Electronic Spectra of Crystalline Guanosine: Transition Moment Directions of the Guanine Chromophore

Leigh B. Clark*

Contribution from the Department of Chemistry, University of California, San Diego, La Jolla, California 92093-0359

Received November 15, 1993®

Abstract: Polarized absorption spectra of single crystals of guanosine dihydrate have been obtained by Kramers–Kronig analysis of reflection data taken from the (001) and (100) crystal faces. Four strong in-plane polarized transitions can be identified. In addition, three features apparently polarized perpendicularly to the planes of the guanine chromophores are observed. The effects of resonance coupling between molecules in the crystal lattice are considered, and a generally consistent picture emerges for the effects of exciton coupling on the crystal spectra. The calculated change of transition moment directions induced by the crystal field is of the order of 10–15°. Consideration of these effects brings the experimental crystal results into better agreement with the results of semiempirical MO calculations for the excited states of the guanine chromophore.

Introduction

This paper about guanosine (Figure 1) is part of a continuing effort to develop transition moment data of the purine bases. Such data are important for interpretation of the optical properties of the many polymers of these bases. The most detailed assignment of transitions of the guanine chromophore dates from a 1975 study of 9-ethylguanine and guanine·HCl single crystals.[1] In that study, an attempt was made to resolve the usual ambiguity in transition moment directions that arises in the interpretation of crystal spectra by required the polarization directions of the protonated and unprotonated versions to be similar. While the correctness of such a procedure may be argued, there were no other guanine-containing crystals of suitable size available for study at that time.

The guanine chromophore is a typical example of the discrepancy that exists between experimental results taken from crystal spectra and the calculated results of various molecular orbital treatments. Both semiempirical[2] and *ab initio* calculations[3] are generally in mutual agreement in regard to the intensities and the transition moment directions of the two lowest energy principal bands (250 and 275 nm); yet these directions disagree with experimental crystal results by 40° or more. For the higher energy region of the spectrum, there is little or no agreement even among the theoretical approaches in detailing the intensity and polarization pattern to be expected in the guanine electronic spectrum. None seem close to predicting the experimental results that have been found mainly from crystal spectra.

This discrepancy has led Woody[4] and later Theiste, Callis, and Woody[5] to consider the effects of the local electrostatic field of the crystals on the free molecule predictions of the MO treatments. Calculations indicate that guanine possesses a large ground state dipole moment and, in addition, exhibits large changes in the dipole moment upon excitation.[6,7] In a crystal lattice the interactions involving the electrostatic field arising from permanent electric dipole moments can lead to significant energy shifts of the bands, and the electrostatic field can mix excited states and thus lead to changes in apparent transition moment directions. These effects are in addition to the usual resonance coupling (exciton mixing) which also lead to energy shifts and intensity mixing. This shifting of intensity among the band components along different crystal axes modifies the apparent free molecule transition moment directions that are deduced from crystal spectra.

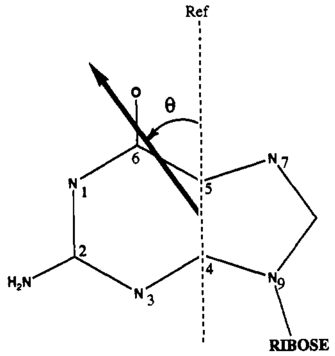

Figure 1. Numbering and angle conventions for the guanine chromophore in guanosine. The C_4–C_5 line is the reference axis. Transition moment directions are positive in a counterclockwise direction from the reference axis. See ref 10.

The results of Theiste, Callis, and Woody[5] for the transition moments of 9-ethylguanine showed that the electrostatic effects indeed cause the apparent transition moment directions calculated for the crystal spectra to be significantly different from those calculated for the free molecule. These calculations resulted in the discrepancy being significantly reduced. Clearly these effects need to be considered in any theoretical treatment of the optical properties of DNA and RNA.

Recently we have been able to prepare single crystals of guanosine dihydrate of a size suitable for spectroscopic measurements. The crystal structures of 9-ethylguanine and guanosine are substantially different, however the relative orientation of adjacent guanine residues linked together into hydrogen-bonded ribbons is nearly identical. The present study was undertaken for several reasons. First, data from a second unprotonated guanine residue were desired for comparison to those obtained earlier for 9-ethylguanine. In this regard, it was hoped that some of the ambiguities of the earlier work might be removed. Second, no polarized spectra of a purine (or pyrimidine) base with a ribose

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® Abstract published in *Advance ACS Abstracts,* May 1, 1994.

(1) Clark, L. B. *J. Am. Chem. Soc.* **1977**, *99*, 3934.

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(4) Woody, R. W. *Biophys. J.* **1984**, *45*, 382a.

