predicted for $\Sigma_N$ are consistent with the experimental ESR data, since the predicted $2s_N$ unpaired electron population is rather small.

Given that the present results indicate that conjugation is far less important to the formamido free radical than it is to the parent formamide system, one would expect that the N-H bond dissociation energy in the latter should be greater than in nonconjugated amines. This expectation is substantiated by STO-3G calculations which predict that the bond energy of formamide is some 7.4 kcal $mol^{-1}$ *greater* than calculated[12] for the reaction $CH_3NH_2 \rightarrow CH_3NH\cdot + H\cdot$. Since the difference in dissociation energies is smaller than is the difference in CN bond rotation barriers, however, there must exist energy changes in the $\sigma$ bonds which occur upon dissociation and which differentially favor the conjugated over the saturated systems.

In conclusion, the ab initio calculations described above predict that the ground state of the formamido radical is almost doubly degenerate. The two lowest states are of identical symmetry in the nonplanar geometry (which is probably preferred by one of them at least), but of different symmetry, $\Sigma$ and $\Pi$, in the planar conformations. Further, the $\Pi$ state is predicted to possess two potential minima which are almost degenerate also, whereas the second geometry for the $\Sigma$ state lies significantly above the first in energy. Presumably the two low-lying states would both be populated except at the lowest temperatures if the predicted energetics are close to correct. It should be emphasized that no final conclusions concerning relative stabilities of closely spaced states can be made from these calculations. More advanced calculations, which would include both polarization functions and configuration interaction, are required before definitive conclusions can be drawn. (In addition, the *relative* stability order presumably can be switched by substituting suitable groups for the hydrogen atoms.) According to present calculations, the allylic form $\Pi_a$ evidently does not correspond to a potential minimum on the energy surface for the ground state. Thus $\Pi_N$ and $\Pi_O$ do not represent merely valence-bond structures which contribute to a resonance hybrid, but correspond closely to the dominant structures at the two geometries which are potential minima on the $\Pi$ surface. The quantum-mechanical reason for this behavior is not clear.

### References and Notes


(1) Research supported by the National Research Council of Canada.
(2) See (a) T. Koenig, J. A. Hoobler, C. E. Klopfenstein, G. Hedden, F. Sunderman, and B. R. Russell, *J. Am. Chem. Soc.*, **96**, 4573 (1974); (b) W. C. Danen and R. W. Gellert, *ibid.*, **94**, 6853 (1972), and references cited therein.
(3) E. Evleth, P. Horowitz, and T. Lee, *J. Am. Chem. Soc.*, **95**, 7948 (1973).
(4) C. C. J. Roothaan, *Rev. Mod. Phys.*, **32**, 179 (1960).
(5) (a) W. J. Hehre, R. F. Stewart, and J. A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969); (b) R. Ditchfield, W. J. Hehre, and J. A. Pople, *ibid.*, **54**, 724 (1971).
(6) W. A. Lathan, W. J. Hehre, L. A. Curtiss, and J. A. Pople, *J. Am. Chem. Soc.*, **93**, 6377 (1971).
(7) C. F. Bender and H. F. Schaefer III, *J. Chem. Phys.*, **55**, 4798 (1971).
(8) W. J. Hehre, R. Ditchfield, L. Radom, and J. A. Pople, *J. Am. Chem. Soc.*, **92**, 4796 (1970).
(9) J. E. Del Bene, G. T. Worth, F. T. Marchese, and M. E. Conrad, *Theor. Chim. Acta*, **36**, 195 (1973).
(10) N. C. Baird and H. B. Kathpal, unpublished calculations.
(11) See K. Van Der Meer and J. J. C. Mulder, *Chem. Phys. Lett.*, **34**, 189 (1975), and references cited therein.
(12) Absolute values for the dissociation energies are not quoted here, as they are likely to be quite inaccurate in calculations which do not include configuration interaction. See, for example, the results in W. A. Lathan, W. J. Hehre, and J. A. Pople, *J. Am. Chem. Soc.*, **93**, 808 (1971).


# Optical Activity of Uridylyl-(3′,5′)-adenosine. Crystal Structures and Solution Conformation


**Neil P. Johnson, Eugene Switkes,* and Thomas Schleich**

*Contribution from the Division of Natural Sciences, University of California, Santa Cruz, California 95064.*

*Received December 17, 1975*


**Abstract:** Circular dichroism and absorption spectra have been calculated for neutral and protonated uridylyl-(3′,5′)-adenosine (UpA) in each of the observed crystalline forms and in two model dinucleotide geometries for the purpose of assessing the importance of crystal structure contributions to the solution conformation of this dinucleotide. Comparison of the calculated optical properties with experimental spectra indicates that the crystal geometries are not the dominant structures in any distribution of conformations which the molecule assumes in aqueous neutral solution. However, one of the crystal structures may be important at low pH. The model dinucleotide geometries give spectral shapes similar to those observed experimentally, but with larger intensities. The optical activity was calculated by both the coupled oscillator and generalized susceptibility methods utilizing monomer parameters from either experimental data or theoretical $\pi$-electron calculations. These methods are compared and the geometric origin of the UpA circular dichroism is discussed.


## I. Introduction

Of the three published dinucleotide crystal structures,[1-4] only that of uridylyl-(3′,5′)-adenosine (UpA) is marked by conformations distinctly different from model RNA[5] or DNA[6] dinucleotide geometries. Two UpA structures, designated UpA 1 and UpA 2, are found in the unit cell and are characterized by open conformations with a distance between bases ($R_{12}$) of 6.8 and 11.8 Å, respectively. By contrast, crystalline adenylyl-(3′,5′)-uridine (ApU) adopts a right-handed incipient helical conformation[3] similar to the RNA 11 form ($R_{12} \approx 4.6$ Å). It has been suggested that the UpA crystal structures are two of seven basic conformations from which the secondary structures of all polynucleotides can be generated[7] and that UpA 1 may be an important component in the structure of tRNA loops.[1,2,7] In this paper we utilize optical criteria to investigate whether the UpA conformations found in the crystal contribute to the conformation(s) inferred to be present in aqueous solution.

