from hydrogen-bond donor solvents to nonhydrogen-bonding solvents.

The following explanation is offered to account for the observed blue shift in the $\pi-\pi^*$ CD bands upon hydrogen bonding of the keto oxygens. The hydrogen-bond forming power of the keto oxygen can, to a first approximation, be estimated by the net charge on the keto oxygen. If changes in charge distribution upon $\pi-\pi^*$ excitation decrease the net negative charge at a keto oxygen which is hydrogen bonded to water, a blue shift would be expected relative to the reference nonhydrogen-bonding state. Several recent theoretical treatments predict that $\pi-\pi^*$ excitation reduces the net negative charge on the keto oxygen in uracil and cytosine. Cytosine nucleosides all exhibited $\pi-\pi^*$ bands that blue shift in water relative to 1,2-dichloroethane (DCE).[29]

Finally a recent study of phosphorescence to fluorescence ratios indicated that there are no closely spaced $n-\pi^*$ and $\pi-\pi^*$ singlets in the 260-mμ spectral region.[14] Further support for the interpretation that all CD bands in uridine arise from $\pi-\pi^*$ transitions is found in the observed red shift of all CD bands upon conjugative substitution on the uracil ring.[51] According to Goodman and associates[52] $\pi-\pi^*$ bands should undergo red shifts and $n-\pi^*$ bands blue shifts when conjugative substituents are placed on aromatic chromophores.

Acknowledgment. The authors thank Mr. William Miles for his excellent technical help.

(51) D. W. Miles, M. J. Robins, R. K. Robins, and H. Eyring, unpublished data.

(52) L. Goodman and R. W. Harrell, *J. Chem. Phys.*, **30**, 1131 (1959), and references therein.

Circular Dichroism of Nucleoside Derivatives. V. Cytosine Derivatives[1]

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Abstract: Circular dichroism data are reported for 12 cytosine nucleoside derivatives. Four CD bands are found in many of the nucleosides examined near 270, 247, 220, and 195 mμ. The CD spectra of a number of cytidine analogs show quite remarkable similarities to the CD spectra of similar type uridine analogs in both the positions and the signs and ellipticities of the Cotton effects. Solvent and structural effects on conformation of both the uridine and cytidine analogs are considered and a diagram is proposed that should be useful in determining the conformation of certain uridine and cytidine derivatives.

Despite much theoretical activity and numerous spectroscopic studies (see paper IV[2] and literature cited therein) on the base components of nucleic acids in the near-ultraviolet region, our understanding of the electronic properties of these systems is far from complete. Several studies have sought to demonstrate that the $\pi-\pi^*$ transitions of the pyrimidine bases are closely related to those in benzene.[2-6] In the present paper and the preceding paper[2] special derivatives of the bases were designed, and their circular dichroism and absorption properties were studied to clarify their spectroscopic and conformational properties. Such investigations, besides the intrinsic interest in the circular dichroism of such systems, provide more complete spectroscopic data for comparison with theoretical treatments now underway in these laboratories of the electronic properties of the purine and pyrimidine bases and for studies on the structure of nucleic acids and related polynucleotides and their oligomers and monomers. The results here presented on cytosine nucleosides give conclusive evidence for four electronic transitions in the cytosine bases above 190 mμ which may be related to the B_{2u}, B_{1u}, and E_{1u} bands of benzene. Clark and Tinoco[4a] found evidence in their absorption study of four absorption bands in cytosine at 276, 237, 204, and 184 mμ. Pentose substitution at N-1 shifts the first three absorption bands to higher frequencies with peaks at 270, 232, and 198 mμ. Analysis of our CD results shows the definite presence of another $\pi-\pi^*$ transition at 220 mμ and indicates that the true location of the apparent absorption band at 237 mμ probably lies above 240 mμ.

This study also relates to the current interest in the conformation of nucleosides and nucleotides in solu-

(1) Supported by Grant No. GM-12862-02 from the National Institutes of Health and Grant No. CA-08109-03 from the National Cancer Institute of the National Institutes of Health.

(2) D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, **91**, 824 (1969).

(3) D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Natl. Acad. Sci. U. S.*, **57**, 1138 (1967).

(4) (a) L. B. Clark and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **87**, 11 (1965); (b) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(5) V. G. Krishna and L. Goodman, *J. Amer. Chem. Soc.*, **83**, 2042 (1961).

(6) D. W. Miles, M. J. Robins, R. K. Robins, and H. Eyring, *Proc. Natl. Acad. Sci. U. S.*, in press.

Table I. Spectroscopic Data on Cytidine Derivatives[a]

Compound	Solvent[b]	B_{2u}	B_{1u}	E_{1ua}	E_{1ub}	λ_1	λ_2	λ_3
Cytidine[c] (**1**)	W	271 (12,700)	...	220 (−11,300)	195 (2000)	271	235	197
6-Methylcytidine[d] (**2**)	W	270 (−1200)	247 (800)	220 (−15,000)	198 (2000)	274	234	198
	AN	275 (−8000)	250 (500)	223 (−10,000)	...	274	237	198
	D	275 (−6000)	...	222 (−8000)	...	274	237	...
6-Methyl-2′-deoxycytidine[d] (**3**)	W	270 (−7000)	248 (−5000)	220 (15,000)	195 (8000)	271	234	198
	D	273 (−9000)	253 (−15,000)	...	...	273	237	...
	AN	273 (−8000)	252 (−10,000)	220 (18,000)	...	273	237	200
1-(2′-Deoxy-α-D-*erythro*-pentofuranosyl)-6-methylcytosine[d] (**4**)	W	270 (6800)	248 (4800)	220 (−13,000)	195 (3000)	271	235	198
	AN	273 (−2500)	252 (5000)	222 (−15,000)	...	274	237	200
1-(β-D-Arabinofuranosyl)-cytosine[c] (**5**)	W	270 (22,000)	...	222 (−15,000)	...	271	235	197
2′-Deoxycytidine[c] (**6**)	W	270 (8500)	Positive shoulder	217 (−12,000)	Small positive at cutoff	270	235	198
2′,3′-O-Isopropylidene-cytidine[c] (**7**)	W	270 (7600)	...	220 (−5000)	...	270	235	197
	DCE	274 (−2300)	254 (1200)	220–225 (−10,500)		275	240	...
5′-Cytidylic acid[c] (**8**)	W	270 (13,000)	...	220 (−13,000)	...	270	235	198
5-Iodo-2′-deoxycytidine[c] (**9**)	W	280 (5000)	Positive shoulder	224 (−1700)	210 (12,000)	284	220	200
5-Bromo-2′-deoxycytidine[c] (**10**)	W	286 (6200)	Positive shoulder	222 (−6300)	204 (12,000)	287	215	202
3-Methylcytidine methosulfate salt[c] (**11**)	W	278 (9000)	...	215 (−9400)	195 (5000)	278	...	202
6-Hydroxycytidine[e] (**12**)	W	267 (500)	...	...	204 (−5000)	267	224	197

