does that for the presumably similar $\beta\beta$DNP excimer. This observation led us to the discovery that $\alpha\alpha$DNP undergoes intramolecular photodimerization. We have isolated the crystalline photodimer and shown that it is an anthracene-like dimer. These findings are reported separately.[22] We found no evidence for photodimerization in $\alpha\alpha$DNE, $\beta\beta$DNP, or $\alpha\alpha$DNB when millimolar solutions in methylcyclohexane were photolyzed with "300-nm light"[23] through a Pyrex filter; the ultraviolet absorption spectra of these solutions (deoxygenated) did not change.

There is much less thermally activated quenching in the unsymmetrical $\alpha\beta$DNP (Figure 8) where the excimer is much less stable than those formed by $\alpha\alpha$- and $\beta\beta$DNP. Similarly the fluorescence spectra of $\alpha\alpha$DNE and $\alpha\alpha$DNB show somewhat more thermally activated self-quenching than is found in the monomer αMN, but the quenching is still much less than is found for the two symmetrical propanes and we conclude that a stable, long-lived excimer is required for self-quenching to occur. This has also been suggested by Stevens and Dubois[24] who found that the self-quenching of naphthalene in dilute solution decreased with increasing temperature.

Conclusions

The observations of intramolecular excimer formation reported here are interpreted as showing that the preferred configuration of the naphthalene excimer is a symmetrical sandwich structure. The activation energy required to achieve this geometry in both $\alpha\alpha$-DNP and $\beta\beta$DNP is a consequence of rotation barriers in the methylene chain joining the naphthalene nuclei. Hirayama's conclusions regarding the geometrical requirements for intramolecular excimer formation in diarylpropanes are essentially verified in these systems.[25]

The thermally activated quenching of the excimer fluorescence of $\beta\beta$DNP does not involve intersystem crossing. Transient photodimer formation is a very good possibility. The dinaphthylpropanes which do not exhibit stable intramolecular excimer formation do not show the pronounced thermally activated quenching that is found in those compounds which do form excimers. Stable excimers appear to be required as intermediates for the self-quenching of aromatic hydrocarbon fluorescence.

(22) E. A. Chandross and C. J. Dempster, *J. Amer. Chem. Soc.*, **92**, 703 (1970).

(23) A Rayonet photochemical reactor containing mercury lamps, each coated with a phosphor having peak emission at 300 nm, was used.

(24) B. Stevens and J. T. Dubois, *Trans. Faraday Soc.*, **62**, 1525 (1966).

(25) NOTE ADDED IN PROOF. We unfortunately neglected to mention, in both the introductory section of this paper and in earlier work,[22] the studies of Leonard, Eisinger, *et al.*,[26,27] on intramolecular interactions in ground and excited states of various dinucleotides and similar compounds. They found intramolecular interactions in these systems to be optimal when the chromophores were linked by a trimethylene chain.

(26) N. J. Leonard, *et al.*, *J. Amer. Chem. Soc.*, **91**, 5855 (1969).

(27) N. J. Leonard, H. Iwamura, and J. Eisinger, *Proc. Nat. Acad. Sci. U. S.*, **64**, 352 (1969).

Polarization of Electronic Transitions in Cytosine

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Abstract: In this paper polarized fluorescence experiments on 5-methylcytosine and specular reflectance measurements on cytosine monohydrate and 1-methylcytosine are reported. The results are brought together with the aim of establishing the directions of the transition moments for the first two singlet–singlet transitions, nominally the 265- and 230-mμ bands. It has been found possible to establish the directions with some fair degree of meaningfulness so that applications to the electronic theory of biopolymers may be envisaged and so that a critique of current π-electron theoretical results for cytosine can be attempted. Indeed such a critique is sketched in here in the latter part of the paper. Some results on thymine are included in passing in an Appendix which is mainly devoted to the problem of working up reflectance data inductively.

In the first section of the paper, experimental methods are briefly outlined. Results are then taken up in subsequent sections according to the experimental techniques employed.

I. Experimental Section

A. Materials. Cytosine was obtained from Nutritional Biochemicals Corp. 5-Methylcytosine (A Grade) was obtained from Calbiochem. 1-Methylcytosine was obtained from Cyclo Chemical Corp. For our purposes the compounds were found not to require further purification.

Isopropyl alcohol was Matheson Coleman and Bell Spectrograde quality and the isopentane was Phillips technical grade, purified by shaking with concentrated H_2SO_4, washing with water, and distilling with sodium hydride.

Cytosine monohydrate crystals were obtained by evaporating about 200 ml of saturated aqueous solution at *ca.* 20°. The crystals were kept immersed in mother liquor until just prior to taking their spectra since they slowly lose water upon standing in air. The crystals were distinctive plates lying on the (100) face.[1]

1-Methylcytosine crystals were obtained by slowly cooling a hot, saturated aqueous solution in a closed weighing dish.