Comparison of the predicted optical properties of UpA 1 and UpA 2 with the solution spectra is important for several reasons. Conformational energy calculations indicate a broad

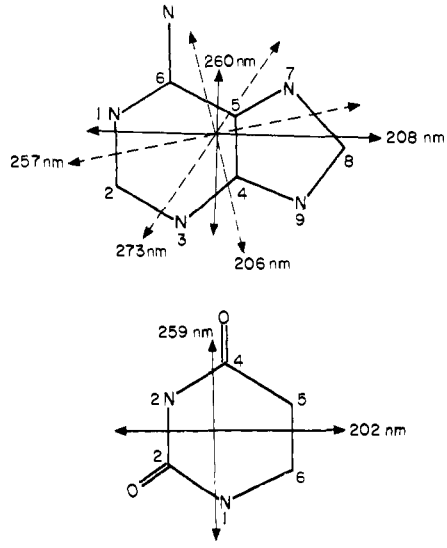


Figure 1. Numbering convention used for adenine and uracil with experimental transition energies and polarizations (— unprotonated, - - - - protonated, see Table I).

global minimum near the RNA polymer geometry with a higher energy for the crystal forms.[8] While these calculations are meaningful for the conformation in the absence of crystal forces, they do not consider interactions of water with the dinucleotide, which, for the uncharged nucleic acid forms, may stabilize the compact RNA and DNA conformations at the expense of the open UpA 1 and UpA 2 structures. Our analysis provides an estimate of the contribution of the UpA 1 and UpA 2 structures to the dinucleotide conformation in aqueous solution. Furthermore, because of the relatively small difference between the optical properties of UpA and its constituent mononucleotides, it has been assumed that UpA is substantially less stacked than other dinucleotides.[9-13] Previous calculations of the circular dichroism (CD) of unprotonated UpA reveal that the major features of the solution spectrum can be predicted from interactions between bases oriented in a stacked, right-handed, incipient helical conformation.[14,15] However, the calculated spectra are larger than the experimental values at room temperature by a factor of 10. The present study explores whether the observed low UpA optical activity could originate from a single solution conformation similar to the UpA 1 or UpA 2 structures. Since in the x-ray study the crystals were grown in an acid solution, we also consider the effects of protonation on the optical activity of UpA. To insure the greatest credibility the calculations were performed by a variety of methods. These include the use of purely experimental monomer transition parameters in addition to theoretical values similar to those of previous studies. In order to estimate the effect of small conformational changes and to illustrate how low optical activity can occur even in a highly stacked structure, we discuss the origin of the CD for each conformation. The specifics of the methods are outlined in section II, the results of the CD calculations and their implications for the structure of UpA are presented in section III, the various methods of calculating CD are compared in section IV, and the calculated optical properties of uracil, adenine, and protonated adenine are discussed in section V.

## II. Calculations

The structures and numbering schemes for uracil and adenine are shown in Figure 1. The circular dichroism was calculated for four conformations: the two crystal structures, UpA 1 and UpA 2; UpA with the bases parallel and ten bases per turn as reported for the B form of DNA;[6] and UpA with 11 bases per turn as reported for RNA 11.[5] We employed the coupled oscillator theory as developed by Johnson and Tinoco (JT)[14] and the generalized susceptibility method (GS).[16,17] Only $\pi$-$\pi^*$ transitions were explicitly included. All electron calculations show low-lying n-$\pi^*$ (250 nm) and higher energy $\sigma$-$\pi^*$ (205 nm) transitions for adenine and uracil.[18] Hug and Tinoco have pointed out that the n-$\pi^*$ transitions contribute to the CD of the mononucleotides, but that interactions among $\pi$-$\pi^*$ transitions of the bases should dominate the CD of the dinucleotide[18] as long as the bases are approximately parallel, as in the four geometries under consideration. Thus the low-energy in-plane transitions should give a reasonable estimate of the near ultraviolet CD spectrum of UpA.

Calculation of the generalized susceptibility is the focus of the GS methods.[16,17] This quantity relates the size and direction of the induced electric (magnetic) moment of the molecule to the perturbing magnetic (electric) field of the radiation. The wave function of the dimer is written as a Hartree product and the monomers interact because the field perturbing each base includes components from both the external disturbance and induced moments on the other base. Monomer interactions are computed by means of the dipole-dipole potential, which may be evaluated with transition moments either from theoretical models or from experiment. We have used the elegant matrix formulation of Harris[16] where, from a single complex susceptibility, one can evaluate absorption, circular dichroism, optical rotatory dispersion, and polarizability. Harris and Schneider[19] have used the GS method to study the optical activity of ApA and UpU. We have followed their method of calculation with the following modifications. In calculations using the particle-in-a-rectangular-box model for the monomer, the box dimensions were calibrated to match the low-energy bands of adenine and uracil and the boxes were positioned so that their centers and longer dimensions corresponded to the center and low-energy transition directions of the bases in the dimer geometries. We have also calculated the monomer susceptibilities using experimental energies, half-widths, transition directions, and oscillator strengths (Table I) for the low-energy $\pi$-$\pi^*$ transitions on each monomer. We solved the polymer matrix equations directly rather than using the specific dimer form.[19] This approach will allow us to consider in future studies the effects of general polymer interactions on optical spectra. The results of the GS calculation using experimental transition properties are presented for each geometry in Figures 2 and 3.