[a] The position of maxima in circular dichroism spectra and absorption spectra are given in mμ units. The molar ellipticities are given in parentheses. [b] Solvents used: W = water at pH 7; AN = acetonitrile; D = dioxane; DCE = 1,2-dichloroethane. [c] Purchased from Calbiochem. [d] M. W. Winkley and R. K. Robins, *J. Org. Chem.*, **33**, 2822 (1968). [e] M. W. Winkley and R. K. Robins, to be submitted for publication.

tion.[6–17] Accurate knowledge of the conformation of the planar purine or pyrimidine ring with respect to the sugar is important in understanding the conformation of dinucleotides and polynucleotides. The conformation of the monomers may be of significance in understanding enzymatic polymerization and related processes observed in biological systems. It is also relevant to the construction of structural models of the nucleic acids. A summary of conformational information deduced from our data on both uracil[2] and cytosine nucleosides is given in the last section. Both types of nucleosides display interesting conformational changes due to solvent and substitutent perturbation.

Experimental Procedures

The references containing the details of the preparation and characterization of the nucleosides of this study or the commercial source are given in Table I. The other experimental details are the same as previously described.[2]

Results and Discussion

A. Spectroscopic Aspects. The absorption spectra of most cytosine nucleoside derivatives listed in Figure 1 are characterized not only by the two well-resolved absorption maxima at around 270 and 198 mμ, but also by the additional absorption in between. A barely resolved absorption peak is usually present at about 235 mμ. Indications of another absorption band can be seen perturbing the long-wavelength side of the very intense 198-mμ absorption peak at about 215 mμ. Representative curves are given in Figure 2. The 270- and 235-mμ absorption bands are both shifted to longer wavelengths by several millimicrons in acetonitrile, dioxane, and 1,2-dichloroethane. Similar solvent effects on the absorption spectrum of cytosine have been reported by Charney and Gellert.[18] In summary the complex absorption spectrum shows definite evidence of three and possibly four rather strong absorption bands compressed in a 70-mμ spectral range. This raises the question as to how close the real band position of the transition, producing much of the absorption in the 230–240-mμ spectral range, coincides with the apparent maximum at 232–235 mμ. While the final results of this study collected in Table I show essentially a 1:1 correspondence between the 270-mμ absorption peak and the long-wavelength CD peak, no CD band is found to exactly match the 235-mμ absorption. The compounds studied are found to give two to four Cotton effects in the near-ultraviolet spectral region down to 190 mμ. In Table I the long-wavelength Cotton effect is listed under the B_{2u} column. The position of the B_{2u} Cotton effect in cytosine nucleosides and the 6-methylcytosine nucleosides is found at 270 mμ correlating exactly with the 270-mμ absorption peak. In the 3-methyl- and 5-halo-substituted derivatives, both the absorption spectrum and the CD spectrum undergo 8–16-mμ shifts to lower frequences. Red shifts are nearly always induced in aromatic systems by both electron-donating and elec-

(7) T. Nishimura, D. Shimizu, and I. Iwai, *Biochim. Biophys. Acta*, 157, 221 (1968).
(8) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, 6, 843 (1967), and references therein.
(9) W. A. Klee and S. H. Mudd, *ibid.*, 6, 988 (1967).
(10) A. E. V. Haschemeyer and A. Rich, *J. Mol. Biol.*, 27, 369 (1967).
(11) D. W. Miles, S. J. Hahn, R. K. Robins, M. J. Robins, and H. Eyring, *J. Phys. Chem.*, 72, 1483 (1968).
(12) F. E. Hruska and S. S. Danyluk, *J. Amer. Chem. Soc.*, **90**, 3266 (1968).
(13) F. Jordan and B. Pullman, *Theor. Chim. Acta*, **9**, 242 (1968).
(14) I. Tinoco, R. C. Davis, and S. R. Jaskunas in "Molecular Associations in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1964.
(15) R. J. Cushley, I. Wempen, and J. J. Fox, *J. Amer. Chem. Soc.*, **90**, 709 (1968).
(16) C. D. Jardetzky, *ibid.*, **82**, 229 (1960).
(17) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *ibid.*, **90**, 1042 (1968).
(18) E. Charney and M. Gellert, *Biopolymers, Symp.* 1, 469 (1963).

