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Electronic Absorption Spectra of Crystals of Thymine, Uracil, and Their Derivatives

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The electronic structures and spectra of thymine, uracil, and their derivatives have been studied by measuring the electronic absorption spectra of the single crystals. In the polarized absorption spectra of the crystal, an unusual absorption band appears at about 285 mμ in the tail of the usual $\pi \rightarrow \pi^*$ transition when the crystal has the C=O···H–N-type hydrogen bond. The direction of the transition moment of this band is found to be along the direction of the hydrogen bond between a pair of C=O···H–N-groups, the nature of which is discussed from several viewpoints. The direction of the transition moment of the first and second $\pi \rightarrow \pi^*$ bands is determined to be inclined at about +38° and +86° from the $C_4 \cdots N_1$ line toward the C_5 atom. The result for the first band is not in perfect agreement with the finding of Stewart and Davidson regarding 1-methylthymine, who regarded this angle as either −14° or +19°.

Thymine and uracil are important base components of DNA and RNA molecules. The electronic structures and spectra of these molecules are significant in studying the conformation and optical properties of poly-nucleotide, since the hypochromic and circular dichroic effects are governed mainly by the nature of the electronic transitions and the interaction of excited states of these base pairs. Although many investigations have appeared on the spectra of DNA bases and these derivatives in solution,[1–5] little work has been done with the polarization of the transition moments. Stewart and Davidson[6] reported on the absorption spectra of single crystals of 9-methyladenine and 1-methylthymine. Chen and Clark[7] measured the polarized reflection spectra of the purine crystal. The present research was undertaken in order to find the polarization of each band by measurements of the absorptions on the single crystals of several of these base components. The polarized crystal absorption spectra of several kinds of crystals of thymine, uracil, and these derivatives were measured; these bases all have the same π-electron framework and, to a first approximation, may have the same polarization property if we disregard the effect of substituent methyl groups. The exciton-type interaction between different excited states (second-order exciton effects) will be disregarded because the transition moments are not very large. The dichroic ratios are used with the oriented gas model to find the directions of two $\pi \rightarrow \pi^*$ transitions in the 220 and 280 mμ regions.

In the crystalline spectra, we have noticed a particular kind of absorption band which is accompanied by a C=O···H–N-type hydrogen bond: The polarization character of this band is rather specific, and its nature will be discussed from several points of view.

Experimental

Materials. The 5-ethyl-6-methyluracil was kindly supplied by Dr. Marsh of the California Institute of Technology, while the 1,3-dimethyl thymine and 1,3-dimethyluracil were synthesized by Dr. Sakabe of our laboratory. All the other compounds were obtained from the Sigma Chemical Company, the California Corporation for Biochemical Research, or the Nakarai Chemical Company. All these compounds were recrystallized from water or ethanol before measurement.

Apparatus. The crystal spectra were recorded at room temperature on a specially-designed microspectrophotometer consisting of: (i) an Olympus polarizing microscope with Zeiss Ultrafluar objectives and a quartz Rochon polarizer, (ii) a Zeiss PMQII monochromater with tungsten and hydrogen sources, and (iii) a 1P28 photomultiplier tube with a standard Zeiss detection system.

Results

1,3-Dimethylthymine. The absorption spectrum of the single crystal of 1,3-dimethylthymine is shown in Fig. 1. The crystal absorption spectrum is similar to that of the solution spectrum, and the $\pi \rightarrow \pi^*$ band is observed at about 270 mμ. The direction of the transition moment can not be discussed with this crystal since the crystal structure has not yet been analyzed.

Calcium Thymidylate. The absorption spectrum of the single crystal of calcium thymidylate has been measured and is shown in Fig. 2-a. The crystal structure analysis has been carried out by Trueblood, Horn, and Luzzati,[8] who showed that the crystal belongs to the space group of C_{2h}-$P2_1$ and that two molecules are included in a unit-cell. The unit-cell dimensions are $a=14.40$, $b=6.87$, $c=9.81$ Å, and $\beta=90°58'$. On the basis of this finding, the projection of molecules onto the developed plane (100) is pictured in Fig. 2-b. A thymine ring is found nearly perpendicular to the bc plane; it is related to another ring with a two-fold screw axis parallel to the b axis. The distance between the two thymine rings is 3.43 Å, and no hydrogen bonding of the C=O···H–N-type is involved between the base rings. The crystalline absorption spectrum

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[1] S. F. Mason, *J. Chem. Soc.*, **1960**, 219; **1954**, 20.

[2] D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers*, **1**, 193 (1963).

[3] K. Nakanishi, N. Suzuki, and F. Yamazaki, *ibid.*, **34**, 53 (1961).

[4] L. B. Clark and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **87**, 11 (1965).

[5] M. Tanaka and S. Nagakura, *Theor. Chim. Acta*, **6**, 320 (1966).

[6] R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255 (1963).

[7] H. H. Chen and L. D. Clark, *ibid.*, **51**, 1862 (1969).