The JT method[14] for calculating optical activity for dinucleotides advances earlier coupled oscillator theories by partitioning the dimer wave function for an excited state into two parts. Monomer states with transition energies at wavelengths above 220 nm are coupled by solving a secular equation constructed from point monopole interaction potentials. The contribution of this part of dimer wave functions to the low-energy CD is calculated from a coupled oscillator expression for rotational strength. The contribution to the CD from higher energy monomer states is obtained from the Kirkwood polarizability expression using polarizabilities arising from transitions below 220 nm. Our CD calculations for UpA used the JT method as published,[14] except for the following alterations: the monomer wave functions described below were used; the transition dipoles were used as calculated rather than scaled to agree with experimental dipole strengths; we included all transitions above 200 nm in the secular determinant; the polarizabilities used to calculate high-energy contributions to the CD were further corrected to include only transitions not considered explicitly; and the CD band shapes were constructed from a weighted sum of Gaussians whose half-widths were determined by fitting the absorption spectra of the monomers to curves centered at the calculated transition energies (Table I). The results of the JT calculations are also presented in Figures 2 and 3.

**Table I.** Results of Molecular Orbital Calculations

| Energy eV (nm) | Oscillator strength | Polarization,[a] deg |
|---|---|---|
| Bailey Parameterization[b] | | |
| Adenine | | |
| 4.638 (267.3) | 0.02 | −25 |
| 4.774 (259.7) | 0.43 | 38 |
| 5.825 (212.8) | 0.50 | −54 |
| 5.993 (206.9) | 0.35 | −94 |
| Protonated adenine | | |
| 4.421 (280.4) | 0.27 | −2 |
| 4.756 (260.7) | 0.15 | −118 |
| 5.504 (225.3) | 0.60 | −94 |
| 6.647 (205.0) | 0.12 | 15 |
| Uracil | | |
| 4.824 (257.0) | 0.33 | −3 |
| 5.647 (219.6) | 0.18 | −70 |
| 5.786 (214.3) | 0.41 | −166 |
| 6.476 (191.4) | 0.63 | −43 |
| Adams and Miller Parameterization[c] | | |
| Adenine | | |
| 4.496 (275.8) | 0.09 | 24 |
| 4.882 (254.0) | 0.38 | 58 |
| 5.726 (216.5) | 0.02 | 10 |
| 5.991 (206.9) | 0.90 | −63 |
| Protonated adenine | | |
| 4.567 (271.5) | 0.37 | 14 |
| 5.146 (240.9) | 0.45 | −96 |
| 5.833 (212.5) | 0.02 | −41 |
| 6.624 (187.2) | 0.37 | −68 |
| Uracil | | |
| 4.751 (261.0) | 0.34 | −10 |
| 5.403 (229.4) | 0.02 | −65 |
| 5.887 (210.6) | 0.41 | −98 |
| 6.410 (193.4) | 0.83 | −23 |
| Experimental adenine[d] | | |
| 4.768 (260) | 0.3 | −3[e] |
| 5.961 (208) | 0.4 | 87[f] |
| 6.703 (185) | | |
| Protonated adenine[g] | | |
| 4.538 (273.2) | 0.08 | −28 |
| 4.823 (257.1) | 0.20 | 100[i] or 92 |
| 6.013 (206.2) | 0.16 | 15 |
| Uracil[d] | | |
| 4.787 (259) | 0.2 | 0[i] or 7[h] |
| 6.138 (202) | 0.3 | 90[i] or −83[f] |

[a] Devoe and Tinoco angle convention; H. Devoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 518–527 (1962). [b] Reference 22. [c] Reference 23. [d] Energies and oscillator strengths from L. B. Clark and I. Tinoco, Jr., *J. Am. Chem. Soc.*, **87**, 11–15 (1965) and ref 32. [e] R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255–266 (1965). [f] Reference 37. [g] Reference 31. [h] W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, **53**, 2164–2172 (1970). [i] Used as experimental polarization in this calculation.

Wave functions for the JT calculation were computed by the PPP method[20] with configuration interaction for all singly excited states. The two-center repulsion integrals were approximated according to Nishimoto and Mataga.[21] Transition dipoles were calculated from the length operator in the point dipole approximation. The geometry for the PPP calculation was an average of the four UpA geometries, ignoring all out-of-plane coordinates. Atoms 1, 4, and 5 of uracil and atoms 4, 5, and 7 of adenine were used to specify the reference planes. Two methods were used to calculate the wave functions;[22,23] they treat the core integrals and protonation differently and use slightly different values of the ionization potentials, electron affinities, and repulsion integrals. Both methods have been used previously to calculate the optical activity of nucleic acids.[24,25] The Adams and Miller method[23] (AM) employs an

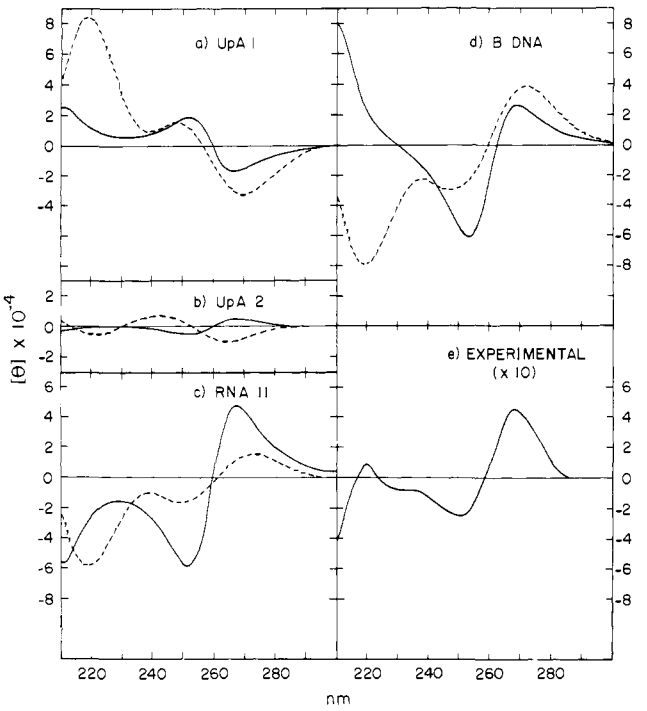


**Figure 2.** Calculated and experimental circular dichroism spectra for unprotonated UpA. (a–d) calculated spectra in the geometries UpA 1, UpA 2, RNA 11, and B DNA, respectively. Spectra calculated using generalized susceptibility method with experimental transition parameters (—). Spectra calculated by coupled oscillator method using Bailey, PPP wave functions (- - - -). (e) Experimental circular dichroism (×10) of UpA pH 7 in aqueous solution. Monomer spectra at the appropriate pH have been subtracted.

