here, it is very difficult to identify the process responsible for the relaxation observed, not to speak of analyzing them in a quantitative manner.

Acknowledgment. The authors acknowledge the generous support of the Center of Materials Research of the University of Maryland and the Office of Saline Water, U. S. Department of the Interior. Computing time was supported by the University of Maryland Computer Science Center under National Aeronautics and Space Administration Grant No. NsG-398.

Stretched-Film Spectra and Transition Moments of Nucleic Acid Bases[1a]

Anthony F. Fucaloro[1b] and Leslie S. Forster*

Contribution from the Department of Chemistry, University of Arizona, Tucson, Arizona 85721. *Received August 3, 1970*

Abstract: The dichroic spectra of adenine, 9-methyladenine, adenosine, guanine, guanosine, cytosine, cytidine, uracil, thymine, and uridine partially oriented in stretched poly(vinyl alcohol) sheets have been recorded in the 215–300-nm region with the aim of determining the relative transition moment directions. A technique has been developed to resolve the ambiguity inherent in the stretched-film method. The 270-nm transition in guanine is perpendicular to the 250- and 220-nm transitions. In cytosine, the 270- and 235-nm transitions are nearly parallel. Evidence for a second transition in the low-energy tail of the adenine 270-nm band is presented.

Theoretical treatments of the hypochromism and circular dichroism of helical polynucleotides commonly depend upon an elucidation of the interactions among the constituent purine and pyrimidine base pairs.[2,3] A detailed knowledge of the energies, intensities, and the directions of the electronic transitions in the individual bases is requisite to any understanding of these interactions.

The spectral properties of these compounds have been extensively studied, and much is known regarding the energies and intensities of the transitions in the 180–300-nm range.[4–9] The vacuum-uv spectra have also been recorded.[10] There are still unanswered questions about possible low-intensity "hidden transitions" situated under the broad envelopes of the intense spectral bands.[4,7,8,9]

There are at least four methods that can be employed to evaluate transition moment directions: (1) polarized spectra of single crystals,[11] (2) fluorescence polarization,[9] (3) spectra of molecules embedded in stretched films,[12–14] and (4) spectra of molecules oriented in external fields.[15] The first method can give absolute transition moment directions if the crystal structure is known. However, crystal interactions may lead to erroneous results.[16] Only relative transition moment directions can be determined with methods 2, 3, and 4.

Experimental determinations of transition moment directions have been few, and in the theoretical work that requires these quantities, reliance on calculated directions has been necessary.[2,3] In view of the difficulties inherent in the experimental estimation of transition polarizations, it is likely that this situation will continue for some time. It is therefore essential that methods for the computation of transition moments be trustworthy. One goal of the present report is to ascertain the dependability of the extant calculations. A second aim of this study is to collect information about the hidden transitions present in the spectra of the purine and pyrimidine bases.

Experimental Section

Stretched-Film Dichroism. Molecules which are dissolved in a poly(vinyl alcohol) (PVA) sheet will tend to orient if the sheet is stretched. Tanizaki[12,13] has developed an expression which relates the amount of stretching of the sheet to the degree of orientation of the molecules. This assumes that the molecule possesses a unique orienting axis which tends to align along the stretch direction of the PVA sheet. In the model, an imaginary sphere formed by the randomly distributed orienting axes of the dissolved molecules is deformed into an ellipsoid of resolution of equal volume when the PVA is stretched. The stretch ratio, R_s, is the ratio of the major and minor axes of the ellipsoid. Tanizaki derived the following expression

$$2r^2 = \frac{2(T-1)+(T+1)R_d}{2T+(T-1)R_d}$$

where

$$T = R_s^2/(R_s^2-1) \times \left[1 - \left\{\frac{\pi}{2} - \tan^{-1}(R_s^2-1)^{-1/2}\right\}(R_s^2-1)^{-1/2}\right]$$

(1) (a) Based on the dissertation of A. F., submitted to the Graduate College of the University of Arizona in partial fulfillment of the requirements for the Ph.D. degree, 1969; (b) Predoctoral Fellow of the National Institute of General Medical Sciences, National Institutes of Health, 1966–1969.