B. Apparatus. 1. Polarized Fluorescence. The essential features of the method used have been described elsewhere.[2]

2. Reflection Spectra. Polarized normal incidence reflection spectra were taken with a spectrometer built by Clark and Gerhold which was a development of an earlier model.[3]

(1) H. A. Rose, *Anal. Chem.*, **27**, 158 (1955).

(2) P. R. Callis, E. J. Rosa, and W. T. Simpson, *J. Amer. Chem. Soc.*, **86**, 2292 (1964).

(3) B. G. Anex and W. T. Simpson, *Rev. Mod. Phys.*, **32**, 466 (1960); L. Clark, Ph.D. Thesis, University of Washington, 1960.
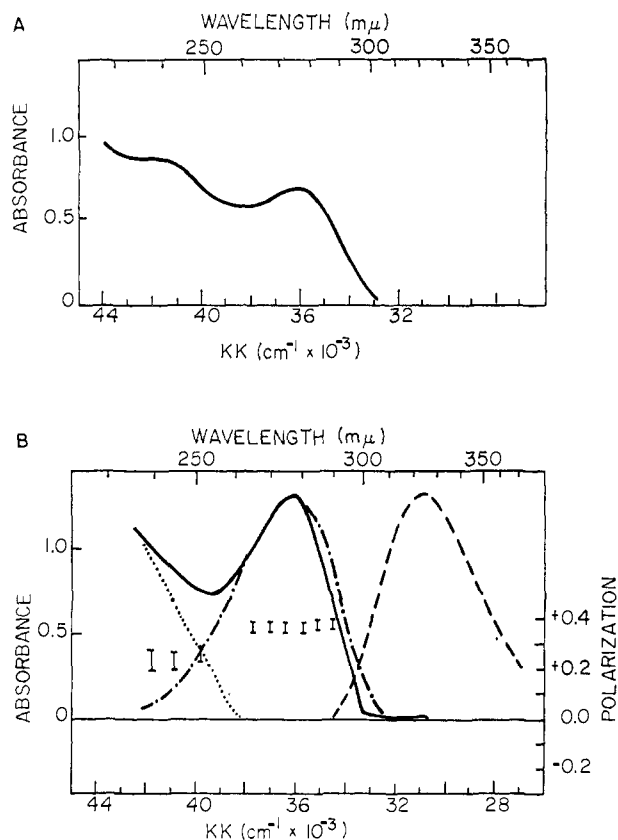
A

B

Figure 1. (A) Room-temperature absorption spectrum of 5-methylcytosine. (B) Absorption, —; fluorescence, ---, and fluorescence polarization (II) of 5-methylcytosine in isopropyl alcohol-isopentane[3-7] at liquid air temperature. The curve (-·-·-·) is the mirror image of the fluorescence and the curve (·····) is the difference of (——) and (-·-·-·).

II. Fluorescence Polarization

A. **Procedure.** 5-Methylcytosine was dissolved in a mixture of isopropyl alcohol–isopentane (3:7) in an amount such that the peak absorbance with 2.5-cm path was ca. 1.5. The solvent mixture forms a clear, rigid glass without cracking at the low temperature customarily employed, 77°K.

Low-temperature (77°K) absorption spectra were taken on a Cary 15 spectrophotometer. A fluorescence excitation spectrum was not taken, but the shape of the fluorescence band was seen to be independent of the frequency of the exciting light in the range 240–290 mμ and was very nearly the mirror image of the first absorption band.

The fluorescence was excited by "vertically" polarized light and was monitored with a 250-mm B & L monochromator set at 325 mμ, the fluorescence maximum. The fluorescence intensity was recorded when an ultraviolet-transmitting polaroid sheet was oriented for optimum transmission of vertically (||) and horizontally (⊥) polarized light, respectively. The process was repeated for ten different wavelengths of exciting light between 250 and 290 mμ. Immediately the fluorescence and absorption procedures were repeated with all experimental conditions the same except for replacing the sample with a glass containing no 5-methylcytosine. Sample and blank values were subtracted yielding the desired absorption curve and fluorescence intensities. The corrections to the fluo-

rescence intensities were always less than 6% and variations in how the corrections were applied did not significantly affect the results.

B. **Results.** In Figure 1B the curve on the right is the fluorescence and the solid curve on the left the low-temperature absorption. The vertical bars give the value of P corresponding to the indicated excitation wavelength

$$P = \frac{I_{||} - I_{\perp}}{I_{||} + I_{\perp}}$$

and $I_{||}$ and $I_{\perp}$ are the fluorescence intensities for the components polarized parallel and perpendicular to the polarization of the exciting light.

C. **Discussion.** The information given by fluorescence polarization measurements is the angle between the transition moment governing absorption and that governing emission. The theoretical limits for P are $+ \frac{1}{2}$ when the absorbing and emitting moments are exactly parallel and $- \frac{1}{3}$ when they are exactly perpendicular. In practice the observed limits are 0.40–0.45 and around -0.20–0.25 owing to various depolarizing effects which one usually does not bother to try to sort out.