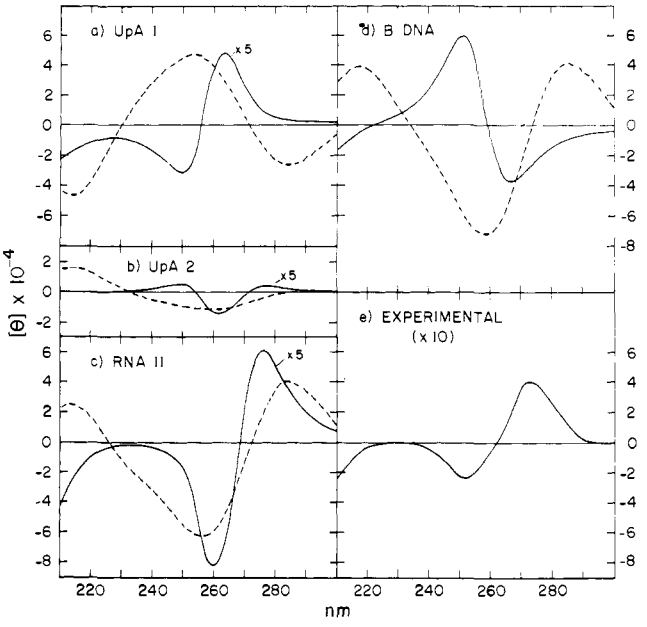


**Figure 3.** Calculated circular dichroism spectra for protonated UpA: a–d as in Figure 2; (e) experimental circular dichroism (×10) of UpA at pH 2 with monomer spectra at the appropriate pH subtracted.

explicitly orthogonalized basis set and computes the core integrals from an empirical expression. The effects of protonation on the AM adenine wave functions are included by adjusting the Slater exponent, ionization potential, and coulomb integral of N(1) in accord with the core charge at the site of protonation.[25] The Bailey method[22] uses an implicitly orthogonalized basis and computes the two-center core integrals for nearest

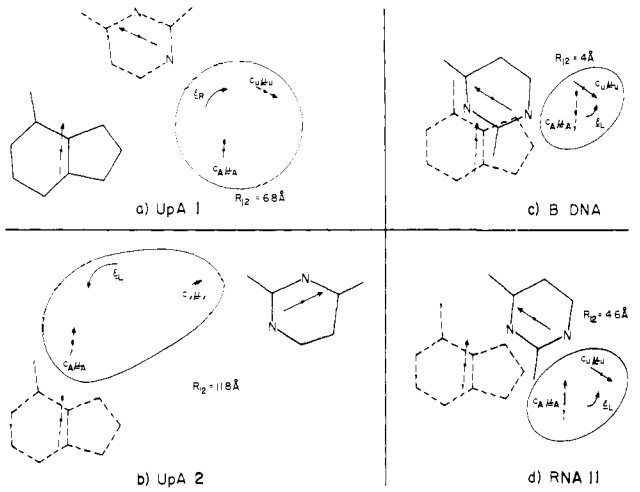


Figure 4. Weighted moment diagrams for unprotonated UpA in the four geometries studied. Bases and transition moments indicated by solid lines lie above those with dotted lines. $R_{12}$ denotes the distance between the centers of the nucleic acid bases. The arrows on the bases are the low-energy experimental monomer transition moments. The encircled region of each panel shows the principal monomer contributions to the lowest energy excited state transition moment of the dinucleotide in the exciton approximation: $\mu_{UpA} = c_A\mu_A + c_U\mu_U$. The curved arrow shows the circular polarization giving larger low-energy absorption by virtue of greater coincidence with the weighted transition moments.

neighbors from the gradient of the overlap integral.[26] In the Bailey PPP calculation the effect of protonation on the $\sigma$ charge density of every atom is included by adjustment of the one-center core integrals.[27] Relevant $\sigma$ charges were obtained from the CNDO results for 9-methyladenine.[28] In the Appendix we list details of the parameterization. Results of the molecular orbital calculations are presented in Table I.

### III. Crystal Structures and Solution Conformation

The experimental solution spectra of neutral and protonated UpA are shown in Figures 2 and 3. The spectra of uridine and adenosine at the appropriate pH have been subtracted from the experimental results to correct for the optical activity due to sugar-base interactions.[14] Although the experimental UpA spectrum is not sensitive to pH, the corrected spectrum shows a slight pH dependence. The circular dichroism of UpA is sufficiently small that uncertainty in the monomer contribution (e.g., due to different glycosyl torsion angles in the monomer and dimer) could introduce errors in the corrected spectra. However, the subtraction procedure still seems the best available to compare spectra calculated using only base-base interactions with experimental results.

The low-energy region of the CD spectra calculated for unprotonated UpA may be compared with experimental results (Figure 2). The calculated spectra for the B DNA and RNA II geometries resemble the neutral solution spectrum as previously reported.[14,15] The sign of the calculated CD of UpA I is opposite to that of the experimental result. For UpA 2 the GS method, but not the JT, gives the correct sign for the low-energy band. Identical conclusions are also reached using the AM wave functions in the JT calculations and the free electron model in the GS calculations. The major differences between the JT and GS results can be traced to differences in the magnitudes and directions of the experimental and theoretical transition moments and will be discussed in section IV.

