Absorption Spectra of Crystals of Cytosine and Its Derivatives

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The electronic spectra of cytosine, one of DNA bases, have been studied. The absorption spectra of single crystals of cytosine and its derivatives are presented, and the direction of the transition moment of the first $\pi\rightarrow\pi^*$ band is determined to be inclined at about $+100^\circ$ from the N$_1$–C$_4$ line toward the C$_5$ atom. The second $\pi\rightarrow\pi^*$ band is shown to have polarization direction at $+30^\circ$ inclined to the reference axis.

Cytosine is one of the important base components of DNA and RNA. The electronic structures and spectra of these molecules are significant in studying the conformation and optical properties of polynucleotides since the hypochromism and circular dichroism are mainly governed by the nature of the electronic transitions and the interaction of excited states of these base pairs. The directions of the electronic transition moments are particularly important in considering the exciton type interactions. The polarization measurements of absorptions on the single crystals are most helpful in order to find these directions. Although many investigations[1] have been presented on the spectra of DNA bases in solution, a few works have been connected with the polarization of the excited state. In the subsequent paper[2] we have presented the absorption spectra of the single crystals of thymine and uracil derivatives and discussed on the directions of the transition moments of two lowest $\pi\rightarrow\pi^*$ bands. In the present paper we are presenting the results of the crystal absorption spectra on cytosine and its derivatives with the probable direction of transition moments.

Experimental

Materials. Cytosine and cytidine sulfate were obtained from Wako Pure Chemical Industries, cytidine was purchases from Biochemica Co. Ltd. and 1-methyl cytosine was obtained from Sigma Chemical Company.

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 M4Q III monochrometer with tungsten and hydrogen sources and (iii) a 1P28 photomultiplier tube with the standard Zeiss detection system.

Results

1-Methyl Cytosine. The absorption spectrum of the single crystal of 1-methyl cytosine is shown in Fig. 1. The crystals obtained on the quartz plate from the ethanol solution were employed for measurement of the polarized spectra. The developed plane of the crystal is assigned to the (100) plane judging from the paper by Mathews and Rich.[3] In this crystal, the first $\pi\rightarrow\pi^*$ band is observed in the vicinity of 285 mμ and is shifted to the longer wavelength as compared to the solution and other crystalline spectra. The second band has a peak below 230 mμ for the two perpendicularly polarized light and its position is not firmly ascertained. The direction of the transition moments of these two bands can not be discussed with the crystal since the coordinates of atoms are not reported.[3]

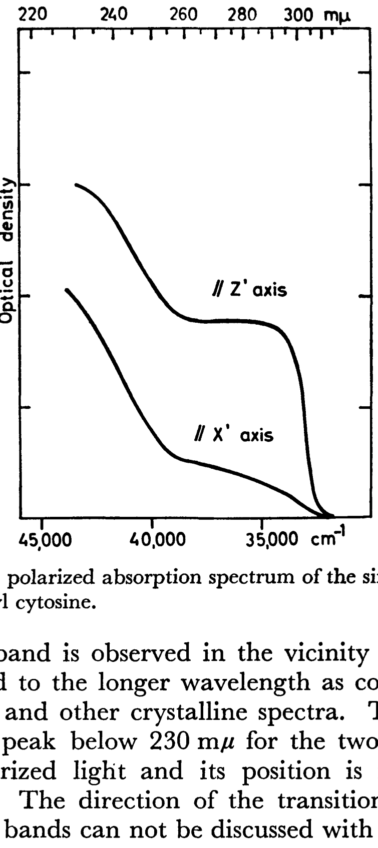

Fig. 1. The polarized absorption spectrum of the single crystal of 1-methyl cytosine.

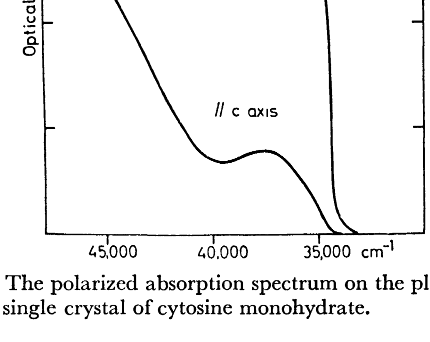

Fig. 2. The polarized absorption spectrum on the plane (100) of the single crystal of cytosine monohydrate.

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[1] S. F. Mason, *J. Chem. Soc.* (London), **1960**, 219, **1954**, 20; D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers,* **1**, 193 (1963); L. B. Clark and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **87**, 11 (1965).

[2] M. Tanaka and J. Tanaka, This Bulletin, to be published.

[3] R. S. Mathews and A. Rich, *Nature,* **201**, 180 (1964).

