Circular Dichroism of Nucleoside Derivatives. IV. Uracil Derivatives[1,2]

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Abstract: Circular dichroism data are reported for 16 uracil nucleoside derivatives especially designed to help determine the nature of the excited states of uridine and their energies. The interpretation of the spectra is facilitated by a comparison of the same electronic transition and its associated Cotton effect in different solvent media and different asymmetric environments. Cotton effects were resolved in the near-ultraviolet CD spectra which indicate the presence of electronic transitions not discovered by other spectroscopic techniques. Four optically active transitions were observed between 185 and 275 mμ.

A problem of considerable current interest in molecular biology is the study of the optical properties of naturally occurring purines and pyrimidines. Many studies, both experimental[3–18] and theoretical,[19–28] have appeared in recent years concerned with this subject which is of fundamental importance in the understanding of energy transfer, hypochromism, photochemistry, optical rotatory dispersion (ORD), and circular dichroism (CD) studies on deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and analogous model systems. On the theoretical side the work of Pullman and his associates[20,21] and the work of Nesbet[23] predict quite well the absorption spectra of uracil and cytosine nucleosides. Experimentally Miles, *et al.*,[3] and Clark and Tinoco[5] have found evidence of additional electronic transitions in the spectra of uracil and cytosine and their nucleosides other than the well-known absorption systems characteristic of these substances. Kleinwachter, *et al.*,[14] Brahms, *et al.*,[9] and Stewart and Davidson[6] were unable to detect the presence of any $n-\pi^*$ transitions in the near-ultraviolet spectral region of uracil derivatives.

This communication and a companion paper[29] present spectroscopic data on a variety of uracil and cytosine nucleosides which feature subtle structural alterations which either render them soluble in both polar and nonpolar solvents or change the relative orientation of the sugar group relative to the base and/or the perturbing potential of the sugar group. The structural changes have little noticeable effect on the absorption spectra characteristic of uracil, thymine, or cytosine bases but introduce important changes in the sign and intensities of the Cotton effects associated with the transitions in the bases. The interpretation of the spectra is facilitated by a comparison of the same electronic transition and its associated Cotton effect in different solvent media and in a variety of asymmetric environments.

Experimental Procedures

The circular dichroism measurements were performed with the Cary Model 6001 circular dichroism accessory to the Cary 60 recording spectrophotometer. To ensure against artifacts in the circular dichroism data, concentrations were chosen to be well under the instrument's peak toleration absorbance level of 3.0, and further dilution of each sample did not alter the shape of the curves. Spectra were frequently taken to 400 mμ and always to 320 mμ in order to check for base-line shifts. The circular dichroism data are expressed as molecular ellipticity (θ). Measurements of CD were made with the use of 5-, 1-, 0.1-, 0.01-, or 0.005-cm cells. Temperature studies were done in a 1-cm cell supplied by Arthur H.

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(1) D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Natl. Acad. Sci. U. S.*, 57, 1138 (1967); D. W. Miles, R. K. Robins, and H. Eyring, *J. Phys. Chem.*, 71, 3931 (1967); and D. W. Miles, S. J. Hahn, R. K. Robins, M. J. Robins, and H. Eyring, *ibid.*, 72, 1483 (1968), respectively, are to be considered papers I, II, and III of this series.

(2) 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.

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

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

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

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

(7) R. F. Stewart and L. H. Jensen, *ibid.*, 40, 2071 (1964).

(8) T. Yamada and H. Fukutome, *Biopolymers*, **6**, 43 (1968).

(9) J. Brahms, J. Pilet, H. Bamany, and V. Chandrasekharan, *Proc. Natl. Acad. Sci. U. S.*, **60**, 1130 (1968).

(10) J. Drobnik and L. G. Augenstein, *Photochem. Photobiol.*, **5**, 13 (1966).

(11) J. Drobnik, V. Kleinwachter, and L. G. Augenstein, *ibid.*, **6**, 147 (1967).

(12) V. Kleinwachter, J. Drobnik, and L. G. Augenstein, *ibid.*, **6**, 133 (1967).

(13) J. Drobnik and L. G. Augenstein, *ibid.*, **5**, 83 (1966).

(14) V. Kleinwachter, J. Drobnik, and L. G. Augenstein, *ibid.*, **5**, 579 (1966).

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

(16) S. F. Mason in "The Pyrimidines," D. J. Brown, Ed., Interscience Publishers, New York, N. Y., 1962, Chapter 13.

(17) K. Nakanishi, N. Suzuki, and F. Yamazaki, *Bull. Chem. Soc. Jap.*, 34, 53 (1961).

(18) B. J. Cohen and L. Goodman, *J. Amer. Chem. Soc.*, 87, 5487 (1965).

(19) C. Nagata, A. Imamura, Y. Tagashira, and M. Kodama, *Bull. Chem. Soc., Jap.*, **38**, 1938 (1965).

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

(21) A. Denis and A. Pullman, *ibid.*, 7, 110 (1967).

(22) Berthod, H. C. Giessner-Prettre, and A. Pullman, *Int. J. Quantum Chem.*, **1**, 123 (1967).

(23) R. K. Nesbet, *Biopolymers, Symp.* 1, 129 (1964).

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

(25) V. A. Kuprievich, *Int. J. Quantum Chem.*, **1**, 561 (1967).

(26) J. Ladik and T. Appel, *Theor. Chim. Acta*, 4, 132 (1966).

(27) K. Nishimoto, *Bull. Chem. Soc. Jap.*, 40, 2493 (1967).