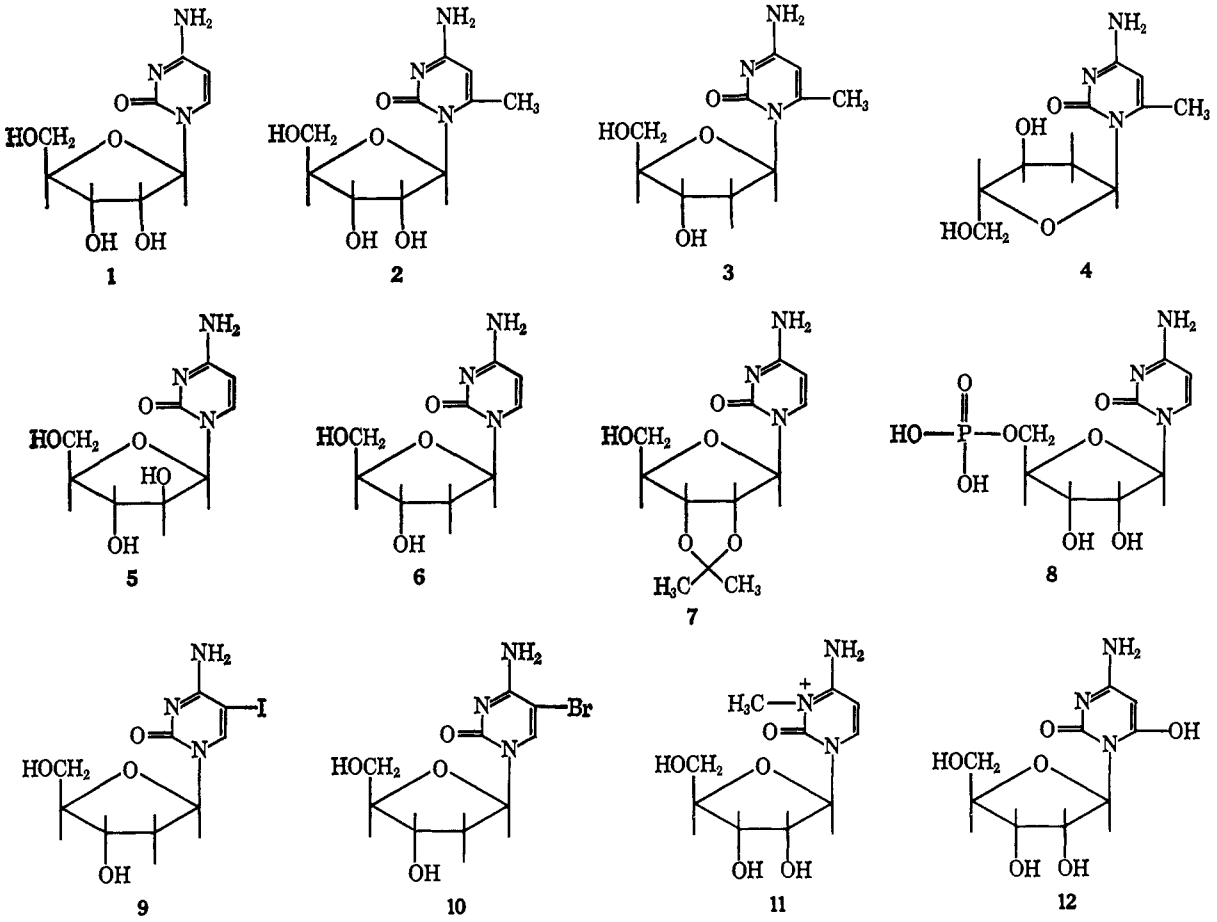

Figure 1. The structural formulas of the cytidine nucleoside derivatives.

tron-withdrawing exocyclic substituents.[14-21] Solvents not capable of strong hydrogen-bonding interactions with the lone-pair electrons of either the aza nitrogen or keto oxygen shift both the absorption and the CD peaks 3–5 mμ to lower frequencies and generally reduce the absorption intensity by about 30%. Attention will be drawn later to interesting changes in the sign and magnitude of the Cotton effects induced by solvents. About half the compounds studied showed Cotton effects in the 245–255-mμ region which are listed under the B_{1u} column. The location of this CD maximum does not appear to be especially solvent sensitive although marked changes in intensity are sometimes observed. These solvent shifts are presumably too small to support any reasonable speculation that the CD-producing transition in this region is of $n-\pi^*$ origin. Indeed, hydrogen-bonding interactions can produce either red or blue shifts on $\pi-\pi^*$ transitions of this magnitude.[19,22,23] All substances display Cotton effects in the 215–225-mμ region which may be correlated with the 215-mμ shoulder in the absorption spectrum. This Cotton effect, listed in the E_{1ua} column, is usually of negative sign and quite large. The extreme absorption of the cytidine chromophore below 200 mμ and the relatively low rotations found in this region makes measurements quite difficult below 210 mμ. In most compounds careful study suggests the presence of a fourth Cotton effect around 195 mμ which is labeled the E_{1ub} Cotton effect. In going from benzene to pyrimidine and finally to cytosine, the observed bands are displaced to different extents and their relative intensities are greatly altered. Moreover new absorption systems may be generated by strongly perturbing exocyclic structural elements. Our present study demonstrates only that there are at least two electronic transitions contained in the 198-mμ absorption system of cytidine that has, in the past, been tacitly assumed to contain a single electronic band. For the sake of convenience the benzene nomenclature shall be retained although the following discussion implies nothing concerning the postulated relationship to the benzene spectrum.

Figure 3 contains the CD spectra of 6-methylcytosine nucleosides including the anomeric pair of 6-methyl-2′-deoxycytidines (**3** and **4**). The approximately mirror image aspects of their CD behavior will be commented on later. Quite well-resolved Cotton effects are noted at 270, 247, and 220 mμ. Extension of measurements down to 190 mμ reveals a fourth CD band occurring at about 195 mμ. The actual energy of the electronic transition producing the 247-mμ CD peak lies closer to 240 than to 250 mμ. The CD band of the same sign at 270 mμ and the CD band of opposite sign at 220 mμ

(19) S. F. Mason in "The Pyrimidines," D. J. Brown, Ed., Interscience Publishers, New York, N. Y., 1962, p 477, and references therein.
(20) J. Petruska, *J. Chem. Phys.*, **34**, 1120 (1961).
(21) P. E. Stevenson, *J. Mol. Spectrosc.*, **15**, 220 (1965).
(22) L. Goodman and R. W. Harrell, *J. Chem. Phys.*, **30**, 1131 (1959).
(23) S. F. Mason, *J. Chem. Soc.*, 1247 (1959), and references therein.

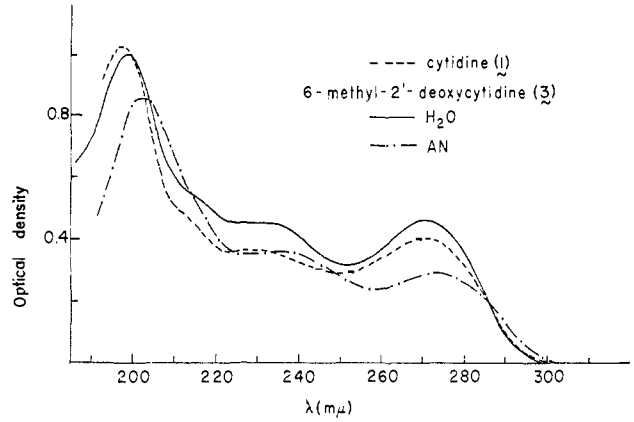

Figure 2. The absorption spectra of cytidine at pH 7 and 6-methyl-2′-deoxycytidine (**3**) at pH 7 and in acetonitrile (AN).