In spite of the ambiguities of the transition moment directions, two conclusions can be drawn regarding the crystal structures and the conformations of UpA in neutral solution. The calculated spectrum of UpA I is inconsistent with the observed solution spectrum; the intensity of the low-energy transition precludes dominant contributions of this structure to any distribution of conformations UpA may assume in solution. Second, UpA 2 cannot be ruled out as a possible solution conformation on the basis of the calculated CD spectra. However, the low-energy absorption band calculated by the GS method for both the B DNA and RNA II geometries is hypochromic (about 11% hypochromicity) as observed experimentally (3.0%),[9] while that calculated for UpA 2 is slightly hyperchromic (about 0.2% hyperchromicity). This result argues that UpA 2 is not the sole solution conformer.

The CD spectra calculated for the protonated dinucleotide are shown in Figure 3. In the B DNA and RNA II conformations the optical activity calculated using theoretical transition polarizations mimics the observed effects of protonation. In contrast the GS calculations using experimental transition data for protonated adenine (Table I) in the UpA I and RNA II geometries reproduce the main features of the experimental spectrum. Although the observed hypochromicity of UpA is undiminished at pH 1,[9] the absorption spectra calculated for the UpA I and RNA II conformers exhibit less than 1% hypochromicity. On the basis of the experimentally derived transition moment data, stacked structures similar to either RNA II or UpA I are consistent with the observed CD spectrum of the protonated molecule. Molecular models show that small conformational changes transform the bases in the RNA II conformation into their relative position in the B DNA geometry. Since the B DNA conformation gives a strongly hypochromic (10%) absorption spectrum and a low-energy CD opposite to that in the RNA II geometry, slight stacking of the RNA II conformation should cause an increased hypochromicity and a further decrease in the intensity of the CD as in the experimental spectrum.

The CD of UpA in the various conformations can be understood in terms of the directions of the transition moments of the nucleic acid bases. Although the direction of a transition moment in each base is ambiguous to rotation through 180°, the relative direction of the monomer moments multiplied by the mixing coefficients of a dimer excited state is fixed. A simple diagram of the weighted low-energy transition moments of uracil and adenine reveals the geometric dependence of the sign and magnitude of the long wavelength CD band. The sign can be determined by comparing the simultaneous coincidence of the electric field direction at each monomer with the relative direction of the weighted moments in the lowest energy orientation. For example, the sign of the lowest energy band will be positive if the weighted transition moments are in a left-handed helical arrangement. The magnitude of the dichroism will depend on: (1) the magnitudes of the weighted transition moments (the extent these transitions mix in the excited state); (2) the component of the second transition perpendicular to the first (this component, on average, distinguishes the effects of left and right circularly polarized light); and (3) the rotation of the circularly polarized light over the length of the molecule (i.e., $2\pi R_{12}/\lambda_{OA}$ where $\lambda_{OA}$ is the wavelength of the dimer transition O → A and $R_{12}$ the distance between bases). These effects are quantitatively expressed in the coupled oscillator equation for rotational strength[14]

$$R_{OA} = -(\pi/2c\lambda_{OA}) \sum_{a,b} \mathbf{R}_{12} \cdot c_1{}^A\mu_{1a} \times c_2{}^A\mu_{2b}$$

where $a$ and $b$ are sums over the transitions on monomer 1 and 2, respectively, $\mu$ designates the transition moment, and $c_i$ the contribution of the monomer excited state to the dimer excited state.

Diagrams of the weighted transition moments (experimental monomer data) of unprotonated adenine and uracil for the low-energy transition in the UpA 1, UpA 2, B DNA, and RNA II geometries are shown in Figure 4. The basis of our results

are clarified in this figure. For UpA 1 the calculated low-energy band is negative because the weighted moments are in a right-handed helical arrangement. Both weighted moments are fairly large and the angle between them is sufficient to expect an intense CD. UpA 2 is an open structure, which leads to little mixing of the uracil transition in the low-energy dimer state. The moments are in a left helical arrangement and, hence, the calculated spectrum will have a small positive low-energy CD band. In B DNA and RNA 11 the moments are in a left-handed helical arrangement with significant mixing and, consequently, the calculated low-energy CD band is reasonably intense and positive.

The relatively low intensity near 260 nm of the unprotonated JT spectrum for RNA 11 (Figure 2) is of special interest. In this geometry the weak intensity is due to the small angle between the principal low-energy transition moments calculated from the Bailey wave functions (section IV). This would be an example of a stacked conformation with a low CD. In contrast, for a dinucleotide in this geometry with identical bases, e.g., ApA or UpU, the relative angle would be the helix increment angle (33°) and the intensity of the CD spectrum would be nearly maximum.

An analogous analysis of Figure 3 is complicated by the presence of two reasonably intense low-energy transitions in protonated adenine. Here the calculated optical activity in the UpA 2 conformation is much lower than that observed experimentally. Figures 1 and 4 may be combined to visualize the relative monomer polarizations for protonated UpA. In the UpA 1, RNA 11, and B DNA geometries the lowest energy protonated adenine transition is approximately perpendicular to the low-energy uracil transition (angles of 88, 82, and 86°, respectively). Thus, there is little mixing of uracil in the lowest energy exciton transition near 273 nm, which has a correspondingly low rotational strength. Although for UpA 1 and RNA 11 this dimer transition has the same circular polarization as in the neutral case (right-handed for UpA 1 and left-handed for RNA 11 as in Figure 4), only in the RNA 11 geometry is it sufficiently strong to dominate the oppositely polarized low-energy band near 260 nm resulting from interaction between the 259 and 257 nm transitions on uracil and protonated adenine. The low-energy CD calculated for the protonated B DNA conformation is determined by this negative 260 nm band. The cancellation of two oppositely polarized CD bands as in the protonated UpA 1 and RNA 11 conformations illustrates a second mechanism by which a stacked dinucleotide can exhibit low optical activity.