(28) J. S. Kwiatkowski, *Theor. Chim. Acta*, 10, 47 (1968).

(29) D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, **91**, 831 (1969).

Table I. Spectroscopic Data on Uridine Derivatives[a]

Compound[b]	Solvent	B_{2u}	B_{1u}	E_{1ua}	E_{1ub}	λ_1	λ_2
Uridine[c] (**1**)	4 *M* HCl	266 (8600)	238 (−4000)	215 (−5000)	...	263	...
	W	267 (8500)	240 (−3700)	215 (−4400)	196 (7600)	262	205
	D	272 (9000)	244 (−5000)	220 (−2200)	...	261	201
	AN	272 (8500)	243 (−5000)	220 (−1500)	200 (9000)	260	200
5-Methyluridine[c] (**2**)	W	272 (5500)	242 (−4000)	217 (−4400)	196 (11,000)	267	205
Thymidine[c] (**3**)	W	272 (4000)	242 (−3700)	215 (−6000)	197 (16,000)	267	205
2′-Deoxyuridine[c] (**4**)	W	267 (6000)	237 (−3600)	215 (−5800)	194 (14,000)	262	204
5,6-Dimethyluridine[d] (**5**)	W	260–270 (−300)	248 (1000)	212 (−10,000)	189 (12,000)	261	200
	D	265–275 (−1000)	...	215 (−12,000)	...	259	...
6-Methyluridine[d] (**6**)	W	260–270 (−300)	250 (1000)	214 (−10,000)	190 (12,000)	268	203
	D	260–270 (−1000)	...	...	...	264	...
	AN	267 (−2800)	...	213 (−12,000)	190 (11,000)	263	209
6-Methyl-3-(β-D-ribofuranosyl)-uracil[d] (**7**)	W	268 (−4300)	242 (6200)	214 (−3800)	195 (−9000)	265	203
	AN	270 (−4300)	243 (5500)	215 (−3500)	195 (−7500)	261	...
	D	273 (−4300)	245 (5700)	...	...	261	...
	DCE	272 (−5000)	246 (6000)	...	...	261	...
3-β-D-Ribofuranosyluracil[e] (**8**)	W	270 (−4000)	244 (5000)	215 (−3000)	195 (−7000)	264	203
5′-Deoxyuridine[f] (**9**)	W	268 (8000)	240 (−4000)	215 (−4000)	194 (8500)	262	204
1-(5-Deoxy-α-L-*lyxo*-pentofuranosyl)uracil[f] (**10**)	W	270 (4000)	243 (−5500)	215 (−7000)	195 (8000)	262	204
1-(2,3-O-Isopropylidene-5-deoxy-(β-D-*erythro*-pent-4-enofuranosyl)uracil[f] (**11**)	W	258 (13,000)	...	214 (10,000)	201 (−9500)	260	199
	n-BuOH	255 (16,500)	...	210 (11,000)	...	258	201
	AN	254 (13,000)	...	211 (10,000)	196 (−10,000)	256	200
	D	255 (16,000)	...	...	...	254	...
	DCE	254 (15,800)	...	...	...	257	...
	CH	255 (16,000)	...	209 (15,000)	...	257	...
1-(5-Deoxy-β-D-*erythro*-pent-4-enofuranosyl)uracil[g] (**12**)	W	266 (8200)	240 (−2800)	213 (−10,500)	193 (27,000)	261	203
	AN	269 (6900)	242 (−1400)	212 (−10,400)	192 (20,000)	258	203
	D	269 (7000)	242 (−1000)	...	...	258	...
	DCE	269 (6800)	243 (−1500)	...	...	258	...
4′-Thiouridine[h] (**13**)	W	263 (−18,000)	240 (S) (8000)	219 (16,000)	205 (24,000)	264	208
	D	263 (−39,000)	240 (S) (10,000)	225 (28,000)	...	265	...
	AN	263 (−32,000)	240 (S) (10,000)	225 (22,000)	204 (28,000)	264	207
Uridine 5′-phosphate[c] (**14**)	W	267 (9000)	239 (−5000)	220 (−6000)	195 (12,000)	262	205
O^2→2′-Cyclouridine[i] (**15**)	W	270 (−1000)	242 (15,000)	217 (−8500)	189 (−8000)	250	222
2′,3′-O-Isopropylidene-O^2→5′-cyclouridine[j] (**16**)	W	268 (2000)	233 (−32,000)	...	193 (68,000)	238	192
	DCE	270 (4300)	235 (−32,000)	...	...	237	...
	AN	270 (4600)	235 (−36,000)	...	189 (60,000)	235	...

[a] The position of maxima in circular dichroism spectra and absorption spectra are given in mμ units. The molar ellipticies are given in parentheses. [b] Solvents are: W = water at pH 7; D = dioxane; AN = acetonitrile; DCE = 1,2-dichloroethane; CH = cyclohexane. [c] Commercial sample. [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. [f] M. J. Robins, J. R. McCarthy, Jr., and R. K. Robins, *J. Heterocycl. Chem.*, **4**, 313 (1967). [g] J. P. H. Verheyden and J. G. Moffatt, *J. Amer. Chem. Soc.*, **88**, 5684 (1966). We wish to thank Dr. J. G. Moffatt for a sample of **12**. [h] B. Urbas and R. L. Whistler, *J. Org. Chem.*, **31**, 813 (1966). [i] A. Hampton and A. W. Nichol, *Biochemistry*, **5**, 2076 (1966). [j] D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 868 (1957).

