chloric acid precipitated the phosphinic acids, $(C_6H_{11})_2P(O)OH$, mp 140–141° [lit.[31] 140–141.5°], and $(C_6H_5)_2P(O)OH$, mp 192–194° [lit.[32] 193–195°]. The lithium salts were precipitated by the dropwise addition of *n*-BuLi in hexane to the acids in petroleum ether and benzene, respectively. The ir spectra were the same as before.

The fluorescence wavelengths of the precipitated salts, together with those of the phosphinates obtained by the slow oxidation of solid $(sec\text{-}C_4H_9)_2PLi$ and $(t\text{-}C_4H_9)_2PLi$, as above, are given in Table II. The butylphosphinates also showed strong bands in the region of 8.80 and 9.50 μ characteristic of the phosphinate structure.

Lithium Salts of Dicyclohexyl- and Diphenylphosphine Oxides. Dicyclohexylphosphine oxide, mp 74–76° [lit.[33] 76–77°], was prepared by air oxidation of the phosphine, by a procedure similar to that described in the literature,[11] and was isolated in anhydrous form by distillation at 142–143° (0.2 mm) through a 13-cm Vigreux column. Its ir spectrum showed principal peaks at 4.40 (ms), 7.56 (m), 7.74 (m), 7.87 (m), 8.27 (ms), 8.45–8.52–8.58 (s, triplet), 8.95 (m), 9.29 (m), 9.59 (m), 10.00 (m), 10.58 (s), 10.69 (m), 10.95–11.02 (w, doublet), 11.35 (m), 11.85 (m), 12.20 (m), 13.33 (m), 13.65 μ (w). Diphenylphosphine oxide, mp 51–53° [lit.[11,32] 53–56°; lit.[34] 51–53°], was prepared from diethyl phosphite and phenylmagnesium bromide as described in the literature.[32] The ir spectrum was essentially the same as the published spectrum.[32]

The lithium salts were precipitated by the dropwise addition of *n*-BuLi in hexane to the phosphine oxides in oxygen-free petroleum ether. The products were collected on a filter and washed with petroleum ether. Chemiluminescence and fluorescence data are given in Table II.[35] The ir spectrum of the cyclohexyl derivative contained principal peaks at 7.75 (m), 7.89 (m), 8.50 (m), 9.04 (m), 10.04 (ms), 10.45 (ms), 11.33 (vs), 11.77 (s), 12.30 (m), 13.40 (m), 13.85 μ (m). The phenyl derivative showed principal peaks at 6.30 (w), 6.97 (ms), 9.15 (m), 9.38 (w), 9.78 (w), 10.04 (w), 10.54 (m), 10.90 (s), 13.42 (ms), 13.60 (ms), 14.41 μ (s).

Slow oxidation of the solid lithium salts of the oxides under nitrogen containing traces of oxygen produced the phosphinates as confirmed by ir spectra identical with those obtained upon slow oxidation of the corresponding phosphides, above.

Silver Salt of Diphenylphosphine Oxide. Diphenylphosphine oxide was prepared as above. Formation of the salt with silver nitrate in 60% ethanol according to the published procedure[14] yielded a hydrate which was dried overnight at 70° *in vacuo*. The ir spectrum of the anhydrous salt showed principal peaks at 6.29 (w), 6.95 (ms), 9.08 (m), 9.37 (w), 9.75 (w), 10.03 (w), 10.55 (s), 13.42 (m), 13.57 (ms), 14.42 μ (s). Taken on a KBr pellet, the ir spectrum contained principal peaks at 3.35 (w), 6.80 (w), 7.01 (ms), 7.28 (w), 8.95 (w), 9.15 (m), 10.62 (s), 13.62 (ms), 14.51 μ (s).

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(35) On generating the lithium salt of dicyclohexylphosphine oxide in ethyl ether, the salt chemiluminesced at 490 mμ instead of 460 mμ, probably due to solvation.

Theoretical Interpretation of the Circular Dichroism of Adenine Nucleosides[1]

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Abstract: We present calculations of the rotational strength of α- and β-deoxyadenosine as a function of sugar-base torsion angle. Our method does not use perturbation theory, but calculates the rotational strength directly from extended Hückel wave functions for the entire nucleoside. Our calculated results show n–π* transitions to have rotational strengths comparable in magnitude with those of π–π* transitions. The geometric feature most influential in determining Cotton effects is the sugar–base torsion angle; sugar puckering and furanose substituents are less important. Comparison of our results with those of coupled oscillator theories indicates that one-electron effects and effects of n–π* transitions are not negligible. For adenine nucleosides, we find agreement with recent experimental data for the four longest wavelength CD bands (270–225 nm) if we assume the nucleosides to be in the anti conformation with the torsion angle near 0°. Departures from enantiomeric behavior of α and β anomers are ascribed to deviations from mirror image relations in the sugar–base torsion angle. We predict adenine nucleosides in syn conformations to have circular dichroism curves quite different from that of deoxyadenosine. Cotton effects arising from interaction of the sugar with the base are large enough to be significant in polynucleotides for certain values of the torsion angle such as that in B form DNA.