In Figure 1B we see that from 290 to 265 mμ P is about 0.4, indicating that the absorption and emission processes occur with parallel moments—as would be expected for the lowest energy band. Below 265 mμ P drops slowly to about $+0.2$ as a second absorption band begins to contribute. If one assumes a decomposition of the absorption which makes the first absorption band (-·-·) have a shape which is the exact mirror image of the shape of the fluorescence (this is a good working assumption in the majority of cases) then the second band is given by the dotted curve (·····). Below 250 mμ the second band would be dominant and the polarization should be rapidly approaching -0.2 to -0.25 if the transition moment for the second band were perpendicular to the first. The fact that P has apparently leveled out at $+0.2$ at 240 mμ strongly suggests that the second transition moment in 5-methylcytosine is far from perpendicular. Quantitative considerations using the equations of Albrecht[4] predict an angle between the two polarization directions of 40 ± 15° as consistent with these data. (We must avoid a too literal view about the quantitative accuracy here, because the importance of depolarizing effects may not be the same for the two transitions.)

III. Polarized Reflection Spectra

A. **Procedure.** Polarized normal-incidence reflection spectra were measured over the range 400–215 mμ for the (001) and (100) faces of cytosine monohydrate and the (100), (010), and (001) faces of 1-methylcytosine. For each crystal face two spectra were taken in which the light was polarized along the two principal directions, respectively.

The faces were easily identified by their morphology due to the work of Rose[1] on cytosine monohydrate and with the aid of a private communication from Dr. Graham Mathews in the case of 1-methylcytosine.

In the reflection apparatus the crystals were mounted on a goniometer head with an azimuthal device so that the principal directions could be brought into align-

(4) A. C. Albrecht, J. Mol. Spectrosc., 6, 84 (1961).

ment with the E vector of the light by a simple rotation. The principal directions were located by rotating until a maximum or minimum reflection was observed. They were independently located with a standard polarizing microscope and for visible light agreement between the two methods was always obtained. The principal directions were independent of wavelength for every face studied except the 010 face of 1-methylcytosine. In this case they were constant from 400 to 280 mμ, rotated 20° between 280 and 260 mμ, and remained constant from 260 to 215 mμ. The procedure followed in taking reflection spectra of the 010 face was to change from one, the long-wavelength setting, to another, the shortwave setting, at 270 mμ—without attempting to follow the change in the 280–260-mμ region.

Reflection coefficients were determined with light from a deuterium lamp which had passed through a 500-mm Bausch and Lomb grating monochromator with slits set at 2 mm. Points were recorded every 5 mμ.

B. Reflection. Theoretical Considerations. The normal-incidence reflection coefficient is given by

$$R = \frac{(n^* - 1)(n - 1)}{(n^* + 1)(n + 1)} \quad (1)$$

where n is the complex refractive index and n^* its complex conjugate. In this paper we shall be making quantitative interpretations of reflection spectra using mainly the Lorentz–Lorenz equation

$$\frac{n^2 - 1}{n^2 + 2} = \frac{1}{3} \sum_r \frac{\omega_{pr}^2}{\omega_{0r}^2 - \omega^2 + i\gamma_r\omega} \quad (2a)$$

and (occasionally) the Sellmeier equation

$$n^2 - 1 = \sum_r \frac{\omega_{pr}^2}{\omega_{0r}^2 - \omega^2 + i\gamma_r\omega} \quad (2b)$$

where ω = the circular frequency of the light, ω_{0r} = the resonant frequency of rth transition, and γ_r = the damping constant for rth transition, which is approximately equal to the half-width of the absorption band. ω_{pr}, the so-called plasma frequency associated with the rth transition, is defined by

$$\omega_{pr}^2 = \frac{4\pi Ne^2}{M} f_{0r} = \frac{16\pi^2 N\omega_{0r}}{h} |\vec{m}_{0r}|^2$$

where N = molecules/cm^3, M, e = mass and charge of the electron, f_{0r} = the oscillator strength of the rth transition effective along the principal direction, and $\vec{m}_{0r}$ = the effective electric dipole transition moment for the rth transition.

In contrast to the absorption, the refractive index, and therefore the reflection, at a given frequency depends on the oscillator strengths of all the transitions. The nature of the denominators in eq 2 gives rise to a well-known phenomenon. As the frequency increases, passing through one of the resonant frequencies, say, the rth, there is an abrupt increase and decrease in n centered about ω_0. This manifests itself in the reflection coefficient R by a parallel increase and decrease. The effect becomes more pronounced as $|\vec{m}_{0r}|^2$ increases and the width over which one observes the effect is given by γ_r. Superimposed upon the abrupt rising and falling of R is a gradual rise occurring because of the terms in (2) referring to all the other tran-

sitions. Practically speaking we do not know what to use for the intensities and frequencies of the higher transitions but at low frequencies, say in the region of the first few electronic transitions, hopefully their effect may be reasonably well simulated by a single term in eq 2 corresponding to an hypothetical very intense transition coming at a high frequency.