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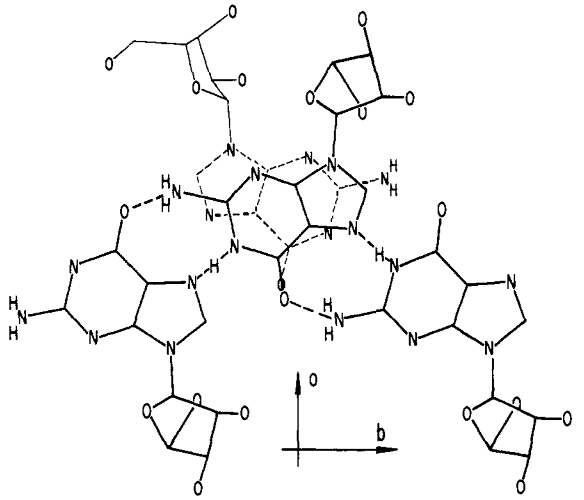

Figure 2. The crystal structure of guanosine dihydrate as projected onto the *ab* crystal plane. The dashed molecule is 3.3 Å below the plane of the other three guanosines. The hydrogen-bonding scheme is indicated. The dashed molecule forms a similar hydrogen-bonded ribbon. The solid and dashed nucleosides represents the two types of molecules of the asymmetric unit.

group attached had ever been reported, and it was of interest to gauge the possible effects of the presence of the ribose group on the transitions of the base chromophore. Third, the consequences of different crystal structures might very well lead to different electrostatic effects (as well as exciton mixing effects) for 9-ethylguanine and guanosine. Comparison of the results might then provide clues for dealing with the interactions between such molecular systems.

Crystal Growth and Structure

All attempts at crystal growth by very slow cooling or evaporation of aqueous solutions at room temperature resulted not in single crystals but in colloidal slurries. However, slow evaporation in an oven of aqueous solutions at elevated temperatures (>60 °C) resulted in long, thin (<100 μm) crystal plates that showed uniform extinction parallel to the long crystal axis. Subsequent indexing using an X-ray diffractometer indicated the large face to be (100) and the *c*-axis to be parallel to the long axis of the crystal. The end face of the crystal was found to be (001). Of the many batches of crystals grown, only a single example was thick enough to permit reflection measurements to be made on this latter face. In this regard the facial dimensions must exceed the spot size of the focused light beam (~80 μm).

The crystal structure of guanosine dihydrate has been worked out by Thewalt, Bugg, and Marsh.[8] The crystals are monoclinic, space group $P2_1$, with cell dimensions a = 17.518 Å, b = 11.502 Å, and c = 6.65 Å, and β = 98.17°. There are two nucleoside molecules and four water molecules per asymmetric unit. Two asymmetric units make up the unit cell. The planes of the purine bases are very nearly parallel to the *ab* crystal plane. The two purine moieties of the asymmetric unit (labeled A and B in the subsequent discussion) are nearly equivalent spectroscopically, however the ribose groups of the two nucleosides assume very different attitudes. These two nucleoside types form individual parallel hydrogen-bonded ribbons along the *b* axis. There is nearly perfect stacking (3.3 Å) of the purine bases along the *c* axis (alternating A and B types). These structural features are depicted in Figure 2.

The natural (001) face of the one crystal of sufficient size for optical measurement was pitted and not of sufficient quality for accurate reflection results. Consequently the face was polished using an ultramicrotome equipped with a diamond knife. The final polishing strokes were made with an advance increment of ~50 Å. The resultant face was smooth and shiny and yielded a *b*-axis spectrum equal to that obtained from the natural (100) face. We conclude from this evidence that no significant loss of structure arose in the polishing procedure.

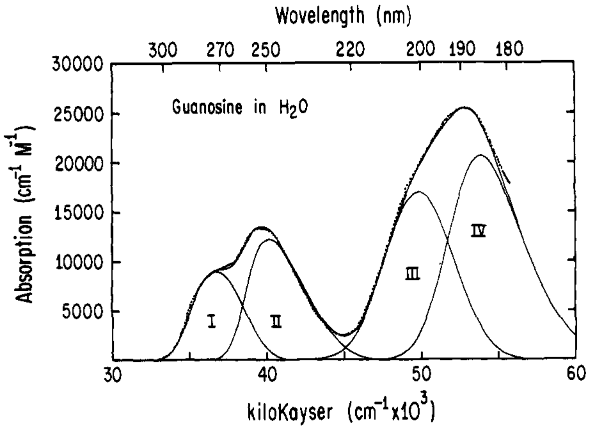

Figure 3. Aqueous solution spectrum of guanosine at pH 6.0 taken with a N_2-flushed Cary 15 and using 0.1 mm pathlength cells. The resolution into four principal transitions (I to IV) is indicated. The four components sum to give the solid curve. The dotted curve is the experimental spectrum. The band contours used are asymmetric and were fitted by a least-squares procedure.

