have presented do give realistic coupled oscillator results. Example **2** demonstrates that a transition moment directed along a saturated carbon–carbon bond can couple with π-electron transitions in a chromophore to a sufficient degree to explain much of the origin of the observed rotational strength, and cannot possibly be construed to be a minor effect. Then it is seen that this method of calculation can also be used to interpret the B_{2u} band in the CD spectra of the cyclouridines. The detailed effects of the hydrogen–oxygen bonds have shown that they can be safely ignored only when the resulting calculated rotational strength is sufficently strong (*i.e.*, an absolute magnitude greater then 1×10^{-40} cgs), and when this condition is not met, great care must be taken in the choice of molecular conformation.

If we consider the nucleosides in particular, there are a number of alterations in the calculation which may improve reliability, but which do not change the overall procedure. First, the molecular orbitals in the chromophore may have substantial room for improvement, particularly since any closed shell Hartree–Fock-type procedure is not very reliable in giving excited state MO's. Furthermore, the Adams–Miller calculation employed here includes only single-electron excited states, and it is widely believed that two-electron excitations contribute significantly to transition moment calculations (although the gradient method of calculation tends to minimize the effect).[31] Inclusion of these latter states in the configuration interaction might well allow a better estimation of the higher energy band rotational strengths than have been obtained thus far. As to the vicinal group, better overall geometries are needed, and an alternative to a dependency on polarizability-based parameters should be developed. Work is in progress to check some of these various possibilities. Our results show, nevertheless, that bond–bond coupling offers a useful description of the electronic–optical properties of the nucleosides with good correlation with experiment. This is further demonstrated in a companion paper which follows, where several vicinal groups are attached to the uracil and cytosine chromophores, occasionally differing by only a single bond.

Finally, it should be noted that if this were the only optical activity calculation available, the absolute configuration of all of the examples considered would have been correctly assigned!

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Circular Dichroism of Nucleoside Derivatives. IX. Vicinal Effects on the Circular Dichroism of Pyrimidine Nucleosides[1]

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Abstract: The circular dichroism data on 38 pyrimidine nucleosides, selected to provide a basis for a reliable index of furanose conformation as a function of its optical activity, are presented. The functional dependency of the B_{2u} Cotton effect on the sugar-base torsion angle and on the specific pentose conformation is determined from theory and compared with experimental data. The theoretical description of the rotational strength as a function of the torsion angle agrees in every respect with available experimental data. Substituent effects on the signed magnitudes of the B_{2u} rotational strength generally correlate quite well with theoretical expectations. The success of the theoretical calculations depend on giving up the Kirkwood–Tinoco coupled oscillator scheme where the transition moment vector is located at the center of gravity of the chromophore and, instead, breaking down the electric transition moment into bond contributions.

In paper VIII of this series,[1] it was shown that an analysis of the $\pi \rightarrow \pi^*$ Cotton effects of cyclopyrimidine nucleosides and several other rigid systems predicts their absolute stereochemical configuration. The method of analysis is an extension of the classical coupled oscillator theory of optical activity of Kuhn,[2] which was reformulated in quantum mechanical terms by Kirkwood.[3] The method resolves the gradient version of the transition dipole moment vector into bond contributions and couples each chromophoric bond component with the far-uv transitions of the vicinal bonds by their dipole–dipole interactions. The totality of the bands in the vicinal bonds are represented by an effective transition moment with the direction of the principle polarizability of the bond

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(3) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).

and an energy based on experimental information now available. In the present paper the theory provides understanding of the relative changes in the B_{2u} Cotton effect of pyrimidine nucleosides induced by substituents. Data on some 38 nucleosides, many of which were specially prepared for this study, are compared with theoretical expectations. These nucleosides are capable of free rotation about the glycosidic bond and interconversion among several puckering modes of the furanose ring. The overall objective of this series of papers (besides providing valuable spectroscopic information) is to establish a reliable index of nucleoside conformation as a function of its optical activity. The implications of furanose conformation on the biological specificity of nucleoside containing enzymes, the structure of nucleic acids in solution, and the cytotoxic activity of nucleoside derivatives have been widely discussed in recent years.[4-7]

An empirical diagram was proposed in paper VI of this series[8] from which one may read the ellipticity of the B_{2u} band of uridine and cytidine nucleosides as a function of the sugar-base torsion angle,[9] ϕ_{CN}. A theoretical basis for this diagram is now given. Besides its intrinsic utility to the nucleoside and nucleotide chemist, this diagram may provide valuable information concerning the furanose conformation in solution.

I. 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 II. The other experimental details are the same as previously described.[8]

II. Theory

(A) Method of Calculation. While the coupled oscillator theory and its exciton modification have been widely applied to molecules with strong coupling between aromatic groups,[10] it has been largely ignored in favor of the one-electron theory for discussions of optical activity of aromatic chromophores attached to far-uv absorbing groups. For Cotton effects assignable to intense absorption bands it is certain, as the calculations in this paper and the preceding paper show, that significant rotatory power is developed by coupling intense near-uv bands with intense far-uv bands by the Kirkwood mechanism.

In aromatic groups the chromophoric transition extends over many atomic nuclei, but certain regions of the chromophore during the act of absorption may be perturbed much more than others. Since the location of the transition moment vector influences the coupled oscillator calculation, emphasizing the regional nature of a transition may significantly improve the calculation. In the uracil chromophore, for example, the low-energy transition is found from molecular orbital calculations to be localized largely in the space surrounding the C_5–C_6 bond. Excellent agreement is achieved between theory and experiment by locating the transition moment vector in this region of the molecule while poor agreement is obtained by locating the transition moment vector at the center of gravity of the chromophore. The transitional region of the chromophore can be emphasized by breaking down the electric transition moment

$$\vec{\mu}_a = \frac{\sqrt{2}\beta_m}{\pi\nu_a}\langle 0|\vec{\nabla}|a\rangle \qquad (1)$$

into bond components

$$\vec{\mu}_{ia} = \frac{\sqrt{2}\beta_m}{\pi\bar{\nu}_a}P_{rs}\vec{\nabla}_{rs} \qquad (2)$$

and then utilizing a bond–bond coupled oscillator scheme to get the rotational strength. The final expression for the rotational strength is

$$R_a = \sum_i\sum_j \frac{2\pi}{hc}\frac{\nu_a\nu_0}{(\nu_b^2 - \nu_a^2)}\mu_{ia}^2\mu_{jb}^2 GF_{ij} \qquad (3)$$

where all symbols are as defined and discussed in the companion paper.

(B) Application to Nucleosides. The Adams–Miller modified Pariser–Parr–Pople molecular orbital theory[11] is applied in straightforward fashion to the base chromophores by using crystal structure data on uridine and cytidine. The valence state data and effective nuclear charges, ionization potentials, and electron affinities are as used in previous calculations.[12-14] Configuration interaction involving single electron excitation between five occupied and the three unoccupied molecular orbitals has been introduced. The bond transition moments for each chromophore treated in this paper are shown in Figure 1 along with their directions.