Thomas Co. Sample temperatures were monitored with the YSI Model 42SC telethermometer or a copper–constantan thermocouple while spectra were being run. Low temperatures were achieved by a specially designed apparatus which circulated ethanol cooled by Dry Ice. Corrections were made for the expansion of the solutions with increasing temperatures.

All solvents used in this study were of spectral grade. The details of the preparation and characterization of the nucleosides have been described in the references given in Table I. Concentration of nucleosides were usually determined by measuring the ultraviolet absorption and using the literature values of the extinction coefficients (see references of Table I). The CD spectra of all uridine derivatives were routinely measured over the widest concentration range permitted by the solubility and rotational and absorption properties. Solutions were allowed to equilibrate for about 24 hr before making the measurement. Experiments in aqueous solution were performed on both buffered and unbuffered solutions at pH 7 with exactly identical results in the accessible spectral range.

Results and Discussion

The longest wavelength transitions occurring in the purine and pyrimidine bases have generally been interpreted as n–π* and π–π* transitions, with σ–π*, σ–σ*, and Rydberg transitions lying at shorter wavelengths and perhaps some miscellaneous unidentified transitions lying in between. Evidence has recently been presented indicating possible correlation of the spectra of purine and pyrimidine bases to the absorption bands of benzene by Mason,[30] Krishna and Goodman,[15] Clark and Tinoco,[5] and Miles, Robins, and Eyring.[3] These authors classify the observed π–π* transitions in the nucleic acid bases into three groups which they name B_{2u}, B_{1u}, and E_{1u} by analogy with the

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(30) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

corresponding nomenclature of the $\pi-\pi^*$ transitions in benzene. The E_{1u} state is doubly degenerate in benzene and certain symmetric derivatives, but is split for nearly all the substituted benzenes. The postulated relationship between the spectrum of benzene and the bases does not suggest similarities in the electronic distribution patterns, *i.e.*, location of nodes and net electronic charge distribution in the excited states. Certain qualitative relationships regarding energies and intensities do appear to exist in studies on sequences of molecules that structurally bridge the gap between benzene and the bases.[5,15,30] Moreover Stevenson[31] has shown that the transitions of benzenes derivatives with strongly perturbing substituents are related to the benzene transitions. Attempts to classify and systematize the transitions of the naturally occurring nucleic acid bases and their derivatives are chiefly aimed at establishing that general correlations involving the spectral characteristics do exist among this series of related compounds. Correlations with the spectrum of benzene, while of minor importance, provide a convenient and well-known nomenclature.

The purine and pyrimidine bases, because of the nonbonding electron pairs of the N and O atoms, also may exhibit $n-\pi^*$ transitions which are polarized perpendicular to the base plane. Application of the McConnell solvent shift test[32] and studies of polarized phosphorescence excitation spectra[14] are frequently useful in distinguishing between $n-\pi^*$ and $\pi-\pi^*$ transitions.

Since a distinct region of absorption in the purine and pyrimidine bases may sometimes contain both $\pi-\pi^*$ and $n-\pi^*$ transitions, the interpretation of the spectra requires careful analysis of the CD and absorption spectra in different solvents of many closely related derivatives of a particular base chromophore. While the occurrence of both positive and negative signs in the CD spectra makes the resolution more definitive than absorption spectra, it is, of course, still possible to "lose" CD bands in places where there are strong positive and negative swings and where a weak CD component is overlaid by one or more stronger adjacent components. Since different asymmetric surroundings may specifically enhance one CD component relative to its adjacent components, a particular optical transition may be rendered strongly optically active in one derivative but be absent or overlaid in another derivative. The total distinct CD extrema observed in a large series of related compounds having essentially identical chromophores should approach the true total of electronic transitions in the spectral region capable of generating observable rotational strengths, provided the possible presences of base-base interactions and solvational, conformational, or tautomeric equilibria can be ruled out.

In Table I we have recorded the pertinent spectroscopic constants uncovered in this study on uridine derivatives of Figure 1. The recorded values are believed to be accurate within 10% in most cases. It is notable that the absorption spectra of all uridine derivatives featured in this study are monotonously similar in band shape, band energies, and band intensity. Two distinct regions of absorption are observed, one located near 260 mμ and the second near 200 mμ. In the CD spectra it is interesting to note the general occurrence of two circular dichroism peaks (usually of opposite sign) in the spectral region spanned by the 260-mμ absorption system, which gives no striking evidence that it contains two overlapping absorption bands. The band does deviate considerably from ideal gaussian behavior, particularly on the short-wavelength side. Similarly two circular dichroism peaks are found in the region of the 200-mμ absorption system.