Table 1. Oscillator Strengths and Positions for Aqueous Guanosine

	f	ν (kK)	λ (nm)
I	0.15	36.8	272
II	0.24	40.6	246
III	0.40	49.9	200
IV	0.48	53.9	186

Experimental Results

Both the UV and VUV instruments used for the polarized reflection measurements have been described previously.[9] For the VUV region the crystal is mounted just outside the vacuum region. The small gap (~4 mm) between the crystal and the bounding LiF window permits a chopper blade to operate and is flushed with dry nitrogen gas. Measurements to about 140 nm can be made with this arrangement.

Guanosine dihydrate crystals exposed to a dry environment show a modest change in spectra with time. This change is apparently due to the loss of water from the crystal surface layers. The effect is reversible, and the original spectra are restored upon bathing the "dehydrated" crystal in a moist gas flow. To minimize these effects, VUV spectra with the dry N_2 flush were taken as rapidly as possible (~2.5 min). Restoration of the surface was carried out between runs. It is estimated that the reflectivities in the further VUV region (below 160 nm) may have changed by not more than ~5% of the magnitude of the correct signal.

Solution Spectrum. The spectrum of guanosine dissolved in water (pH 6) is shown in Figure 3. There appear to be four principal electronic transitions down to ~170 nm [60 kK (1 kK = 10^3 cm^{-1})]. Oscillator strengths and band positions for this spectrum are given in Table 1. The bands are labeled I to IV as in the earlier work with 9-ethylguanine. Resolution of both two-band regions was accomplished with a least-squares procedure employing a special asymmetric band, curve fitting routine. This procedure is somewhat arbitrary, and there is, of course, uncertainty in the oscillator strengths. However the resolution and band shapes shown in Figure 2 appear reasonable and are

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[8] Thewalt, U.; Bugg, C. E.; Marsh, R. E. *Acta Crystallogr.* **1970**, *B26*, 1089.

[9] Zaloudek, F.; Novros, J. S.; Clark, L. B. *J. Am. Chem. Soc.* **1985**, *107*, 7344.

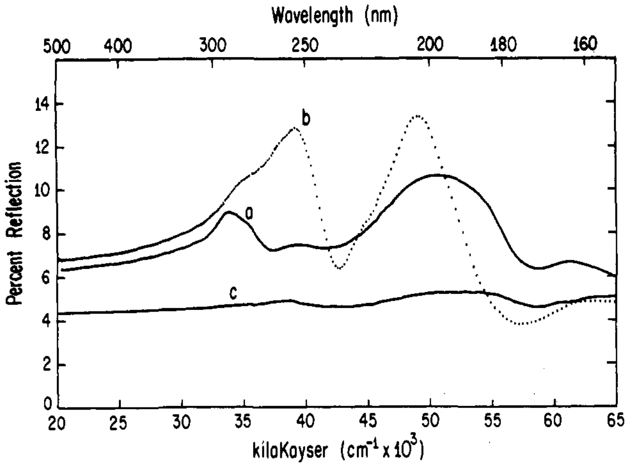

Figure 4. Reflection spectra for incident radiation polarized along the *a*, *b*, and *c* axes of the (001) and (100) crystal faces of guanosine dihydrate.

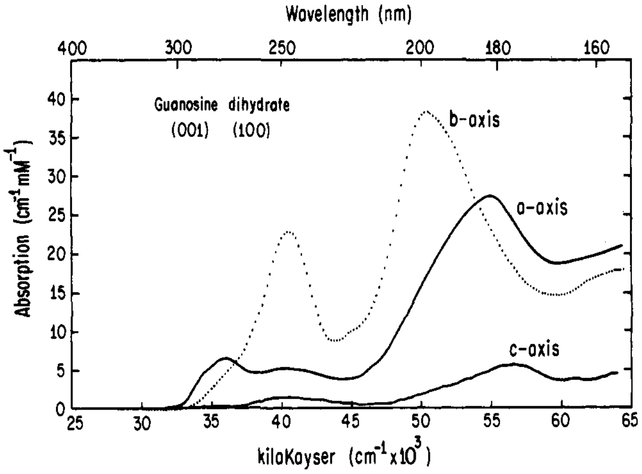

Figure 5. Absorption spectra for radiation polarized along the *a*, *b*, and *c* axes of the (001) and (100) faces. These curves are derived from the reflection spectra shown in Figure 2 by Kramers–Kronig analysis.