[8] K. N. Trueblood, P. Horn, and V. Luzzati, *Acta Crystallogr.*, **14**, 965 (1961).

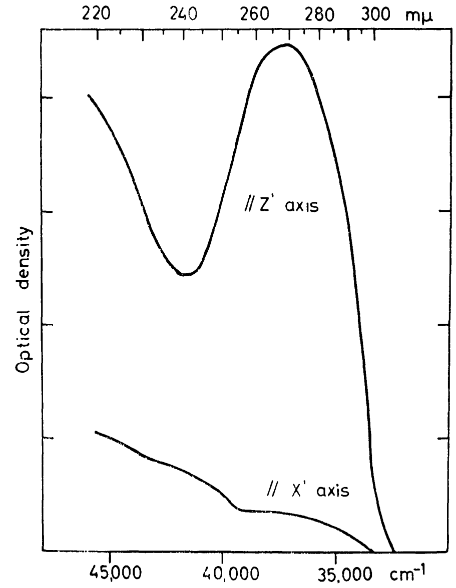

Fig. 1. The polarized absorption spectrum of the single crystal of 1,3-dimethylthymine.

is measured on the bc plane. In the *c* axis spectrum, two peaks are observed at 275 and 220 mμ but the *b*-axis spectrum has only a peak at 280 mμ in the region between 330 and 220 mμ. Each peak shifts to a longer wavelength as compared with the solution spectrum, but no extra band is observed. The dichroic ratio for the first $\pi\rightarrow\pi^*$ band in the vicinity of 280 mμ is determined from the areas under two perpendicularly-polarized absorption curves and it gives $I_c : I_b = 10 : 1$. If θ is the angle which the transition moment projected onto the bc plane makes with the *c* axis, then the polarization ratio ($I_c : I_b$) is equal to $\cot^2\theta$. From the crystal structure data, we may be able to find the relation of the molecular frame to the *b* axis and, finally, to determine the direction of the transition moment in the molecular plane. By this method, the transition moment of this band is determined to lie at either $+79°$ or $+97°$ inclined to the reference axis, which is settled along the line joining the C_4 atom and its para partner (N_1) as is shown in Fig. 3. Here, the positive sign of the angle indicates clockwise rotation around the center of the ring. The dichroic ratio of the second $\pi\rightarrow\pi^*$ band is about 6 : 1 ($I_c : I_b$) as measured from the observed band intensities. From these results, the transition moment to the reference axis is estimated as being inclined to $+82°$ or $+94°$. These angles are found to be more or less likely for the first and second bands, but this result is not reasonable from a theoretical point of view. The accuracy of the measurement is poor, because the molecular plane is sharply tilted to the developed crystalline plane. In such a configuration an accurate determination will be rather difficult, but it will provide some auxiliary information.

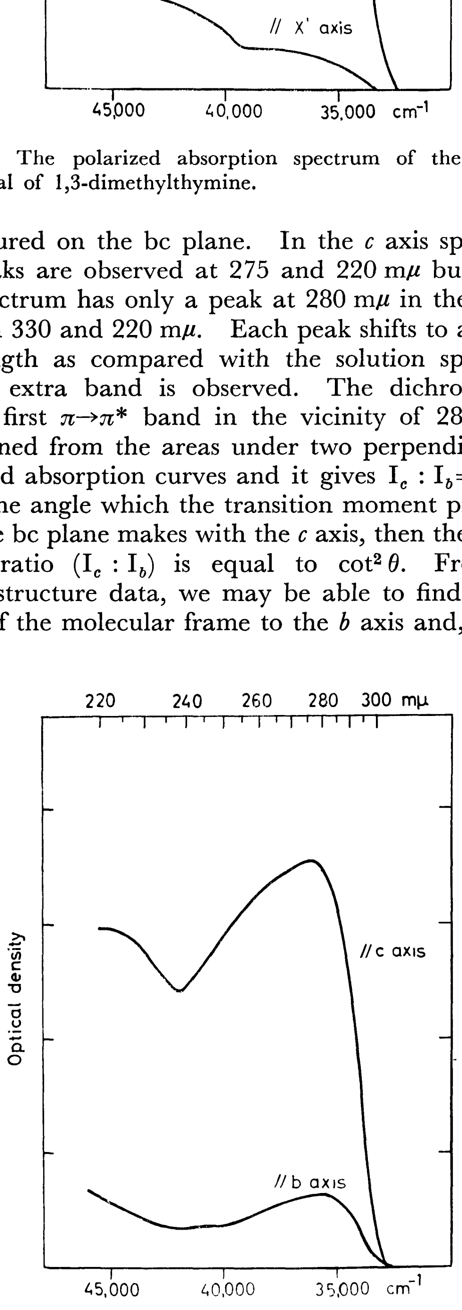

Fig. 2-a. The polarized absorption spectrum in the (100) plane of the single crystal of calcium thymidylate.