In Table I we have continued the usage of the benzene group theory symbols when referring to the excited states of the uridine and cytidine derivatives. The long-wavelength CD extrema, generally occurring in the 260–270-mμ region, are listed in the B_{2u} column. The molecular ellipticity of the B_{2u} Cotton effect varies in the uridine derivatives from a positive 16,500 (**11** in butyl alcohol) to $-39{,}000$ (4′-thiouridine (**13**) in dioxane.) Its wavelength maximum position varies from 255 mμ in **11** to 272 mμ in **7** in 1,2 dichloroethane (DCE). Toward the short-wavelength end of the spectrum (the E_{1u} region) all uridine derivatives exhibit two CD maxima, one around 210–220 mμ and another below 200 mμ. We shall refer to the two short-wavelength Cotton effects as the E_{1ua} and E_{1ub} Cotton effects, respectively (in order of increasing frequency). The E_{1u} Cotton effects are generally of opposite sign. In between the E_{1u} and B_{2u} spectral region the B_{1u} transition is located. A CD extremum is found in all uridine derivatives in the 237–247-mμ region. While solvent effect studies (see next section) do not unambiguously demonstrate that this Cotton effect is of $\pi-\pi^*$ origin, this does appear to be the most likely conclusion when all CD, absorption, fluorescence, and phosphoresence[14] data are considered together. The B_{1u} Cotton effect is generally smaller and of oppositive sign from the B_{2u} Cotton effect. Substance **7** gives the most intense B_{1u} band with a molecular ellipticity of 6200 in water (we should note, however, that 4′-thiouridine (**13**) exhibits a shoulder at 240 mμ with a molecular ellipticity of 10,000). Uridine (**1**) exhibits the most intense negative B_{1u} band with a molecular ellipticity -5000 at 243 mμ in acetonitrile.

Critical examination of the data of Table I reveals that the B_{2u} CD maximum is always to the red of the absorption maximum when the B_{1u} Cotton effect is of opposite sign. When the B_{1u} Cotton effect is absent or possibly of the same sign, the B_{2u} Cotton effect lies to the blue of the absorption maximum. For example, the first, absorption maximum in compounds **11** and **12** lies at 260 and 261 mμ, respectively, whereas the $B_{2\mu}$ CD maximum lies at 268 and 255 mμ, respectively. In **12** the presence of a negative Cotton effect at 240 mμ doubtlessly moves the apparent maximum of the $B_{2\mu}$ Cotton effect toward the red. In the case of **11** the likely presence of a small positive B_{1u} Cotton effect (see Solvent Studies section) in the 240-mμ region would be expected to blue shift the B_{2u} maximum. An exact 1:1 correspondence between the prominent maximum of the absorption spectra and the CD peaks is not expected in substances exhibiting complex CD spectra. Wellman, *et al.*,[33] have established that two overlapping gaussian curves of op-

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(31) P. E. Stevenson, *J. Mol. Spectrosc.*, **15**, 220 (1965).

(32) H. McConnell, *J. Chem. Phys.*, **20**, 700 (1952).

(33) K. M. Wellman, P. H. A. Laur, W. S. Briggs, A. Moscowitz, and C. Djerassi, *J. Amer. Chem. Soc.*, **87**, 66 (1965).