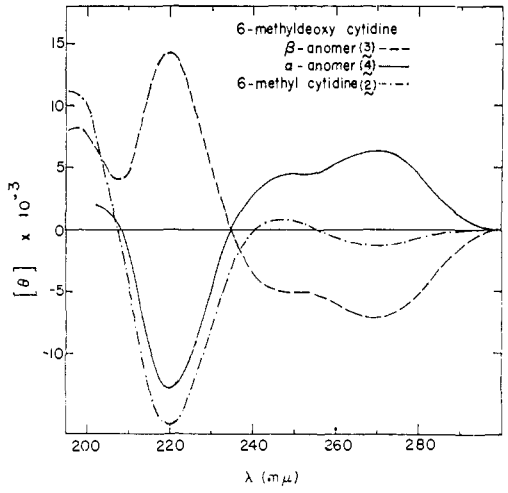

Figure 3. The circular dichroism curves of 6-methylcyctidine (**2**), 6-methyl-2′-deoxycytidine (**3**), and 1-(2′-deoxy-α-D-*erythro*-pentofuranosyl)-6-methylcytosine (**4**) in water at pH 7.

produce a red shift of the intermediate band. Presumably the same electronic transition producing the apparent CD band at 247 mμ is responsible to a large extent for the apparent absorption maximum at 235 mμ. Generally the 235-mμ absorption band of cytidine has been taken as corresponding to the B_{1u} band of benzene.[1a]

Protonation studies[3] suggested an alternative explanation. However, in going from neutral to acid solution such drastic alterations are seen in the absorption spectra as to question the validity of comparisons between the protonated and unprotonated states.

Figure 4 contains the CD spectra of cytidine (**1**), 2′-deoxycytidine (**6**), and two 5-halogenated deoxycytidine derivatives (**9** and **10**). The B_{2u} Cotton effect is large and positive in all cases, but the B_{1u} Cotton effect occurs only as a small positive perturbation in the 260–240-mμ region. The E_{1ua} and E_{1ub} Cotton effects are clearly present in the halogenated derivatives. The data of Figure 4, in general, nicely corroborate the findings presented in Figure 3, *i.e.*, that four $\pi-\pi^*$ electronic transitions are found in the CD spectra of cytidine derivatives down to 190 mμ. Halogenation at position 5 shifts all four CD bands to lower frequencies. These data suggest that all four CD bands arise from $\pi-\pi^*$

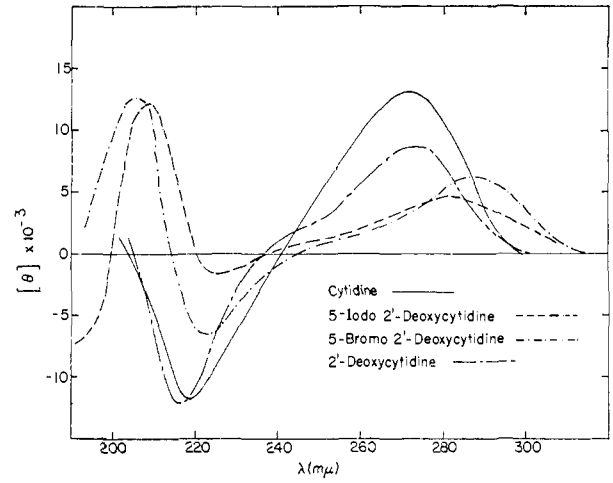

Figure 4. The circular dichroism curves of cytidine, 2′-deoxycytidine (**6**), 5-iodo-2′-deoxycytidine (**9**), and 5-bromo-2′-deoxycytidine (**10**) in water at pH 7.

transitions. Mason[19] has noted that the $n-\pi^*$ and $\pi-\pi^*$ transitions in pyrimidines undergo shifts to the blue and red, respectively, upon halogenation. These CD data suggest that the assignments of Clark and Tinoco[1a] may not be quite correct. They observed absorption bands at 276, 237, 204, and 184 mμ in cytosine which they related to the B_{2u}, B_{1u}, and E_{1u} bands of benzene. In cytidine the first three bands are shifted a few millimicrons to higher frequency with peaks at 270, 235, 198 mμ, and the fourth peak could not be reached at 180 mμ. Our CD band at 222 mμ does not correspond to any obvious absorption peak but does correspond to a slight shoulder at about 215 mμ in the absorption spectra of cytidine. It appears that the first four $\pi-\pi^*$ transitions of cytidine occur near 270, 240, 220, and 195 mμ. The absorption peak at 184 mμ in cytosine may be a fifth $\pi-\pi^*$ band in this chromophore.

Solvents can cause sign reversals of the B_{2u} Cotton effect and may significantly alter the magnitude of the other CD bands as well. Solvent studies were limited to the 6-methylcytidines and isopropylidenecytidene as most of the other compounds are only sparingly soluble in acetonitrile, dioxane, and 1,2-dichloroethane. The effect of 1,2-dichloroethane on isopropylidenecytidine (**7**) is found in Figure 5. In neutral aqueous solutions of isopropylidenecytidine, only the B_{2u} and E_{1ua} Cotton effects are resolved with positive and negative signs, respectively. Dichloroethane reverses the sign of the B_{2u} Cotton effect, resolves the B_{1u} component which appears at 253 mμ, and enhances the magnitude of the E_{1ua} Cotton effect. Acetonitrile greatly increases the negative B_{2u} Cotton effect of 6-methylcytidine (**2**) as shown in Figure 5. A similar effect is observed for the α anomer of 6-methyl-2′-deoxycytidine (**4**) where the B_{2u}, B_{1u}, and E_{1u} bands are resolved in water but acetonitrile reverses the sign of the B_{2u} Cotton effect as seen in Figure 6. Addition of small amounts of water regenerates the general shape of the CD curve found in pure water solutions. These effects are suspected to be due primarily to conformational changes which will be discussed below. It is also of interest to note that both acetonitrile and dioxane produce an interconversion of the intensity of the B_{2u} and B_{1u} Cotton effects of the α anomer (**4**) as seen in Table I.