(2) H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 518 (1962).
(3) W. C. Johnson, Jr., and I. Tinoco, Jr., *Biopolymers*, **7**, 727 (1969).
(4) L. B. Clark and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **87**, 11 (1965).
(5) J. W. Longworth, R. O. Rahn, and R. G. Shulman, *J. Chem. Phys.*, **45**, 2930 (1966).
(6) V. Kleinwächter, J. Drobnik, and L. Augenstein, *Photochem. Photobiol.*, **6**, 133 (1967).
(7) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).
(8) W. Voelter, R. Records, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 6163 (1968).
(9) P. R. Callis, E. J. Rosa, and W. T. Simpson, *ibid.*, **86**, 2292 (1964).
(10) T. Yamada and H. Fukutome, *Biopolymers*, **6**, 43 (1968).
(11) R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255 (1963).
(12) Y. Tanizaki, *Bull. Chem. Soc. Jap.*, **32**, 75 (1959).
(13) Y. Tanizaki, *ibid.*, **38**, 1798 (1965).
(14) Y. Tanizaki and S. Kubodera, *J. Mol. Spectrosc.*, **24**, 1 (1967).
(15) H. Labhart, *Tetrahedron, Suppl.*, **2**, 223 (1963).
(16) H. H. Chen and L. B. Clark, *J. Chem. Phys.*, **51**, 1862 (1969).

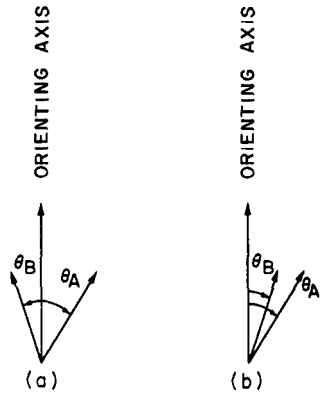

Figure 1. Relative dispositions of transition moments **A** and **B**: a, on opposite sides of the orienting axis; b, on the same side of the orienting axis.

$$R_d = D_{||}/D_\perp$$

$$r = \cot^{-1}\theta$$

$D_{||}$ and $D_\perp$ are the measured optical densities of the partially oriented molecules with the incident radiation parallel and perpendicular to the stretch axis and θ is the angle between the transition moment and the orienting axis. The assumption that the orienting axis is unique can be tested (*vide infra*).

The ambiguity inherent in this method is illustrated in Figure 1. The angle between the orienting axis and the transition moment can be evaluated in magnitude only. Thus, if the transition moment vector for a particular transition, **A**, is placed to the right of the orienting axis as shown in Figure 1, the vector corresponding to a transition at a different energy, **B**, can lie on the same side of the orienting axis as **A**, or the two may be on opposite sides of the orienting axis. The data obtained with a single species cannot be used to distinguish the two possibilities, but if the orienting axis is changed by a substituent which *does not change the transition moment directions*, it is possible to resolve this ambiguity. Either $\theta_A + \theta_B$ or $\theta_A - \theta_B$ will be unaltered by substitution.

In this treatment, coplanarity of the transition moments and orienting axis has been assumed. The orienting axis of planar molecules will satisfy this requirement if the transitions are π–π^*.[14] If a substituent destroys the planar symmetry of the molecule in the sense that the orienting axis and the transition moments are not coplanar, this simple procedure for resolving the ambiguity becomes invalid. A case in point is the cytosine–cytidine pair. The sugar group of the cytidine does not appreciably interfere with the electronic structure of the π system but does destroy the total planar conformation of the molecule. If, however, the absorption spectra of the compound and its nonplanar derivative display three or more distinct transitions, it is possible to resolve the ambiguity by means of a cone analysis which is described elsewhere.[1a]

A direct procedure for the testing of the uniqueness of the orienting axis can be employed.[17–19] The distribution of partially oriented molecules is equivalent to a fraction (f) of molecules totally oriented and a fraction ($1 - f$) randomly oriented. For a totally oriented sample the dichroic ratio would be

$$R_{d\infty} = \epsilon_{||}/\epsilon_\perp$$

and, for a randomly oriented sample (as in an unstretched sheet)

$$R_d = \frac{{}^1/_3(\epsilon_{||} + 2\epsilon_\perp)}{{}^1/_3(\epsilon_{||} + 2\epsilon_\perp)} = 1$$

where $\epsilon_{||}$ and $\epsilon_\perp$ are the extinction coefficients of the totally oriented sample. For a sample which possesses partial orientation

$$R_d = \frac{f\epsilon_{||} + {}^1/_3(1-f)(\epsilon_{||} + 2\epsilon_\perp)}{f\epsilon_\perp + {}^1/_3(1-f)(\epsilon_{||} + 2\epsilon_\perp)}$$

(17) R. D. B. Fraser, *J. Chem. Phys.*, **21**, 1511 (1953).
(18) R. D. B. Fraser, *ibid.*, 24, 89 (1956).
(19) M. Beer, *Proc. Roy. Soc., Ser. A.*, **506**, 136 (1956).