The optical activity of polynucleotides is reasonably well understood, at least on a qualitative theoretical basis, and a number of useful empirical techniques have been developed based on this understanding.[2a] Unfortunately, no comparable theoretical basis has yet been developed for mononucleosides. Some empirical rules relating nucleoside optical activity to conformation have been proposed but they have limited applicability.[2a]

The reason for our poorer understanding of the optical activity of mononucleosides over that of their polymers lies in the different nature of the perturbation giving rise to the Cotton effects in the two cases. In oligonucleotides and polymers it is the interaction of similar chromophores (base–base interaction) which dominates the CD spectrum, while in mononucleosides it is the weak perturbation of the nonchromophoric sugar on the nucleoside base which gives rise to the observed Cotton effects.

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(1) Research supported by National Science Foundation Grant No. GP-20053.

(2) (a) C. A. Bush in "Basic Principles of Nucleic Acid Chemistry," P. O. P. Ts'o, Ed., Academic Press, New York, N. Y., in press; (b) D. W. Miles, W. H. Inskeep, M. J. Robbins, M. W. Winkley, R. K. Robbins, and H. Eyring, *J. Amer. Chem. Soc.*, **92**, 3872 (1970).

Recently Miles, *et al.*,[2b] used a coupled oscillator technique to calculate the $\pi-\pi^*$ Cotton effect induced in pyrimidine nucleosides. The far-ultraviolet transitions in the sugar were approximated by bond polarizabilities. The $\pi-\pi^*$ transitions of the base were distributed among bond-centered oscillators whose magnitudes were calculated in a transition gradient formalism. The results of this calculation were in reasonable agreement with experiment for a number of pyrimidine nucleosides. This agreement was taken as evidence for the validity of the assumption that one-electron Cotton effects and $n-\pi^*$ transitions could be ignored.

In a theoretical treatment similar to that of Miles, *et al.*,[2b] Teng, *et al.*,[3] calculated rotational strengths for two $\pi-\pi^*$ transitions in both purine nucleosides and pyrimidine nucleosides. Their method treats the $\pi-\pi^*$ transition as a point dipole rather than as a distribution of dipoles at the bonds as was done by Miles, *et al.*[2b] Nevertheless, the results of these two calculations are similar for pyrimidine nucleosides and in agreement with experiment. The results of Teng, *et al.*,[3] on purines are not in as good agreement with experiment as are their results for pyrimidines.

Even if the Kirkwood polarizability term was calculated correctly, a possible criticism of the two theories described above is their neglect of $n-\pi^*$ transitions in the bases. It is probable that Cotton effects of $n-\pi^*$ transitions are not negligible in polynucleotide optical activity.[4] Moreover, a recent series of experiments studying the anomeric pairs of the D-pentafuranosides of adenine suggests that six transitions can be found between 280 and 196 nm.[5] It seems quite likely that some of these bands are not $\pi-\pi^*$ transitions and that attempts at theoretical interpretation of these new and detailed data should include $n-\pi^*$ transitions.

Although one can incorporate $n-\pi^*$ Cotton effects into existing calculations based on perturbation theory, we will take a different approach in this work. We do not use perturbation theory at all, but calculate the rotational strength by brute force methods from a semiempirical wave function for the entire nucleoside.[6] Since this wave function has both σ and π electrons, it will include both $n-\pi^*$ and $\pi-\pi^*$ transitions.

Description of the Method

A. Optical Activity Calculation. The Rosenfeld equation tells us that if we know the complete wave function of a molecule in its ground (o) and excited (a) states, we may calculate the rotational strength for the transition between them. $\boldsymbol{\mu}_{oa}$ is the electric transi-

$$R_{oa} = Im(\mathbf{m}_{ao} \cdot \boldsymbol{\mu}_{oa}) \qquad (1)$$

tion dipole moment and $\mathbf{m}_{oa}$ is the magnetic transition dipole moment. Direct calculations of the rotational strength using eq 1 are rare due to the difficulty of obtaining wave functions for large, unsymmetrical molecules. We use the recently developed method of Gould and Hoffmann,[6] in which extended Hückel wave functions are constructed for the molecule of interest and the operators in eq 1 are calculated for a single configuration transition, o to a. The transition moment, $\boldsymbol{\mu}_{oa}$, is calculated in a gradient formalism in the method of Gould and Hoffmann.[6] The electric dipole or position method is equivalent to the gradient (transition momentum) for exact wave functions, but they may differ for approximate functions as we will see below.