The effective transition moment chosen to represent carbon–carbon bonds and carbon–oxygen bonds in the sugar residue are described in the preceding paper. Again carbon–hydrogen bonds are neglected because Denbigh's polarizability data[15] indicate that their contribution is less than 20% of the contribution of the carbon–oxygen bonds, which are relatively small contributors also. The oxygen–hydrogen bonds are neglected because their conformation is not yet well defined in nucleosides and because inclusion of this bond seldom appreciably affects the calculated results. For a chloro substituent an effective transition moment of 3.34 is calculated from Denbigh's polarizability data. We take its frequency, ν_b, at 2.3×10^{15}. The C–C double bond is represented by an effective transition moment of 3.7 D and a frequency of 1.54×10^{15}. These values are derived from absorption data on norbornene,[16] which simulates to a considerable

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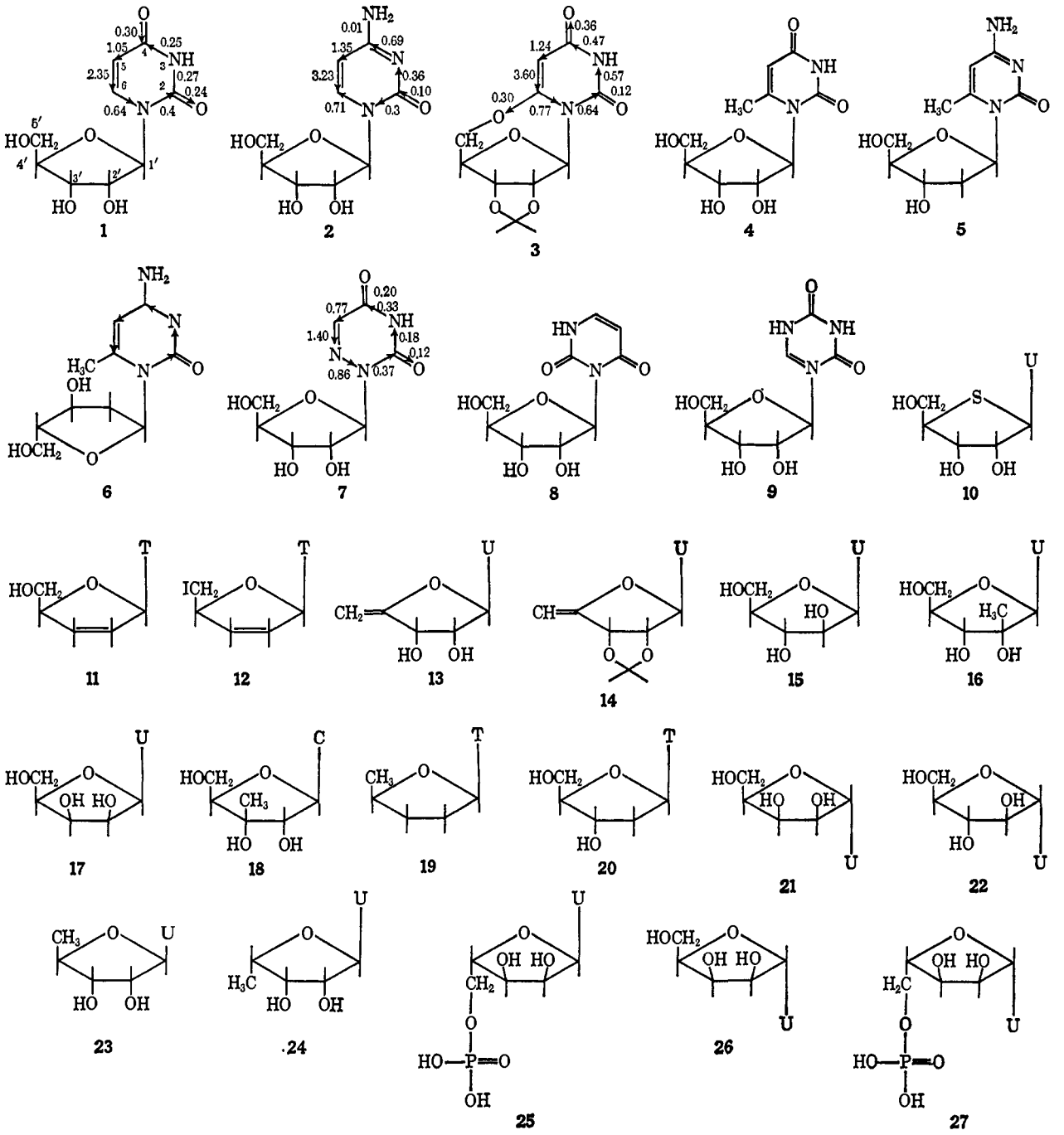

Figure 1. The structural formulas of many of the compounds studied in this paper. Other compounds discussed are simple derivatives of uridine or cytidine. The SCF-CI bond transitional moments are given for uridine (**1**), cytidine (**2**), 5′,6-anhydro-2′,3′-O-isopropylidene-6-hydroxyuridine (**3**), and 6-azauridine (**7**) (in Debyes).

extent the furanose structure, *i.e.*, pentane ring system and alkyl substituents at C′-1 and C′-4. The three intense absorption bands of the N–V type exhibited by the C–S–C chromophore in tetrahydrothiophene[17] at 208, 180, and about 160 mμ and transition dipole moments at 2.5, 2.5, and 3.5 D, respectively, are represented by an effective transition moment of 4.3 D at 185 mμ for each bond.

(C) Conformational States. In the crystalline and solution states of pyrimidine nucleosides the furanose ring is not planar. To avoid extensive eclipsing either C′-2 or C′-3 is displaced about 0.5 Å out of the plane formed by the other four ring atoms with the displacement being on the same side (*endo*) of the furanose ring as the base.[18] The 3′-*endo* puckered form has been implicated by nmr as dominating in solution with considerable conformational purity[18] while both 3′-*endo* and 2′-*endo* forms are found in the crystalline states. Besides interconversion between the puckering modes the nucleosides are conformationally labile about the glycosidic link between the pentose and the base. Molecular models indicate that the base could exist in two ranges of the torsion

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Table I. Comparison of the Computed and Experimental Spectra

Structure		B_{2u}	$f(r)$	$f(\nabla)$	B_{1u}	$f(r)$	E_{1ua}	$f(r)$	E_{1ub}	$f(r)$
Uridine	Calcd	4.81	0.34	0.17	5.49	0.03	5.99	0.46	6.49	0.81
	Exptl	4.74	0.20[a]	0.20[a]	5.17		5.65		6.33	0.30[a]
Cytidine	Calcd	4.26	0.10	0.12	5.33	0.07	6.04	1.11	6.62	0.23
	Exptl	4.58	0.20[a]	0.20[a]	5.15	0.16	5.68	0.60	6.36	
O^6-5′-Hydroxy-cyclouridine	Calcd	4.80	0.37	0.25	5.62	0.25	6.08	0.16	6.46	0.76
	Exptl	4.72	0.28	0.28	5.24		5.65		6.47	0.36
6-Azauridine	Calcd	4.3	0.19		4.77	0.16	6.13	0.49	6.55	0.78
	Exptl	4.71	0.12	0.12						