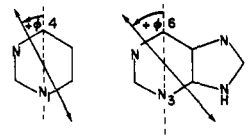

Figure 2. Conventions for transition moment directions of purines and pyrimidines (ref 20).

Substitution and rearrangement gives

$$f = \frac{(R_d - 1)(R_{d\infty} + 2)}{(R_{d\infty} - 1)(R_d + 2)}$$

where $R_{d\infty} = 2r^2$. For molecules which possess a unique orienting axis, f will be a function only of the stretch ratio and will be independent of wavelength. For the molecules studied here, the f values, at a given stretch ratio, are constant to 2% over the entire range of wavelengths.[1a]

Sample Preparation. The solute and PVA powder were codissolved in water and transferred onto a glass plate. After air drying the solution for 36 hr, the PVA sheet was torn from the plate. Two pairs of marks were made on the sheet with a pen at 1-cm intervals, one pair parallel and one pair normal to the stretch direction. After stretching, the intervals between the two marks of each pair were remeasured. The ratio of the long (a) and short intervals (b) is the stretch ratio, R_s. Tanizaki assumed that an imaginary sphere distorts into an ellipsoid of revolution of equal volume. The following should then hold

$$(4\pi/3)(1)^3 = (4\pi/3)(ab^2)$$

This expression was verified experimentally, within 5%.

The thickness and uniformity of thickness of each sheet were determined before and after stretching, with a micrometer. The thickness is a measure of the radius of the sphere and semiminor axis of the ellipsoid, respectively. Hence, the ratio of the thickness before and after stretching should equal the ratio of the shortened interval before and after stretching. Again, this was verified, within 7%.

Spectral Measurements. All measurements were made on a Cary 14 spectrophotometer with a Glan-Taylor polarizer in the sample beam. The sample was secured in a rotatable holder and the optical densities were measured with the stretch axis parallel and perpendicular to the plane of the polarized light. To eliminate base-line drift and other instrumental fluctuations, both parallel and perpendicular measurements were made in a manual mode, at 2.5-nm intervals in the absorption regions and 10-nm intervals in the absorption windows. No film was in the reference beam. The absorption of the calcite polarizer limits the measurements to $\lambda \geq 215$ nm, but all of the qualitative features of the spectra have been checked down to 190 nm with MgF_2 polarizers (Rehovoth Instruments) in a Cary 15 spectrophotometer. To correct for PVA and polarizer absorption and reflection, identical measurements were carried out on pure PVA samples of varying thickness and ratio.

Results

The conventions for describing the polarization directions follow DeVoe and Tinoco (Figure 2).[20] Fig-

Table I

Compound	θ_A, deg	θ_B, deg	θ_C, deg
Adenine	45.8	48.8	
9-Methyladenine	43.7	50.9	
Adenosine	44.9	50.4	
Guanine	51.2	41.8	43.6
Guanosine	48.5	46.4	45.0
Uracil	47.2	50.5	
Thymine	48.6	47.2	
Uridine	46.2	49.7	
Cytosine	46.1	44.5	49.3
Cytidine	44.8	45.7	49.3

(20) H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 500 (1962).

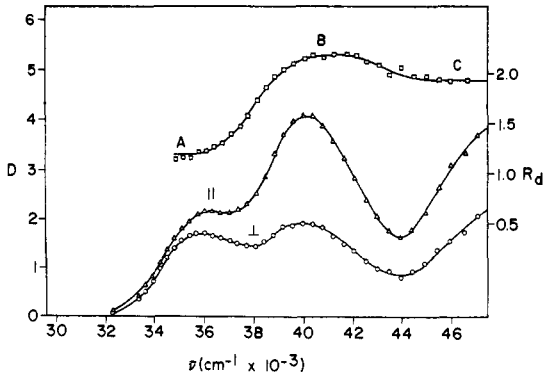

Figure 3. Spectrum of guanine in stretched PVA: Δ, polarizer parallel to stretch direction; O, polarizer perpendicular to the stretch direction; □, $R_d = D_\parallel/D_\perp$ (the curve represents R_d *vs.* $\bar{\nu}$ as averaged over several spectra). D is measured on an arbitrary scale.