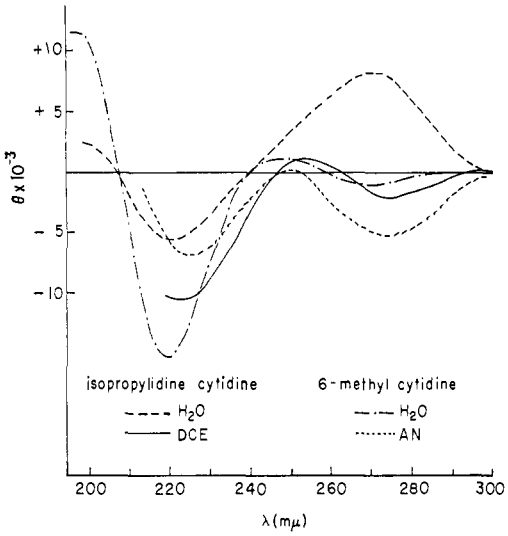

Figure 5. The solvent effects on the circular dichroism spectra of 2′,3′-O-isopropylidenecytidine (**7**) and 6-methylcytidine (**2**). The water curves were run at pH 7.

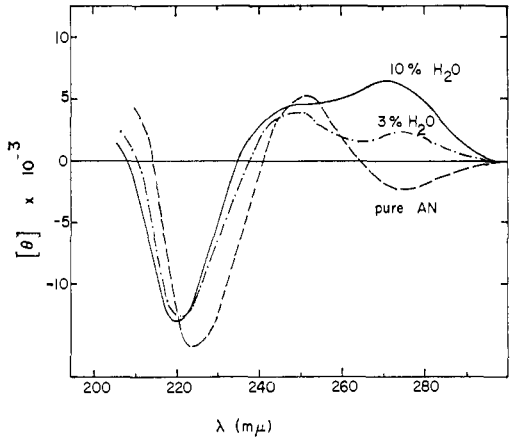

Figure 6. The circular dichroism spectra of 1-(2′-deoxy-α-D-*erythro*-pentofuranosyl)-6-methylcytosine (**4**) in acetonitrile–water solvent systems.

B. Conformational Aspects. A very general occurrence found in comparison of ref 2 with Table I of this paper and with published ORD measurements[8] is that uracil nucleosides and cytosine nucleosides having identical pentose moieties and identical substituents at the 5 and 6 positions of the heterocyclic ring give B_{2u} Cotton effects of the same sign and of the same magnitude within a factor of 2 or 3. The usually smaller B_{1u} Cotton effect does not appear to have a regular behavior in both uridines and cytidines, but the E_{1ua} Cotton effect does and is negative in all structurally similar uracil nucleosides and cytosine nucleosides. Generally the E_{1ub} Cotton effect is positive. In Figure 7 we compare the CD curve of 6-methyluridine with that of 6-methylcytidine (**2**) and the CD curve of 5-bromo-2′-deoxyuridine with 5-bromo-2′-deoxycytidine (**10**). It is quite remarkable that the four Cotton effects exhibited by the 6-methyl-substituted uridines and cytidines are of the same sign and nearly equal in magnitude. Similarly,

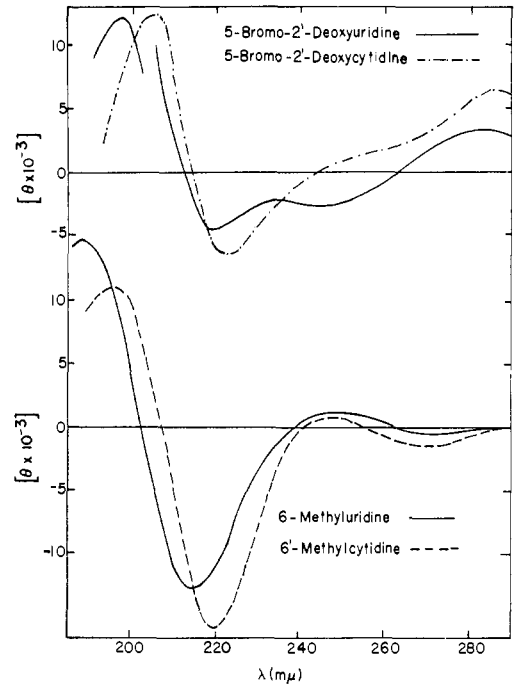

Figure 7. A comparison of the circular dichroism spectra of 5-bromo-2′-deoxyuridine and 5-bromo-2′-deoxycytidine (top) and 6-methyluridine and 6-methylcytidine (bottom) all in water at pH 7.

it is found that the B_{2u}, E_{1ua}, and E_{1ub} Cotton effects in the 5-bromo-2′-deoxy derivatives of uridine and cytidine are positive, negative, and positive, respectively, for both types of base derivatives (see Figure 7). The B_{1u} Cotton effect is positive in 5-bromo-2′-deoxycytidine and negative in 5-bromo-2′-deoxyuridine. Crystal structure data[10] (5-bromo-2′-deoxyuridine, $C_{2'}$ *endo* $\phi_{CN} = -43°$; 5-bromo-2′-deoxycytidine, $C_{2'}$ *endo* $\phi_{CN} = -62°$) indicate that quite similar crystal conformations exist for the deoxyuridines and deoxycytidines. It appears quite reasonable to assume that structurally similar uracil and cytosine nucleosides in solution have approximately the same relative populations of conformations about the glycosidic bond with definite ranges of the torsion angle strongly preferred. At least the naturally occurring cytosine and uracil nucleosides have the same preferred conformation which is *anti* according to recent experimental and theoretical studies. On the basis of proton and fluorine magnetic resonance spectra of nucleosides in solution, it has been suggested that C-3′ is *endo* and the sugar–base conformation is *anti* in pyrimidine nucleosides.[15–17] Haschemeyer and Rich[10] have analyzed the nature of the steric barriers to rotation about the glycosidic bond. For pyrimidine nucleosides they found an allowed range of ϕ_{CN} of about -80 to $-50°$ based on normal van der Waals radii. Jordan and Pullman[13] have evaluated the total electronic energy in different conformations of both uridine and cytosine and have found the *anti* range preferred. Tinoco, *et al.*,[14] have performed calculations on the intermolecular (van der Waals–London) interactions between the two rings in uridine, cytidine, adenosine, and guanosine and have found that the first three nucleosides prefer the *anti* conformations. The observed Cotton effects are, of course, the weighted average of the CD of the main rotameric component and all other ro-