Indeed it has been found possible to simulate observed reflection curves fairly closely over the region of the first two absorption bands of cytosine by adjusting the intensities, positions, and widths of the various bands as they appear in ω_p^2, ω_0, and γ, respectively, in a truncated version of eq 2.

$$\frac{n^2 - 1}{n^2 + 2} = \frac{1}{3}\left(\frac{\omega_{p1}^2}{\omega_{01}^2 - \omega^2 + i\gamma_1\omega} + \frac{\omega_{p2}^2}{\omega_{02}^2 - \omega^2 + i\gamma_2\omega} + \frac{\omega_{p3}^2}{\omega_{03}^2 - \omega^2} \right) \quad (3)$$

Here the third term effectively simulates the sum of effects from all transitions coming at higher frequency than ω_{02}.

For the so-called oriented gas model the probability that a given molecule in the crystal will absorb light polarized along a given principal direction is proportional to the square of the projection of the appropriate transition moment onto this direction. The n and n^* values, and hence the reflection coefficient, are obtained for the crystal from the sum of such projections over all the molecules in a unit cell. Beer's law holds in general only for light polarized along a principal direction, and all possible optical information concerning the behavior of plane-polarized light propagating normal to a given face depends on information obtained when the light is polarized along the two principal directions.

C. Reflection. Practical Considerations. Cytosine is a planar molecule, and in the two kinds of crystals studied here the planes of all the molecules are parallel.[5,6] Furthermore, there exists a face for each crystal studied which is nearly parallel to the molecular planes. Since it is virtually a certainty that the transition moments for the intense transitions under consideration lie in the plane, the problem of determining their directions is greatly simplified.

For 1-methylcytosine the molecular planes are nearly parallel to the ac face.[7] There are two molecules per unit cell, related by inversion through a point, so that the direction of the transition moment for a particular transition is the same for each molecule of the crystal (one "optical" molecule per unit cell). The angle, θ, which the moments make with the c' principal direction is defined by $m_{a'}^2/m_{c'}^2 = \tan^2\theta$ (the c' principal direction is the one closest to the c crystal axis, viewing the ac face). Thus there are two possible transition moment directions consistent with the ratio $m_{a'}^2/m_{c'}^2$ (which ratio is what one can observe). An experiment yielding independent information is necessary to resolve this ambiguity. (Having but one optical molecule per unit cell should lead to no ambiguity about θ unless the strong principal direction differs from the transition moment direction all through the transition—as can happen with a weak transition.)

(5) B. G. Anex and A. V. Fratini, *J. Mol. Spectrosc.*, 14, 1 (1964).
(6) G. A. Jeffry and Y. Kinoshita, *Acta Crystallogr.*, 16, 20 (1963).
(7) F. S. Mathews and A. Rich, *Nature*, 201, 179 (1964).
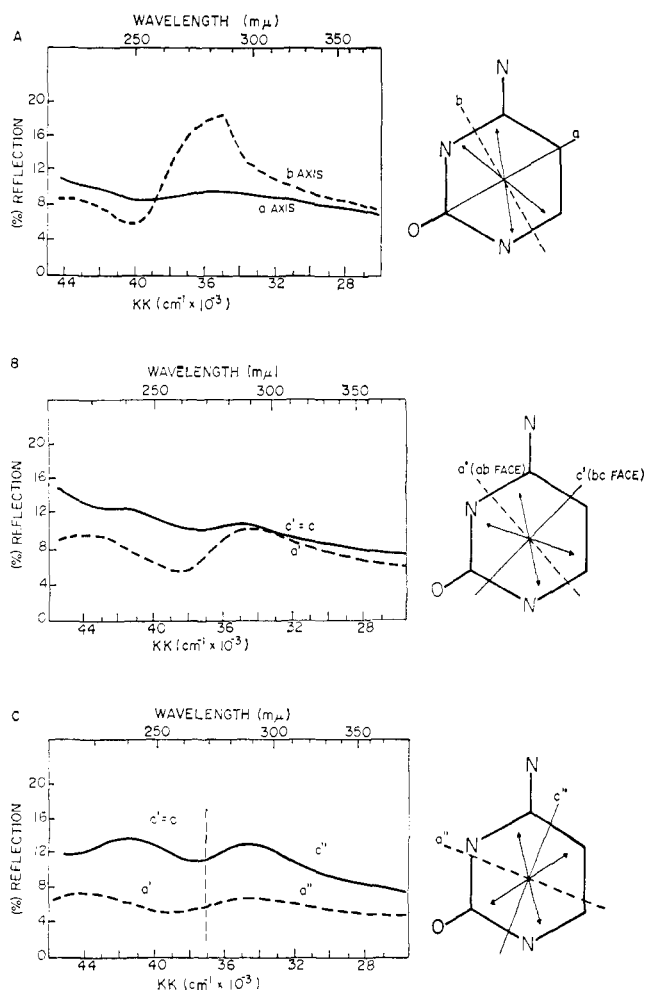
A