[a] Experimental oscillator strengths for these compounds are taken from H. Berthod, C. Geissner-Prettre, and A. Pullman, *Int. J. Quantum Chem.*, **1**, 123 (1967). Otherwise experimental oscillator strengths are calculated from the expression, $f = (7.67 \times 10^{-9})\,\bar{\nu}_i(\epsilon_i\Delta_i/\lambda_i)$, where ϵ_i is the molar extinction coefficient at the curve maximum, λ_i is the wavelength of the ith maximum, Δ_i is the half-band width at ϵ_i/e, and $\bar{\nu}_i = 1/\lambda_i$.

angle, the *anti* range centered near −30° and the *syn* range centered near 150°. The implications of furanose conformation has stimulated[19] several recent theoretical and experimental studies which indicate the *anti* range is usually preferred.[4-7,21-24] Tinoco[21] assigns each atom a charge, a polarizability, an ionization energy, and a steric repulsion parameter to determine the functional dependency of the total molecular energy of purine and pyrimidine nucleosides on the torsion angle. His results on cytidine and uridine justify our adoption of the probabilities of 0.4, 0.2, 0.2, 0.1, and 0.1 for molecular conformations at −30, −20, −40, −10, and −50° in the calculations on nucleosides which contains the basic furanose skeletal structure and no C-6 ring substituent. The C′-2 and C′-3 hydrogens and the O′-1 oxygen make contact with the carbonyl oxygen and the carbon-6 hydrogen of the base during rotation about the glycosidic link. Most uridine nucleosides discussed in this paper have these common steric features or possess even greater potential for steric interactions.

III. Results and Discussion

Figure 1 shows the structural formulas of key compounds emphasized in the discussion and includes the SCF-CI bond transitional moments, μ_{ia}, for the B_{2u} band of uridine (**1**), cytidine (**2**), 5′6′-anhydro-2′,3′-O-isopropylidene-6-hydroxyuridine (**3**), and 6-azauridine (**7**). Table I presents the calculated energies and oscillator strengths, $f(r)$, of the first four π–π^* states and makes comparisons with available experimental data. Table I also contains the oscillator strength, $f(\nabla)$, of the B_{2u} band. The symbol ∇ indicates that the oscillator strength is calculated using the dipole velocity formula for the transition dipole moment instead of the dipole length formula. In order to introduce maximal empirical content into rotational strength calculations the transition moments arising from the MO calculations are modified by a factor that corrects each theoretical value to its experimental value. Thus in the calculations of the rotational strength each theoretical bond transition moment, μ_{ia}, of uracil, cytosine, 6-hydroxyuracil, and 6-azauracil, is multiplied by the factors 1.15, 1.67, 1.3, and 1.04, respectively, to give the experimental oscillator strength of Table I with, however, the direction of the total transition moment remaining unchanged. The circular dichroism results are collected in Table II and include relevant data previously reported from this laboratory and several interesting compounds found in the literature. Generally four Cotton effects are found above 190 mμ.[25] It is convenient to again refer to these bands in terms of the corresponding benzene group theory symbols, *e.g.*, B_{2u}, B_{1u}, E_{1ua}, and E_{1ub} in order of increasing energy. This usage is illustrated in Figure 2. Table II also contains the experimental and theoretical rotational strengths.

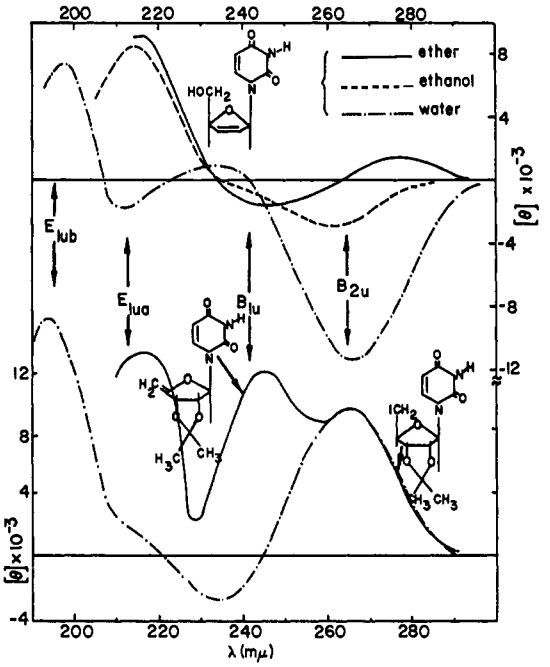

Figure 2. Circular dichroism spectra of compound **11** in ether, ethanol, and water (at pH 7) (top); circular dichroism spectra of compound **14** in EPA and 2′,3′-di-O-acetyl-5′-deoxy-5′-iodouridine (in water at pH 7) (bottom).

(19) The importance of the torsion angle is emphasized in structural considerations of the B form of DNA where Landridge[20] has described three models which differ in the relative distance of the polynucleotide chains from the helical axis. These changes are brought about by altering the folding of the polynucleotide backbone through a change in the torsion angle which cannot unambiguously be derived from the type of diffraction data obtained from these structures.

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Table II. Circular Dichroism Data and Theoretical Results[a]