A major strength of this direct method is that it calculates the exact rotational strength for the wave functions under consideration. The method has two major weaknesses. First, the semiempirical wave functions are probably poor approximations of the true wave functions of large molecules. Moreover, we have no effective way of measuring the extent of this deficiency. Second, since the method does not employ perturbation theory, it is difficult to assign a physical interpretation to the calculated Cotton effects. One can discern $n-\pi^*$ from $\pi-\pi^*$ transitions in the nucleoside, but it is difficult to say whether the Cotton effects arise from coupling or static field effects or which geometrical features of the perturbing sugars are most important in inducing them.

Gould and Hoffmann[6] have tested their method on the $n-\pi^*$ transitions of ketones and on 1,3-dienes. Their results on the coupled chromophore case of 1,3-dienes were confusing, but their results on ketones were reasonably encouraging. They were able to calculate the correct sign for the Cotton effects and the magnitudes were within a factor of two to four. The ketone case approximately corresponds to our nucleoside case except that we will calculate both $\pi-\pi^*$ and $n-\pi^*$ rotational strengths.

B. Geometry of the Nucleoside. All the calculations we describe here are for deoxyadenosine. There are two reasons for choosing this compound over adenosine. First, it is smaller (85 orbitals) and, second, there are steric interactions of the 2′-hydroxyl of α-adenosine with the purine base for certain values of the torsion angle, ϕ_{CN}. Steric contacts of the sugar with the aromatic base cause difficulties in the calculation (see below).

In order to be able to modify the deoxyribose geometry and the sugar–base torsion angle ϕ_{CN} easily, we introduce the geometry in terms of bond distances, bond angles, and dihedral angles. The bond angles and bond distances used in all cases are those found in 5′-AMP.[7] The dihedral angles for the nucleoside having 3′-endo puckering were those of 5′-AMP which have been calculated from the data of ref 7 by Sundaralingam.[8] For the sugar in the 2′-endo puckering, we used the dihedral angles calculated by Sundaralingam for 6-thiopurine riboside.[8] In certain cases we modified dihedral angles defining exocyclic atoms from those found in the crystal in order to avoid steric contacts. Such changes do not greatly modify calculated rotational strengths.

In crystals such as that of 5′-AMP, some deviations from planarity of the aromatic base occur and in addition the anomeric carbon, C-1′, is not in the base plane.[7] These deviations introduce chirality into the chromophore which leads to erratic results in our calculation of the rotational strength. Therefore, we assume that these deviations are not present in solution, and that C-1′ is in the base plane. For this geometry, our definition of the sugar–base torsion angle (ϕ_{CN}) as the

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(3) N. H. Teng, M. S. Itzkowitz, and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, 93, 6257 (1971).

(4) C. A. Bush, *J. Chem. Phys.*, **53**, 3522 (1970).

(5) J. S. Ingwall, *J. Amer. Chem. Soc.*, **94**, 5487 (1972).

(6) R. R. Gould and R. Hoffmann, *ibid.*, 92, 1813 (1970).

(7) J. Kraut and L. H. Jensen, *Acta Crystallogr.*, 16, 79 (1963).

(8) M. Sundaralingam, *Biopolymers*, 7, 821 (1969).

C-8–N-9–C-1′–O-1′ dihedral angle is equivalent to other definitions based on average planes.[9]

The extended Hückel parameters in our calculation are the conventional ones used by Hoffmann[10] in azines. The Slater exponent for hydrogen is 1.3.

Results for Deoxyadenosine

A. Ultraviolet Transitions. We give the energies for four calculated transitions in deoxyadenosine in Table I. Since the sugar is a modest perturbation of the chromophoric base, we see general correspondence between the highest filled and lowest empty molecular orbitals given in Table I and the results of a similar calculation on methyladenine.[4] The magnitudes and directions of the calculated transition moments differ in the two calculations since the present method uses transition gradients while the earlier work used point monopoles in a position dipole calculation. These two methods are equivalent for exact wave functions; the observed differences arise from the well-known inequivalence of the two methods for approximate functions.[11]

Table I. Calculated Transitions and Experimental Assignments

Transition	Energy, eV	μ_{calcd}, D	Polarization,[a] deg	Assignment, nm
$n-\pi_1^*$	3.37	0.49	Z	270
$\pi-\pi_1^*$	3.17	3.48	65	260
$\pi-\pi_2^*$	3.56	2.68	−6	250
$n-\pi_2^*$	3.76	0.78	Z	225

[a] Polarization relative to line from N-1 to C-2. Positive angle measured toward N-3. This polarization angle is similar to the convention of DeVoe and Tinoco (H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, 4, 500 (1962)).