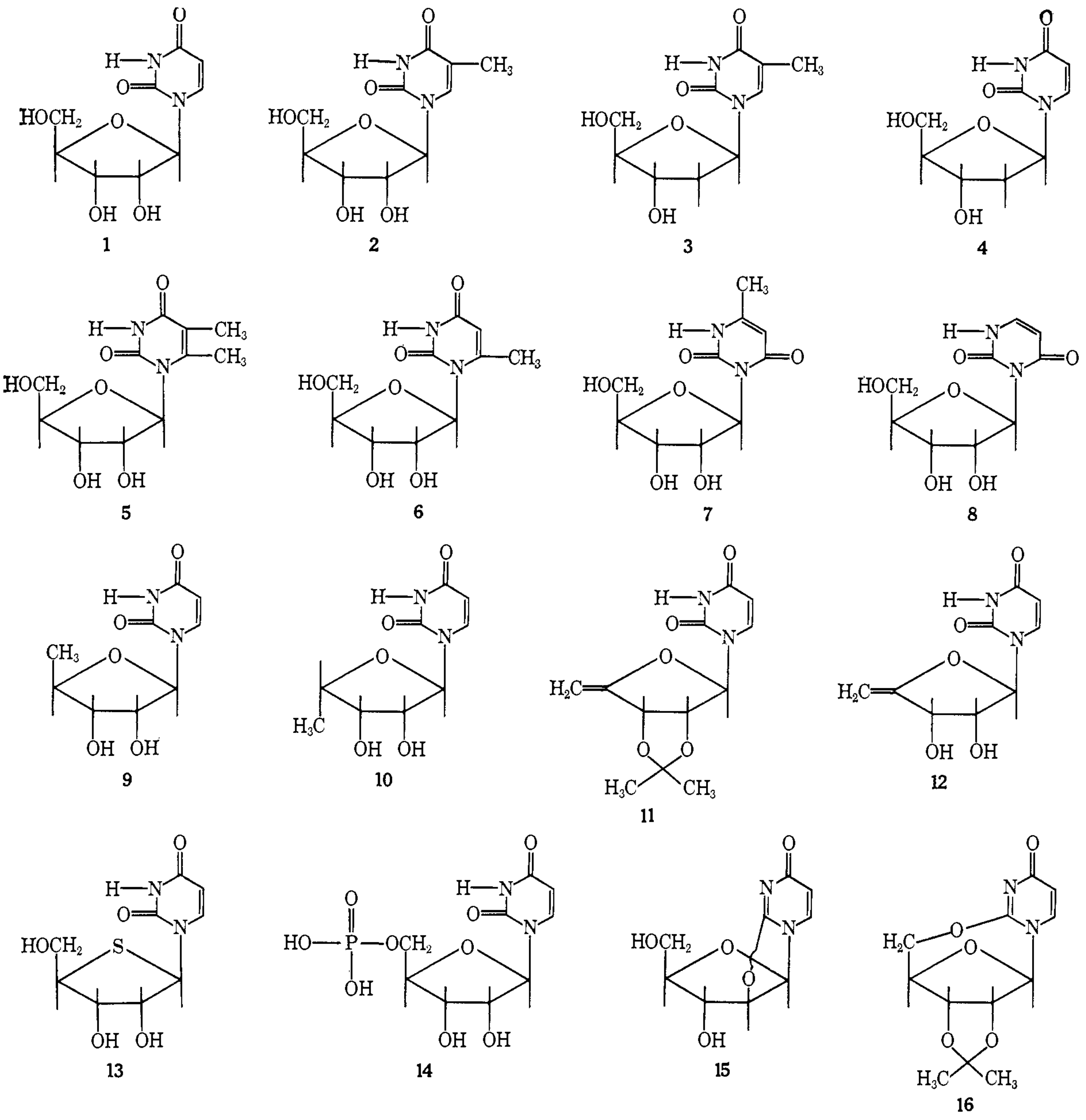

Figure 1. The structural formulas of the uridine nucleoside derivatives.

posite sign give a resultant curve with peaks shifted in position from the true position of either component bands.

The CD spectra of aqueous solutions of 5-methyluridine (**2**), thymidine (**3**), and uridine (**1**) are illustrated in Figure 2. Representative solvent-effect curves often included with each figure will be discussed separately in another section of this paper. Four Cotton effects are observed in the spectral region (300–185 mμ) which contains the B_{2u}, B_{1u}, and E_{1u} bands of benzene. The presence of a CD band generally implies the existence of a corresponding absorption band and *vice versa.* However, an equilibrium involving two species arising from association effects, solvational, conformational, and tautomeric equilibrium may account for the appearance of two oppositely signed CD absorptions centered around a single absorption maximum. One requirement for an equilibrium hypothesis is an increase in one species relative to the other on going to lower temperature. Equilibrium effects in this study seem unlikely since the first three CD bands of uridine in methanol *all* increase in intensity about 15% as the temperature is lowered from 45 to $-60°$. Association effects seem unlikely since the CD curves of uridine at 2×10^{-5} and 2×10^{-2} *M* are almost identical. The two absorption systems of uridine peak at 262 and 203 mμ in aqueous neutral solution. Both the absorption and CD bands of thymidine and 5-methyluridine are red shifted several millimicrons relative to the corresponding band of uridine by the perturbing effects of the 5-methyl substituent.

Figure 3 contains the CD spectra of 5,6-dimethyluridine (**5**) in water and acetonitrile. Very similar results are obtained for 6-methyluridine (**6**). Molecular models suggest that the 6-methyl substituent should drastically alter the base–sugar conformation, *i.e.*, favor

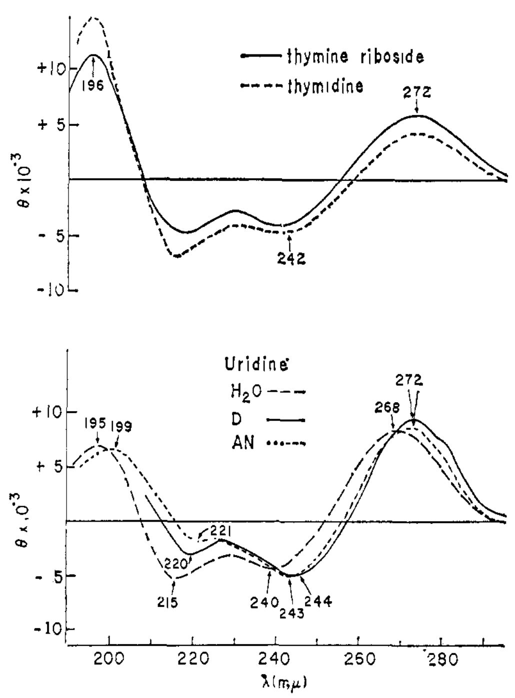

Figure 2. (Top) The circular dichroism curves of thymine riboside (**2**) and thymidine (**3**) in water at pH 7. (Bottom) The circular dichroism curves of uridine (**1**) in water (H_2O) at pH 7, dioxane (D), and acetonitrile (AN).

another range of the torsion angle, ϕ_{CN}, which describes the relative orientation of the sugar with respect to the base.[34] Current models of optical activity[35-41] show explicitly that it is the nature and orientation of the sugar substituents relative to the transition dipole moment, which attend the optical transition under consideration, which in turn determines the sign and magnitude of the Cotton effect. Conformational analysis of both uridine and cytosine nucleosides are discussed in ref 29. It is particularly interesting to compare the CD curve of 5,6-dimethyluridine (**5**) with the CD curve of uridine and thymidine. The B_{2u} and B_{1u} Cotton effects of 5,6-dimethyluridine (**5**) and 6-methyluridine (**6**) are much smaller and carry the opposite signs, while the E_{1u} Cotton effects carry the same signs and, indeed, nearly the same magnitude as the corresponding bands in uridine or thymine riboside.

The circular dichroism curves of **7**–**10** again suggest the presence of at least four electronic transitions above 190 mμ in the uracil chromophore. A different site of base–sugar attachment (*i.e.*, the N-3 hydrogen and N-1 base–sugar bonds of uridine have been interchanged) is featured in **7** and **8**. Substances **9** and **10** are epimeric isomers at C-4′, having the D and L configuration, respectively. The CD spectra of **7** (Figure 4) feature interesting changes in sign and magnitude of the Cotton effects, but relatively little change has occurred in band positions. Both the signs and magnitudes of the B_{2u} and B_{1u} bands have been reversed in comparison with the CD spectrum of uridine. Of some interest also is the fact that both short-wavelength Cotton effects, E_{1ua} and E_{1ub}, are negative.