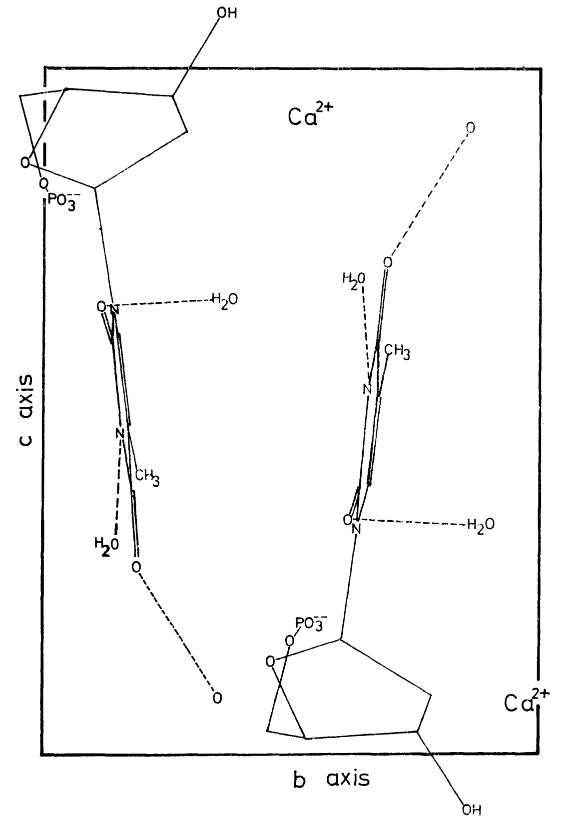

Fig. 2-b. Projection of calcium thymidylate molecules onto the (100) plane.

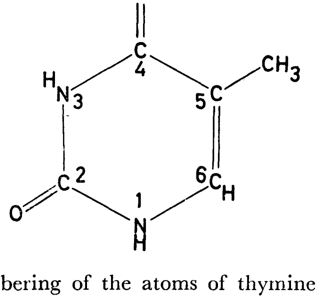

Fig. 3. Numbering of the atoms of thymine molecule.

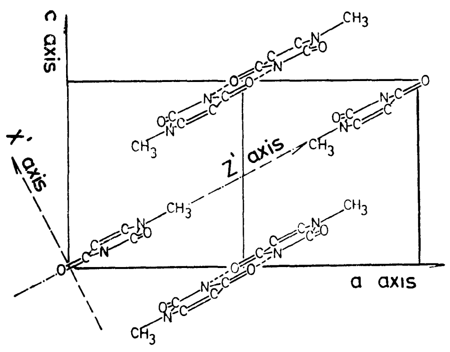

Fig. 4-a. Projection of 1-methylthymine molecules onto the (010) plane.

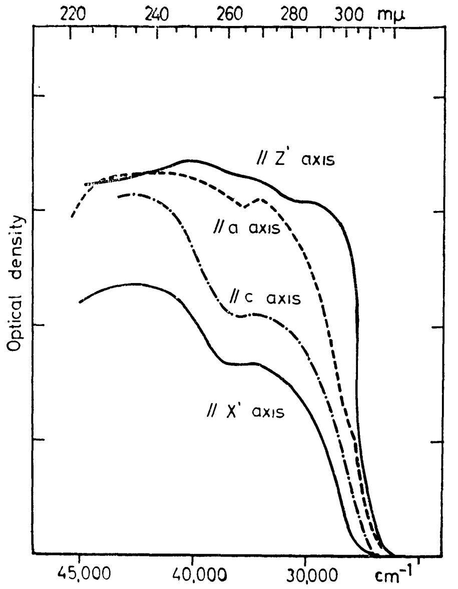

Fig. 4-b. The polarized absorption spectrum in the (010) plane of the single crystal of 1-methylthymine.

1-Methylthymine. Hoogsteen[9] analyzed the crystal structure of 1-methylthymine and showed that the crystal belongs to the space group of C_{2h}-$P2_1/c$, including four molecules in a unit-cell. The unit-cell dimensions are $a=7.53$, $b=12.09$, $c=7.60$ Å, and $\beta=89°58'$. The projection of molecules onto the developed cleavage plane (010) is pictured in Fig. 4-a. The molecules are arranged in layers closely parallel to the (102) plane and nearly perpendicular to the ac plane. The molecules are joined together in pairs by two C=O···H–N-type hydrogen bonds around a center of symmetry. The absorption spectra have been measured along the a and c crystal axes as well as along the Z' and X' optical axes, as is shown in Fig. 4-b. Here, the optical axis Z' is placed within the (102) plane and the X' axis is normal to its plane. Stewart and Davidson[6] measured the polarized absorption spectrum with the (102) plane, in which they used a crystal cut by a diamond knife of ultramicrotome. Our spectra are different from their spectrum in that an extra peak appears in the vicinity of 310 mμ. This band is nearly perfectly polarized along the Z' axis. Therefore, the direction of the transition moment must be along the direction of the hydrogen bond. Accordingly, the origin of this band might be intimately connected with the existence of the hydrogen bond of a C=O···H–N-type. This absorption characteristic is also found with other types of crystals containing the C=O···H–N-hydrogen bond between the base rings; we will discuss its nature in a later section. The next peak is found in the 270 mμ region; this peak may be assigned to the first $\pi\rightarrow\pi^*$ band. In the determination of the dichroic ratio of these bands, two methods are employed. One is to subtract the extra band from the observed spectral curve, regarding it as of different electronic origin. The other method is to include it into a single $\pi\rightarrow\pi^*$-type transition. By the first method the observed dichroic ratio is about 6 : 1 ($I_{z'}$: $I\ '$) and the direction of the transition moment