B

C

Figure 2. (A) Polarized reflection spectra taken along the two principal directions of the *ab* face of cytosine monohydrate. (B) Polarized reflection spectra of 1-methylcytosine taken along the *a'* (in-plane) principal direction of the *ab* face and the *c* axis in the *bc* face. (C) Polarized reflection spectra taken along the principal directions in the *ac* face of 1-methylcytosine. The diagram at the right of each spectrum indicates the orientations of the principal directions relative to the molecules in the crystal. The dashed spectra correspond to light polarized along the dashed principal direction. In the *ac* face the principal directions rotate, and below 260 mμ the principal directions are close to those in Figure 2B. The double-headed arrows represent possible transition moment directions for the 265-mμ band consistent with the data. These were obtained as outlined in the Appendix in the case of Figures 2A and 2B, but in Figure 2C it was merely assumed that the intensity along *c''* was twice that along *a''*.

Because of the fact that the moment directions are parallel for the two molecules in the unit cell of the 1-methylcytosine it is possible to obtain the required information by making observations on a face of the crystal different from *ac*. The requirement is that there be a principal direction parallel to the molecular planes whose direction is significantly different from either the *a'* or *c'* principal directions. In the *ac* face the *c'* principal direction makes an angle of 20° with the *c* axis for wavelengths between 400 and 280 mμ, *i.e.*, the region where the first transition should contribute maximally to the reflection. The molecular planes contain the *c* axis so that for the *bc* face (an "edge-on" face) the in-plane principal direction is parallel to *c*, indeed different from *c'* and perhaps significantly so. In the other edge-on face, the *ab* face, the in-plane

principal direction makes an angle of 22° with the *a* axis and makes an angle of 86.5° with the *c* axis.

Thus by careful trigonometry, in principle we should be able unambiguously to assign a transition moment direction for the intense long-wavelength transition of cytosine from experiments on 1-methylcytosine alone. Very conveniently, we may avoid relying on this procedure by obtaining further independent information from the cytosine monohydrate crystal. (We assume that the transition moment directions for the transitions considered here are not affected significantly by a single methyl substitution.) Because cytosine monohydrate is monoclinic, by symmetry, the *b* axis must always be a principal direction. There are four molecules in the unit cell. They are related by combinations of translation, a 180° rotation about *b*, and reflection through a plane perpendicular to *b*, and all four have their molecular planes containing *b*. Thus the *b* principal direction makes the same angle with each molecule in the unit cell. Specifically, this direction is parallel to the line connecting the two ring nitrogens, N_1 and N_3. The *a* axis is only 17° from all four molecular planes so that the molecules are nearly parallel to the *ab* face. The transition moments of the several molecules in the unit cell are not mutually parallel, but they all have identical projections (neglecting sign) onto the principal directions in the *ab* face.

D. Results. 1. Polarization of the First Transition (265 mμ). In Figures 2A–C are displayed three pairs of reflection spectra. The spectra in Figure 2A were taken with light polarized along the two principal directions of the *ab* face of cytosine monohydrate. Those in Figure 2C are for the *ac* face of 1-methylcytosine. These two parts of Figure 2 show faces which are nearly parallel to the molecular planes. The two spectra shown in Figure 2B were taken along the "in-plane" principal directions of the *ab* and *bc* faces of 1-methylcytosine (the edge-on faces). The spectra along the principal directions perpendicular to the molecular planes in the latter two faces (not shown in Figure 2B) gave a constant reflection coefficient of about 4%, as would be expected. To the left of each pair of spectra is an illustration showing the orientation of the relevant principal directions with respect to the molecules of the crystal as obtained by combining the present optical data with the published crystal structures. The relevant principal directions in Figure 2B are ones related to the *a'* and *c'* of Figure 2C. The solid spectra correspond to the solid principal direction, the dashed spectra to the dashed principal direction. The two double-pointed arrows in each illustration give the orientations of the two possible transition moments for the first transition (265 mμ) consistent with the observed ratio of intensities along the two principal directions, as obtained through the more or less exhaustive application of eq 3.

Let us first look at Figure 2A. Clearly the transition moment for the first transition lies close to the *b* principal direction but not parallel to it because some intensity is observed along the *a* direction. A quantitative estimate is that it makes an angle of 21° with the *b* direction. By the convention adopted by Devoe and Tinoco (Figure 3), the direction will be specified by the counterclockwise angle it makes with the line connecting N_1 and C_4. Thus the *b* direction (dashed line) is

+30° and the possible transition moment directions are +9 and +51°.