tamers about the C-1′ to chromophore bond. It would, indeed, be quite fortuitous for the ordinary cytosine and uracil nucleosides and their 5-substituted analogs to give quite comparable positive B_{2u} and negative E_{1u} Cotton effects, for their 6-aza analogs to have nearly identical CD curves with large negative B_{2u} Cotton effects[8,24] and large positive E_{1u} Cotton effects,[24] and for 6-methyluridine and 6-methylcytidine to have almost identical CD curves with small negative B_{2u} Cotton effects if similar conformational weightings were not characteristic of each cytidine–uridine pair. Nearly similar Cotton effects for the B_{2u} and E_{1u} transitions for each pair also suggest similarities in the polarization directions of these transitions.

The interesting changes in conformational weighting induced by structural and solvent effects suggest, upon rather careful study with molecular models, a diagram of the expected molecular ellipticity of the B_{2u} Cotton effect as a function of the torsion angle. This diagram should be useful in helping determine the anomeric configuration in uracil and cytosine nucleoside derivatives. The diagram is given in Figure 8. It was constructed by the following assumptions and considerations.

1. We assume that cytidine and its 6-methyl derivative have nearly identical B_{2u} transition moment vectors. This assumption is supported by the identical energies and intensities of the B_{2u} band (within experimental error) as shown by the absorption study summarized in Table I. It is well known that methyl substitution has but a small perturbing effect on aromatic systems in general.

2. The preferred conformation of cytidine and isopropylidenecytidine is assumed in the *anti* range, *i.e.*, at $\phi_{CN} = -30 \pm 30°$. The experimental and theoretical evidence cited above supports this assumption. Cytosine arabinoside with its 2′-OH group on the same side of the pentose ring as the base has a larger B_{2u} Cotton effect than cytidine. The 2′-OH group provides steric hindrance favoring conformations toward $-60°$ rather than 0°. The α anomer of cytidine, which gives B_{2u} Cotton effects of opposite sign and usually larger magnitudes,[7,8] is assumed to take *anti* conformations near $\phi_{CN} = 0°$. In this α anomer, the steric interaction of the C-2 oxygen and the C-2′ hydroxyl prohibits conformations in the -30 to $-90°$ range of the torsion angle.

3. Examination of molecular models reveals that the sugar–base conformation in 6-methylcytidine is limited to the relative narrow range of torsion angle of 90–150°. The major steric interactions are those of the furanose oxygen, C-2′ substituents, C-3′ substituents, and C-5′ substituents with the 6-methyl and 2-keto ring substituents. Molecular models must be made to appreciate the rather severe steric effects of these interactions.

4. The allowed conformation for 6-methyl-2′-deoxycytidine is $135 \pm 45°$. The absence of the 2′-hydroxy group permits a somewhat wider range of torsion angle for the 2′-deoxy analog of 6-methylcytidine than for 6-methylcytidine itself.

5. Molecular models indicate that 1-(2-deoxy-α-D-*erythro*-pentofuranosyl)-6-methylcytosine (**4**) is sterically restricted to a conformation centered at $-130 \pm 45°$.

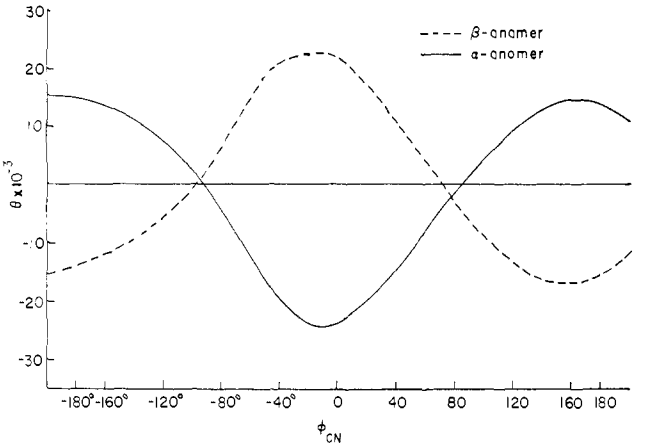

Figure 8. The molecular ellipticity of the B_{2u} Cotton effect as a function of the torsion angle, ϕ_{CN}, for α and β anomers of uridine and cytidine derivatives. The origin of this diagram and its limitations are discussed in the text.

6. It is not likely that hydrogen bonding in aqueous solution of the sugar hydroxyl hydrogen with the unshared electron pair of the keto oxygen or with the π-electron cloud of the base chromophore is a factor.[8] However, in dichloroethane, dioxane, and acetonitrile a 2-keto to 5′-OH hydrogen bond in the β anomer and a 2-keto to 3′-OH hydrogen bond in the α-deoxy anomer should be competitive with the solvent–sugar hydroxy interaction.

7. The molecular ellipticity is expected to change slowly and in a regular manner as the torsion angle is varied. Theoretical plots of the calculated rotational strength against the torision angle support this assumption[11,25] and show that nodes (sign changes) are spaced approximately 180° apart.