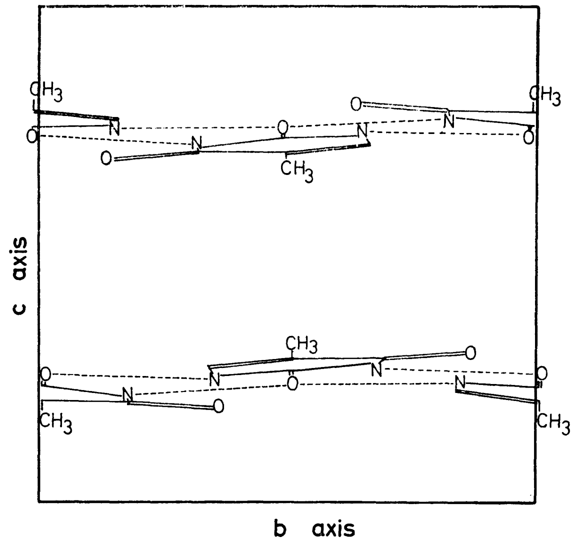

Fig. 5-a. Projection of thymine molecules onto the (100) plane.

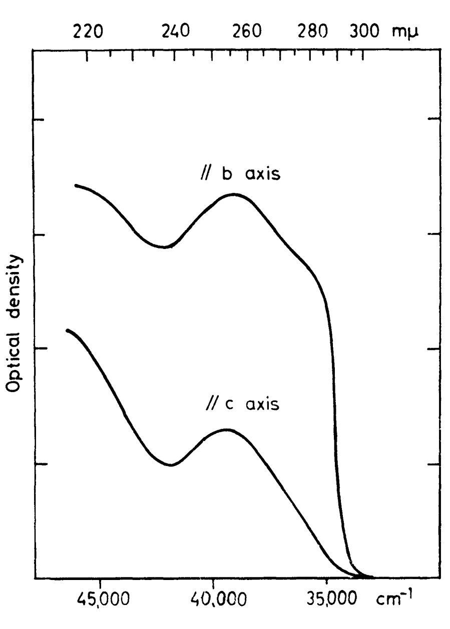

Fig. 5-b. The polarized absorption spectrum in the (100) plane of the single crystal of thymine.

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[9] K. Hoogsteen, *Acta Crystallogr.*, **16**, 28 (1963).

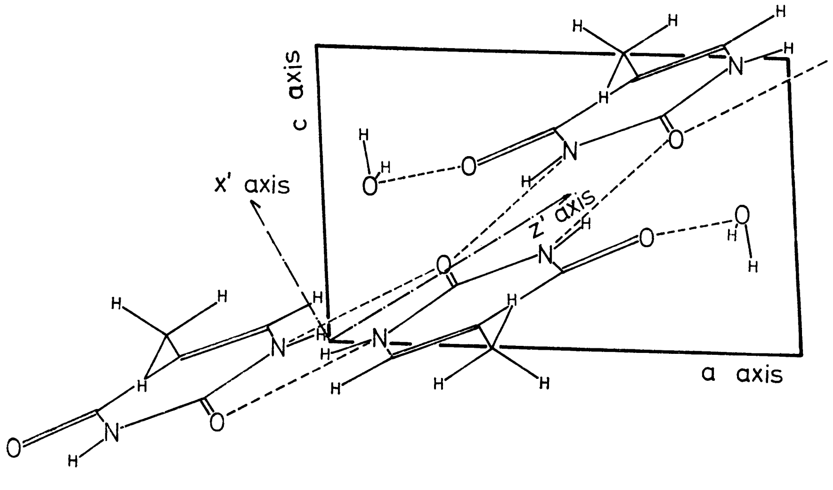

Fig. 6-a. Projection of thymine monohydrate molecules onto the (010) plane.

is deduced to be either +84° or +103° to the C_4-N_1 reference axis. By the latter method, the angle is found to be +80° or +110°. The second $\pi\to\pi^*$ band exists in the region of 235 mμ, with the dichroic ratio of 1.5 : 1 ($I_{z'}$: $I_{x'}$). From this value, the transition moment is determined to lie at +87° or +96° inclined to the reference axis. In this case also, the first and the second bands show similar polarizations; this must be due to the conditions of the developed plane being unfavourable for the measurement of the first band.