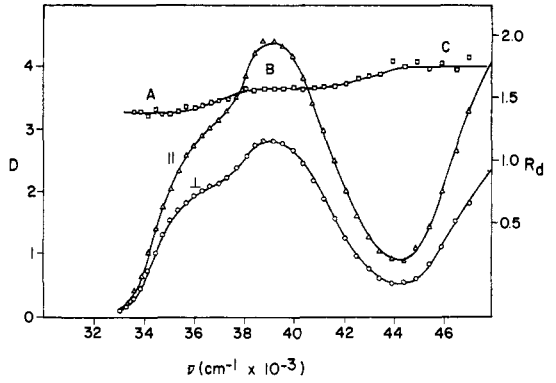

Figure 4. Spectrum of guanosine in stretched PVA. Symbols as in Figure 3.

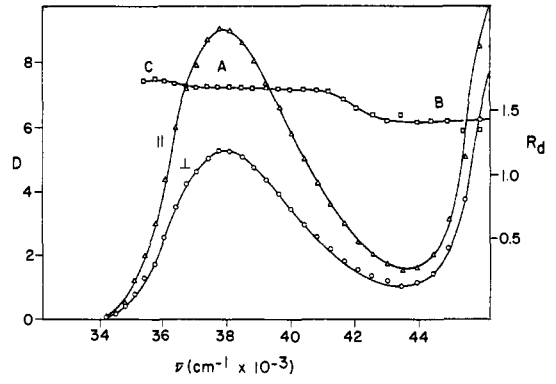

Figure 5. Spectrum of adenine in stretched PVA. Symbols as in Figure 3.

ures 3–9 show representative dichroic spectra. The R_d curves were constructed to reflect the data for all the dichroic spectra measured and therefore are not always the best fit for the particular spectrum shown. Table I summarizes the results for all the compounds studied. The orienting axes for the nucleosides are, in general, not coplanar with transition moments, while those for the bases are coplanar. The regions of suspected hidden transitions are omitted and will be treated in the Discussion section.

Adenine. It is seen from Table I that the possible transition moment direction schemes are, for adenine

(1) $\theta_A + \theta_B = 94.6°$

(2) $|\theta_A - \theta_B| = 3.0°$

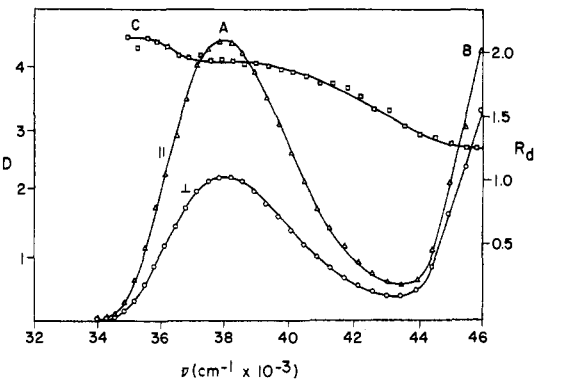

Figure 6. Spectrum of 9-methyladenine in stretched PVA. Symbols as in Figure 3.

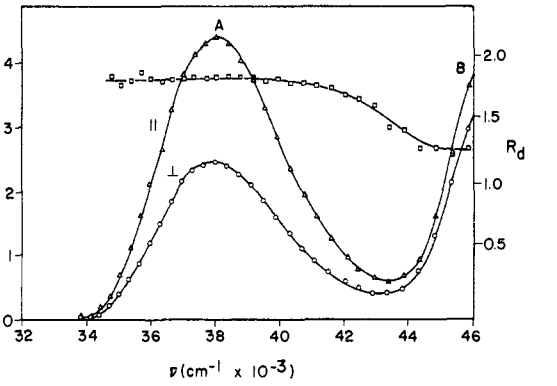

Figure 7. Spectrum of adenosine in stretched PVA. Symbols as in Figure 3.