A lower nonbonding orbital (called σ_2 by Bush[4]) does not appear as a pure chromophore orbital in these calculations. It is mixed with several orbitals which extend over the sugar. The perturbing effect of the sugar in reducing the symmetry of the molecule is evident in the delocalization of most of the lower energy orbitals, spreading them out over the sugar as well as the base. Among higher excited orbitals, on the other hand, some are found to be localized π^* orbitals on the base.

We also indicate in Table I an assignment of these calculated transitions to the lowest four bands resolved in CD by Ingwall.[5] There are two obvious difficulties in this assignment. First, the calculation indicates that the lowest energy transition is a $\pi-\pi^*$, while experiments indicate that the lowest energy transition at 270 nm is most likely an $n-\pi^*$ transition. Thus, our assignment reverses the energy order from that calculated. Secondly, the polarization of the longest wavelength $\pi-\pi^*$ transition (260 nm) is thought to be close to 0° not 65° as we calculated.[12] In spite of these possible objections, the assignment of Table I will allow us to make a reasonable interpretation of the CD of adenosine pentafuranosides. The fact that all three of the longest wavelength CD bands have the same sign of the rotational strength makes this interpretation possible (see below).

B. Conformational Energies. Extended Hückel calculations for deoxyadenosine in a number of conformations provide the conformational energy as a function of sugar–base torsion angle. In principle these energies could be used to make conformational maps, indicating energy minima.[13] In fact, there are several reasons for our choosing not to represent these energies as a conformational map.

We find that steric contacts closer than the closest extreme values given by Ramachandran[14] tend to cause excessive mixing of the sugar and base molecular orbitals, destroying the simple classification of the chromophore σ and π orbitals. The transitions from these orbitals have extremely high rotational strengths and we consider them to be computational artifacts. Therefore, for each value of ϕ_{CN}, we take only sugar conformations not having extreme contacts. In order to save computer time, we do a simple hard sphere steric test on each conformation before carrying out the molecular orbital calculation. It is obvious from the conformational calculations that changes of the sugar puckering from 2′-endo to 3′-endo change the allowed values of ϕ_{CN}.[9–15] A survey of crystallographic data shows that it is necessary to change from 3′-endo puckering to 2′-endo when rotating the torsion angle into the syn range.[16]

We also allowed the glycosyl bond angle (C-8–N-9–C-1′) to deviate to 120° from 128° (its value in 5′-AMP) in order to relieve steric contacts for some torsion angles. As a result of these changes in geometry, our energies as a function of ϕ_{CN} are somewhat erratic. In general we found much lower barriers than those of Jordan and Pullman[13] who used a fixed 3′-endo conformation. We found 2′-endo conformations to be generally lower in energy than 3′-endo conformations by about 4 kcal/mol. The lowest energy for 2′-endo was at $\phi_{CN} = 120°$, and local minima at -50 and $-120°$ were 2.2 and 3.0 kcal higher. The positions of these minima are in approximate agreement with those of a calculation of nonbonded interactions.[15]

For α anomers, we again find 2′-endo conformations to have generally lower energies. We find a broad minimum at $\phi_{CN} = -20$ to $+10°$ and another minimum of similar energy for $\phi_{CN} = 180°$. These results differ from those calculated for α ribonucleosides since the presence of the 2′-OH makes a substantial difference in the case of the α-anomeric nucleosides.[15b]

C. Rotational Strengths. Since one of the major motivations for this study was to determine the dependence of optical activity on nucleoside conformation, we report the rotational strengths of the four electronic transitions as a function of torsion angle, ϕ_{CN}. The results for β-deoxyadenosine are given in Figures 1 and 2, while those for the α anomer are given in Figures 3 and 4. For certain torsion angles, calculations could be carried out for both 2′- and 3′-endo sugars. The figures indicate that the rotational strengths do not depend strongly on sugar puckering. Neither

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(9) A. E. V. Haschemeyer and A. Rich, *J. Mol. Biol.*, 27, 369 (1967).

(10) R. Hoffmann, *J. Chem. Phys.*, 40, 2745 (1964).

(11) A. E. Hansen, *Mol. Phys.*, 13, 425 (1967).

(12) R. F. Stewart and L. H. Jensen, *J. Chem. Phys.*, 40, 2071 (1964).