Compound	B_{2u}	B_{1u}	E_{1ua}	E_{1ub}	R (exptl)	R(theor)
Uridine (**1**)	267 (9.2)	240 (−4.0)	215 (−5.0)	196 (8.0)	8.6	4.5
Cytidine (**2**)	271 (12.7)	Shoulder	220 (−11.3)	195 (2.0)	11.9	5.9
5′,6-Anhydro-2′,3′-O-isopropylidene-6-hydroxyuridine[f] (**3**)	266 (41.0)	233 (−21.0)	213 (−23.0)	191 (33.0)	38.5	16.0
6-Methyluridine[g] (**4**)	265 (−0.3)	250 (1.0)	214 (−10.0)	190 (12.0)	−0.3	Small negative
6-Methylcytidine[g]	270 (−1.2)	247 (0.8)	220 (−15.0)	198 (2.0)	−1.1	Small negative
6-Methyl-2′-deoxycytidine[g] (**5**)	270 (−7.0)	248 (−5.0)	220 (15.0)	195 (8.0)	−6.6	−3.2
1-(2′-Deoxy-α-D-*erythro*-pentofuranosyl)-6-methylcytosine[g] (**6**)	270 (6.8)	248 (4.8)	220 (−13.0)	193 (3.0)	6.4	3.8
6-Azauridine[h] (**7**)	257 (−10.0)		205 (14.0)		−9.4	−2.0
6-Azauridine triacetate[h]	257 (−12.0)		204 (13.0)		−11.2	−2.0
6-Azacytidine[b]	ϕ_i = −5450[a]				−8.9[e]	−1.3
3-(β-D-Ribofuranosyl)uracil[g] (**8**)	270 (−4.0)	244 (5.0)	215 (−3.0)	195 (−7.0)	−3.8	−1.5 (−90°) −1.1 (+90°)
β-Pseudouridine[h] (**9**)	274 (−2.0)	242 (2.0)	220 (2.0)		−1.9	−4.0
4′-Thiouridine[i] (**10**)	263 (−18.0)	240 (sh) (8.0)	219 (16.0)	205 (24.0)	−17.0	−11.0
1-(2,3-Dideoxy-β-D-*glycero*-pent-2-eno-furanosyl)-thymine[j] (**11**)	266 (−11.5)	235 (1.0)	212 (−1.8)	197 (7.5)	−10.8	
Compound **11** in ether	276 (1.5)	246 (−1.5)	214 (9.0)		1.4	Positive at 150°
i-(2,3,5-Trideoxy-5-iodo-β-D-*glycero*-pent-2-eno-furanosyl)thymine[j] (**12**)	266 (−11.0)	231 (1.0)	209 (−5.0)		−10.3	
Compound **12** in ether	266 (−12.0)	230 (4.0)			−11.3	
1-(5-Deoxy-β-D-pento-4-enofuranosyl)uracil[g] (**13**)	266 (8.2)	240 (−2.8)	213 (−10.5)	193 (27.0)	7.7	11.0
1-(2,3-O-Isopropylidene-5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)uracil[g] (**14**)	258 (13.0)		214 (10.0)	201 (−9.5)	12.2	
1-(β-D-Arabinofuranosyl)cytosine	270 (22.0)		222 (−15.0)		20.7	8.0
1-(β-D-Arabinofuranosyl)uracil[k] (**15**)	266 (22.0)	235 (−6.5)	215 (−9.7)	194 (20.0)	20.7	6.6 7.6
2′-C-Methyl-5-fluorouridine[c] (**16**)[a]	ϕ_i = 13,100[a]				22.0[e]	6.5 6.7
2′-Deoxyuridine[g]	267 (6.0)	237 (−3.6)	215 (−5.8)	194 (14.0)	5.6	5.3 3.0
2′-Chloro-2′-deoxyuridine[l]	266 (8.3)	238 (−3.5)	215 (−4.0)	196 (11.0)	8.0	3.8
1-(β-D-Lyxofuranosyl)uracil[d] (**17**)	ϕ_i = 12,300[a]				20.6[e]	6.0
3′-C-Methylcytidine[c] (**18**)	ϕ_i = 3300				5.4[e]	4.5
1-(2,3,5-Trideoxy-β-D-*glycero*-pentofuranosyl)-thymine (**19**)	273 (3.7)	242 (−3.8)	219 (−2.7)	200 (10.0)	3.5	3.3
Thymidine[h] (**20**)	272 (4.0)	242 (−3.7)	215 (−6.0)	197 (16.0)	3.8	3.3
2′,3′-Di-O-acetyl-5′-deoxy-5′-iodouridine[m]	265 (8.8)	234 (−1.2)		196 (10.0)	8.3	4.5
2′,3′-Di-O-acetyluridine[m]	266 (9.0)	236 (−2.8)	215 (2.5)	194 (15.2)	8.5	4.5
3′-O-Acetylthymidine[m]	274 (4.2)	241 (−2.9)	215 (−3.3)	194 (14.0)	3.9	4.5
1-(α-D-Lyxofuranosyl)uracil[d] (**21**)	ϕ_i = −1600[a]				−2.7[e]	−3.5
1-(α-D-Arabinofuranosyl)uracil[d] (**22**)	ϕ_i = −3800[a]				−6.4[e]	−2.9
5′-Deoxyuridine[g] (**23**)	268 (8.6)	240 (−4.5)	215 (−4.5)	194 (9.4)	8.3	4.5
1-(5-Deoxy-α-L-*lyxo*-pentofuranosyl)uracil[m] (**24**)	270 (4.3)	243 (−5.5)	215 (−7.0)	195 (8.0)	4.1	3.0
1-α-L-Ribofuranosyl 5′-phosphate[d] (**25**)	ϕ_i = 9900[a]				16.6[e]	5.6
1-(α-D-Lyxofuranosyl)uracil[d] (**26**)	ϕ_i = −1600[a]				−2.7[e]	−3.5
1-(β-L-Ribofuranosyl)uracil 5′-phosphate[d] (**27**)	ϕ_i = −3500[a]				−5.6[e]	−4.5
5′-Deoxy-5′-iodo-2′,3′-O-isopropylideneuridine[n]	265 (5.0)	235 (−0.7)		208 (+5.0)	4.7	4.5

[a] The position of maxima in the circular dichroism spectra are given in mμ units. The molar ellipticities × 10^{-3} are given in parentheses. The experimental rotational strengths, R(exptl), are calculated from the expression, $R_i = (1.23 \times 10^{-42})(\theta_i\Delta_i)/\lambda_i$, where θ_i is the molar ellipticity at the maximum of the curve, λ_i and Δ_i are defined in Table I. All measurements were made in water at pH 7 unless otherwise indicated. The ORD data represent the molecular rotation, ϕ_i, at the first extrema of the B_{2u} band. [b] Data are taken from ref 31. [c] Data are taken from ref 32. [d] Data are taken from ref 33. [e] The rotational strengths for compounds where only ORD data are available are estimated from the expression, $R = R'\phi_i'/\phi_i$, where R and R' represent the rotational strength of the compound and reference compound (uridine or cytidine), respectively, and ϕ_i' or ϕ_i represents the molecular rotation at the first extrema. [f] B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, **34**, 1390 (1969). [g] See ref 25 and 26. [h] Commercial source. [i] M. W. Winkley and R. K. Robins, *J. Chem. Soc.*, 791 (1969). [j] J. P. Horwitz and J. Chua, "Synthetic Procedures in Nucleic Acid Chemistry," W. W. Zorback and R. S. Tipson, Ed., Academic Press, Inc., New York, N. Y., 1968, p 383. [k] I. Wempen, and J. J. Fox in footnote *j*, p 369. [l] J. F. Codington, I. Doerr, D. V. Pragg, A. Bendich, and J. J. Fox, *J. Amer. Chem. Soc.*, **83**, 5030 (1961). [m] M. W. Winkley, in press. [n] B. F. West in footnote *j*, p 313.

(A) Ribofuranosyl Pyrimidines. The emphasis of this section is on exploration of the functional dependence of the rotatory strength of some ribofuranosyl pyrimidines on the torsion angle. Experimentally, this is done by the study of model compounds which are limited to different ranges of the torsion angle by steric or long-range coulombic forces. Theoretically the torsion angle is a variable in the calculation. The positions of the atoms in the 3′-*endo* conformation of uridine and cytidine are taken from the X-ray work of Trueblood, *et al.*,[26] and Furberg, *et al.*,[27] whereas the work of Harris and MacIntyre[28] and Sundaralingam and Jensen[29] are used for the 2′-*endo* conformations. Figure 3 shows the rotatory

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strength *vs.* torsion angle behavior predicted for the B_{2u} transition for both common puckered modes of uridine and cytidine. We focus attention on the 3′-*endo* curves, as the nmr study of Jardetsky[18] indicates that uridine and cytidine favor this conformation in solution. When the 3′-*endo* curves of uridine and cytidine are compared with our empirical plot presented in paper VI of this series, it is noted that the theoretical and empirical curves are remarkably similar in shape and nodal positions. The empirical plot embraces considerable CD data on uridine and cytidine derivatives and, consequently, represents at least a coarse-grained representation of the dependency of B_{2u} optical activity on the torsion angle. The present work gives the empirical plot a solid theoretical basis.