In Figures 2B and 2C the dashed principal directions are 42 and 70°, respectively. If the +9° possibility of Figure 2A is the correct one we should see intensity progress from being mainly along the dashed direction to being mainly along the solid one as the dashed direction swings from +30 to +70° (Figures 2A–C in order). Clearly this is the case. Indeed, the crudest conceivable interpretation, that of assuming equal intensity along the two principal directions in Figures 2B and 2C, places the moment direction between −1 and +25°, respectively. But there is definitely more intensity in one curve or the other in the two cases and one case is the reverse of the other, so that one may safely narrow the limits somewhat. Again, the 9° double-headed arrow in Figure 2A finds a counterpart from among the double-headed arrows in Figures 2B and 2C, whereas the 51° arrow perhaps has a counterpart in Figure 2B, but not in Figure 2C. Applying the semiquantitative procedure described in the Appendix and averaging over the three parts of Figure 2 one obtains +12 ± 3° for the direction of the transition moment for the first transition. The error limits involved in the application of the procedure given in the Appendix are low—often just a few degrees. However, a weak reflectance such as along b in Figure 2A could be caused by something like Franck–Condon forbiddenness even if no intrinsic allowed intensity along b were to exist. For this and similar reasons error limits of the order of 10° or so could well turn out to be more realistic.

2. Polarization of the Second Transition (230 mμ). Earlier in this paper it was pointed out that the polarized fluorescence spectrum of 5-methylcytosine suggests that the 230-mμ transition is far from being perpendicular to the first, or 265-mμ transition. In the cytosine monohydrate crystal the anomalous reflection due to the second transition seems to show up entirely along the b principal direction (Figure 2A). Thus the direction of this moment is closer to the b axis than to the a axis, and we see again that the two transitions must be approximately parallel. Simulation of the two curves in Figure 2A using the Sellmeier and Lorentz–Lorenz formulas (see Appendix) leads to the conclusion that the 230-mμ transition moment lies within 26° of the b axis so that its direction, according to our convention, could have any value between +4 and +56°.

Turning now to the data from the 1-methylcytosine crystal (Figures 2B and 2C) we first note that in the region of the second transition (below 270 mμ) the two sets of data are not really independent. This is because the principal directions for the ac face at wavelengths shorter than 260 mμ are shown in Figure 2C but unhappily have rotated so as to be essentially parallel to those in Figure 2B. The similarity of the two sets of spectra reflect this fact; that they are not identical is not well understood at present, although the high frequency part of Figure 2B (dotted curve) is being pulled down by the contribution from the high-frequency part of the first transition. At any rate it is noted that the intensities along the two principal directions are approximately equal in Figure 2C. Because of the lowness of the intensities it is not possible to get a precise estimate of the intensity ratio, but it appears unlikely that there is more than twice
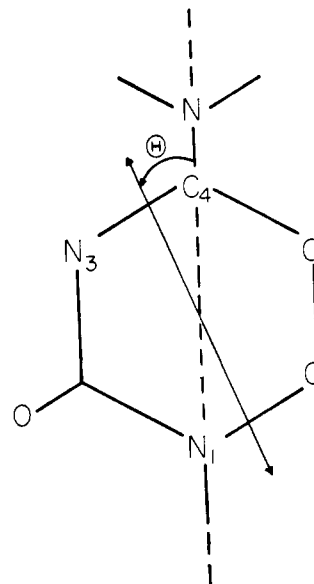

Figure 3. Devoe and Tinoco's convention[11] for specifying transition moment directions, which is used throughout this paper.

as much intensity along one direction as along the other. This limits the possible directions of the transition moment as lying between +75 and +103° or between −11 and +9°. Certainly, if there are no large effects due to methyl substitution and the different crystal environments, the latter possibility must be the correct one since it is consistent with the cytosine monohydrate result and with the result from the polarized fluorescence, that the first and second transitions are more nearly parallel than perpendicular (we have learned earlier that the first transition is polarized $ca.$ +12°).

In summary, then, polarized fluorescence coupled with crystal reflectivity measurements interpreted via an oriented gas model yield a direction of +12 ± 3° for the first transition (265 mμ) and place the second transition (230 mμ) between −11 and +9°. The fact that by polarized fluorescence the second transition is apparently found not to be exactly parallel to the first allows us to favor the −11° limit. A guess here would be to put the second transition at −1° (+5–10°).

IV. Discussion

The assumption concerning methyl substitution which allowed us to use cytosine monohydrate and 1-methylcytosine more or less interchangeably may well be true. The oriented gas model is, however, admittedly naive and conclusions based on its use must be regarded as tentative. That the model is appropriate as a first approximation is indicated by the fact that crystal spectra of these crystals and of other DNA bases[8] retain the essential features of their solution spectra. On the other hand certain simple theoretical models indicate that even comparatively weak interactions can lead to significant changes in polarization with little change in band shape.[9,10] Perhaps it is best to conclude only that the first transition (265 mμ) in cytosine has a transition moment direction in the neighborhood of +12° and that the second transition

(8) R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255 (1963).
(9) W. C. Johnson and W. T. Simpson, *ibid.*, **48**, 2168 (1968).
(10) H. H. Chen and L. B. Clark, *ibid.*, **51**, 1862 (1969).