Cytosine. The absorption spectrum of the single crystal of cytosine monohydrate has been measured and is shown in Fig. 2. The crystal structure analysis has been carried out by Jeffrey and Kinoshita[4] and it has been shown that the crystal belongs to the space group of $P2_1/c$ and four molecules are included in a unit cell. The unit cell dimensions are $a=7.80$, $b=9.84$, $c=7.68$ Å, and $\beta=99°42'$. Single crystals of cytosine monohydrate were obtained by slow evaporation from dilute aqueous solution as monoclinic plates tabularly on the (100) plane. The projection of molecules onto this developed plane (100) is pictured in Fig. 3. According to this figure, the cytosine molecules are hydrogen-bonded into parallel ribbons extending in the b axis direction. Adjacent ribbons are related by the c glide symmetry and the perpendicular separation of the planes of the ribbons is 3.2 Å. The crystalline absorption spectrum has been measured on the bc plane. As shown in Fig. 2, cytosine is a weakly coupled system in this crystal and no large frequency shifts and splitting are observed for the bands in the crystal relative to their positions in the solution spectrum. The first $\pi\to\pi^*$ band has a peak at 265 mμ in both the b and c axes spectra. In the second $\pi\to\pi^*$ bands, only a tail of the band was measured but a peak may be located in the vicinity of 220 mμ in both the b and c axes spectra. The observed dichloric ratio for the $\pi\to\pi^*$ band at 265 mμ is determined from the areas under the two perpendicularly polarized absorption curves and (I_b/I_c) may be seen to be equal to $\cot^2\theta$. Here θ is the angle between the transition moment and the c axis. From the crystal structure data we may now determine the relation of the molecular frame to the b axis and finally the relation of the transition moment to the molecular frame. According to the above method, the transition moment of this band is determined to lie at either +25° or +100° inclined to the reference axis which designates the line joining the nitrogen (N_1) and its para partner (C_4) (clockwise rotation denoted as positive). The dichroic ratio of the second $\pi\to\pi^*$ band is about 1.5 : 1 (I_b/I_c) judging from the observed region. The angle between the transition moment and the reference axis is determined by the dichroic ratio to be either +45° or +80°.

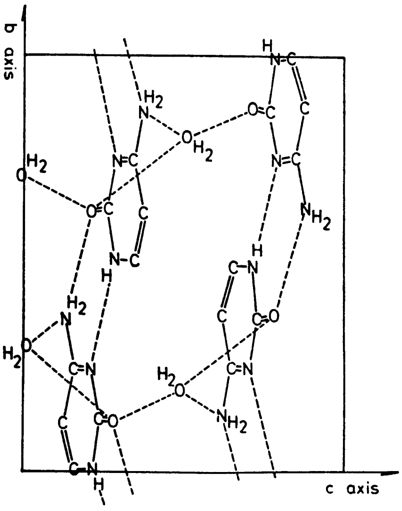

Fig. 3. The projection of cytosine molecules onto the developed plane (100).

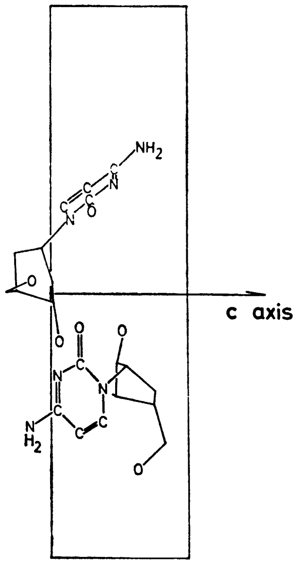

Fig. 4. The projection of cytidine molecules onto the developed plane (110).

Cytidine. Furberg, Petersen, and Rϕmming[5] analyzed the crystal structure of cytidine and showed that the crystal belongs to the space group of $P2_12_12_1$ and there are four molecules in the unit cell. The unit cell dimensions are $a=13.99$, $b=14.79$, and $c=5.12$ Å. The crystal is obtained by evaporating slowly a solution of cytidine in an alcohol-water mixture and has the shape of prism elongated along the c axis. The projection of molecules onto the developed plane (110) is pictured in Fig. 4. Two cytosine rings in the unit cell are nearly parallel to this developed plane and other two cytosine rings are located perpendicularly. The crystalline absorption spectrum has been measured along the directions parallel or perpendicular to the c axis on the

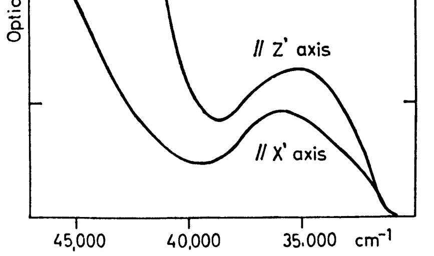

Fig. 5. The polarized absorption spectrum on the plane (110) of the single crystal of cytidine.

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[4] G.A. Jeffrey and Y. Kinoshita, *Acta Crystallogr.*, **16**, 20 (1963).

[5] S. Furberg, C. S. Petersen, and C, Rϕmming, *Acta Crystallogr.*, **18**, 313 (1965).