As seen from the μ_{ia} values recorded in Figure 1 the $A_{1g} \rightarrow B_{2u}$ transitions of both uridine and cytidine are largely localized in the molecular region in the vicinity of the C_5–C_6 bond. The R–ϕ_{CN} plots of both nucleosides, consequently, differ appreciably in amplitudes but not in location of nodes. Calculations[30] show that 5 substituents such as methyl, chloro, bromo, iodo, or hydroxy appreciably lower the amplitudes of the R–ϕ_{CN} plots but, again, the nodal positions are not greatly affected. Thus the practical application of these diagrams to nucleoside derivatives of unknown stereochemistry extends to uracil and cytosine bases with methyl, chloro, bromo, iodo, and hydroxy exocyclic substituents at the 5 carbon and to sugars including methoxyl, amino, acetamido, deoxy, methyl, alkyl, chloro, bromo, iodo, and acetate modifications. (Applications involving sugar structural changes involves the use of Table III which will be discussed later.) In applying these correlations it is necessary to recognize that exocyclic sugar substituents replacing hydroxyls generally do not decrease the steric interactions which favor the *anti* range but, if anything, increase the stability of *anti* conformers. While our theoretical procedure, as outlined in section II.C, is keyed to Tinoco's probability curves,[21] quite useful results may be attained by merely taking the R value at $\phi_{CN} = -30°$ from the diagrams of Figure 3. Comparison of the cytidine and uridine curves (and the curves of their 5-substituted analogs[30]) at $\phi_{CN} = -30°$ explains the similarity in the signed magnitudes of the B_{2u} band for structurally related uridine and cytidine derivatives as noted in paper VI of this series (see, for example, Figure 7 of paper VI).

The experimental and calculated rotational strengths, $R_{B_{2u}}$, of uridine and cytidine differ by a factor of 2 in both cases. This agreement is, of course, quite good. It should be emphasized that because of unavoidable use of approximate wave functions and polarizability data (which are probably reliable on a relative basis but not in any absolute sense), exact agreement with experiment is not to be expected of our calculations. What can be expected is that the relative changes in magnitude of rotations and the relative configurations of the compounds investigated should agree with those observed experimentally as is the case for most of the substances here considered.

(30) D. W. Miles, W. H. Inskeep, and H. Eyring, in preparation.

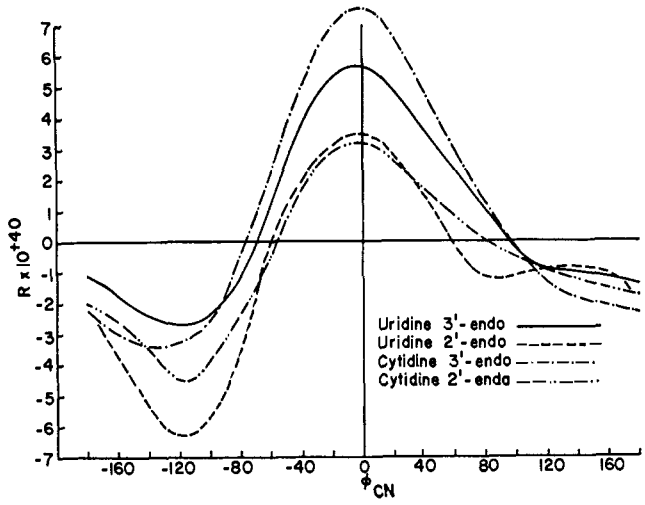

Figure 3. The dependency of the B_{2u} rotational strength on the torsion angle and the specific puckered conformation of the ribose ring as calculated by the bond–bond coupled oscillator theory.

Most ORD and CD measurements previously reported[31,32] on β-D-pentofuranosyl pyrimidines exhibit positive B_{2u} Cotton effects regardless of configuration at C-2′, C-3′, or C-4′, and α-D-pentofuranosyl pyrimidines exhibit negative B_{2u} Cotton effects. We next consider a series of uridine and cytidine derivatives, which, in contrast to the above, give a negative B_{2u} Cotton effect for β anomers and a positive B_{2u} Cotton effect for α anomers. For example, we have synthesized several 6-methylpyrimidine nucleosides which show negative B_{2u} Cotton effects. Examination of CPK molecular models suggest that the sugar-base conformation in 6-methylcytidine and 6-methyluridine (**4**) is limited to the 90–150° range of the torsion angle. Theoretically, the sign reversal is predicted for either puckered representations of uridine or cytidine. In addition the theoretical plot of Figure 3, which increases in a negative sense with increasing ϕ_{CN}, suggests that a larger absolute value for the molecular ellipticity would occur upon formation of a hydrogen bond between the keto oxygen and the 5′-OH group. This interaction draws the sugar-base torsion angle nearer 180°. Experimentally a larger negative molecular ellipticity is found for the B_{2u} band of 6-methyluridine (**4**), 2′-deoxy-6-methylcytidine (**5**) and 6-methylcytidine in dioxane and acetonitrile.

In analogous fashion one can explain the positive B_{2u} Cotton effect of the α anomer of 6-methyl-2′-deoxycytidine (**6**) in water and the reversal of sign brought about by dioxane and acetonitrile (see Figure 6 of paper VI of this series). Models show that **6** favors a conformation centered around −135° but hydrogen bond formation between the 3′-OH and the keto oxygen of the pyrimidine stabilizes the conformation at about −80°. A rotational strength torsion angle diagram for the enantiomer of cytidine can be mentally constructed by reflecting the cytidine curve and reversing the signs of the abscissa scale. If this is done one sees that the curve for the enantiomer of cytidine will be positive around −130° but negative

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(32) T. Nishimura, B. Shimizu, and I. Iwai, *Biochim. Biophys. Acta*, **157**, 221 (1968).

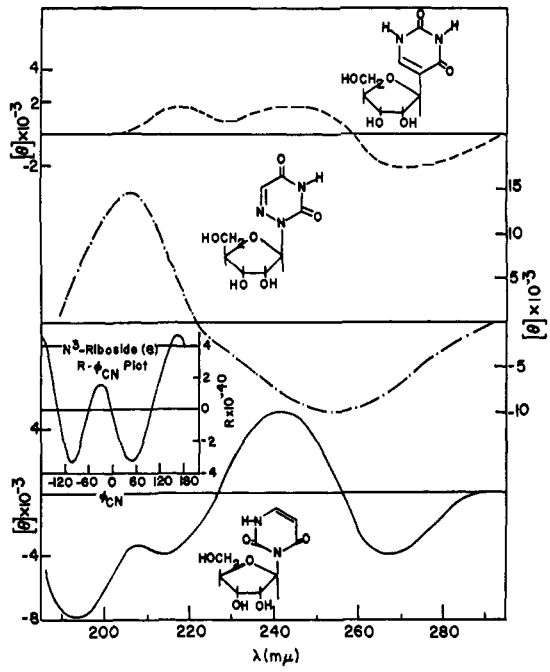

Figure 4. The circular dichroism spectra of pseudouridine (**9**) in water at pH 7 (top); the circular dichroism spectra of 6-azauridine (**7**) in water at pH 7 (middle); the circular dichroism spectra of 3-(β-D-ribofuranosyl)uracil (**8**) in water at pH 7 (bottom). The insert depicts the B_{2u} rotational strength plotted against the torsion angle.