consistent with solution spectra of 9-ethylguanine in which III and IV are somewhat more clearly resolved.[1]

Crystal Spectra. The normal incidence reflection spectra taken with incident light polarized along the *a*, *b*, and *c* axes of the (001) and (100) crystal faces are shown in Figure 4. The corresponding absorption curves obtained through Kramers–Kronig analysis of the reflection data are shown in Figure 5. The spectra are not very appealing in that they are diffuse and show little structure. Individual components of transitions I and II can be resolved with reasonable confidence; however the situation is much less clear for transitions III and IV. Here, only single peaks appear along both the *a* and *b* axes, and there are no apparent shoulders, inflections, etc. The shape of the strong *a*-axis band centered at 55 kK (band IV) suggests a substantial component of band III may be mixed in here. The very low intensity found polarized perpendicularly to the planes of the guanine bases (*c* axis) is in line with the customary belief that the bulk of the intensity should arise from in-plane polarized, $\pi \rightarrow \pi^*$ transitions.

Oriented Gas Analysis of Crystal Spectra. The simplest approach to the interpretation of crystal spectra in terms of free molecule properties presumes that the crystal is composed of oriented, noninteracting molecules. The various transition moments of the chromophore simply project onto the crystal axes and provide their corresponding component of the oscillator strength polarized along that axis. This type of analysis will in all cases be approximate owing to the neglect of the effects of intermolecular interactions, but it does form the starting point for the subsequent detailing of the latter effects. The results from the earlier study of 9-ethylguanine were obtained using this model for the crystal spectra. Reasonable resolutions of the spectra into individual components are shown in Figure 6, and numerical values for these resolutions are given in Table 2.

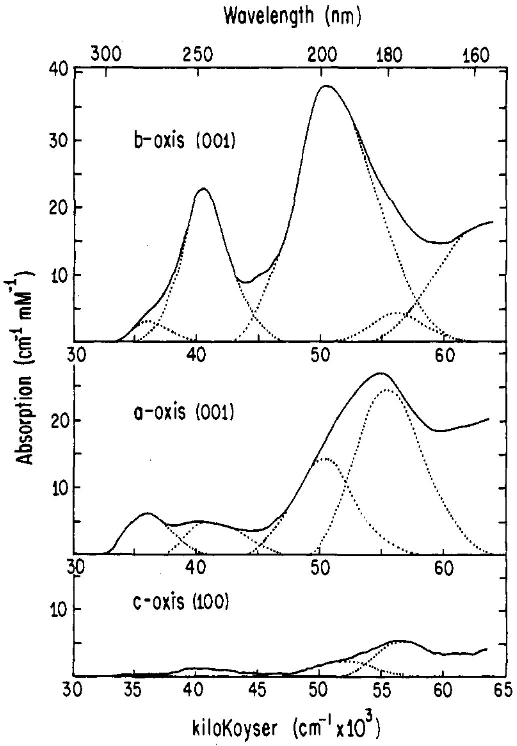

Figure 6. Resolution of the absorption spectra into individual band components for the oriented gas analysis of dichroic ratios. The magnitudes of the *a*-axis component (III) at 50 kK and especially the *b*-axis component of IV are very uncertain and are meant to represent reasonable maximum values for the intensities of these band components.

Table 2. Oscillator Strengths[a] and Band Positions (kK) of the Crystal Spectra

	a	*b*	*c*
I	0.12 (36.0)	0.05 (36.0)	0.001 (36.0)
II	0.11 (41.0)	0.49 (40.5)	0.04 (40.5)
III	0.40 (50.5)	1.37 (50.5)	0.04 (51.0)
IV	0.86 (55.5)	~0.16[b] (56.2)	0.15 (56.2)

[a] All values are for the resolutions shown in Figure 6. Oscillator strengths are evaluated with the formula $f = 1.085 \times 10^{-9}\ \epsilon\ d\nu$ where ϵ is the molar extinction coefficient and ν is in cm^{-1}. [b] Very uncertain.

Transition I. The resolutions for transition I shown in Figure 6 yield oscillator strength values of the band components which when randomized give an overall $f = 0.06$. This value is less than 40% of the value found from the aqueous solution spectrum (0.16). The dichroic ratio f_a:f_b is 2.4, and this value is consistent with an in-plane transition polarized at either +60° or −24° according to the DeVoe–Tinoco convention (positive counterclockwise from the C_4–C_5 reference axis).[10] See Figure 1 for this convention and the numbering system. These directions differ appreciably from the corresponding choices reported for 9-ethylguanine (+35° or −7°).[1] The apparent significant loss of intensity suggests that crystal interactions are not negligible.