Utilizing the assumed regular behavior of the molecular ellipticity *vs.* torsion angle plot and correlating the experimental molecular ellipticities of cytidine, 2′,3′-O-isopropylidenecytidine in dichloroethane, 6-methylcytidine in water and acetonitrile, cytosine arabinoside and uracil lyxoside,[7] and α- and β-6-methyl-2′-deoxycytidine in water and dioxane, one arrives at the diagram of Figure 8. The fact that the molecular ellipticity of the B_{2u} Cotton effect of 6-methylcytidine, 6-methyluridine,[2] 5,6-dimethyluridine,[2] and 6-methyl-2′-deoxycytidine all increase in a negative sense in dioxane or acetonitrile provides additional values that aid in the construction of the diagram. Hydrogen-bonding interactions of the keto oxygen with the 5′-OH tend to draw the sugar–base torsion angle toward 180° although steric limitations are imposed upon 6-methylcytidine and 6-methyluridine. Similarly, isopropylidenecytidine gives a negative Cotton effect in 1,2-dichloroethane (see Figure 5), whereas it has the characteristic positive Cotton effect in water. Presumably the formulation of the same keto to 5′-OH hydrogen bond, which would change the conformational weighting from the *anti* range to the *syn* range, could account for this solvent effect. The β curve of Figure 8 suggests that cytosine and uracil nucleosides tend to have positive Cotton effects when in the

(24) D. W. Miles, M. J. Robins, R. K. Robins, and H. Eyring, unpublished data.

(25) D. W. Miles, Ph.D. Thesis, University of Utah, Salt Lake City, Utah, 1967.

anti range and negative Cotton effects when in the *syn* range.

The α curve is drawn so as to fit the data of ordinary α-cytosine and α-uracil nucleosides which give negative Cotton effects[7,8,26] with conformations presumably around 0°, and our finding that the α anomer of 6-methyl-2′-deoxycytidine (**4**) gives a large positive Cotton effect with conformations favoring a range around −135°. In dioxane or acetonitrile the B_{2u} Cotton effect becomes small negative (see Figure 6). It therefore seems necessary to postulate that in these solvents the 3′-OH is hydrogen bonded to the 2-keto oxygen of the pyrimidine stabilizing the sugar–base torsion angle at −80°. This provides another point on the diagram (see Figure 8). This diagram encompasses Ulbricht's[8] rule which relates the sign and magnitude of the B_{2u} Cotton effect in pyrimidine nucleosides to their conformation and is more specific and useful since it relates the sign and magnitude of the B_{2u} Cotton effect to the torsion angle. Ulbricht's[8] rule is: The sign of the Cotton effect (B_{2u}) will be positive if (1) the nucleoside has a preferred conformation due to restricted rotation about the glycosidic bond and (2) a line from the $C^4{=}O$ (or $C^4{-}NH_2$) group passing through the $C^2{=}O$ group passes from above to below the plane of the furanose ring provided that the chromophore is not twisted to such an extent that the line passes through $C^{5'}$. ("Above" is defined as the same side of the furanose ring as $C^{5'}$.)

This empirical rule is based on the experimental ORD curves of pyrimidine β-nucleosides, which give positive B_{2u} Cotton effects, and pyrimidine α-nucleosides, which give negative B_{2u} Cotton effects, and ORD data on four cyclouridine nucleosides, which are locked into specific *syn* and *anti* conformations. The naturally occurring pyrimidine nucleosides were assumed to adopt the *anti* conformation in which the 2-carbonyl group is directed away from the sugar. The CD curves of O^2-2′-cyclouridine and 2′,3′-O-isopropylidene-O^2-5′-cyclouridine which form the basis for Ulbricht's rule do *not* themselves fulfill the conditions of this rule (see Figure 9). A fixed conformation at about $\phi_{CN} = 30°$ is found in molecular models of O^2,2′-cyclouridine. According to condition 2 of Ulbricht's rule this compound should give a positive B_{2u} Cotton effect. The long-wavelength Cotton effect exhibited by this compound, presumably the B_{2u} Cotton effect, is negative. A large positive Cotton effect is found at 240 mμ (B_{1u}) which obscures the presence of the B_{2u} Cotton effect at 270 mμ in the ORD spectra. Close examination of the ORD curve of this compound shows that the Cotton effect mistakenly identified by Ulbricht as the B_{2u} is centered about 15 mμ toward higher frequency than the B_{2u} Cotton effect of uridine. Cyclization does not appear to have markedly changed the energy of the π–π^* transitions but does cause changes in the intensity distribution among the bands. The B_{2u} band appears as a perturbation on the lower frequency side of the now much more intense B_{1u} band. The B_{1u} band is not resolved in the absorption spectra of ordinary uracil nucleosides.[2] Also of interest is the presence of an absorption band at 220 mμ. In ref 2 it is found that uridine gives a negative Cotton effect at about 220 mμ where no absorption band is readily discernible. In this cyclo analog of uridine,

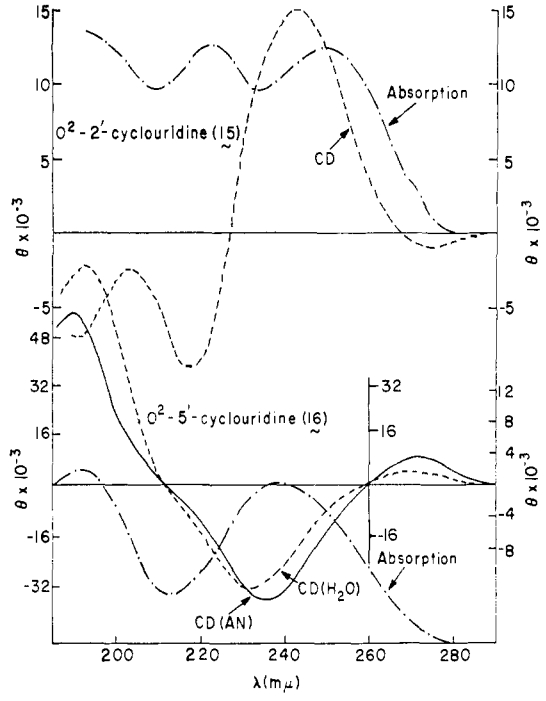

Figure 9. The circular dichroism curve of O^2-2′-cyclouridine (**15**) in water at pH 7 (top) and the circular dichroism curves of 2′,3′-O-isopropylidene-O^2-5′-cyclouridine (**16**) in water at pH 7 and acetonitrile (bottom). The Arabic numbers refer to structures **15** and **16** of paper IV of this series. The absorption curves are also included for comparison, but the ordinate scale is not provided.

changes in intensity distribution have resolved a 222-mμ absorption band, the E_{1ua} band.