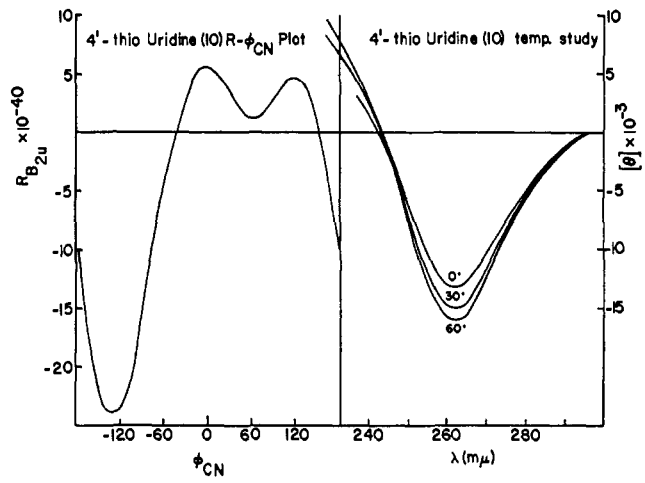

Figure 5. The temperature effect on the B_{2u} Cotton effect of 4'-thiouridine in water at pH 7 (left) and a portion of the B_{2u} rotational strength–torsion angle plot (right).

at −80°. Of course compound **6** is *not* the enantiomer of cytidine but actual computations on **6** show this explanation remains valid.

A negative Cotton effect is found for 6-azauridine (**7**) and 6-azacytidine (Figure 4) in spite of the β configuration at C-1′. Our molecular orbital calculations indicate that the negative sign does not arise from chromophoric effects as only modest changes in the relative magnitudes of the μ_{ia} vectors are calculated for the replacement of C-6 by nitrogen (see Figure 1). Computations show that the rotational strength changes of 6-azauridine and uridine with torsion angle have magnitude differences but identical nodal positions. The negative sign can be explained by the hypothesis that the lone pairs of N-6 and the keto oxygen of the pyrimidine ring interact with the lone pair of electrons of the ether oxygen of the pentose to stabilize conformations at around −90 or 90°; at −90 and 90° a negative Cotton effect and a node point are predicted.

A similar explanation is suggested for the negative B_{2u} Cotton effect exhibited by 3-(β-D-ribofuranosyl)uracil (**8**) as shown in Figure 4. In the N^3-ribosides there is no preferred *syn* or *anti* conformation since keto groups occupy both *ortho* positions to the glycosidic nitrogen. Coulombic repulsions between lone pair electrons on the furanose and keto oxygens again suggest a preferred conformation at $\phi_{CN} = \pm 90°$. The different site of base-sugar attachment requires a permutation of each vicinal chromophoric bond pair in the computational procedure. An altogether unique R–ϕ_{CN} plot results (see insert of Figure 4), but a negative sign is predicted at both $\phi_{CN} = \pm 90°$. The calculated rotational strength given in Table II is within a factor of 2 of experiment.

β-Pseudouridine (**9**) also exhibits a negative B_{2u} Cotton effect as seen in Figure 4. In regard to pseudouridine it is noted that the atoms C_5, C_4, N_3, C_2, N_1, and C_6 occupy the same relative positions to the sugar moiety as N_1, C_2, N_3, C_4, C_5, and C_6, respectively, of the uridine structure. On the other hand, the overall structure of pseudouridine is similar to uridine and the same range of torsion angle is probably preferred. When the correct bond permutations are entered into the computational procedure, a negative Cotton effect is predicted for pseudouridine in the *anti* range, although the predicted magnitude is too large by a factor of 2.

In summary the theoretical results on the 6-methyl and 6-aza analogs, on N^3-ribosides and pseudouridine predict the effect of altering the disposition of the vicinal chromophoric bond pairs on the B_{2u} Cotton effect.

(B) Unsaturated Nucleosides and 4′-Thiouridine. Among the uridine derivatives covered in this study are compounds **10–14** which contain thioether and unsaturated functions in the furanose ring. The signed magnitudes of Cotton effects exhibited by **10** and **11**, as displayed in Figure 2 and Figure 5, bear no resemblance to the typical positive, negative, negative, positive pattern of many uridine derivatives.[25] Both conformational and substituent effects are involved here. Models show that almost the entire range of the torsion angle is accessible to the 2′,3′ unsaturated compounds as the hydrogen atom on C-2′ is in the plane of the furanose ring and makes no contact with the base during rotation about the glycosidic bond. However, a known conformation does exist for compound **11** in nonpolar solvents due to hydrogen bond formation. Figure 2 shows a progressive reversal in signs of all four Cotton effects with progressive changes in hydrogen bond donor properties of the solvent. In ether the sign pattern is totally reversed for the B_{2u}, B_{1u}, E_{1ua}, and E_{1ub} bands. In water a negative, positive, negative, positive pattern is observed. These sign reversals are *not* observed for compound **12**, which also contains the 2′,3′ double bond but lacks a hydrogen bond donor substituent on the 5′ carbon. In both ether and water the CD curves of **12** are superim-

posable as suggested by the molecular ellipticities tabulated in Table II. Models show that hydrogen bonding interactions of the keto oxygen with the 5′-OH tend to fix the sugar-base torsion angle at about 150° (the *syn* range). $R_{B_{2u}}$ calculated for this conformation is in good agreement with the observed $R_{B_{2u}}$ in ether. Calculations suggest a conformation in the −70 to −150° range exists in water. Electronic repulsion involving the lone-pair electrons on the keto function and the π electrons of the double bond would favor such a conformation for **12** in both solvents and for **11** in water. The hydrogen bond energy for **11** could offset the repulsion forces in nonpolar solvents.

Positive B_{2u} Cotton effects are exhibited by compounds **13** and **14** which have C_4'–C_5' double bonds. The CD curve of **14** in ether–pentane–alcohol mixture is given in Figure 2. The C-5′ nucleus and its substituents are the only sugar atoms with coordinates significantly different from the corresponding atoms of uridine. The steric and other factors which determine that uridine is *anti* are probably still present in these compounds. It is found that the double bonds greatly alter the rotational strength-torsion angle diagrams relative to uridine but that for compounds **13** and **14** positive B_{2u} Cotton effects are still predicted for conformation in the *anti* range.

The sulfur atom of 4′-thiouridine accentuates the steric and polarizability capabilities of the furanose ring. Steric considerations alone suggest that the conformation is still *anti* but ranges on the low side of −30° are less likely because of the steric effects of the larger sulfur atom. Figure 5 shows both experimental and theoretical results on 4′-thiouridine. Good agreement is attained between theory and experiment when the theoretical values are averaged over the −30 to −80° range. The nature of the R–ϕ_{CN} curve of Figure 5 and the steric effects of the sulfur suggest that the B_{2u} Cotton effects should increase in absolute magnitude with increasing temperature. The positive and small negative contributing ranges of the torsion angle are prohibited by steric interactions of sulfur and the C-6 hydrogen. The large negative contributing ranges are more or less acessible as the torsional oscillations are increasing by raising the temperature. The temperature study included in Figure 5 shows that a larger negative molecular ellipticity is observed for the B_{2u} band at higher temperatures.