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(14) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, 23, 283 (1968).

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(16) S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, 92, 4963 (1970).

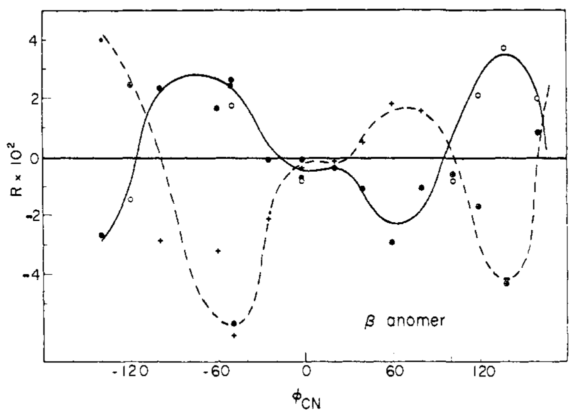

Figure 1. Rotational strength of β-deoxyadenosine in Debye–Bohr magnetons as a function of sugar–base torsion angle for the two long-wavelength transitions: (- - - -) n–π_1^*; (——) π–π_1^*. Points are calculated for n–π_1^* with the sugar in 3′-endo puckering (+) and 2′-endo puckering (⊕) and for the π–π_1^* with the sugar in 3′-endo puckering (●) and for 2′-endo puckering (○).

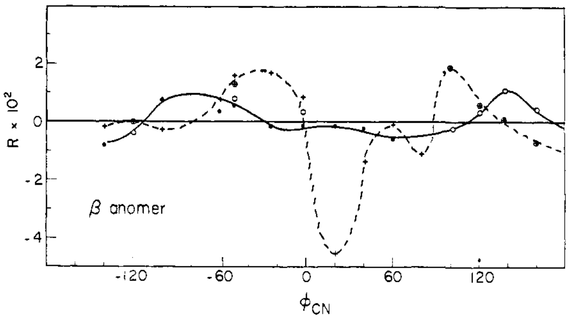

Figure 2. Rotational strength of β-deoxyadenosine in Debye–Bohr magnetons as a function of sugar–base torsion angle for the two short-wavelength transitions: (- - - -) n–π_2^*; (——) π–π_2^*. Points are calculated for n–π_2^* with the sugar in 3′-endo puckering (+) and 2′-endo puckering (⊕), and for the π–π_2^* with the sugar in 3′-endo puckering (●) and for 2′-endo puckering (○).

do the rotational strengths depend strongly on rotation about exocyclic bonds such as C-4′–C-5′ as long as the rotations do not cause steric contacts. The plots of rotational strength *vs.* ϕ_{CN} are not nearly as smooth as those found by perturbation theory.[2,3] It is difficult to draw a line showing the dependence of rotational strength on torsion angle and the lines in Figures 1–4 should be taken as approximate. Nevertheless, our results indicate that the rotational strength depends strongly on the torsion angle, ϕ_{CN}.

One rather surprising result of these calculations is that the rotational strengths of the n–π^* transitions are at least as large as those of the π–π^* transitions. This fact suggests that neglect of n–π^* transitions in the perturbation theory treatments may not be justified.[2,3]

Figures 1 and 3 show that for certain regions of the ϕ_{CN} range, the n–π_1^* and π–π_1^* tend to appear in signed pairs. On the other hand, one does not usually observe signed pair Cotton effects in the 260–270-nm region in adenine nucleosides.[5] This observation could be interpreted as either an indication that this theory is incorrect or that values of the torsion angle associated with the signed pair effect do not occur in adenine nucleosides in solution. Another possibility is that adenine nucleosides in solution exist in an equilibrium mixture of conformations.

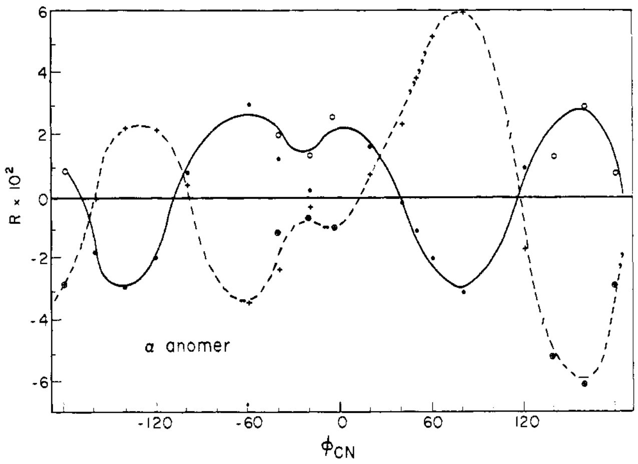

Figure 3. Rotational strength of α-deoxyadenosine in Debye–Bohr magnetons as a function of sugar–base torsion angle for the two long-wavelength transitions: (- - - -) n–π_1^*; (——) π–π_1^*. Points are calculated for n–π_1^* with the sugar in 3′-endo puckering (+) and 2′-endo puckering (⊕) and for the π–π_1^* with the sugar in 3′-endo puckering (●) and for 2′-endo puckering (○).