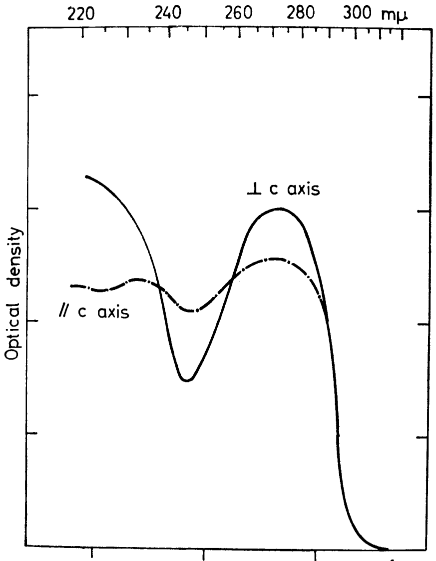

Fig. 6. The polarized absorption spectrum of the single crystal of cytidine sulfate.

plane (110) as shown in Fig. 5. The peak of the first $\pi\rightarrow\pi^*$ band exists at 272 mμ and the observed dichroic ratio is about 1.1 : 1 ($I_{\perp c}/I_c$) and the direction of the transition moment is deduced to be either +15° or +105° to the N_1—C_4 reference axis. The second $\pi\rightarrow\pi^*$ band exists in the 230 mμ region and its dichroic ratio is 1.4 : 1 ($I_{\perp c}$: I_c). By this ratio its transition moment is determined to lie at either +20° or +90° inclined to the reference axis.

Cytidine Sulfate. The absorption spectrum of the single crystal of cytidine sulfate is shown in Fig. 6. In this crystal, Davydov splittings are observed. The first band has a peak at 288 mμ for the light polarized parallel to the *c* axis and at 280 mμ for the light polarized perpendicular to the *c* axis and Davydov splitting is about 1000 cm^{-1}. The second band has a peak at 228 and 220 mμ for the light polarized parallel and perpendicular to the *c* axis, respectively. Davydov splitting of this second band is about 1600 cm^{-1}. Unfortunately the direction of the transition moment can not be discussed with this crystal because the crystal structure has not been analyzed so far.

Discussion

The absorption spectra of single crystals of cytosine and its derivatives show two absorption $\pi\rightarrow\pi^*$ bands corresponding to the 220 and 260 mμ bands in solution in the measured region. The observed dichroic ratios of the 220 and 260 mμ bands of these crystals are summarized in Table 1. There are in general two in-plane directions consistent with a dichroic ratio measured on a single crystal and data from a single crystal face will not in general be sufficient for a unique assignment of polarization directions. Therefore, the spectra of a few of cytosine derivatives were measured in order to determine the directions of the transition moments of two $\pi\rightarrow\pi^*$ bands but these directions could not be uniquely assigned from the observed ratios. As shown in Table 1, the transition moment of the first 260 mμ band is determined to be inclined at +20° or +100° from the N_1—C_4 line toward the C_5 atom and that of the second 220 mμ band at +30° or +85°. On the other hand, Nagata *et al.*,[6] Berthod, Giessner, and Pullman[7], and Morita and Nagakura[8] calculated the electronic states of cytosine molecule by the P-P-P method and discussed the directions of the transition moments. Particularly, Berthod, Giessner, and Pullman[7] decided the transition moments of two $\pi\rightarrow\pi^*$ bands to be inclined at +95° and +47° with respects to the N_1–C_4 axis. Accordingly, it is reasonable that the transition moments of the first and second bands are uniquely speculated to be inclined at +100° and +30° from the N_1–C_4 line toward the C_5 atom as shown in Fig. 7.

TABLE 1. OBSERVED DICHROIC RATIOS AND DIRECTIONS OF THE TRANSITION MOMENTS

		First band		Second band	
Cytosine monohydrate	I_b/I_c	7	+ 25° or+100°	1.5	+45° or+80°
Cytidine	$I_{\perp c}/I_c$	1.1	+ 15° or+105°	1.6	+20° or+90°

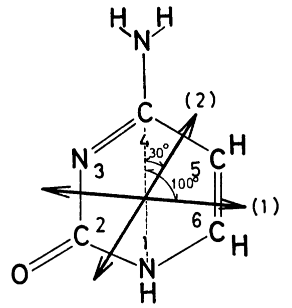

Fig. 7. The directions of the transition moments of the first (1) and second (2) $\pi\rightarrow\pi^*$ bands.

In the polarized absorption spectra of the crystal of thymine and uracil derivatives,[2] an usual absorption band appears at about 285 mμ in addition to the usual $\pi\rightarrow\pi^*$ transition when the crystal has the C=O···H–N hydrogen bond. The direction of the transition moment of this band is along the direction of the hydrogen bond between a pair of C=O···H–N groups. In the crystal of cytosine monohydrate, the cytosine molecules are hydrogen-bonded through N–H···N and C=O···H–N into parallel ribbons extending in the *b* axis direction but no hump was observed in the vicinity of 280 mμ.

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6) C. Nagata, A. Imamura, Y. Tagashira, and M. Kodama, This Bulletin, **38**, 1638 (1965).

7) H. Berthod, C. Giessner-Prettre, and A. Pullman, *Theoret. Chim. Acta*, **5**, 53 (1966).

8) H. Morita and S. Nagakura, *Theor. Chim. Acta*, **11**, 279 (1968).

Note added in proof: Recent paper by P. R. Callis and W. T. Simpson, *J. Amer. Chme. Soc.*, **92**, 3593 (1970), discusses the polarization of cytosine absorption bands.