Transition II. Once the *a*-axis component of I is resolved from the spectrum, the *a* component of II is easily distinguishable. The dichroic ratio (f_b/f_a) is 4.4, and this value corresponds to possible transition moment directions of −56° or −92° (+88°). A value of −72° was reported in the earlier work on 9-ethylguanine. In that work, however, a distinct band component for transition II could be identified along but one of the principal axes of the (111) face of 9-EG. It should be noted, however, that a substantial

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

component of II could easily have been obscured in the spectrum polarized along the opposite principal axis. A more appropriate tack in the 9-EG work would have been to estimate the maximum, plausible f number of the obscured component and simply assert a corresponding range of possible polarization directions. Reanalysis of the old 9-EG data along these lines yields a range of possible directions from about −50° to about −94° for transition II. The appearance of distinct components along both the *a* and *b* axes for II in guanosine is the key to restricting the possible directions to the two values given above for guanosine.

Transitions III and IV. The two strong transitions in the 180–200-nm region appear as single, broad, diffuse bands polarized individually along the *a* and *b* crystal axes. Along either axis there is no clearcut resolution into individual components of III and IV even though significant intensity of each transition may occur polarized along both axes. The appearance of the *a*-axis spectrum in this region suggests an appreciable component of III, and the resolution shown in Figure 6, although arbitrary, is not implausible. The resolutions shown give a dichroic ratio f_b:f_a of 2.8 and yield corresponding transition moment direction choices of −50° or +86°. The situation is nearly hopeless for transition IV. If there is no component of IV along the *b* axis, then the transition is polarized at $\theta = +18°$. If a modest component of IV is buried in the *b*-axis spectrum, then a reasonable assessment of the corresponding range for θ is −8° to +44°. This range is consistent with a maximum possible dichroic ratio f_a:f_b of ~8.

c-Axis Spectrum. Since the *c* axis is nearly perpendicular (~98°) to the planes of the bases, one expects to observe very small component intensities from in-plane polarized transitions. On the other hand, perpendicularly polarized bands like $n \rightarrow \pi^*$ transitions should appear almost entirely along the *c* axis. Furthermore, in-plane transitions directed more or less along the short molecular axis ought to be more prominent along the *c* axis than in-plane transitions polarized generally along the long molecular axis (*b* in the crystal). The 98° angle between the *a* and *c* axes provides that about 2% of the intensity found along the *a*-axis component should be observed along the *c* crystal axis. The observed *c*-axis intensity in the vicinity of I is in accord with this expectation. However, at higher frequencies considerably more intensity is observed than can be accounted for by the simple projection of in-plane polarized transition moments. The fact that the two principal bands at 40 and 57 kK along with the broad shoulder at 51 kK appear at positions close to those of transitions II, IV, and III respectively does not clarify the assignments. Either (1) we are witnessing genuine perpendicularly polarized transitions at these three positions, or (2) the nominal $\pi \rightarrow \pi^*$ transitions of guanosine are not strictly polarized in the molecular plane, or (3) crystal interactions are mixing the intensities seriously so as to confuse the issue (*vide infra*).

Discussion of Oriented Gas Results

When the results deduced from the crystal spectra are combined with the fluorescence polarization study of 9-EG and guanine by Callis, Rosa, and Simpson[11] and the linear dichroism study of guanine and guanosine in stretched films of Matsuoka and Nordén,[12] then removal of some of the ambiguities is possible as the latter authors have themselves concluded. Both of these studies indicate that I and II have directions that make a reasonably large angle with each other (60–90°), and thus two possible combinations for I and II are selected. Furthermore, the LD of guanosine changes in significant ways from that of guanine. Matsuoka and Nordén attribute these changes to a shift of the molecular orientation axis relative to the stretching direction. The orientation axis of guanine was determined by infrared dichroism measurements to be approximately at +32°. Ribose substitution to form guanosine will presumably shift the orientation axis in the stretched films toward a smaller positive angle and toward the now longer (molecular) structural axis. The conclusion reached in the stretched film work was that transition I must be polarized not far from the short molecular axis (C_4–C_5) while transition II is directed near the long molecular axis. Consequently the −24° choice for I and the −88° choice for II are selected. The results are summarized in Table 3.