(C) Configuration at Asymmetric Centers of the Sugars. The circular dichroism of compounds **1–4** and **15–27** shows the relationship of positive Cotton effect centered at around 270 mμ with *R* chirality at C-1′ and negative Cotton effect with *S* chirality at C-1′ (with essentially constant *anti* conformation). The relationship holds regardless of substitution of methyl, hydroxyl, chloro, etc., for hydrogens at centers more remote than C-1′ in the sugar ring. The correlation of absolute stereochemistry at asymmetric sites of the sugar with circular dichroism can be expected to be useful only if all examples possess roughly the same relative populations of rotational conformations about the bond from C-1′ to the chromophore. In addition the specific sugar conformations must not be grossly different. It is worthwhile to assume initially that substituents do not alter conformations significantly and see how far one can go in correlating experiment with theory. We begin by considering the contributions of each vicinal bond to the total rotational strength of the B_{2u} transition of uridine. Theoretical values are averaged over the *anti* range as discussed in section II.C and they are tabulated in Table III. These values are applicable in near quantitative

Table III. Bond Contributions for Common Sugar Substituents in *anti* Conformation[a]

Substituent	$R_{B_{2u}}$	Substituent	$R_{B_{2u}}$
C_1'–C_2'	4.0	O_1'–C_1'	0.1
C_2'–C_3'	1.1	C_2'–O_2' (*cis*)	1.3
C_3'–C_4'	0.1	C_3'–CH_3' (*cis*)	2.5
C_4'–C_5' (*cis*)[b]	0.7	C_2'–O′ (*trans*)	−0.8
C_4'–C_5' (*trans*)	−0.3	C_3'–O′ (*cis*)	−0.6
C_4'–O_1'	−0.7	C_3'–O_2' (*trans*)	0.04
		C_5'–O_5'	0.01

[a] Calculation based on crystal structure data of ref 26. [b] The term *cis* means the substituent is on the same side of the sugar ring as the base residue. The term *trans* has the opposite meaning.

fashion to cytidine 3′-*endo* and thymine riboside. It is noted that for 1-(β-D-ribofuranosyl)uracil (**1**), which has *R* chirality at C-1′, the furanose ring proper makes the major contribution of 4.6×10^{-40} cgs unit to the rotational strength. The smaller contributions of most exocylic ring substituents indicate that configuration at other sugar sites will have only a modifying effect on the rotation unless substituents introduce steric effects or have intense absorption bands in the near-uv. An example of sign reversal is given by 4′-thiouridine (**10**) discussed in the preceding section. Nucleosides with *S* configuration, of course, would acquire a -4.6×10^{-40} cgs contribution from the five furanose bonds. The above expectations are well established experimentally. Circular dichroism and ORD data on anomeric nucleosides at C-1′ show positive and negative signs for *R* and *S* configuration, respectively with near equality in absolute magnitudes.

Configuration at C-2′ profoundly affects the magnitude of the B_{2u} Cotton effect but not the sign. The absolute magnitude of B_{2u} Cotton effects of 1′,2′-*cis*-pyrimidine nucleosides are always larger by a factor of 2 or more than those of 1′,2′-*trans* anomers. Figure 6 compares the CD curves of several 2′-*cis* derivatives, *i.e.*, 1-(β-D-arabinofuranosyl)uracil (**15**) and 2′-C-methyluridine (**16**) with uridine and 5′,6-anhydro-2′,3′-O-isopropylidene-6-hydroxyuridine (**3**). One may consult Table II to compare the Cotton effects of uridine with those of the 2′-*trans* derivatives, 2′-deoxyuridine and 2′-chloro-2′-deoxyuridine. As seen from Table III the 2′-*cis* contribution has the same sign as the total contribution of the furanose ring proper, whereas the 2′-*trans* contribution has the opposite sign. Approximately a 50% increase in absolute magnitude is predicted for a *trans* → *cis* exchange of hydroxyl for hydrogen. Thus our theory accounts for about ½ of the experimental increment. Our calculations are for the 3′-*endo* conformation. However, in analogy with the nmr results of Walton, *et al.*,[33] on 2′-C-methyladenosines, the conformation is very likely 3′-*endo*-2′-*exo*. Coordinates for this conformation may improve our results although models do indicate that the interconversion from 3′-*endo* to 3′-*endo*-2′-*exo* involves but a slight twist

(33) E. Walton, S. P. Jenkins, R. F. Nutt, F. W. Holly, and M. Nemes, *J. Med. Chem.*, 12, 306 (1969).

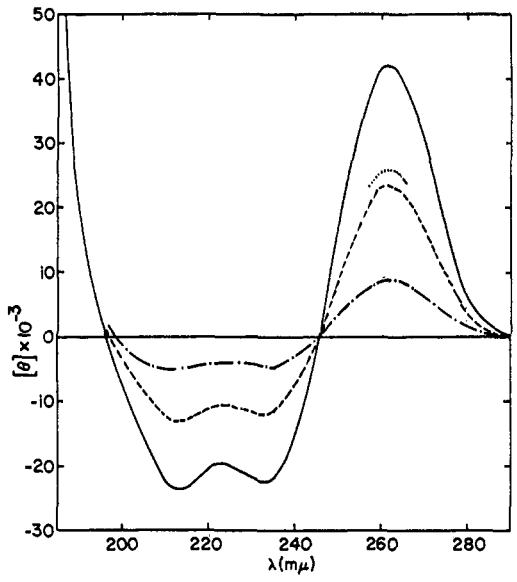

Figure 6. The circular dichroism spectra of 5′,6-anhydro-2′,3′-O-isopropylidene-6-hydroxyuridine (**3**) (——), 1-β-D-arabinofuranosyluracil (**15**) (----), and uridine (·····) all in water at pH 7. In addition the molecular ellipticity of 2′-C-methyluridine as estimated from ORD data is given by the dotted line.

of the C_2'–C_3' bond. *cis*-Oriented substituents at C-2′ may also sterically interact with the pyrimidine ring with the possibility of narrowing or altering the allowed torsion angle range and/or building up the population of the *anti* range at the expense of the *syn* range. We intend to return to this problem in more detail after computations of one-electron effects now in progress in this laboratory are completed. For the present the twofold increment is attributed in about equal amounts to the new steric and coupling potentials introduced by the *cis* substituent. It is significant to note that, while the B_{2u} Cotton effect of the O^6-5′-cyclouridine derivative **3** exceeds that of uridine by a factor of 4.5, nearly 80% of this increment on a relative basis is accounted for by theory.

trans substituents at C-2′ are predicted to contribute negatively to the B_{2u} Cotton effect according to Table III. In agreement with this expectation is the smaller B_{2u} Cotton effect exhibited by 2′-chloro-2′-deoxyuridine relative to uridine. However, 2′-deoxyuridine has a significantly smaller B_{2u} Cotton effect than uridine, despite the abstraction of the negative contribution. As far as steric effects on the torsion angle, it makes no difference whether the sugar is ribose or deoxyribose when the conformation is 3′-*endo*. No close contacts are made by O-2′ during rotation. The puckering, however, of the furanose ring presumably results from torsional forces tending to relieve the short contacts between the substituents attached to adjacent carbon atoms. Deoxyuridine lacks adjacent hydroxyls and probably does not maintain the 3′-*endo* conformation. Our theoretical results are compatible with a change in puckered conformation to the 2′-*endo* or a more nearly planar ribose ring (see Figure 3). In Table II we compared our calculations on 2′-deoxyuridine for both puckered conformation. Good agreement relative to the reference compound uridine is achieved for the 2′-*endo* conformation.