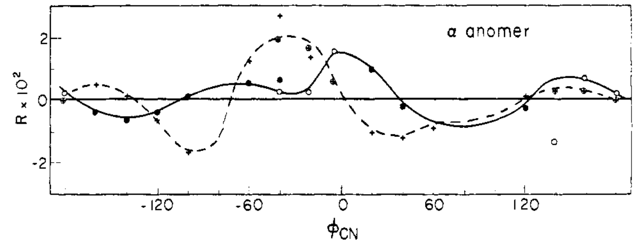

Figure 4. Rotational strength of α-deoxyadenosine in Debye–Bohr magnetons as a function of sugar–base torsion angle for the two short-wavelength transitions: (- - - -) n–π_2^*; (——) π–π_2^*. Points are calculated for n–π_2^* with the sugar in 3′-endo puckering (+) and 2-endo puckering (⊕) and for π–π_2^* with the sugar in 3′-endo puckering (●) and for 2′-endo puckering (○).

Discussion

A. Origin of Cotton Effects. In spite of the fact, mentioned above, that physical interpretation is not one of the main virtues of the direct method for calculating rotational strengths, nevertheless we will attempt to relate our results to more common perturbation theory studies on the same systems. Two extreme points of view have been expressed on the perturbation theory calculation of Cotton effects in aromatic chromophores perturbed by an asymmetric substituent. The first point of view is expressed by Deutsche, *et al.*,[17] who take the one-electron approach. They point out that only by mixing of π–π^* states with n–π^* states can optical activity be induced in the aromatic chromophore absorption bands. The second point of view ignores the n–π^* transitions completely and calculates the coupled oscillator effects between π–π^* transitions of the aromatic ring and the far-uv transitions of the asymmetric perturber.[2,3] The partial success of the coupled oscillator method on nucleosides indicates that it cannot be completely incorrect. Yet our results can be interpreted as evidence that this approach is not completely adequate and that neither a purely one-electron nor a purely coupled oscillator point of view is appropriate for nucleosides.

(17) C. W. Deutsche, D. A. Lightner, R. W. Woody, and A. Moscowitz, *Annu. Rev. Phys. Chem.*, 20, 407 (1969).

B. Comparison of Calculated and Experimental Rotational Strengths. The order of magnitude of our calculated rotational strengths is in quite good agreement with experiment. The experimental values of the absolute value of rotational strength range from 0.004 to 0.03 DBM.[5] The calculation yields some values as high as 0.06 DBM, but it is probable that those values of ϕ_{CN} are either unlikely or averaged over several conformations.

In general agreement with the experimental observation that purine β-nucleosides have negative Cotton effects in the 270–250-nm range, we calculate all three of the long wavelength bands to be negative for torsion angles of -25 to $+25°$ which is close to the preferred anti range of ϕ_{CN}. It is also convenient that since all three bands are negative, these conclusions do not rest on the detailed assignment of transitions outlined in Table I above.

If we therefore accept the electronic assignments of Table I, we can compare our calculations with the data of Ingwall.[5] In addition to the small negative Cotton effects in the 270–250-nm region, ara-, lyxo-, and xyloadenosine have negative bands at 225 nm while riboadenosine has a positive band at that wavelength. One can see from Figures 1 and 2 that we get agreement if we assume that ϕ_{CN} is in the anti region and ranges from -20 to $+10°$. The three long-wavelength bands are calculated to be negative in that range and the rotational strength of n–π_2* is a strong function of ϕ_{CN}, passing through zero near 0°. For riboadenosine, we estimate ϕ_{CN} to be -10 to $-20°$ and for ara-, lyxo-, and xyloadenosine, we estimate a small positive torsion angle. A torsion angle of $+10°$ would not be unreasonable as may be seen in the nonbonded energy plots of Wilson and Rahman.[15a] For deoxyadenosine they find broad energy minima for torsion angles between -50 and $+20°$.

In the above discussion, we have assumed that the molecular framework of deoxyadenosine is a good model for adenine attached to several different sugars. This comparison is valid if the hydroxyl groups influence the optical activity mainly by sterically determining ϕ_{CN} rather than by direct optical perturbation. Figures 1–4 indicate that sugar puckering is not crucial in determining Cotton effects. In addition, we have done some calculations in which only exocyclic bonds were rotated which indicate minimal influence of substituents. But our strongest argument for the predominating influence of the furanose ring comes from comparison of our results for α and β anomers.