Thymine. The crystal structure of thymine anhydrate has been analyzed by Ozeki, Sakabe, and Tanaka[10] in our laboratory. The crystal belongs to the space group of C_{2h}–$P2_1/c$ and includes four molecules in a unit-cell. The unit-cell dimensions have been found to be as follows; $a=12.87$, $b=6.83$, $c=6.70$ Å, and $\beta=105°$. The projection of molecules onto the cleavage plane (100) is pictured in Fig. 5-a. The plane of the molecule is nearly parallel to the (001) plane and is included at 3.3° and 4.1° to the a and b axes respectively. The molecules are connected by two hydrogen bonds of the C=O···H–N-type. The crystalline absorption spectrum has been measured on the (100) plane; it is shown in Fig. 5-b. In the spectrum parallel to the b axis, an extra hump is observed at about 285 mμ and no component is detected along the c axis. Accordingly, this band may be related to the hydrogen bond of the C=O···H–N-type; it will be discussed in a later section, The peak of the first $\pi\to\pi^*$ band exists at 255 mμ in both the b- and c-axes spectra, and the observed dichroic ratio is about 3.6 : 1 (I_b : I_c). The direction of the transition moment is determined to be either +50° or +68° (or, alternatively, +52° or +66°) to the C_4–N_1 axis. In the second $\pi\to\pi^*$ band, a maximum is observed at 220 mμ in the b axis spectrum, but no peak is found in the c-axis spectrum down to 220 mμ. The dichroic ratio is determined to be 2 : 1 (I_b : I_c), and the transition moment is estimated to be either +55° or +63° for the reference axis.

Thymine Monohydrate. The crystal structure of thymine monohydrate was analyzed by Gerdil.[11] The crystal belongs to the space group of C_{2h}–$P2_1/c$ and includes four molecules in a unit-cell. The unit-cell dimensions are $a=6.08$, $b=27.76$, $c=3.82$ Å, and $\beta=94°19'$. The projection of molecules onto the developed plane (010) is pictured in Fig. 6-a. The hydrogen bonds of the C=O···H–N-type extend along the direction of the Z' optical axis. The polarized absorption spectrum was measured in the (010) plane; it is shown in Fig. 6-b. The spectrum has an extra band at about 285 mμ in addition to two $\pi\to\pi^*$ bands at 265 and 220 mμ. The extra band is polarized along the direction of the Z' axis, which is the direction of the maximum refractive index. The observed dichroic ratios for two $\pi\to\pi^*$ bands at 265 and 220 mμ are about 3.4 : 1 and 1.4 : 1 ($I_{z'}$: $I_{x'}$) respectively. The angles between the transition moment and reference axes are determined to be +24° or +81° (or,

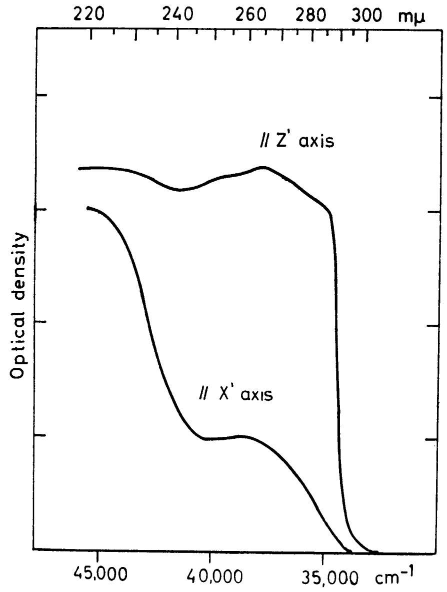

Fig. 6-b. The polarized absorption spectrum in the (010) plane of the single crystal of thymine monohydrate.

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[10] K. Ozeki, N. Sakabe, and J. Tanaka, *Acta Crystallogr.*, **B25**, 1038 (1969).

[11] R. Gerdil, *ibid.*, **14**, 333 (1961).

alternatively, +35° or +78°) for the first band and +43° or +76° for the second band.

1,3-Dimethyluracil. The absorption spectrum of the single crystal of 1,3-dimethylthymine is shown in Fig. 7. The crystal absorption spectrum is similar to the solution spectrum, and the first $\pi\rightarrow\pi^*$ band is observed at about 265 mμ. The peak of the second $\pi\rightarrow\pi^*$ band exists below 220 mμ. The direction of the transition moments of these two bands can not be discussed on the basis of the crystal spectra, since the crystal structures have not yet been analyzed.

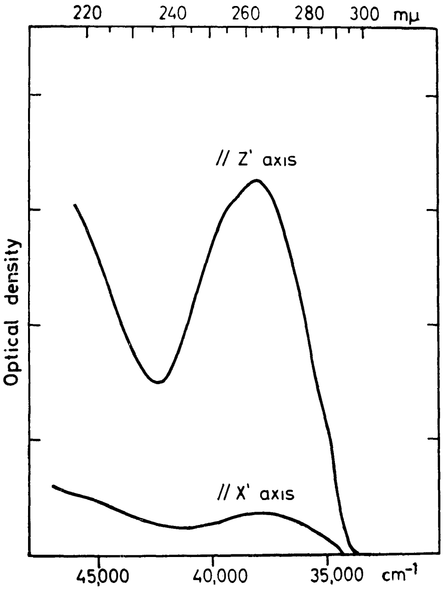

Fig. 7. The polarized absorption spectrum of the single crystal of 1,3-dimethyl uracil.

