orbitals at nitrogen to be more important in F_3NO than in $(CH_3)_3NO$.

Finally it may be noted that this viewpoint is not fundamentally different from the alternative[4,20] that ascribes the stability of F_3NO, and the differing properties of NO bonds in F_3NO and in the organoamine oxides, to the double bonding resulting from the back-donation of lone pairs on oxygen into antibonding σ orbitals associated with the NF bonds. The relation between these bonding models follows since the antibonding σ orbitals would presumably be expected to be associated with substantial 3d orbital contributions, and, as we noted earlier, the 3d orbitals with π symmetry with respect to the NO bond have appreciable σ character in the NF bonds.

Acknowledgments. We thank the National Research Council of Canada for financial grants, Dr. W. B. Fox of Allied Chemical Corporation for the generous gift of a sample of F_3NO, Professor K. Hedberg for communicating his electron diffraction results, Dr. R. C. Catton for his help with the computations, and Professor C. A. McDowell for his interest in this work.

Circular Dichroism of Nucleoside Derivatives. X. Influence of Solvents and Substituents upon the Cotton Effects of Guanosine Derivatives

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Abstract: The effects of certain substituents and solvents on the circular dichroism (CD) spectra are reported for a number of guanine nucleoside derivatives from 320 to 200 nm. Both theoretical and empirical analyses of the data suggest that the anti conformation predominates in aqueous solution, but that the syn conformation is preferred in alcoholic solvents, at low pH in water, and when the heterocycle carries a large substituent on carbon 8 of the imidazole ring. Theoretical optical calculations based on the bond–bond coupled oscillator theory are included to check the validity of the theory with experimental data. The interaction of guanine nucleoside derivatives with actinomycin is also reported and the data suggest that the anti conformation is necessary for complex formation.

A fundamental aspect of the conformational analyses of nucleic acids and their fragments concerns the allowed conformational states of the individual nucleotide or nucleoside monomers. In particular the relative position of the sugar and the base about the glycosidic bond, as described by the torsion angle, ϕ_{CN}, has received a great deal of attention.[1-4] Steric considerations lead to two extreme conformations as demonstrated by **1** and **5** in Figure 1, which are designated anti and syn, respectively. Crystallographic studies have pointed to the almost exclusive presence of the anti conformation.[5] A number of exceptions, however, are known: deoxyguanosine, which is syn in a mixed crystal with 5-bromodeoxycytidine;[6] 3′,5′ cyclic AMP, which can exist simultaneously in the anti and syn conformations in the same crystal structure;[7] 8-bromoguanosine;[8] 8-bromoadenosine;[8] and 3′-*O*-acetyladenosine.[9] Several independent calculations using the criteria of potential energy conclude that in purine nucleosides the free energy difference between the syn and anti states is small.[10-12] Finally, several nmr studies have implicated the anti conformation as predominating in solutions.[13,14]

We now report the effects of substituents, pH, and solvent on the CD spectra of certain guanosine derivatives. Despite measurements on numerous derivatives under diverse conditions, much of the data can be understood in terms of two basic spectra which may approximate the characteristic spectra of a pure anti conformational distribution and a pure syn conformational distribution. The circular dichroism curve of a guanosine derivative having a conformational distribution entirely in the syn range is likely quite well represented by the CD spectra of the six derivatives featuring a bulky 8 substituent. To provide a guide for interpretation of the data in terms of molecular con-