Table 3. Summary of Oriented Gas Results for In-Plane Polarized Bands

	possible directions (deg)	chosen direction (deg)
I	−24 to +60	−24
II	−56 or +88	+88
III	−50 or +86	+86
IV	−8 or +44	(−8 to 44)

The linear dichroism study of Matsuoka and Nordén[12] and the earlier data given by Fucoloro and Forster[13] show a slight decrease in the reduced dichroism for guanine moving from transition II into the region of transition III. While these measurements cannot penetrate deeply enough into the UV to witness the absorption maximum of III, they do, in our opinion, go far enough to indicate the observed dichroism change is owed to III and not to some underlying weak feature. This result for guanine implies that III is polarized very similarly to II and the +86° value for III of guanosine is indicated. In fact, the changes in the LD between guanine and guanosine coupled with the presumed change in orientation axes between these two molecules quite clearly imply that II and III are polarized in the same general direction and that the angle that III makes to the reference axis (C_4–C_5 axis) is somewhat (~10°) less than that of transition II. On the basis of the above evidence, transition III is assigned to lie at +86° (or −94° with reversal of phase). The earlier result for 9-EG asserted that III was much like II. However, with the present interpretation the derived angles for III of 9-EG should be revised to be consistent with that given here for guanosine.

Transition IV shows a discernible strong component only along the *a* axis. The fact that a modest *b*-axis component could lie obscured along the *b* axis leaves us with little recourse but to assign an angular range of +44° to −8°. This result is not inconsistent with the spectra taken from 9-EG crystals.

If the strong $\pi \rightarrow \pi^*$ bands are nominally polarized in the molecular plane, then the bands appearing at 41, 51, and 57 kK polarized along the *c* crystal axis must arise from transitions polarized perpendicular to the molecular plane. If this is the case, then these features mark the locations of $n \rightarrow \pi^*$ transitions or transitions involving other σ-type orbitals of modest intensity. This conclusion is not subverted by consideration of the effects of intermolecular interactions (*vide infra*).

Effects of Intermolecular Interactions

Interactions between molecules in the crystal result in mixing of states with the consequent energy shifts and band to band intensity borrowing. The mixing coefficients are different for the two principal axes of any given crystal face, and in addition vary from face to face. Given two asymmetric units per unit cell, there will be two types of factor group states per free molecule state. Since there are two molecules in the asymmetric unit, there will be two states of each of the two symmetry species arising from each free molecule state. However, since the guanine chromophores are almost equivalent spectroscopically (i.e. interchanged with a reflection in the *ac* plane plus translation), we expect half of the formally allowed $|\mathbf{k}| = 0$ crystal transitions to carry little if any intensity. In the calculations that follow, we have ignored all terms in the interaction Hamiltonian except those of transition dipole–transition dipole nature (i.e. exciton

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[11] Callis, P. R.; Rosa, E. J.; Simpson, W. T. *J. Am. Chem. Soc.* **1964**, *86*, 2292.

[12] Matsuoka, Y.; Norden, B. *J. Phys. Chem.* **1982**, *86*, 1378.

[13] Fucaloro, A. F.; Forster, L. S. *J. Am. Chem. Soc.* **1971**, *93*, 6443.

Table 4. Model Spectrum

	ν (kK)	f	θ (deg)
I	36.0	0.15	-12
II	41.0	0.24	80
III	50.0	0.40	70
IV	53.0	0.48	-10
V[a]	(70.0)	(0.25)	(80)
VI[a]	(70.0)	(0.25)	(-10)
$n\pi^*$ (1)	42.0	0.01	$\perp$
$n\pi^*$ (2)	51.0	0.01	$\perp$
$n\pi^*$ (3)	57.0	0.05	$\perp$

[a] Arbitrary high-energy bands, not observed experimentally.

Table 5. Comparison of Oscillator Strengths of the *a* and *b* Components of the In-Plane Transitions[a]

	I	II	III + IV
a	0.34 (0.11) 0.11	0.16 (0.13) 0.12	1.58 (1.60) 1.26
b	0.11 (0.05) 0.05	0.56 (0.56) 0.49	1.1 (1.1) 1.4
c	0.01 (0.003) < 0.001		

[a] The three numbers for each entry are in order (1) the oriented gas projections of the model spectrum, (2) the calculated crystal intensity, and (3) the observed crystal intensity. Mixing of III and IV does not permit clear identification of crystal components so the sum intensities are given. Only the values for I are given for the c-axis since the weak components of the in-plane bands are presumably obscured by stronger $n \rightarrow \pi^*$ intensity (see text).

exchange) in the point dipole limit. Thus, any effects arising from higher multipoles (i.e. distribution of the transition dipole about the molecule) and permanent dipole moments will be ignored. Although dropping the latter term may be an important omission, our intention here is to gauge the effects of this limited crystal field on the spectrum of the guanine chromophore.