Sodium Uridine-5'-phosphate. The absorption spectrum of the single crystal of sodium uridine-5'-phosphate has been measured; it is shown in Fig. 8. The crystal structure is not known, but there seems to be no hydrogen bond of the C=O···H–N-type between the bases. The absorption spectrum is similar to that in solution, as expected, and no anomalous extra band is observed.

Uracil. The optical property of this crystal was studied by Gilpin and McCrone,[12] while the crystal structure was analyzed by Parry.[13] The crystal belongs to the space group of C_{2h}–$P2_1/a$ and includes four molecules in a unit-cell. The cell dimensions are a=11.82, b=12.35, c=3.62 Å, and β=120°. The projection of molecules onto the ac plane is pictured in Fig. 9-a. The molecules are arranged in a hydrogen-bonded layer parallel to the (001) plane. In this crystal, one oxygen atom (O_8) forms two hydrogen bonds with nitrogen atoms in the neighbouring molecules. The crystalline absorption spectrum was measured on the ac plane by means of a polarized light; the results are shown in Fig. 9-b. For the spectrum on the ac plane, an extra peak is observed along the direction of the Z' optical axis, which is related to the hydrogen bond of the C=O···H–N-type. The peak of the first $\pi\rightarrow\pi^*$ band exists at 255 mμ, and the dichroic ratio is about 1.4 : 1 ($I_{z'} : I_{x'}$). Accordingly, the angle between the transition moment and the reference axis is estimated to be +19° or +23° for this plane. For the second $\pi\rightarrow\pi^*$ band, a peak

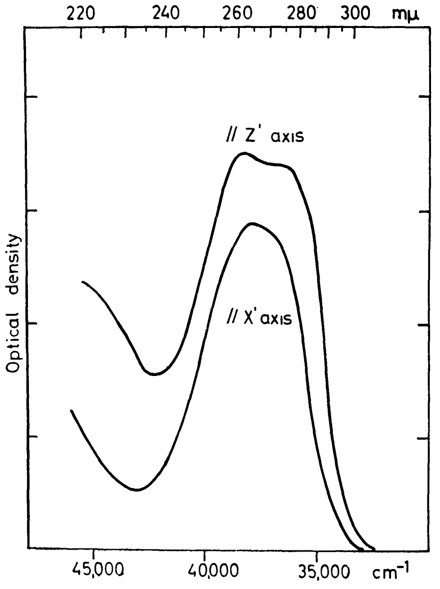

Fig. 8. The polarized absorption spectrum of the single crystal of sodium uridine-5'-phosphate.

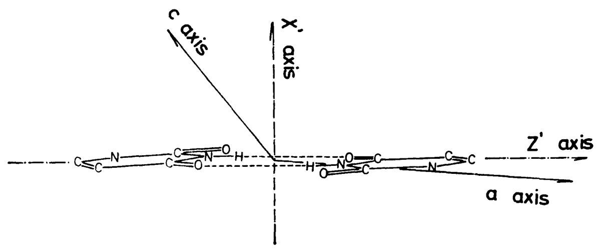

Fig. 9-a. Projection of uracil molecules onto the (010) plane.

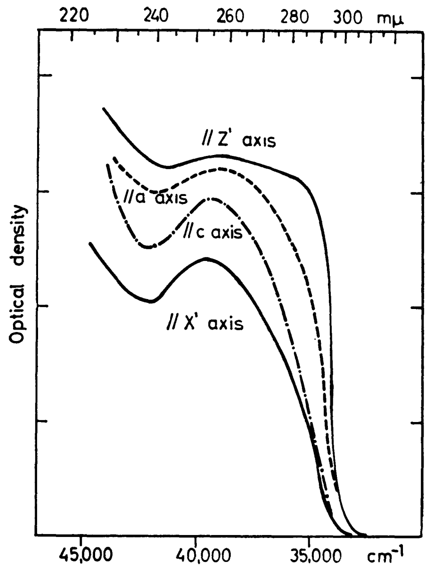

Fig. 9-b. The polarized absorption spectrum in the (010) plane of the single crystal of uracil.

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[12] V. Gilpin and W. C. McCrone, *Anal. Chem.*, **22**, 368 (1950).

[13] G. S. Parry, *Acta Crystallogr.*, **7**, 313 (1954).