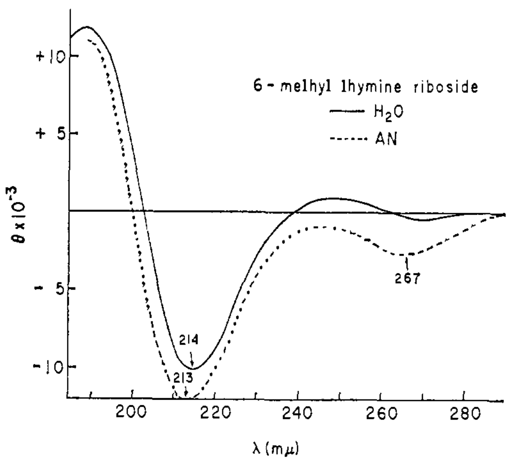

Figure 3. The circular dichroism curves of 6-methylthymine riboside (**5**) in water at pH 7 and in acetonitrile (AN).

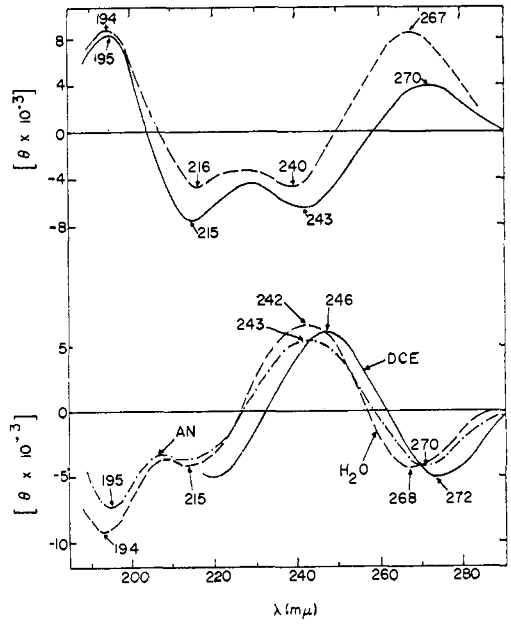

Figure 4. (Top) The circular dichroism curves of the 4′ epimers **9** (- - - -) and **10** (——) in water at pH 7. (Bottom) The circular dichroism curves of **7** in water at pH 7, in acetonitrile (AN), and in 1,2-dichloroethane (DCE).

Among the substances included in this study are compounds containing the 4′–5′ double bond and the 4′–1′ thioether function which are known to generate additional absorption bands below 230 and 210 mμ, respectively. The thioether function in particular may have transitions producing Cotton effects that overlap the ultraviolet CD spectra of uridine itself. Figure 5 contains the CD spectra of **11** and **12**. Compound **12**, which contains the 4′–5′ double bond but is otherwise structurally similar to uridine, produces a CD curve similar to that of uridine with respect to sign and the position of the four CD extrema, but it does feature interesting

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(34) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).
(35) J. A. Schellman, *Accounts Chem. Res.*, **1**, 144 (1968).
(36) D. W. Miles, S. F. Hahn, R. K. Robins, M. J. Robins, and H. Eyring, *J. Phys. Chem.*, **72**, 1483 (1968).
(37) E. G. Hohn and O. E. Weigang, *J. Chem. Phys.*, **48**, 1127 (1968).
(38) R. W. Woody and I. Tinoco, Jr., *ibid.*, **46**, 4927 (1967).
(39) D. J. Caldwell and H. Eyring, *Rev. Mod. Phys.*, **35**, 577 (1963).
(40) W. J. Kauzmann, J. Walter, and H. Eyring, *Chem. Rev.*, **25**, 339 (1940).
(41) H. Eyring, H. Liu, and D. J. Caldwell, *ibid.*, **68**, 525 (1968).

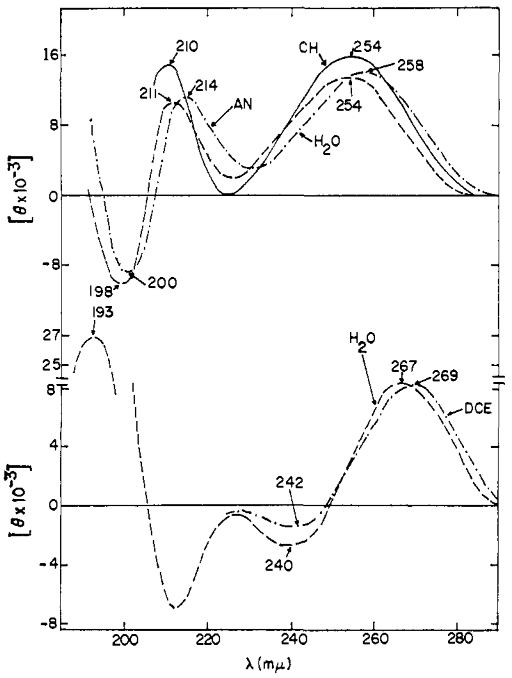

Figure 5. (Top) The circular dichroism curves of **11** in water at pH 7, cyclohexane (CH), and acetonitrile (AN). (Bottom) The circular dichroism curves of **12** in water at pH 7 and in 1,2-dichloroethane (DCE).

