crystal absorption spectra were simulated by including both ground-state and excited-state electrostatic interactions. The results indicated that the crystal field effects are different for 9MA and 6MAP. Moreover, the strong transitions within the 215 nm band were predicted to mix, leading to changes in intensity and rotations of the transition moment directions.[7] For 6MAP, the agreement with experiment was improved for transitions I and II when the crystal field effects were taken into account. However, in the case of 9MA, there was no significant improvement. The very similar transition moment directions for transitions I and II of our and Clark's investigations indicate that the crystal field and exciton effects are relatively small for these two transitions. The discrepancy between the INDO/S gas-phase polarizations and the experimental polarizations for transitions I and II seems, therefore, not to be due to exciton effects but must have another explanation. It is noteworthy that the discrepancy between experiment and theory for the two lowest transitions in adenine is not a general problem for the theoretical methods used in this work. Differences in the molecular orbitals and in the mixing of the excited configurations[52] lead to different results and we find that the CIS and CASPT2 results are in better agreement with experiment than the INDO/S results. Thus, we believe that disagreement between INDO/S theory and experiment regarding the polarizations of transitions I and II has to be ascribed to a small imbalance in the mixing of excited configurations in the INDO/S calculation. However, the overall performance of the INDO/S method must be regarded as satisfactory since it predicts transition energies and oscillator strengths in good agreement with experiment for both 7MA and 9MA.

Finally, we comment on the results from previous stretched film studies of the adenine UV spectrum. Fucaloro and Forster[10] reported the dichroic spectra of 9MA and Ado partially oriented in stretched PVA which resulted in the first relative transition moment assignments for the adenine chromophore. However, their molecular orientation model has later been shown unphysical,[21,53,54] and the proposed transition moment directions of Fucaloro and Forster are not in agreement with the ones presented in this work. The polarized UV spectrum of the free base of adenine was also reported by Fucaloro and Forster[10] and later by Matsouka and Nordén who used IR dichroism to assess the orientation parameters.[9] However, as clearly demonstrated in the present study the results obtained for adenine must be interpreted in terms of contributions from the 7-H and 9-H tautomers of adenine (Figure 6), which make the results less useful for the understanding of the spectral properties of the 9-substituted adenine chromophore.

Position of Lowest n → π* Transition: Implications for Photophysics of Adenine. 2-Aminopurine (2AP) is a structural isomer of adenine, but exhibits drastically different photophysical properties, with a strong fluorescence and no phosphorescence, in contrast to adenine for which both fluorescence and phosphorescence have very low quantum yields. Adenine and 2AP in turn are contrasted by purine which shows no fluorescence but has approximately unit quantum yield for phosphorescence. In 9-methylpurine (9MP), it is well established that the lowest transition is an n → π* transition centered at about 34 500 cm⁻¹.[16] In a recent film dichroism study of the electronic transition moments of 2-aminopurine chromophore, we found a low-lying $(S_0 → S_2)$ n → π* transition centered at about 36 000 cm⁻¹, thus, being about 3300 cm⁻¹ higher in energy than the lowest π → π* transition $(S_0 → S_1)$.[17] INDO/S calculations for 9MP and 2-amino-9-methylpurine (2A9MP) yield the same ordering of the lowest π → π* and n → π* transitions as observed experimentally.[8,17] The calculations generally predict the energy of the lowest n → π* transition to be too low, with a discrepancy to the experimental findings by 500 cm⁻¹ for 9MP and 1900 cm⁻¹ for 2A9MP. If it is assumed that the error for 9MA is somewhere between these two values, the lowest n → π* transition in 9MA can be expected at an energy of 36 800–38 300 cm⁻¹. Hence, the lowest n → π* transition in the 9-substituted adenine chromophore would be nearly degenerate with the lowest π → π* transition occurring at 36 700 cm⁻¹ (Table 4). If this is the case, a vibronic interaction between these close-lying nπ* and ππ* states, termed "the proximity effect" by Lim,[55] could explain the extremely efficient nonradiative deactivation of the lowest singlet state in adenines.[50,51] However, other deactivation pathways may be present as recently proposed for 6DMAPr (N^6,N^6-dimethyladenosine).[56] This compound shows dual fluorescence in aprotic solvents, probably due to the formation of a twisted intramolecular charge transfer (TICT) state involving the dimethylamino group.

Conclusions

From this study of electronic spectra of adenine derivatives, the following has been learned:

(i) The UV spectra of 7MA and 9MA have both been interpreted in terms of five π → π* transitions whose energies, intensity distributions, and transition moments have been determined from the combination of LD^r and absorption spectra, supported by FA and MCD spectra. Evidence for a weak π → π* transition (III) at about 240 nm is presented.

(ii) In the case of the 9-substituted adenine chromophore, the polarizations of the two π → π* transitions (I and II) responsible for the 260 nm band are found to be essentially the same as those determined by Clark[5] for crystalline samples. However, for the strong π → π* transition IV, the polarization $(−21°)$ differ significantly from that determined by Clark $(−45°)$ which could indicate that the crystal field or exciton mixing affects this transition in the crystalline samples.

(iii) Methylation of the amino group in Ado does not perturb the transition moments appreciably. Nor does solvent polarity seem to have any significant effect on the transition moment directions in the compounds studied.

(iv) Semiempirical INDO/S, *ab initio* CIS/6-31G(d), and *ab initio* CASPT2 calculations all predict the basic features of the

(52)) For both transitions I and II, the dominating excited configurations are common to the methods (7-H-adenine: I (HOMO → LUMO) and II (HOMO → LUMO + 1); and 9-H-adenine I (HOMO → LUMO + 1) and II (HOMO → LUMO)). However, other configurations also contribute significantly.

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adenine spectrum. As to predicting transition moment directions, the *ab initio* methods seem superior to the INDO/S method regarding the first two $\pi \rightarrow \pi^*$ transitions.

(v) On the basis of the very good agreement between calculated (INDO/S) and observed transition energies of the lowest n $\rightarrow \pi^*$ transition for the 9-substituted purine and 2AP chromophores, we are able to confidently position the lowest n $\rightarrow \pi^*$ transition in 9MA very close to the lowest $\pi \rightarrow \pi^*$ transition. The proximity of the first $^1n\pi^*$ and $^1\pi\pi^*$ states in

adenine could play an important role for the efficient nonradiative deactivation of the lowest excited $^1\pi\pi^*$ state.

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