The strategy is to assume a model spectrum (*viz.* energies, oscillator strengths and transition moment directions) for the chromophore and perform the usual calculation of crystal energies and wave functions by diagonalizing the usual secular determinant. For the present case, this matrix will have dimension equal to (4 sites × number of molecular transitions). Dipole lattice sums are evaluated with the Ewald–Kornfeld procedure[14] for unit vectors (1 Å) directed along the *a*, *b*, and *c* crystal axes. Any arbitrary dipole lattice sum can be evaluated from these sums.[15] In addition to gauging the magnitude of the possible effects, it was hoped that such calculations might select (or confirm) particular choices of the transition moment directions from the various possible cases. Of interest also is the predicted magnitude of mixing of in-plane polarized $\pi \rightarrow \pi^*$ transitions with $n \rightarrow \pi^*$ states.

A constraint on all calculations was that the starting oscillator strengths of the four dominant transitions should be fixed at the values taken from the solution spectrum (Table 1). Input energies were allowed to shift by ±1 kK relative to the values in the solution spectrum in order to account for the usual uncalculable crystal shift. Finally, the transition moment directions were varied in order to tailor the output to obtain as close a fit as possible to the observed spectrum. Various models using combinations of the alternate choices for the assignments of the polarization directions were evaluated. It was quickly determined that the observed intensity along the *c* crystal axis could not arise from crystal field mixing of in-plane polarized bands. Consequently, we have assumed three perpendicularly polarized transitions at 41, 51, and 56 kK, and these transitions account nicely for the observed intensity along *c*. Finally, we have artibrarily included additional bands of long and short axis polarization at higher energy (~75 kK) to account minimally for higher energy, absorption intensity that must certainly be present. The presence of these latter transitions affects the calculated results for I and II hardly at all, however they do borrow modest intensity from III and IV.

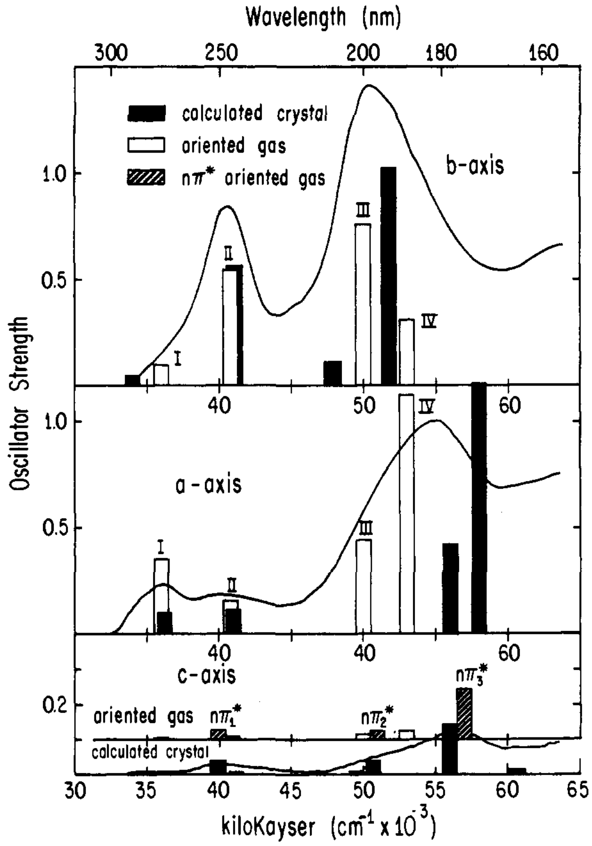

Figure 7. Comparison of the spectra along the *a*, *b*, and *c* axes of the oriented gas projections of the model spectrum and the calculated spectra after mixing with the crystal field. The open bars represent oscillator strength components using oriented gas projections. The crystal field-induced mixing yields the crystal states represented by the solid bars. The two situations for the *c* axis have been separated and the ordinate axis expanded for clarity. Only transitions with significant intensity are displayed in the *a*- and *b*-axis panels.

The transition moment directions of the various bands were then allowed to vary so as to obtain a reasonably close fit to the experimental spectra. Although the directional choices of transitions I, II, and III were fairly certain, various other combinations were evaluated. That combination yielding the best fit is given as the model free molecule parameters in Table 4. The results of the crystal calculations showing the effects of crystal field mixing are also presented in Table 5 and shown graphically in Figure 7. Alternate combinations of transition moment directions degrade the best fit, although given the approximations involved, the degradation is not severe enough to rule out unequivocally the alternate possibilities.

The best fit calculation shows some successes and some failures. The most prominent failure is the calculated red shift of the *b* component of I. The calculated Davydov split (relative to the *a*-axis component of the *ab* face) of ~2000 cm^{-1} is independent of the available choices of polarization directions for the bands and is not observed in the crystal spectrum. The breakup of transition I into several vibronic components so as to shift from strong coupling to the weak coupling limit does not change the outcome in regard to the location of intensity. On the other hand, the pronounced hypochromism (loss in excess of 60% of total intensity) of I along both the *a* and *b* axes, relative to the oriented gas projections, is a strong success. While the calculated intensities of the *a*- and *b*-axis components of I and II agree well with the experimental spectra, agreement is less clear for bands III and IV. Although the general observed bulk intensity is reproduced, modest changes in input energies have a profound effect on the relative intensities of the components of III and IV of the *a* axis. Clearly these two transitions are strongly mixed

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(14) Kornfeld, H. *Z. Phys.* **1924**, *22*, 27.