Assuming that the monomer spectra have been approximately subtracted from the experimental spectrum there seem to be three possible explanations for the low intensity of the room-temperature UpA circular dichroism: (1) UpA is highly stacked, but the small CD is due to the coincidence of the low-energy monomer transition moments (section IV) or cancellation of oppositely signed exciton bands; (2) UpA is unstacked, e.g., as in the UpA 2 conformation; or (3) UpA has no preferred secondary structure, but exists in a continuous range of conformations[17,29] or in a mixture of conformations with intense oppositely signed bands, e.g., as a combination of the UpA 1 and RNA 11 conformations at neutral pH. The first possibility seems unlikely, since in order for a stacked structure to have coincident moments either UpA assumes a stacked conformation greatly different from B DNA or RNA 11 or a large error must exist in the observed polarization of the low-energy monomer transition moments. Cancellation of exciton bands may occur when there are two low-energy transitions on one base, each oppositely signed as was calculated for protonated UpA; such cancellations should not be important for unprotonated UpA. The second possibility can probably be ruled out on the basis of NMR evidence. At neutral pH uridine proton resonances H(5), H(6), and H(1′) of UpA occur upfield

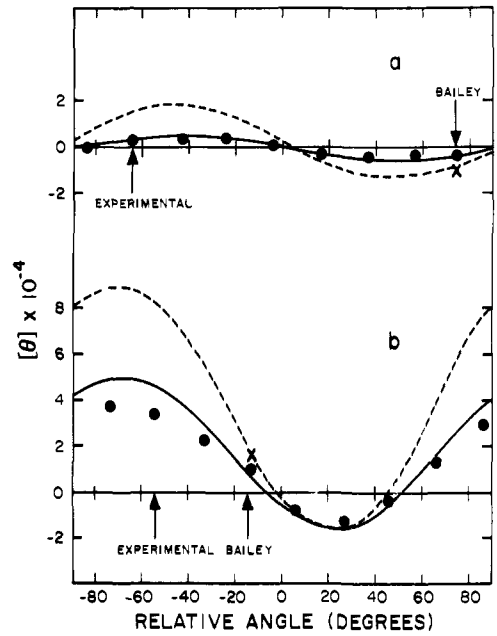


**Figure 5.** The intensity of the low-energy maximum in the calculated CD spectrum of unprotonated UpA as a function of the adenine polarization (see text); the relative angle is the angle between the low energy adenine and uracil moments: (a) UpA 2 conformation; (b) RNA 11 conformation. The following results are summarized in the figure: (—) generalized susceptibility calculations as in Figure 2; (- - -) Tinoco coupled oscillator calculation using transitions above 200 nm on uracil and neutral adenine from Bailey wave functions and a dipole interaction potential, but ignoring the polarizability correction; (• • •) Tinoco coupled oscillator calculation using experimental transition parameters and 10-nm half-width Gaussian bands; (×) intensity of a complete Tinoco method calculation as in Figure 2. The relative angles in the two geometries for the experimental and molecular orbital results are indicated by arrows.

relative to those of free uridine,[13] probably the result of adenine ring current effects, which would become negligible at distances greater than 7 Å.[30]

Thus these spectroscopic considerations suggest that UpA exists in a range of conformations in solution and that the crystal structures are not dominant solution conformers. These results are an independent verification of the conclusions drawn from in vacuo potential energy calculations.[8]

## IV. Comparison of Methods

In the theoretical spectra of Figures 2 and 3 the GS method employed experimental transition moments, while the JT method used calculated polarizations and oscillator strengths (Table I). Since there is a large discrepancy between the theoretical and experimental adenine transition moments (section V), we have calculated the intensity of the low-energy CD band for unprotonated UpA by both methods as a function of the polarization of the adenine moments (Figure 5). Polarization was changed by rotating all moments together in the plane of adenine about its center and does *not* represent a conformational change such as unstacking. This study leads to several conclusions. First, the similarity of the curves from the JT and GS calculations suggests that differences in the magnitudes and polarizations of the transition moments are the primary cause of discrepancies between the two methods. For example, both methods would give the same sign for the low-energy CD of UpA 2 if the principal long wavelength theoretical and experimental transition moments had the same polarization. Discrepancies previously reported between GS and JT calculations of the optical activity of ApA as a function of the helix increment angle[19] originate in the choice of geometric models rather than inherent differences in the two methods. Second, the unprotonated RNA 11 spectrum as calculated by the JT

**Table II.** Ionization Potentials, $I$ (eV), One-Center Coulomb Repulsion Integrals, $\gamma$ (eV), and Slater Exponents, $\zeta$, Used in the Molecular Orbital Calculations[a]

| | $\dot{C}$ | $\dot{N}$ | $\ddot{N}_{amino}$ | $\ddot{N}_{pyrrolic}$ | $\ddot{N}_{lactam}$ | $\dot{O}$ |
|---|---|---|---|---|---|---|
| $I$ | 11.16 | 14.12 | 25.73 (28.78) | 25.00 (28.78) | 26.77 (28.78) | 17.70 |
| $\gamma$ | 11.13 (10.80) | 12.34 | 16.76 (16.47) | 16.76 (16.47) | 16.76 (16.47) | 15.23 |
| $\zeta$ | 3.25 | 3.9 | 3.9 (4.25) | 3.9 (4.25) | 3.9 (4.25) | 4.55 |

[a] When the parameters used in the two methods differ the values appropriate to the Adams and Miller calculations are given in parentheses.

method illustrates how low CD intensity can arise from nearly parallel transitions, as described in section III. Finally, disagreement between experimental and theoretical transition polarizations may lead to serious ambiguities in the sign and magnitude of the CD.

Estimates of the errors in polarized reflectance studies for nucleic acid crystals suggest that the experimental polarizations should be within 20° of the actual values for the isolated molecule.[31] PPP (ref 22, 32, and Table I) and recent CNDO-Cl calculations[18] give results for adenine which are outside of this range. We conclude that experimental values are presently the most reliable source of transition energies, intensities, and polarizations and that optical activity calculations which bypass explicit use of wave functions should be pursued. Previous workers have used combinations of experimental polarizations and calculated monopoles to compute the CD of polynucleotides.[33-35] Our application of the GS method and analogous JT calculations using experimental monomer transition parameters indicate that use of dipole-dipole interaction potential is sufficiently accurate for the geometries considered here. For example, the primary differences between the monopole and dipole results in Figure 5 can be traced to larger values of the calculated oscillator strengths (Table I).