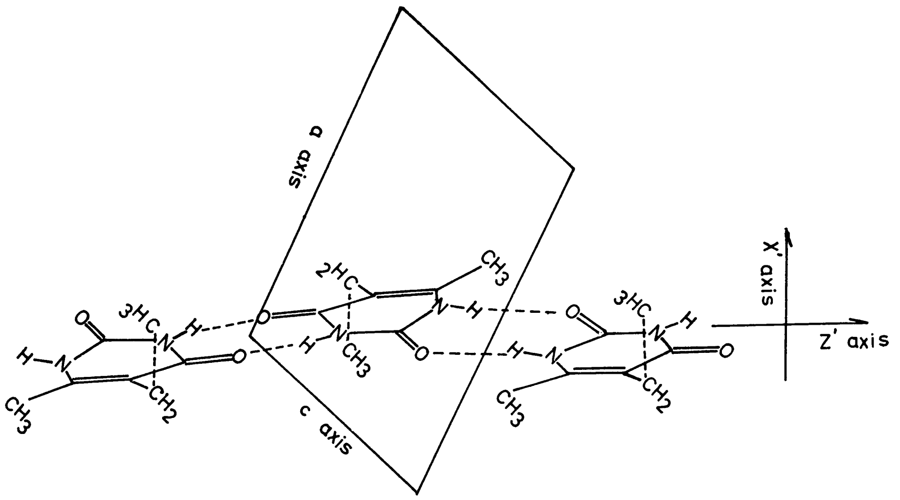

Fig. 10-a. Projection of 2-ethyl-5-methyluracil onto the (010) plane.

was not observed and so it would be difficult to obtain the dichroic ratio and to determine the direction of the transition moment.

2-Ethyl-5-methyluracil. Reeke and Marsh[14] had analyzed the crystal structure of 5-ethyl-6-methyluracil and had shown that the crystal belongs to the space group of $P\bar{1}$ and includes two molecules in a unit-cell. The projection of molecules onto the developed cleavage plane (010) is pictured in Fig. 10-a. Molecules are held together by C=O···H–N-type hydrogen bonds to form chains along the [102] direction. The absorption spectrum has been measured on the ac plane; it is shown in Fig. 10-b. In the polarized absorption spectra parallel to the Z' optical axis, an extra peak has been observed at about 290 mμ, and it has no component along the X' axis. Therefore, this band may be connected with the hydrogen bond of the C=O···H–N-type. The peak of the first $\pi\rightarrow\pi^*$ band is supposed to lie at 265 mμ; the observed dichroic ratio is about 9 : 1 ($I_{z'}$: $I_{x'}$), and it gives the direction of the transition moment to be either +45° or −60° to the reference axis. In the second $\pi\rightarrow\pi^*$ band, no peak is observed down to 220 mμ, but the dichroic ratio may be determined to be 3 : 1 ($I_{z'}$: $I_{x'}$) from the intensities in the measured region. Therefore. the transition moment is estimated to be either +35° or +100° for the C_4–N_1 axis.

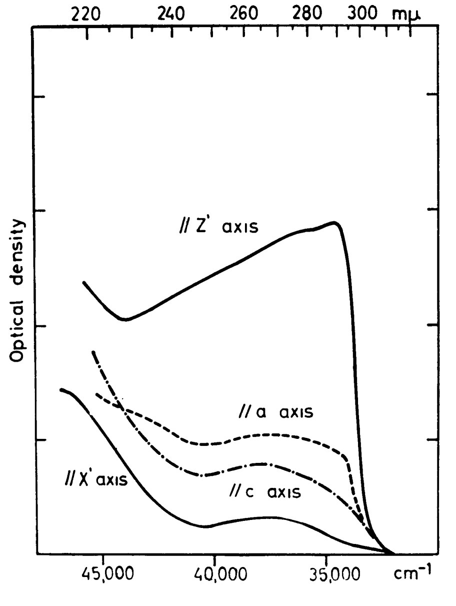

Fig. 10-b. The polarized absorption spectrum in the (010) plane of the single crystal of 2-ethyl-5-methyluracil.

Discussion

The directions of the transition moments obtained from these measurements are tabulated in Table 1. The values for the first and the second $\pi\rightarrow\pi^*$ bands are rather scattered, and a careful consideration is needed to determine the average angle. First of all, the accuracy of the dichroic measurement with each crystalline plane is not sufficient enough to decide the angle within an error of ±10°, because several bands overlap in this region. Secondly, the estimation of the angle is particularly difficult when the molecular plane is sharply tilted to the developed crystalline plane and the transition moment projected on the crystalline plane is placed nearly parallel to the line at which the molecular plane intersects the crystalline plane. For this reason, the results on calcium thymidylate and 1-methyl thymine are disregarded for the determination of the direction of the transition moment of the first $\pi\rightarrow\pi^*$ band; the results with four other crystals yielded the average value of +38°, with a probable error of ±14°. This value is not in exact agreement with the value obtained by Stewart and Davidson,[6] who regarded this angle as −14° or +19° on 1-methylthymine. The second $\pi\rightarrow\pi^*$ band will be perpendicular to the first $\pi\rightarrow\pi^*$ band because of the orthogonal relation, and the transition moment of the second band is placed perpendicularly to the intersecting line in the cases of calcium thymidylate and 1-methylthymine. Therefore, the results with five crystals are estimated to be +86°. The above-mentioned results are shown in Fig. 11. The deviations

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[14] G. N. Reeke, Jr., and R. E. Marsh, *Acta Crystallogr.*, **20**, 703 (1966).