C. α and β Anomers. α and β anomeric nucleosides are often discussed as though they were optical enantiomers. In fact they are nearly true mirror images. If, instead of ribofuranose, the nucleoside contained tetrahydrofuran, the only surviving asymmetry is at C-1′ and the anomers become true enantiomers. Departure from enantiomeric behavior of α and β pairs indicates the influence of the furanose hydroxyl groups.

There are two ways in which the 2′- and 3′-hydroxyls and the 4′-CH_2OH can influence the optical activity of nucleosides. First, these groups may influence the conformation, modifying the puckering of the sugar and the torsion angle. Second, the hydroxyl groups could act as direct optical perturbations. We may show this second effect to be small by examining Figures 1–4.

If one ignores the 3′ and 4′ substituents of deoxyadenosine, the α anomer becomes the exact enantiomer of the β anomer having ϕ_{CN} of the opposite sign. We may compare Figures 1 and 3 for the long-wavelength transitions of the α and β anomers and Figures 2 and 4 for the short-wavelength transitions by a 180° rotation of the figures about the origin. This procedure, which reverses the sign of both ϕ_{CN} and of the rotational strengths, demonstrates the approximate validity of considering the α and β anomers as enantiomers in the optical sense.

The tendency of α-anomeric nucleosides in the solid state to have torsion angles approximately opposite in sign to those of β nucleosides has been pointed out by Sundaralingam.[18] The quasienantiomeric behavior of α and β anomers in optical activity has been pointed out by Ulbricht[19,20] and is also clearly recognizable in the data of Ingwall[5] in the wavelength region longer than 240 nm. However, Ingwall's data for the 225-nm band and the shorter wavelength band do not always follow enantiomeric relations. For example, the 225-nm band and the shorter wavelength bands of both α- and β-adenosine are negative. We can easily understand the departure of α- and β-adenosine from enantiomeric behavior in the 225-nm band by noting the strong dependence of the rotational strength of n–π* (225 nm) on torsion angle in the region near 0° (see Figures 2 and 4). Our interpretation of these deviations from the enantiomeric behavior of α and β anomers is that, as a result of steric effects of the 2′-hydroxyl as well as furanose pucker, ϕ_{CN} is not exactly opposite in sign in the α and β anomers such as xyloadenosine and adenosine. We conclude that while the α and β anomers are approximate enantiomers in the optical sense, their conformations may deviate from mirror images. Therefore, one may observe departures from enantiomeric CD curves.

D. Comparison of These Results with Those of π–π* Coupled Oscillator Theories. The direct method for calculating rotational strengths differs substantially from the perturbation theories used by Miles, *et al.*,[2] and Teng, *et al.*[3] Moreover, these two coupled oscillator theories differ in that the former, as does our present study, uses a gradient formalism for the transition moments while the latter uses a point transition dipole calculated from monopoles. Yet in spite of their respective deficiencies outlined above, all three theories give rotational strengths for nucleosides which are in general agreement. Furthermore, our results agree with Miles, *et al.*,[2] that the position of the furanose ring is the main factor influencing the rotational strength. The substituents and sugar puckering exert their influence mainly by determining the sugar–base torsion angle, ϕ_{CN}.

For the purposes of empirical correlation of the rotational strengths with torsion angle, it is important to know into how many sectors the ϕ_{CN} curve is divided by sign changes in the rotational strength. One can see that Figures 1–4 exhibit a quadrant rule for the rotational strengths predicted by the present theory for deoxyadenosine. In contrast, the perturbation theory

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of Miles, *et al.*,[2] predicts a simple bisector rule for pyrimidines. It is not clear that this is a fundamental disagreement between perturbation theory and the direct method used in the present study; certain of the curves calculated by Miles, *et al.*,[21] for guanosine as well as certain of the results of Teng, *et al.*,[3] show quadrant behavior.

The good agreement with experiment found for pyrimidines by Miles, *et al.*,[2] might seem surprising in light of our result that n–π* transitions and one-electron effects are not negligible. In fact, Miles, *et al.*,[2] chose uracil nucleosides as a test case for the coupled oscillator theory because a simple consideration of their electronic structure would indicate that n–π* transitions might not be important. We interpret the good agreement with experiment found by Miles and Eyring[2] as an indication that n–π* transitions are less important in pyrimidines than in purines, at least in the 260-nm spectral region.