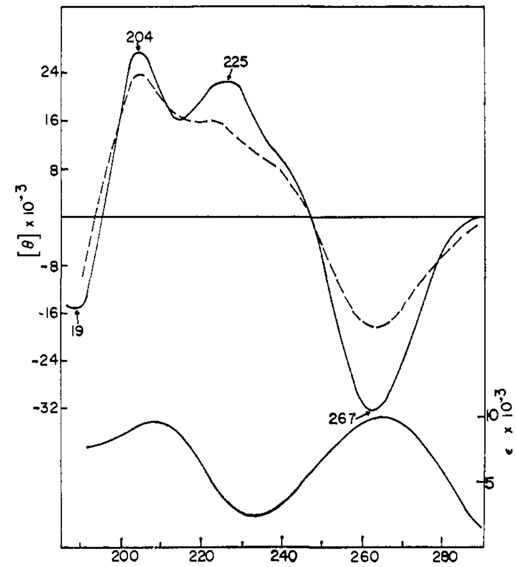

Figure 6. (Top) The circular dichroism curves of **13** in water at pH 7 (- - - -) and in acetonitrile (——). (Bottom) The absorption curve of **13** in water at pH 7.

and significant changes in magnitudes of the bands. Results of solvent studies are also given in Figure 5. A CD spectrum that is remarkably different from those of both uridine and **12** is obtained from **11**, which has a 2′,3′-O-isopropylidene group in addition to the 4′-5′ double bond. The positive CD bands found at 258 and 215 mμ and the negative CD band found at 199 mμ may be correlated with B_{2u}, E_{1ua}, and E_{1ub} electronic transitions of uracil. The absorption spectra is shifted to 259.5 and 199 mμ. The B_{1u} component is missing in the CD spectra of this compound but is probably of small magnitude and is overlaid by the two large positive CD bands at 258 and 215 mμ.

Generally the presence of an isopropylidene group in nucleosides has only a small effect on the magnitude of the CD bands.[42] The coupling of the π–π* transitions in the double bond with a particular base transition is capable of generating large contributions to the rotational strength.[36] It is quite likely, and examinations of molecular models tend to support this idea, that the isopropylidene group on **11** forces the double bond into a different angular alignment with the base transition dipoles than that existing in **12**. Changes in base–sugar conformation and/or specific sugar conformation could also be contributing factors. The B_{2u} Cotton effect occurs at 262 mμ in 4′-thiouridine (**13**) (see Figure 6), and the B_{1u} Cotton effect is seen as a positive shoulder in the 235–245-mμ region. Below 230 mμ three other CD extrema are observed, but the complexity introduced by the cyclic dialkyl sulfide structural element precludes assignment attempts. Dialkyl sulfide has a low intensity absorption band usually observed only as an inflection near 230 mμ. A stronger band occurs at 210 mμ.[43]

The rigid cyclouridine nucleoside, O^2-2′-cyclouridine (**15**), also gives four Cotton effects at approximately the same wavelengths as those found in uridine itself (compare Figure 2 with Figure 9 of ref 29). Changing the keto oxygen to alkoxy at position 2 of the heterocyclic ring and perhaps torsional strain as well causes changes in the intensity distribution in the absorption spectrum of O^2-2′-cyclouridine (**15**) resolving the B_{1u} and E_{1u} bands which are not clearly discernible in the absorption spectra of uridine and most of its analogs. Absorption bands are found at 240, 222, and 194 mμ with a shoulder at 270 mμ.

In summary it has been shown that a maximum of four CD bands are present in the CD spectrum of uridine (unless other chromophores are present) and its closely related derivatives, while only two bands are seen in the absorption spectrum. In O^2-2′-cyclouridine (**15**) all four bands are found in both the CD and absorption spectrum. It is interesting to note that the theoretical treatment of uracil by Nesbet[23] and thymine by Denis and Pullman[21] both predict four π–π* bands are present above 195 mμ in these substances. These theoretical treatments also predict that the intensity of the B_{1u} band will be much less than the B_{2u} band.

Solvent Studies

The solvent studies reported herein provide evidence concerning the possibility that one or more of the electronic transitions observed by the CD technique may be due to transitions of the n–π* type rather than π–π*. The study of electronic spectra in hydrogen-bonding and nonhydrogen-bonding media has been used to characterize n–π* transitions[15,16,32,44–47] and to distinguish

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(42) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, 6, 843 (1967).

(43) H. H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1962, p 474.

(44) G. J. Brealey and M. Kasha, *J. Amer. Chem. Soc.*, 77, 4462 (1955).

(45) S. F. Mason, *J. Chem. Soc.*, 1240 (1959), and references therein.

(46) N. S. Bayliss and E. G. McRae, *J. Phys. Chem.*, 58, 1002 (1954).

(47) G. C. Pimentel, *J. Amer. Chem. Soc.*, 79, 3323 (1957).

them from π–π* transitions. Uridine and its derivatives contain no lone-pair (nonbonding) electrons on nitrogens since either a hydrogen or a sugar residue is attached to both nitrogens. Both hydroxy groups are almost completely in the keto form in these compounds at neutral and acidic pH. Since the ketonic oxygens are known to give rise to n–π* transitions involving excitation of the nonbonding electron on the keto oxygen to the antibonding π-oribital in *p*-benzoquinone and related compounds,[32,48,49] it would seem that similar transitions may be present in the spectra of uridine and its derivatives, although several studies[6,9,14] have been unable to detect n–π* transitions in the near-ultraviolet spectral region of uracil derivatives.