-10°
(4→6)
$\mu = 1.16$ Å
-42°
(5→7)
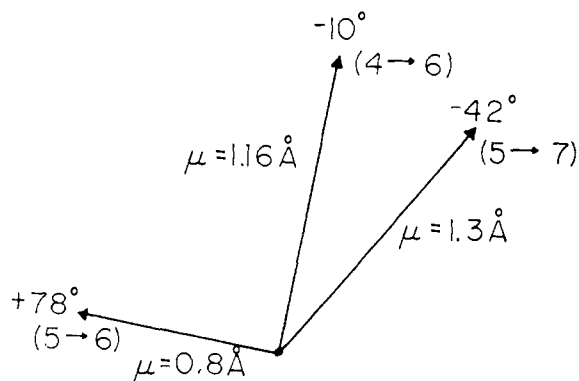$\mu = 1.3$ Å
+78°
(5→6)
$\mu = 0.8$ Å

Figure 4. Typical result for the first three transitions moments from HMO or SCF calculations. This particular result was from an HMO calculation using a simple choice of parameters: all β's equal and all α's = 0 except $\alpha_{N_1} = \alpha_{N_4} = \beta$ and $\alpha_0 = 0.5\beta$.[19] This result is not significantly different from that reported by others in several instances.[14,16,19]

(230 mμ) has a moment negatively rotated by a few degrees so as to be nearly but not exactly parallel to the first.

It is hoped that these directions will find use in connection with exciton treatments of optical and other properties of biopolymers.

One reason for measuring electric dipole transition moments is to provide experimental information about the ground and excited state wave functions. It is important to compare predictions made using approximate quantum mechanical treatments with experiment for properties other than energy and energy differences, since energy is insensitive to small variations in the wave function. The DNA bases represent a particularly difficult problem with respect to calculations, even those which are heavily calibrated (the semiempirical type) owing to the total lack of symmetry and the presence of several types of heteroatoms within each base. At the same time the transition moment directions assume a more interesting (and perhaps more effective) role as a gauge of wave function accuracy for these molecules since the directions are not fixed by symmetry.

Because of their biological importance the DNA bases have been the subject of a considerable number of semiempirical π-electron calculations within the past few years.[11-20] Most of the calculations fall into three categories: (1) simple Hückel molecular orbital (HMO) calculations, (2) Roothaan-type self-consistent field calculations using the simplifications of Pariser and Parr and of Pople (SCF-MO), (3) SCF-MO calculations including some configuration interaction to improve the description of excited states (SCF-MO-CI). (In addition several calculations which take into account more than just the π electrons have been carried out

recently (see ref 21 and references therein). However, these have dealt only with the ground state.)

As concerns the transition moments of cytosine, methods 1 and 2 yield, by and large, uniform results for the three lowest excitations. These are typified graphically in Figure 4. The transitions may be labeled using serial numbers of participating π MO's.

The lowest energy transition (5 → 6) has a direction of around +80° while the second and third transitions are nearly parallel and (roughly) perpendicular to the lowest, coming at ca. −10 and −40°, respectively. The (4 → 6) state is lower than the (5 → 7) state. The transition lengths are all about equal but usually correspond to about twice the observed intensities. Thus, not surprisingly, the HMO and SCF-MO methods do not give a satisfactory account of the transition moments of cytosine.

There have been three published calculations in the third category. Berthod, et al.,[14,15] seem to have taken particular care in selecting a set of empirical parameters which give reasonable results with respect to energy for a variety of heteroatom-containing molecules. In their SCF-MO results for cytosine before CI the first three transitions have moment directions of +85, −16, and −47°, respectively, not much different from the directions in Figure 4. The latter two transitions have intensities twice that observed for the second transition, while the first has a magnitude corresponding to an intensity half that observed.

After configuration interaction the lowest transition moment direction rotates 17° clockwise to +68° with its length remaining constant. The second transition moment direction rotates 13° counterclockwise to −3° and becomes equal in length to the first. While this in itself still does not represent especially good agreement with experiment, the effect of the configuration interaction is definitely to improve the agreement with experiment; i.e., it brings both moments toward +12° and gives them equal intensity. This result suggests that if the mixing were a little stronger this method could well predict the first two moments to be nearly parallel and as having the right general direction, and thus to be in reasonable agreement with experiment.