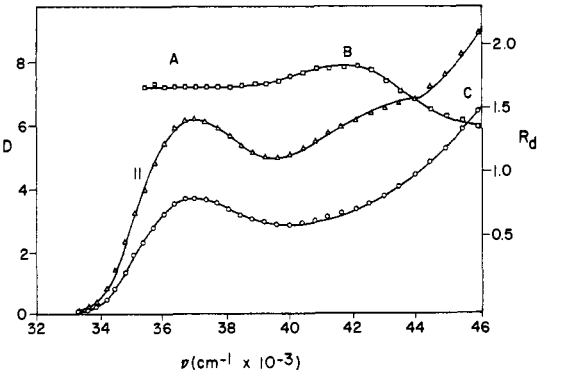

Figure 8. Spectrum of cytosine in stretched PVA. Symbols as in Figure 3.

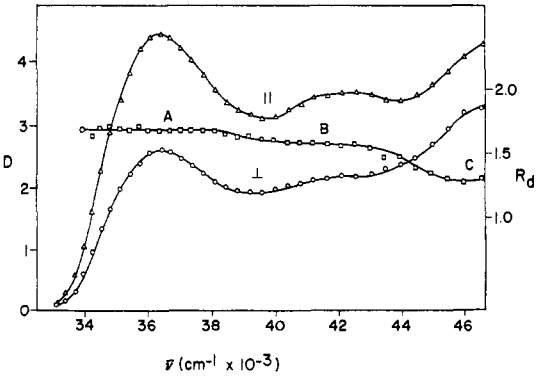

Figure 9. Spectrum of cytidine in stretched PVA. Symbols as in Figure 3.

and for 9-methyladenine

(1) $\theta_A + \theta_B = 94.6°$

(2) $|\theta_A - \theta_B| = 7.2°$

Assuming no perturbation of the transition moment by the methyl group, the correct choice is $\theta_A + \theta_B = 94.6°$; *i.e.*, the A and B transitions are nearly perpendicular.

Guanine. Since the guanine system possesses three distinct transitions and guanosine is nonplanar, a cone analysis is required to resolve the ambiguity among the four possible transition moment direction schemes of guanine. They are

(1) $\theta_A + \theta_B = 93.0°$; $\theta_A + \theta_C = 94.8°$

(2) $\theta_A + \theta_B = 93.0°$; $|\theta_A - \theta_C| = 7.6°$

(3) $|\theta_A - \theta_B| = 9.4°$; $\theta_A + \theta_C = 94.8°$

(4) $|\theta_A - \theta_B| = 9.4°$; $|\theta_A - \theta_C| = 7.6°$

This analysis leads directly to the choice of (1) as the correct scheme.

Cytosine. The four possible transition moment direction schemes are

(1) $\theta_A + \theta_B = 90.6°$; $\theta_A + \theta_C = 95.4°$

(2) $\theta_A + \theta_B = 90.6°$; $|\theta_A - \theta_C| = 3.2°$

(3) $|\theta_A - \theta_B| = 1.6°$; $\theta_A + \theta_C = 95.4°$

(4) $|\theta_A - \theta_B| = 1.6°$; $|\theta_A - \theta_C| = 3.2°$

Since the cytosine system possesses three distinct transitions and cytidine is nonplanar, the cone analysis can be used to resolve the ambiguity. However, schemes 2 and 3 are both reasonable if allowance for experimental error is made. This will be discussed below.

Uracil. The transition moment direction schemes for this system are, for uracil

(1) $\theta_A + \theta_B = 97.7°$

(2) $|\theta_A - \theta_B| = 3.3°$

and for thymine

(1) $\theta_A + \theta_B = 95.8°$

(2) $|\theta_A - \theta_B| = 1.4°$

No choice can be made in this case and, with only two transitions, the cone analysis is inapplicable. The failure of the method in this case can be easily rationalized. Methyl substitution on uracil changes the direction of **A** by at least 19° [21] and it would be surprising if the angle between **A** and **B** was left unchanged.

Discussion

Information about the transition moment directions in the nucleic acid bases is fragmentary and scattered. Callis has collected the results available in 1966.[22] The major additions to the literature in the interim have been the CD[23-26] and MCD[8] spectra.

In the absence of single-crystal studies, only relative polarizations can be inferred, and the possible influence of intermolecular interactions requires a cautious attitude toward the single crystal interpretations. We will, nevertheless, assume the validity of the absolute polarization directions, when available, in relating our relative polarization results to the molecular framework.

Guanine. The cone analysis yields an unambiguous result for guanine, wherein the A band is roughly perpendicular to the B and C bands, in accord with the photoselection studies of the A and B bands.[9,27] No absolute polarization data are available for guanine or its derivatives.