Spectral shifts due to hydrogen-bonding interaction are usually much larger than shifts due to dispersion, dipole–dipole, and dipole–polarizability forces. Both the strength of the hydrogen bond[44] and the magnitude of the Franck–Condon stabilization energy,[47] w', act together to produce a large blue shift (shift to higher frequency) on the n–π* transitions when a hydrogen bond is formed between the solvent molecule and the n electrons. The magnitude of the blue shift of n–π* transitions vary for different molecules. For acetone and quinone which contain keto oxygens, the blue shifts are moderate, 2100 and 1600 cm^{-1}, respectively, on changing from a hydrocarbon solvent to water.[32] Hydrogen-bonding interactions cause shifts to the red or to the blue of a few hundred wave numbers on π–π* transitions although shifts up to 700 cm^{-1} have been recorded.[45,50] The McConnell shift test is not, therefore, completely unambiguous in the cases where both π–π* and n–π* transitions are blue shifted by hydrogen-bond formation, particularly if the association constant of the solute molecule with water is very small. The electron pair on the carbonyl groups of uridine are capable of strong hydrogen-bonding interactions with hydrogen-bond donors such as water and other Bronsted acids, but little interaction is expected between the carbonyl group and solvents such as acetonitrile and dioxane. These latter solvents are capable of acting only as hydrogen-bond acceptors although the polar nature of acetonitrile may give rise to a preferential orientation of acetonitrile around the uracil base and thus have a higher concentration than average in the neighborhood of the solute molecule. The blue shift of n–π* transitions is expected to parallel the strength of the solvent–solute interaction. Both relatively nonpolar dioxane and polar acetonitrile generally give quite similar effects on the spectrum. The change from water to dioxane or acetonitrile should give rise to large shifts in n–π* transitions of uridine and its analogs.

Compound **11** is sufficiently soluble in water, alcohols, acetonitrile, dioxane, and methylcyclohexane to permit measurements in 5×10^{-4} to 5×10^{-5} *M* solutions. No concentration effects were found in this range. Representative solvent effect curves are given in Figure 5, and the critical spectroscopic constants are given in Table I. The low-energy absorption band and all CD peaks of **11** undergo 2- to 4-mμ shifts to lower frequencies in the presence of hydrogen-bond donor solvents, H_2O, and methanol. Variations in the intensities of the CD bands in the series of solvents roughly parallel the magnitude of the solvent dipole moment. For example, a polar solvent like acetonitrile which is not expected to hydrogen-bond strongly with the solute molecules n electrons, has approximately the same effect on intensities of the CD bands as does water but causes frequencies shifts similar to cyclohexane. Direct application of the solvent shift test supports the π–π* assignment of the B_{2u}, E_{1ua}, and E_{1ub} bands. In the region of the minimum between the B_{2u} and E_{1ua} CD bands, one sees significant differences in the CD pattern induced by solvent changes. In water the molecular ellipticity at the minimum is over 5000; in cyclohexane the molecular ellipticity is near zero. The minimum occurs close to the B_{1u} region of the spectra.

One possibility is that a "blue shift" band lies under the dominant CD band in cyclohexane and is shifted to higher energy upon hydrogen-bond formation with water. Thus this one set of curves could be cited in support of an n–π* assignment for the transition producing the Cotton effects in the 240-mμ region in uracil nucleoside derivatives. However, alternative explanations do exist such as solvent-induced changes in band shape, intensities, energies, and relative vibrational contributions of both the B_{2u} and B_{1u} CD bands. Relatively minor shifts in energy coupled with small intensity changes could easily produce the lower minimum upon changing from water to cyclohexane.

Comparison of solvent effects on compounds **1**, **7**, and **12** of Figure 2, 4, and 5 (which give quite similar results) with solvent studies on **11** (Figure 5) reveal another interesting difference. In all cases a similar red shift is observed in the absorption spectrum in going from aprotic solvents like dioxane, acetonitrile, and 1,2-dichloroethane to alcohols and finally to water (see Table I). The size of the shift parallels the expected hydrogen-bond donor ability. Similar effects are noted for **12**. However, shifts in CD peaks parallel the shifts in absorption peak only for **11**. In substances **1**, **7**, and **12** the absorption peaks shift to lower frequencies while the CD peaks shift to higher frequencies upon hydrogen-bond formation. In the CD and absorption studies on cytosine nucleoside derivatives this problem does not arise as both the absorption and CD bands shift in the same direction with solvent.[29] The size of the blue shift is about 700 cm^{-1} in going from 1,2-dichloroethane to water. It should be pointed out that apparent blue shifts of almost this magnitude sometimes arise from variations in the intensity of overlapping negative and positive CD bands as demonstrated by Figure 4 (top). Compounds **9** and **10**, which are DL epimers at the 4′-carbon, give the same absorption spectra within experimental error, but different CD peak positions are noted. Apparently increasing the magnitude of the B_{1u} Cotton effect relative to the B_{2u} Cotton effect gives rise to an apparent shift of both bands to lower frequencies. In uridine, dioxane and acetonitrile both enhance the intensity of the B_{1u} band relative to the B_{2u} band. Thus the real frequency shift, at least in uridine, is much too small to support any serious speculation about possible n–π* origins of the B_{2u} and B_{1u} Cotton effect. It is not completely clear why the CD band peaks and absorption peaks shift in opposite directions upon changing

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(48) P. E. Stevenson, *J. Mol. Spectrosc.*, 17, 58 (1965), and references therein.

(49) S. Nagakura and A. Kuboyama, *J. Amer. Chem. Soc.*, 76, 1003 (1954).

(50) S. F. Mason, *J. Chem. Soc.*, 1247 (1959), and references therein.

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.

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(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-

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(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.