According to condition 2 of Ulbricht's rule, the rigid compound, 2′,3′-O-isopropylidene-O^2-5′-cyclouridine, should give a negative B_{2u} Cotton effect because the chromophore is now twisted so that the line does pass through $C^{5'}$. In Figure 9 it is seen that this compound gives a small positive Cotton effect (B_{2u}) at about 270 mμ. (Because of the presence of the intense negative B_{1u} Cotton effect at lower wavelength, the true maximum of the B_{2u} Cotton effect lies toward 260 mμ.) In this cyclouridine analog the conformation is rigidly fixed at $\phi_{CN} = 110°$. In summary the cyclouridine analogs examined here do not support Ulbricht's rule or the diagram of Figure 8. Other exceptions to Ulbricht's rule will be noted below.

There is some question of the usefulness of the cyclo compounds as models because model building shows that these systems are under considerable torsional strain. The effect of such strain shows up in the comparison of the absorption spectra of O^2-2′-cyclouridine and O^2-5′-cyclouridine given in Figure 9. Although the chromophores should be identical, the absorption spectrum is quite different. It is difficult to predict the effect of this strain on the CD properties of these substances.

Comparative data on uracil and cytosine nucleoside derivatives offer an interesting challenge in predicting rotational strengths or devising empirical rules governing the sign and magnitude of the Cotton effects. Tinoco[27] has provided the following equation for calculat-

(26) I. Fric, J. Smejkal, and J. Farkas, *Tetrahedron Lett.*, 75 (1966).

(27) I. Tinoco, Jr., *Advan. Chem. Phys.*, **4**, 113 (1962).

ing the influence of all transition moments in the ribose group on the rotational strengths of the base transition

$$R_{B_{2u}} = \frac{\pi \nu_a \nu_0^2 \mu_{i0a}^2 (\alpha_{33} - \alpha_{11})(V_{ij})_{ei} \times \hat{e}_j \cdot \vec{r}_{ij}}{c(\nu_0^2 - \nu_a^2)} \quad (1)$$

$$V_{ij} = 1/r_{ij}^3 \left\{ e_i \cdot e_j - \frac{3(\hat{e}_i \cdot \vec{r}_{ij})(\hat{e}_j \cdot \vec{r}_{ij})}{r_{ij}^2} \right\}$$

where $\hat{e}_i$ is the unit vector in the direction of the electric dipole moment, μ_{i0a}, of the B_{2u} transition, and ν_a is its frequency; e_j is a unit vector in the direction of the principle axes of polarizability of the ribose group and ($\alpha_{33} - \alpha_{11}$) is the anisotropy of the ribose group; $\vec{r}_{ij}$ is the distance from i to group j and can be approximated by a frequency around 1×10^{15}. In cytosine nucleosides the direction of the B_{2u} moment and the principle axes of polarizability of the ribose group and its anisotropy are unknown parameters. These latter difficulties can be circumvented by using known bond polarizabilities and anisotropies.[11,28]

We have observed that the pair of anomeric 6-methyl-2′-deoxycytidines (**3** and **4**) give near-mirror-image circular dichroism curves (see Figure 3). This observation may be of considerable importance in the understanding of the origin of optical activity in nucleosides in general. Ignoring the 2′, 3′, or 4′ substituents on the pentose ring, it is found upon examination of molecular models that reflection of the ring atoms of the β anomer at $\phi_{CN} = 90°$ generates the α anomer at $\phi_{CN} = -90°$. In fact any β structure in the 90–180° range has its mirror image in the −90 to −180° range. The near-mirror-image CD properties of the anomeric pair suggest, in view of eq 1, that the major contribution to the rotational strength arises from the interactions of the transitions in the pentose ring proper with the B_{2u} moment of the base. Changes in the principle axes of polarizability of the ribose due to 2′, 3′, and 4′ substituents is expected to depend on the polarizability of the substituent and the site of substitution. Variations of relatively nonpolar substituents in the 2′, 3′, or 4′ positions appear to have little effect on the amplitude of the ORD curves[7–9] unless the substituent is bulky and steric factors become important. Hence, these substituents apparently do not change the polarizability properties of the ribose ring. Moderately polarizable substituents in the sugar ring such as a $C_{2'}$–$C_{3'}$ double bond[8] or a 4′-sulfur ring atom[2] reverse the sign of the B_{2u} Cotton effect and thus violate Ulbricht's rule as stated. Steric factors may also be involved, but significant changes in the polarizability properties of the pentose ring would occur.

The present results demonstrate, in agreement with the conclusions of Fric, *et al.*,[26] that the stereochemistry of pyrimidine nucleosides may be assigned from comparison of ORD or CD spectra only in those cases in which the unknown and reference compounds have approximately similar B_{2u} transition moment directions, similar electronic structures in the pentose ring, and similar conformational patterns about the sugar–chromophore bond. These requirements appear to be met in most cytosine and uracil nucleosides containing relatively nonpolarizable substituents in or on the sugar residue and with methyl, chloro, bromo, and iodo substituents on the base chromophore. The B_{2u} Cotton effect of 5-hydroxyuridine, although still positive, has a very small magnitude. Certain uracil nucleosides with strongly perturbing substituents such as $-NH_2$ and $-N_2^+$ groups at position 5 on the uracil base give negative Cotton effects at long wavelength.[24] Thus compounds with substituents that give rise to large changes in the electronic structure of the base chromophore may exhibit optical transitions contrary to empirical predictions. This study has exposed several other exceptions to Ulbricht's rule,[8] namely the 6-methyluracil and 6-methylcytosine nucleosides, which most likely involve changes in the torsion angle. Exceptions noted for the 6-aza and 6-hydroxy analogs of uridine and cytidine[8] probably involve both conformational and electronic changes. Therefore, the application of ORD and CD to configurational analysis of nucleosides and their derivatives should be made with caution. The exceptions to Ulbricht's rule *as stated* are of sufficient number to almost completely preclude its predictive usefulness. Diagram 8 is intended to apply to uridine and cytidine derivatives (excluding cyclonucleosides and other strained chromophores) which contain relatively nonpolarizable substituents on or in the pentofuranose residue and weakly perturbing substituents on the uracil and cytosine nucleus.

(28) D. W. Miles, R. K. Robins, and H. Eyring, *J. Phys. Chem.*, 71, 3931 (1967).