Indeed upon examination of the SCF-MO-CI results it is apparent that the theoretical prediction for the second transition moment in cytosine will be rather sensitive to the choice of the empirical parameters used. This is because the (4 → 6) and (5 → 7) configurations, being nearly degenerate, tend to mix strongly and as it turns out in a manner such that the resultant transition moment is close to the vector difference of the individual moments (phases as portrayed in Figure 4). Thus the resultant intensity should be low and the transition moment direction quite different from either of the directions for the transitions to the individual excited configurations. Such a situation actually obtains in the CI calculations of Nesbet[17] and of Ladik and Appel.[18] In their results the first two moments are nearly parallel in the direction of ca. +90° and the second transition is from 4 to 50 times less intense than the first. (It is tempting to relate the parameter sensitivity of the relative intensities of the first two bands of cytosine to the observed environmental sensitivity of the intensity

(11) H. Devoe and I. Tinoco, J. Mol. Biol., 4, 518 (1962).
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ratio of these bands. For example, compare the absorbance at 240 mμ relative to that at 280 mμ in the absorption spectrum of 5-methylcytosine in isopropyl alcohol–isopentane (3:7) at 25° with that at 77°K, shown in Figures 1A and 1B.)

Appendix

Estimation of Dichroic Ratios from Reflection Data.

Here we describe our inductive (the Kronig–Kramers method may be described as deductive) method for obtaining dichroic ratios for crystals from their specular reflection. The method is demonstrated for the case of 1-methylthymine and the results are compared with those obtained from the thin-crystal absorption spectra of Stewart and Davidson.[8]

Thymine has an isolated absorption band coming at ca. 36 kK. Therefore in this example we use a two-term formula instead of the three-term formula like eq 3. The parameters in the second term are supposed to simulate the sum of terms due to all transitions coming at higher frequency than the first. The Sellmeier rather than the Lorentz–Lorenz formula is used here. The procedure followed was first to vary ω_{p1}, ω_{01}, γ_1, ω_{p2}, and ω_{02} to effectively simulate the reflection curve along the more intense principal direction ("p" axis), and to pay close attention to fitting amplitude-wise rather than frequency-wise. The spectrum along the other principal direction should be characterized, hopefully, by the same values of ω_{01} and γ_1 but different ω_{p1}, ω_{p2}, and ω_{02}. Thus when one has found a ratio $(\omega_{p1}^2)_a/(\omega_{p1}^2)_p$ which best fits the experimental curves he has found $|\vec{m}_i|_a^2/|\vec{m}_i|_p^2 = \tan^2 \theta$, where θ is the angle $\vec{m}_1$ makes with the p axis.

Looking now at Figure 5 we see the results of carrying out such a procedure for 1-methylthymine, a crystal for which the absorptions are known (solid curves).

Most of the intensity is along the p axis and values of ω_{p1}, ω_{01}, and γ_1 of 21, 35.5, and 4 kK were used to produce the curve (-·-·-) from the Sellmeier formula. The simulated curves for the a-axis spectrum use $\omega_{p1} = 5$ (----) and 7 kK (·····). From general ap-
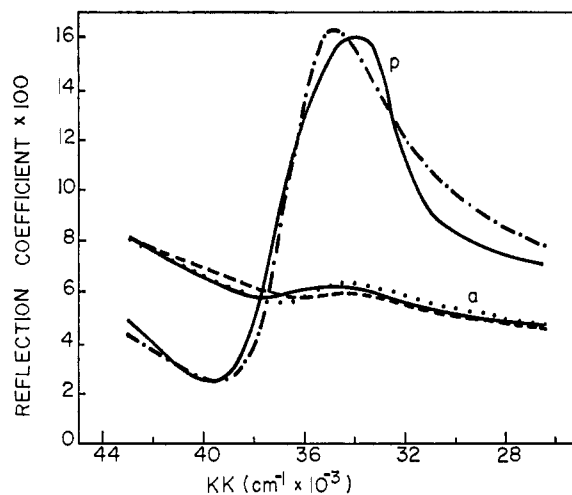

Figure 5. Experimental (—) and theoretical polarized reflection spectra of the 102 face of crystalline 1-methylthymine. The theoretical curves are based on the Sellmeier formula and differ only in the values taken for ω_{p1}, namely, 5 (---), 7 (····), and 21 KK (-·-·-).

pearances one would say the "a" axis spectrum was matched by $\omega_{p1} = 6 \pm 1$ kK.

Thus θ lies between $\tan^{-1} |^5/_{21}|$ and $\tan^{-1} |^7/_{21}|$. That is, one infers by this method that the transition moment makes an angle between 14.5 and 18° with the p axis. The result is in almost perfect agreement with the result of Stewart and Davidson (16.5 ± 2°).[8] There is a flavor of empiricism here which is not entirely un-warranted. It is to be noted that the a-axis (low-intensity) theoretical curves have the reflection "hook" occurring over a narrower frequency range than experimentally observed, and the plan here is to leave matters where they stand. Indeed it is found that if the shape of the hook is brought into agreement by increasing γ_1 and concomitantly ω_{p1} for the a-axis spectrum the ω_{pa}/ω_{pp} ratio is altered, undesirably, it is felt, so as to give $\theta = ca.$ 23°. The method described here was used for making the quantitative estimates from the cytosine spectra referred to in the text of this paper.