## V. Molecular Orbital Results

In this section we will discuss the results of the molecular orbital calculations (Table I). It should be pointed out that Bailey has previously calculated the electronic properties of neutral adenine and uracil by her method.[24] We have used slightly different geometries consistent with the conformations of interest. Molecular orbital results by the AM method for these molecules have not been published to our knowledge.

From Table I energies, oscillator strengths and polarizations evidently compare favorably with experimental data, except for the polarization of adenine. Poor adenine polarization is a common result of PPP calculations;[24,32] it may reflect low $\pi$ character of the C(6)-N(6) bond, which is suggested by the noncoplanarity of N(6) in the crystal structures of many adenine derivatives.[1,2,36] Disagreement between the CD spectra calculated by the JT and GS methods can be traced to different polarizations of the adenine transitions (section IV).

We predict that the low-energy absorption band of unprotonated adenine is composed of two transitions, in agreement with previous theoretical[24,32] and experimental[37,38] results. The second transition has been difficult to observe experimentally and there has been speculation whether there are two nearly degenerate transitions or the hidden transition is relatively weak.[37] Our results support the latter explanation. This weak second transition plays a more important role when the molecule is protonated (see below). Although two transitions have been observed in the low-energy absorption band of 6-azauracil,[39] we do not predict two low-energy transitions for uracil.

Both PPP methods show that the calculated absorption spectrum of adenine has red shifted on protonation in agreement with experimental observations. The two methods treat protonation differently (see Appendix). Both parameterizations lead to a twofold increase in the core parameter at the site of protonation on adenine, N(1), which results in a large increase in the electron density at this atom as expected. Examination of the wave functions reveals that the lowest energy transition is to the same state for both protonated and unprotonated adenine. For this transition the large transition monopole[18] at N(1) in neutral adenine appears at C(2) in the protonated molecule and, consequently, the oscillator strength increases. This result was found for both parametrizations. According to these PPP results, a change of transition energy does not cause the observed red shift of adenine on protonation; rather, it is primarily a result of increased intensity of the previously hidden transition. Consequently, the long wavelength compent of the adenine 260-nm absorption band can be observed in the spectrum of protonated adenine.[31]

CNDO-Cl results[18] show that protonation increases the intensity of one of the low-energy transitions, but that the energy ordering of the excited states also changes. Although these all-electron calculations probably treat the quantum mechanical basis of the electron distribution with more rigor, we have utilized PPP wave functions which, in general, are calibrated to better reproduce $\pi$-$\pi^*$ transition energies.

## VI. Conclusions

Our comparison of the calculated absorption and circular dichroism of UpA with the experimental room temperature optical properties indicates that neither structure found in crystalline UpA can be the dominant solution conformation of unprotonated UpA, but that the structure of UpA I may be important at low pH. The low intensity of the UpA circular dichroism spectrum was investigated theoretically; and with the aid of ancillary NMR data, we suggest that UpA exists in a range of conformations in solution. These purely spectroscopic considerations independently confirm the major conclusions of the in vacuo conformational energy calculations of UpA.[8]

Once the differences between experimental and calculated transition moment directions have been taken into account, similar CD spectra are calculated using a Tinoco coupled oscillator calculation with theoretical monopole interactions, a generalized susceptibility calculation with a free-electron monomer model, and coupled oscillator and generalized susceptibility calculations which include only experimental transition moments for transitions at wavelengths greater than 200 nm. The latter approximation suggests a reliable way to calculate dinucleotide circular dichroism without recourse to wave functions. PPP calculations on neutral and protonated adenine indicate that the observed 260 nm adenine absorption band is composed of two transitions. The red shift observed on protonation is a result of enhanced intensity of the previsouly weak lower energy component.

**Acknowledgment.** This work was supported by the Petroleum Research Fund, administered by the American Chemical

Society, and the Research Corporation through grants to E.S. and by a grant from the National Science Foundation (BMS 75-17114) to T.S. We wish to express special gratitude to Drs. D. W. Miles and W. C. Johnson for copies of computer programs and to Dr. E. A. Dratz for helpful discussions.

## Appendix

**Details of the Molecular Orbital Parameterization.** Ionization potentials, one-center core integrals, and Slater exponents employed in PPP calculations using methods of Bailey[22] and Adams and Miller[23] are presented in Table II. For entries with two values the number in parentheses is the Adams and Miller value. Two-center coulomb repulsion integrals were calculated by the Nishimoto and Mataga formula[21] and overlap integrals by the Mulliken formula.[40] In the Bailey method the core integrals are

$$H_{rr} = -I_r$$

$$H_{rs} = (\hbar^2/mR)(\mathrm{d}S_{rs}/\mathrm{d}R) \qquad \text{(ref 26)}$$

where $S$ is the overlap integral and $R = |\mathbf{R}_r - \mathbf{R}_s|$, and effects of protonation are included through the influence of the change in the $\sigma$ charge, $\Delta Q^\sigma$, on the one-center core integral[23]

$$\Delta H_{rr} = (Q_{H^+})(\gamma_{rH^+}) - \sum_{s \neq H^+} \Delta Q_s{}^\sigma \gamma_{rs}$$

with $\gamma_{rH^+} = 14.352$ eV,[41] where $H^+$ is the added proton.