Børresen[27] has argued for a third transition in the A and B regions of the guanine spectrum based upon photoselection studies of protonated guanine and guanosine. Our results give no indication of this, and MCD spectral studies of guanosine[8] suggest the presence of only two transitions in this region.

Cytosine. The cone analysis for the cytosine system leads to two possible polarization schemes. In both cases the B and C bands are approximately perpendicular. If the results of the polarized specular reflection studies of cytosine and 1-methylcytosine,[22] which indicate approximate parallel polarizations for the A and B bands, are applicable to the isolated molecules, the ambiguity is removed and the correct relative scheme becomes

$$|\theta_A - \theta_B| = 1.6°; \theta_A + \theta_C = 95.4$$

This conclusion is consistent with the photoselection studies of 5-methylcytosine[22] which indicate that the A and B bands are clearly not perpendicular.

Figure 10 shows the absolute polarization scheme for cytosine. The B and C bands are related to the A-band polarization as determined by .single-crystal studies.[22] Since our results give only the relative polarizations, there is an uncertainty in the orientation of the B and C bands with respect to the A band. The angles can be measured either clockwise or counterclockwise from the A band.

There is controversy regarding the origin of the B band of cytosine. Some workers consider this transition to be a nitrogen $n \rightarrow \pi^*$ type[24] because of the large blue shift upon protonation. However, the large value of R_d for the B band of cytosine precludes an allowed nitrogen $n \rightarrow \pi^*$ transition. Although carbonyl-localized transitions ($n \rightarrow \pi^*$ or $n \rightarrow \sigma^*$) cannot be ruled out by polarization arguments, it is unlikely that carbonyl-localized transitions could possess such large intensities. Even considering extensive mixing with intramolecular charge-transfer states, an intensity of two orders of magnitude less than the observed value is predicted.[28] Hence, it is likely that the B transition of cytosine is $\pi \rightarrow \pi^*$ in nature. On the basis of CD studies of 2′-deoxycytidine, Miles, *et al.*,[29] have revised their assignment for this transition and now agree that its origin is probably $\pi \rightarrow \pi^*$.

(21) W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, 53, 2164 (1970).
(22) (a) P. R. Callis, Ph.D. Thesis, University of Washington, 1966; P. R. Callis and W. T. Simpson, *J. Amer. Chem. Soc.*, 92, 3593 (1970).
(23) D. W. Miles, R. K. Robins, and H. Eyring, *J. Phys. Chem.*, 71, 3931 (1967).
(24) D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U. S.*, 57, 1138 (1967).
(25) D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, 91, 824 (1969).
(26) D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *ibid.*, 91, 831 (1969).
(27) H. C. Børresen, *Acta Chem. Scand.*, 21, 920 (1967).
(28) M. Vala, Jr., and J. Tanaka, *Bull. Chem. Soc. Jap.*, 41, 2548 (1968).
(29) D. W. Miles, W. H. Inskeep, M. J. Robins, M. W. Winkley, R. K. Robins, and H. Eyring, *Int. J. Quantum Chem., Symp.*, 3, 129 (1969).

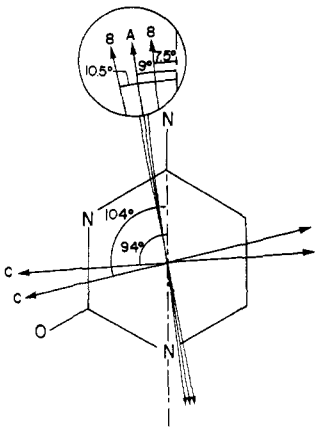

Figure 10. The transition moment directions in cytosine. The two possible orientations of B and C are indicated.

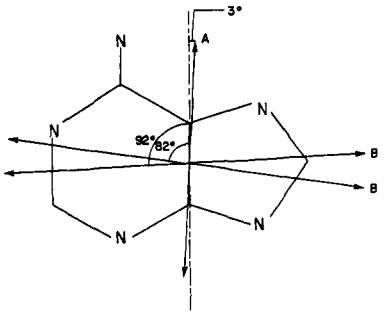

Figure 11. The transition moment directions in adenine. The two possible orientations of B are indicated.