In the case of purine nucleosides, the agreement of coupled oscillator theory with experiment is not as clear. For adenosine, Teng, *et al.*,[3] find agreement with experiment for anti conformations having ϕ_{CN} between −10 and −90°. On the other hand, the resolution of experimental CD spectra by Ingwall[5] shows that there are several bands in the near-ultraviolet CD of adenosine derivatives which are likely to be of n–π* origin. Moreover, Teng, *et al.*,[3] do not find agreement with experiment for the case of guanosine. Indeed, it is just adenine and guanine that show most pronounced n–π* bands in polymer CD as well. n–π* bands seem to be present in the 270–290-nm region for poly A and poly G.[4] Also, the π–π* calculation of Johnson and Tinoco[22] for dinucleosides generally does not show good agreement with experiment for dinucleosides containing guanine. One might summarize this situation by saying that π–π* theories agree with experiment for pyrimidines and not so well for the case of purines, especially guanine.

E. Syn and Anti Nucleosides. Clearly one of the major objectives of this study must be to relate the CD to the conformational feature most important in determining it, the torsion angle ϕ_{CN}. This angle is conventionally discussed in terms of the allowed ranges called syn and anti. These conformations can be quantitatively assigned from examination of crystal structures. The anti form ranges from +10 to −90° and syn may be either 140 or −120°.[16]

The CD of all the adenosine pentafuranosides we have discussed above could be conveniently correlated with theory for ϕ_{CN} in the anti range, near 0°. For nucleosides in syn conformations, examination of Figures 1–4 will show that the predicted CD should be quite different. Large differences between syn and anti conformation for adenosine are also predicted by the calculation of Teng, *et al.*[3] Similarly, for pyrimidines, Miles, *et al.*,[2] calculate differences in sign of the Cotton effects for syn and anti conformations in agreement with experiment on pyrimidine nucleosides.

It is difficult to verify these expectations about differences in CD between *syn*- and *anti*-adenosine nucleosides. The crystal structure of 3′-*O*-acetyladenosine is syn with $\phi_{CN} = 140°$.[16] Yet the CD bands in the 260-nm region are all negative as in adenosine.[23] In the crystal of 3′–5′ cyclic AMP, two values of ϕ_{CN} are found. One is syn (102°) and the other is anti (−50°). Yet the optical activity again shows negative bands in the 260-nm region.[24] Infrared studies indicate 2′–3′-isopropylideneadenosine is syn in nonpolar solvents due to internal hydrogen bonds.[25] The optical activity shows this compound also to have negative CD bands in the 260-nm region in aqueous solution.[24] In spite of the crystallographic and infrared evidence, we conclude that under the conditions of the CD measurement, all three nucleosides are in an anti conformation. Apparently these three compounds have syn conformations in crystals and nonpolar solvents as a result of hydrogen bonds which are not formed in water solution.

F. Application to Polynucleotides, RNA, and DNA. Teng, *et al.*,[3] point out that the B form of DNA has $\phi_{CN} = -86°$ which is somewhat different from that in most free nucleosides. In the A form of DNA, which is similar to RNA, the value (−14°) is not so different from the value we assign to β-adenosine nucleosides in solution. Examination of Figures 1 and 2 reveals that for $\phi_{CN} = -86°$, we would predict Cotton effects for adenosine in B-form DNA which would be rather different from those in the free nucleoside adenosine.

In a commonly used empirical calculation of polynucleotide Cotton effects, one sums the monomer curve plus base–base interactions as reflected in dinucleosides.[26,27] Our results indicate that for adenosine this procedure may be adequate for RNA geometry but is less likely to be correct for DNA geometry where the same method has also been applied.[28]

We might also ask if the interaction of the sugar with the base could be contributing Cotton effects in synthetic polynucleotides. The rotational strengths observed in nucleosides, especially adenosine, are substantially smaller than those observed in their polymers. Typically, the adenosine effects have rotational strengths of −0.01 DBM while most polymer rotational strengths are at least an order of magnitude larger. On the other hand, for $\phi_{CN} = -86°$, we predict contributions of the order of 0.05 DBM. Cotton effects of this magnitude could be observed, for example, in the 280-nm region of poly dA ($R_{280} = 0.013$ DBM) and possibly even in poly rA ($R_{280} = 0.09$ DBM).[29] The effects we calculate here for n–π* transitions are quite comparable to polymer n–π* transitions calculated previously in this laboratory.[4]

Acknowledgments. The author wishes to thank Dr. Roald Hoffmann for sending the extended Hückel and rotational strength programs and also extends thanks to Dr. Joanne Ingwall for data prior to publication, for helpful discussions, and for assistance in preparing the manuscript.

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