Configuration at C-3′ is considered next. Theory predicts according to Table III a modest decrease in the absolute magnitude for 1′,3′-*cis* nucleosides relative to their 1′,3′-*trans* isomers. Approximately a 10% decrease is expected for a *trans* → *cis* exchange involving hydroxyl and hydrogen and a 20% decrease for methyl–hydrogen exchange. Decreases of 15 and 45% are observed for **17** and **18** relative to **15** and **2**, respectively. Similarly a decrease is noted for 1-(α-L-ribofuranosyl)-thymine 5′-phosphate relative to 1-(α-L-xylofuranosyl)-uracil.[32] Incidently the experimental CD measurements on ***19*** and ***20*** agree with the expectations of Table III that 3′- and 5′-hydroxy substituents have negligible effects on B_{2u} Cotton effects. Similarly, concerning the negligible contribution predicted for 5′ substituents (assuming that crystal conformation is retained in solution), the B_{2u} bands of uridine, 5′-deoxyuridine, 2′,3′-di-O-acetyl-5′-deoxy-5′-iodouridine, and 2′,3′-di-O-acetyluridine are all nearly superimposable. The presence or absence of a 3′-acetyl (see data on 3′-O-acetylthymidine and thymidine in Table II) or a 5′-phosphate group (**25**) also has little noticeable effect on the B_{2u} Cotton effect.

Experimental data available on several *S*-configuration nucleosides show that an increase instead of the predicted decrease results for the *trans* → *cis* exchange of hydroxyl for hydrogen. For example, the Cotton effect exhibited for **22** is larger by about 20% in a negative sense than the corresponding Cotton effect measured for **21**. The discrepancy may indicate that slightly different conformations about the glycosidic bond are adopted for *S* nucleosides relative to *R* nucleosides. Moreover the crystal structure data on *R*-configuration nucleosides may not adequately describe the *S*-configuration nucleosides (for which there are no X-ray data available). More data on C-1′ anomers are needed before firm conclusions may be drawn.

Changes in configuration at C-4′ significantly affect the magnitude of the Cotton effect. Theory predicts a substantial increase in absolute magnitude for 1′,4′-*cis* nucleosides relative to their 1′,4′-*trans* isomers. By referring to the CD data on the 4′ epimers, **23** and **24** or **1** and **25**, or **26** and **27**, one may note the experimental verification of this prediction for compounds with ribose or lyxose sugars. However, 1-(β-D-arabinofuranosyl)-thymine (*cis*) is reported[32] to give a smaller B_{2u} ORD amplitude than 1-(α-L-xylofuranosyl)thymine (*trans*). In ribose or lyxose sugars the 2′- and 3′-hydroxy functions are *cis* to one another, whereas in arabinose and xylose they are *trans*. Specific effects on ribose conformation involving adjacent hydroxyls may be involved here.

The results of Table III are intended to apply to uridine derivatives (excluding 6-methyl, 6-aza, and other 6-substituted analogs) which contain the exocyclic sugar substituents listed in the table. For more facile comparison one could double the bond contributions of Table III since the computed values are often too small by a factor of 2. At present, however, the experimental–theoretical correlations justify only a qualitative use of the results of Table III. On a qualitative basis the bond contributions of Table III are applicable to cytidine and to most 5-substituted analogs of uridine and cytidine. The results can be applied in approximate fashion to other sugar substituents by comparing values of

$$\frac{\nu_b \mu_{jb}^2}{\nu_b^2 - \nu_a^2}$$

for the substituent and the reference substituent of Table III. For example, a C_3'–S (*cis*) bond is predicted to contribute substantially in a negative sense to the rotation (about eight times more than the -0.55×10^{-40}-cgs contribution of the C_3'–O (*cis*) bond). Hence 3′-thioxylofuranosyluracil is predicted to exhibit a much smaller positive B_{2u} Cotton effect than 1-(β-D-xylofuranosyl)uracil. On the other hand 3′-mercapto-3′-deoxyuridine should give a B_{2u} Cotton effect only slightly larger than the corresponding Cotton effect of uridine since eight times the contribution of a C_3'–O *trans* bond, *i.e.*, $8 \times 0.04 \times 10^{-40}$, still does not add an appreciable amount of the total B_{2u} rotation. Similarly 2′-thioarabinofuranosyluracil is expected to have a very large positive B_{2u} Cotton effect where as the 2′-*trans* isomer may well have a negative B_{2u} Cotton effect. Thus the configuration at C-2′ and C-3′ for a sulfur substituent should be rather easily determined by CD measurements.

In summary, the coupled oscillator term in the general expression for the rotational strength describes on a relative basis most of the changes in the B_{2u} Cotton effect due to changes in the nature and geometry of the vicinal oscillators. The agreement is indeed very good for *R*-configuration nucleosides for which X-ray data are available, but several exceptions are noted for *S*-configuration nucleosides, which may serve to indicate that the sugar-base conformation or the specific sugar conformation is influenced by the C-1′ configuration. Although there are a few instances where an individual vicinal bond has shown a divergent contribution from experimental data, this has never been sufficiently pronounced to lead to a wrong assignment of the absolute configuration. In the three cases where the theoretical vicinal contribution of one bond differed in sign from the experimental value, the exact configuration is problematical due to lack of X-ray data on these structures.

Inclusion of contributions from the other terms to the rotational strength may improve the calculations but, on a practical basis, the results of this paper show that many problems regarding nucleoside stereochemistry may be solved by appealing solely to the bond–bond coupled oscillator theory. The success of the theoretical calculations reported here depend on giving up the Kirkwood–Tinoco method where the base chromophore is considered as a single oscillator. In the bond–bond coupled oscillator theory each transition of the base chromophore is resolved into contributions from the individual bonds. Thus the geometry of the chromophore and its disposition relative to the vicinal oscillators are introduced into the calculations. This procedure is essential for the success of the theory. Several previously published methods were tried without notable success, including the method in which the transition of the base chromophore is resolved into transition monopoles to calculate the potential.[10]

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Spin Label Studies of Oriented Smectic Liquid Crystals[1] (A Model System for Bilayer Membranes)

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Abstract: Smectic liquid crystals with bilayer structure can be homogeneously oriented between two parallel glass surfaces. Spin labels dissolved in this system are found to undergo a rapid, anisotropic motion. The esr spectrum depends on the orientation of the sample with respect to the applied magnetic field. The formulas necessary for a quantitative evaluation of the spin label spectra are derived. Amphiphilic spin labels and steroid spin labels are used to investigate the different regions of the bilayer. The amphiphilic spin labels indicate an exponential decrease in the degree of order, when the distance between the carboxyl group and the spin label group is increased. In spite of this flexibility, the amphiphilic materials of the bilayer are found to be in an almost extended configuration. The behavior of the spin labels in phospholipid dispersions is very similar to that in the liquid crystalline model system.

In recent years the spin labeling technique has been introduced as a method in the elucidation of the structure of biological membranes.[3] Progress in this field has been made by the synthesis of spin labels which show a rapid and highly anisotropic motion in biological membrane systems.[4,5] It has been suggested that the anisotropic motion of these labels can be attributed to their incorporation into a bilayer structure.

Here we wish to report spin label experiments in

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