Adenine. The dichroic analysis of the spectra of adenine and 9-methyladenine clearly demonstrates that the polarizations of the A and B bands are approximately perpendicular. These results are consistent with the single-crystal study of 9-methyladenine which indicates that the A and B bands are polarized at least 45° from each other.[11] Figure 11 shows a reasonable polarization scheme for adenine. The absolute orientation of the A band was obtained from the 9-methyladenine single-crystal study of Stewart and Davidson.[11]

Theoretical treatments of adenine predict two $\pi \rightarrow \pi^*$ transitions in the 260-nm spectral region.[30-34] Based on this, workers have attempted to find and characterize this so-called hidden transition. The existence of the second transition has been demonstrated in recent MCD studies of adenine[8] which clearly show two transitions under the A band, one on either side of the absorption maximum. However, the positions and intensities have not, as yet, been determined.

A change in dichroism along the short-wavelength tail of the A band in adenine, 9-methyladenine, and adenosine is observed. It is unlikely that this is due to B-band overlap, since this would require an inordinately long red tail. Callis, *et al.*,[9] have also observed this change in polarization in their photoselection study of adenine. They concluded that this was probably caused by the presence of a hidden transition under the short-wavelength tail. They have estimated that this hidden transition would have a maximum extinction coefficient about one-tenth that of the intense 260-nm transition. This analysis is consistent with the correlation studies of Clark and Tinoco[4] and Mason.[7]

The dichroism increases on the long-wavelength tail of both the adenine and 9-methyladenine spectra. The fluorescence polarization results also exhibit this increase.[22] This increase in R_d cannot be ascribed to the presence of a nitrogen $n \rightarrow \pi^*$ transition since out-of-plane polarization would result in a decrease in the dichroic ratio. It is possible that this change in dichroism is caused by the presence of the $\pi \rightarrow \pi^*$ hidden transition under the long-wavelength tail. This assignment is supported by the fluorescence lifetime studies of adenylic acid (AMP), where the observed lifetime is some 140 times greater than the lifetime calculated from the absorption intensity, while the observed and calculated lifetimes of the other nucleotides differ by a factor no greater than 5.[35] The fluorescence excitation spectrum of adenine exhibits a marked enhancement at 280 nm, on the red side of the main 270-nm maximum,[5] strengthening arguments for this interpretation.

It becomes evident that a case can be presented for placing the hidden transition on either side of the band peak. These apparently conflicting results can be resolved by considering the two transitions to be nearly degenerate and possessing intensities that are not necessarily greatly disparate, as has been previously assumed (the intensities of the two lowest energy transitions of guanine, for example, differ by a factor of 2 at most). This interpretation would explain the three dichroic ratio regions observed in adenine and 9-methyladenine: on the long-wavelength tail (region C), transition a predominates; in the A (central) region of the band, there is extensive overlap of transitions a and a′ with concomitant mixed polarization; and finally on the short-wavelength tail, transition a′ predominates.

For adenosine, there is no change in dichroism along the long-wavelength tail. This could result from transition a red shifting and/or transition a′ blue shifting. Along the short-wavelength tail of adenosine, a much less pronounced dichroic ratio change is observed, and this is the expected result if the two transitions are more nearly degenerate. Moreover, the fluorescence spectrum of adenosine is red shifted and is significantly different in intensity and structure from the adenine fluorescence. This implies that the shifting has resulted in a reversal of transitions a and a′.

The disparity between the observed and calculated fluorescence lifetimes of adenine and its derivatives might be partly due to the fluorescence originating from only one of the states, while the calculated lifetime is based upon the total intensity of the two transitions.

Reliability of Calculated Transition Moments. The ease with which semiempirical calculations of the Pariser–Parr–Pople type can be made has led to a profusion of attempts to fit the spectra of the nucleic acid bases.[30-34] The calculated transition energies are somewhat sensitive to the parameter choice, and the transition intensities are more so.[34] Johnson and Tinoco,[3] using the SCF coefficients provided by Berthod,

(30) H. Berthod, C. Giessner-Prettre, and A. Pullman, *Int. J. Quantum Chem.*, **1**, 123 (1967).
(31) T. L. Kunii and H. Kuroda, *Rep. Comput. Cent. Univ. Tokyo*, **1**, 227 (1968).
(32) V. A. Kuprievich, *Int. J. Quantum Chem.*, **1**, 561 (1967).
(33) J. Ladik and K. Appel, *Theor. Chim. Acta*, **4**, 132 (1966).
(34) M. L. Bailey, *ibid.*, **16**, 309 (1970).
(35) J. Eisinger, *Photochem. Photobiol.*, 7, 597 (1968).