TABLE 1. DIRECTION OF THE TRANSITION MOMENTS DETERMINED FROM THE OBSERVED DICHROIC RATIOS

Compound		First $\pi\rightarrow\pi^*$ band (I)[a]		First $\pi\rightarrow\pi^*$ band (II)[b]		Second $\pi\rightarrow\pi^*$ band	
			angle[c]		angle[c]		angle[c]
Calcium thymidylate	$I_c : I_b$	10 : 1	+79° or + 97°			6 : 1	+82° or + 92°
1-Methyl thymine	$I_{z'} : I_{x'}$	6 : 1	+84° or +103°	7 : 1	+80° or +110°	1.5 : 1	+87° or + 96°
Thymine	$I_b : I_c$	3 : 1	+52° or + 66°	3.6 : 1	+50° or + 68°	2 : 1	+55° or + 63°
Thymine monohydrate	$I_{z'} : I_{x'}$	3.4 : 1	+35° or + 78°	4.4 : 1	+24° or + 81°	1.4 : 1	+43° or + 76°
Uracil	$I_{z'} : I_{x'}$	1.5 : 1	+19° or + 23°	1.9 : 1	+16° or + 26°		
2-Ethyl-5-methyl uracil	$I_{z'} : I_{x'}$	9 : 1	+45° or − 60°	14 : 1	+48° or − 55°	3 : 1	+35° or +100°

a) The hump in the vicinity of 285 mμ is thought as another extra band which is assumed to have a line shape of gaussian type.
b) The hump in the vicinity of 285 mμ is thought as part of the same band.
c) The angle which the transition moment makes with the line joining the nitogen (N_1) and its para partner (C_4) (clockwise rotation denoted as positive in Fig. 3)

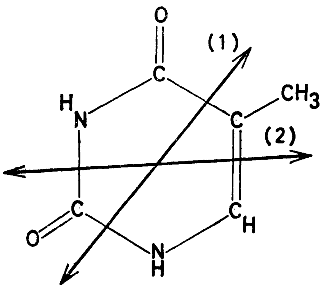

Fig. 11. Directions of the transition moments of the two $\pi\rightarrow\pi^*$ bands in the vicinity of 260 mμ (1) and 220 mμ (2).

between the observed values and average values may be interpreted by the mixing effect of the crystal field. It is now necessary to develop the experimental and theoretical studies of this effect.

Finally, let us discuss the nature of the additional band. Two prominent characteristics are noticed with this extra band; one is its polarization parallel to the direction of the hydrogen bonding, and the second is that it is observed as a hump on the tail of the first π—π^* band. We can imagine several explanations for this band; a charge-transfer point of view associated with a strong hydrogen bonding is stressed in a previous short communication.[15] Another explanation, which seems much more reasonable, is based on the consideration of the potential curve for the excited and the ground states. It is well known that the potential function of the hydrogen bonding has two minima along the direction of the hydrogen-stretching coordinate. It is very probable that the excited state has a displaced potential minimum relative to that of the ground state, as is shown in Fig. 12, because the N–H bond of the excited molecule may have a stronger acidity or hydrogen-donating power than the normal molecule, or the carbonyl group may

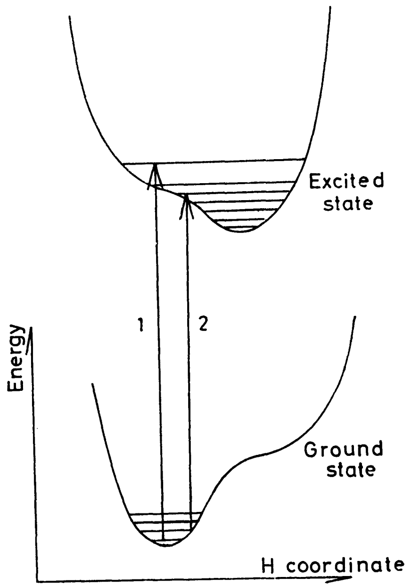

Fig. 12. The potential curve along the hydrogen bond.

have a stronger accepting power. For this particular potential problem, the vibrational structure of the electronic transition must be greatly modified. Specifically, the lower energy transitions are accompanied by a large displacement of the hydrogen coordinate and a large vibrational Frank-Condon factor, which causes the polarization to be along the direction of the hydrogen bonding. An alternative idea to explain this pattern is that the energy of the first $\pi\rightarrow\pi^*$ excited configuration is modified by the perturbation of the charge-transfer excited configuration; this may be regarded as a kind of vibronic perturbation in the excited state. These considerations are really suggestive of the shape and the electronic structures of the excited hydrogen bonding system in connection with the photochemical changes and mutations induced by ultraviolet light and radiation in DNA bases.

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15) J. Tanaka and M. Tanaka, *Nature*, **213**, 68 (1967).