The Adams and Miller method employs a Löwdin basis set

$$\mathbf{H}^\lambda = \mathbf{S}^{-1/2}\mathbf{H}\mathbf{S}^{-1/2}$$

$$H_{rs} = \tfrac{1}{2}(H_{rr} + H_{ss})[S_{rs} - 0.0855R_{rs} + 0.24639] - n_r(S_{rs}/4)(\gamma_{rr} + \gamma_{rs}) - n_s(S_{rs}/4)(\gamma_{ss} + \gamma_{rs})$$

where $n_r$ is the number of electrons contributed to the $\pi$ system by atom $r$ and the last two terms in the square bracket are ignored for interatomic distances $R_{rs} > 2.882$ Å. To include protonation in this method, the parameters of adenine N(1) are changed to $I = 26.12$ eV, $\gamma = 15.44$ eV, and $\zeta = 4.35$.[25,42]

## References and Notes

(1) J. L. Sussman, N. C. Seeman, S. H. Kim, and H. M. Berman, *J. Mol. Biol.*, **66**, 403–421 (1972).
(2) J. Rubin, T. Brennan, and M. Sundaralingham, *Biochemistry*, **11**, 3112–3118 (1972).
(3) J. M. Rosenberg, N. C. Seeman, J. J. P. Kim, F. L. Suddath, H. B. Nicholas, and A. Rich, *Nature (London)*, **243**, 150–154 (1973).
(4) R. O. Day, N. C. Seeman, J. M. Rosenberg, and A. Rich, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 849–853 (1973); S. F. Stellman, B. Hingerty, S. B. Broyde, E. Subramanian, T. Sato, and R. Langridge, *Biopolymers*, **12**, 2731–2750 (1973).
(5) S. Arnott, M. H. F. Wilkins, W. Fuller, and R. Langridge, *J. Mol. Biol.*, **27**, 535–548 (1967).
(6) R. Langridge, D. A., Marvin, W. E., Seeds, A. R. Wilson, C. W. Hooper, M. H. F. Wilkins, and L. D. Hamilton, *J. Mol. Biol.*, **2**, 38–64 (1960).
(7) S. H. Kim, H. M. Berman, N. C. Seeman, and M. D. Newton, *Acta Crystallogr., Sect B*, **29**, 703–710 (1973).
(8) S. B. Broyde, S. D. Stellman, B. Hingerty, and R. Langridge, *Biopolymers*, **13**, 1243–1259 (1974).
(9) M. W. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, **20**, 29–38 (1966).
(10) N. P. Johnson and T. Schleich, *Biochemistry*, **13**, 981–987 (1974).
(11) R. C. Davis, Ph.D. Thesis, University of California, Berkeley, 1967.
(12) R. C. Davis and I. Tinoco, Jr., *Biopolymers*, **6**, 223–242 (1968).
(13) P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, *Biochemistry*, **8**, 997–1082 (1969).
(14) W. C. Johnson, Jr. and I. Tinoco, Jr., *Biopolymers*, **8**, 715–731 (1969).
(15) M. L. Bailey, *Biopolymers*, **11**, 1091–1102 (1972).
(16) R. A. Harris, *J. Chem. Phys.*, **43**, 959–970 (1965).
(17) A. D. McLachlan and M. A. Ball, *Mol. Phys.*, **8**, 581–595 (1964).
(18) W. Hug and I. Tinoco, Jr., *J. Am. Chem. Soc.*, **95**, 2803–2813 (1973); **96**, 665–673 (1974).
(19) A. S. Schneider and R. A. Harris, *J. Chem. Phys.*, **50**, 5204–5215 (1969).
(20) R. Pariser and R. G. Parr, *J. Chem. Phys.*, **21**, 466–471 (1953); J. A. Pople, *Trans. Faraday Soc.*, **49**, 1375–1385 (1953).
(21) K. Nishimoto and N. Mataga, *Z. Phys. Chem. (Frankfurt am Main)*, **12**, 335–338 (1957).
(22) M. L. Bailey, *Theor. Chim. Acta*, **13**, 56–64 (1969).
(23) O. W. Adams and R. L. Miller, *Theor. Chim. Acta*, **12**, 151–165 (1968).
(24) M. L. Bailey, *Theor. Chim. Acta*, **16**, 309–315 (1970).
(25) D. W. Miles, W. H. Inskeep, L. B. Townsend, and H. Eyring, *Biopolymers*, **11**, 1181–1207 (1972).
(26) J. Linderberg, *Chem. Phys. Lett.*, **1**, 39–41 (1967).
(27) M. L. Bailey and J. P. M. Bailey, *Theor. Chim. Acta*, **16**, 303–308 (1970).
(28) F. Jordan and D. H. Sostman, *J. Am. Chem. Soc.*, **94**, 7898–7902 (1972).
(29) D. Glaubiger, D. A. Lloyd, and I. Tinoco, Jr., *Biopolymers*, **6**, 409–414 (1968).
(30) C. Giessner-Prettre and B. Pullman, *J. Theor. Biol.*, **27**, 87–95 (1970).
(31) H. H. Chen and L. B. Clark, *J. Chem. Phys.*, **58**, 2593–2603 (1973).
(32) H. Berthod, C. Giessner-Prettre, and A. Pullman, *Int. J. Quantum Chem.*, **1**, 123–137 (1967).
(33) C. L. Chech, W. Hug, and I. Tinoco, Jr., *Biopolymers*, **15**, 131–152 (1976).
(34) D. S. Moore and T. E. Wagner, *Biopolymers*, **12**, 201–221 (1973).
(35) R. S. Studdert and R. C. Davis, *Biopolymers*, **13**, 1405–1416 (1974).
(36) K. Shikata, T. Ueki, and T. Mitsui, *Acta Crystallogr., Sect. B*, **29**, 31–38 (1973).
(37) A. F. Fucaloro and L. S. Forster, *J. Am. Chem. Soc.*, **93**, 6443–6449 (1971).
(38) W. Voelter, R. Records, E. Bunneberg, and C. Djerassi, *J. Am. Chem. Soc.*, **90**, 6163–6170 (1968).
(39) J. N. Brown, L. M. Trefonas, A. F. Fucaloro, and G. G. Anex, *J. Am. Chem. Soc.*, **96**, 1597–1598 (1974).
(40) R. S. Mulliken, C. A. Reike, D. Orloff, and H. Orloff, *J. Chem. Phys.*, **17**, 1248–1267 (1949).
(41) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289–3296 (1966).
(42) D. W. Miles, personal communication.