Giessner-Prettre, and Pullman,[30] computed different transition moment directions. In the zero-differential-overlap approximation, the "monopole" calculation in ref 3 is identical with the method used in ref 30. We have no explanation for this discrepancy. The experimental moments are compared in Table II with transition moments calculated by two different parameter choices, and it is evident that confidence in the theoretical moments is unwarranted. The discrepancies are particularly apparent for guanine and cytosine. The computed angle between the A and B guanine transitions is 74 or 66°, compared to the experimental 87°. In cytosine the angle between A and B is calculated as 71 or 51°. The experimental value is 2°. Even if we assume that the single-crystal results are unreliable and cannot be used to choose between the two alternatives presented by the cone analysis, the disagreement remains, since in both instances B and C are nearly perpendicular (87°), compared to the calculated difference, ≃40°.

Table II

Compound	Transitions	φ, deg: Exptl	φ, deg: *a*	φ, deg: *b*
Adenine	A	−3	37	?
			52	94
	B	82 or 92	98	48
Uracil	A	−19	9	81
			9	133
	B	71	50	34
Cytosine	A	9	68	50
	B	7.5 or 10.5	−3	−1
	C	94 or 104	136	141
Guanine	A	φ[c]	110	106
	B	(φ + 90)	36	40
	C	(φ + 90)	126	125

[a] Reference 30. [b] Reference 31. [c] The absolute directions in guanine are unknown.

Conclusion

Stretched-film spectra and fluorescence polarization measurements provide essentially the same qualitative information, and wherever fluorescence polarization information is available we have found essential agreement between the results of the two methods. It is easier to extract quantitative information about the relative transition moment directions from the stretched-film spectra. Higher resolution is also possible in absorption than in excitation spectra. Of course, in the absence of fluorescence, the fluorescence-polarization technique is inapplicable.

The calculation of transition moment directions by semiempirical procedures is fraught with uncertainty, and sufficient experimental information on the nucleic acid bases is not yet available to use in the calculations of interactions between the bases.

Acknowledgments. We thank Dr. Kurt Rosenheck of the Polymer Department, Weizmann Institute, Rehovot, Israel, for providing the MgF_2 polarizers. This work was supported by research grants from the U. S. Public Health Service (No. GM-13218 and EY-00064).

Establishment of an Optical Scale for Lewis Basicity in Inorganic Oxyacids, Molten Salts, and Glasses

J. A. Duffy* and M. D. Ingram

Contribution from the Department of Chemistry, University of Aberdeen, Aberdeen AB9 2UE, Scotland. *Received March 10, 1971*

Abstract: Nephelauxetic effects (measured from uv spectra) in the outer s and p orbitals of the probe ions Tl^+, Pb^{2+}, and Bi^{3+} are used for setting up scales of Lewis basicity for oxide systems. Values of Lewis basicity, expressed as "optical basicities," are assigned to a variety of oxide media including oxyanion glasses and oxyacids, thereby making it possible to compare the basicity of media for which comparisons based upon the Brønsted–Lowry or Lux–Flood concepts are not feasible. A critical evaluation of optical basicity is made in comparison with the conventional Lux–Flood approach to basicity in oxyanion media. The relevance of electronegativity to optical basicity is also considered. Scales for molten halides are also proposed, and from the optical basicities for the LiCl–KCl (41 % KCl) melt and the NaCl–$AlCl_3$ (67 % $AlCl_3$) melt, it is apparent that chloride is affected in a similar way to oxide by highly polarizing cations, but to a lesser extent.

The properties of solvent systems are often regarded in terms of acid–base behavior which, for protonic solvents, can be expressed quantitatively by referring to a scale such as pH or the Hammett acidity function.[1] For aprotic solvents, the expression of acidity or basicity in quantitative terms is less straightforward. One of the most general approaches to acid–base behavior is that due to Lewis,[2] although in the field of molten salt and glass chemistry, it has not proved very useful, and its application has been entirely qualitative.[3] The reason that it has not been applied quantitatively is that, until

(1) L. P. Hammett, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1970, pp 267–272.

(2) G. N. Lewis, *J. Franklin Inst.*, **226**, 293 (1938).

(3) W. A. Weyl and E. G. Marboe, "The Constitution of Glasses," Vol. 1, Interscience, New York, N. Y., 1962, Chapter 5.