(15) A listing of these lattice sums can be obtained by writing the author.

in the crystal, and qualitative agreement between the calculated results and experimental spectra are probably all that can be hoped for. The calculations indicate that the *c*-axis components of the $\pi \rightarrow \pi^*$, in-plane polarized transitions actually show decreased intensity under the crystal field. The presence of the three separate perpendicularly polarized bands is therefore required to match the observed *c*-axis intensities.

The crystal field induced angular shifts of the apparent directions of transitions I and II are +12° and −8°, respectively. Corresponding values for III and especially for IV are not meaningful owing to strong mixing between these two transitions and consequent ambiguity in identifying the parentage of the crystal bands.

Conclusions

The bulk of evidence suggests that I is polarized at modest negative angles while II is polarized generally along the long molecular axis. The exciton mixing calculations modify the free molecule transition moment directions by an amount on the order of 10° or so. If the effects of the static electrostatic field of the 9-EG structure are similar to those in guanosine, then a generally consistent pattern of polarization directions is obtained. It should be mentioned that the hydrogen-bonding scheme, between the guanine bases in both 9-EG and guanosine dihydrate crystals are nearly identical. If the dominating interaction terms occur between adjacent members of the hydrogen-bonded ribbons, then the general effects for the two structures may be similar. A comparison of the results of the 9-EG calculations and the present exciton mixing calculations is shown in Figure 8. There is general agreement in the directions of polarization for I, II, and III. Furthermore, the semiempirical MO results appear in somewhat better agreement with the experimental findings than has been previously noted.

The presence of the ribose group does not seem to affect the transition moment directions of the guanine chromophore in any significant manner, for the suitably adjusted directions of 9-EG are near to those found here for guanosine. Such was the expected outcome.

The assignment of the three $n \rightarrow \pi^*$ transitions is far from certain. The presence of these transitions must be assumed in order to account for the observed *c*-axis intensity. However, the fact that they appear at positions nearly identical to the $\pi \rightarrow \pi^*$ bands creates considerable doubt in their assignment as $n \rightarrow \pi^*$ transitions. Theoretical calculations place a number of $n \rightarrow \pi^*$ transitions in the spectrum, however the calculated oscillator strengths are much smaller than those necessary to account for the observed *c*-axis crystal spectrum. Irrespective of the origin of this absorption intensity, its existence has bearing on structural conclusions drawn from the interpretation of, for example, the linear dichroism spectra of polynucleotides.

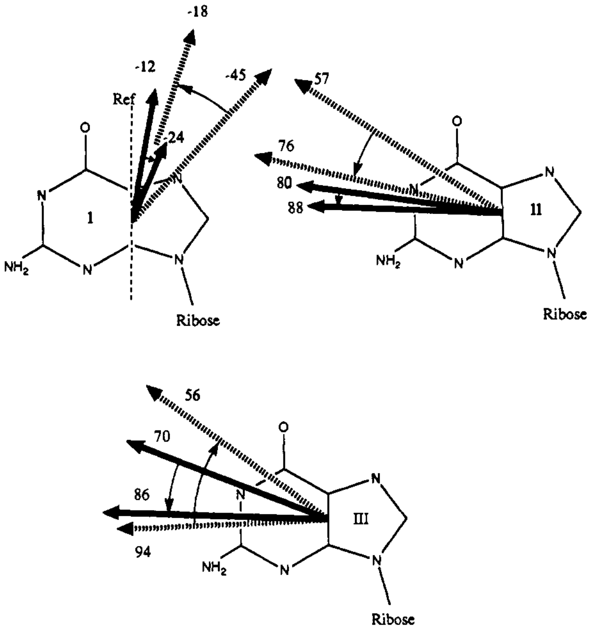

Figure 8. The effects of crystal field-induced mixing on the transition moment directions for I, II, and III (clockwise from upper left). The solid arrows are from the present study of guanosine. The arrow direction of the arcs connecting the two solid vectors represents the apparent change in the transition moment vectors owed to exciton mixing. The broken vectors are the theoretical directions reported for 9-EG. Again the connecting arcs indicate the change from the free molecule semiempirical MO results upon including the electrostatic crystal field (see ref 5).

Acknowledgment. This work was supported by the National Institute of General Medical Sciences, Grant GM38575.