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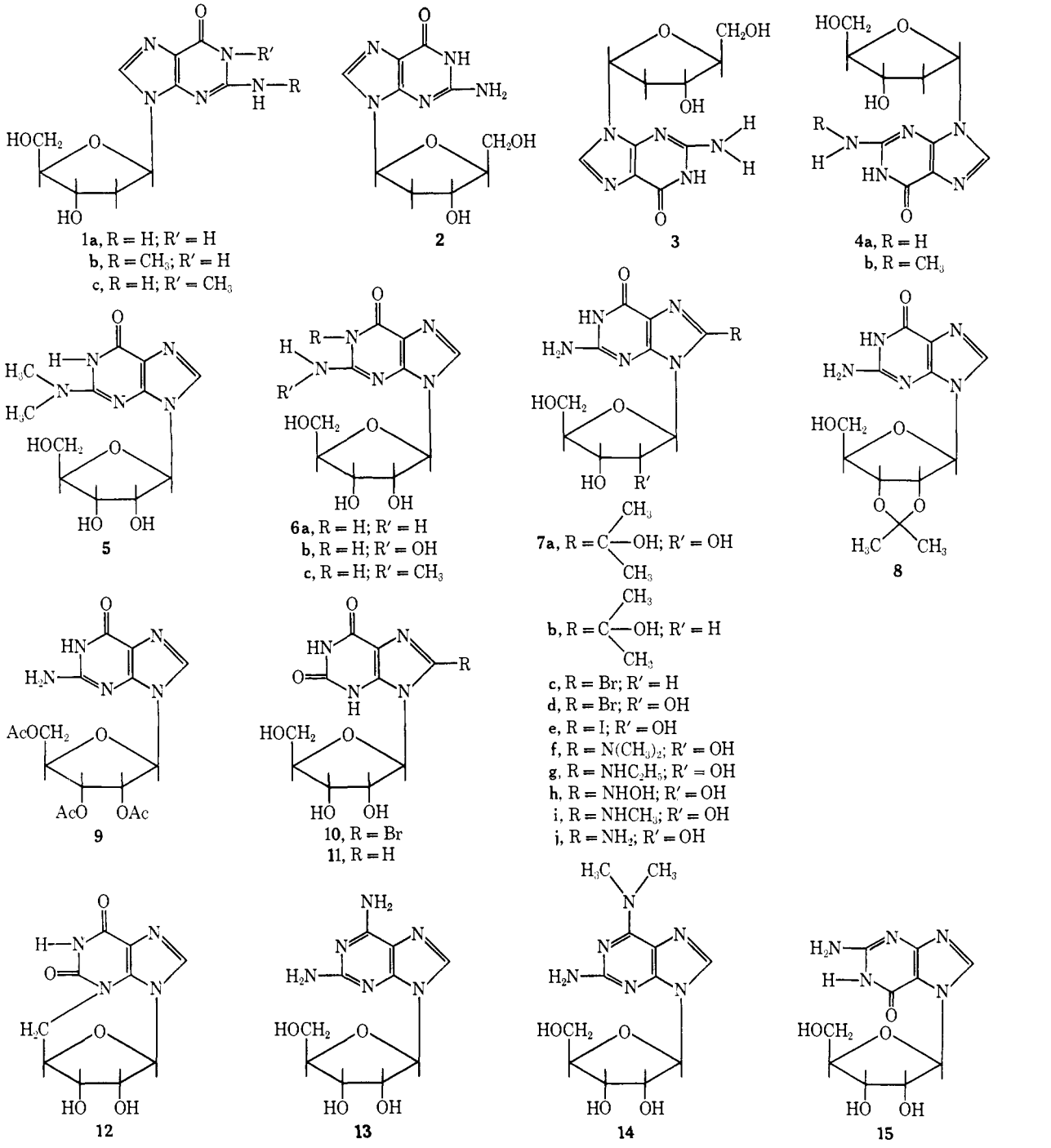

Figure 1. Structural formulas for the molecules included in this study. Compound **1** is drawn with the base in the anti conformation. All other compounds are drawn in the syn conformation in order to conserve space. Numbering system used in referring to substituents and atoms is indicated. Source of compounds: **1a–4b**, ref 20; **6a**, **8**, **9**, and **11**, Sigma Chemical Co.; **7a** and **7b**, H. Steinmaus, I. Rosenthal, and D. Elad, *J. Amer. Chem. Soc.*, **91**, 4921 (1969); **5** and **6c**, J. F. Gerster and R. K. Robins, *ibid.*, **87**, 3752 (1965); **12**, R. E. Holmes and R. K. Robins, *J. Org. Chem.*, **28**, 3483 (1963); **7d**, R. A. Long, R. K. Robins, and L. B. Townsend, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N. Y., 1968, p 228; **7c** and **7e–j**, R. A. Long, R. K. Robins, and L. B. Townsend, *J. Org. Chem.*, **32**, 2751 (1967), and references cited therein; **14**, J. F. Gerster, B. C. Hinshaw, R. K. Robins, and L. B. Townsend, *ibid.*, **33**, 1070 (1968); and **15**, R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, **90**, 2661 (1968).

formation, we have joined our bond–bond coupled oscillator theory[15–17] with Lakshminarayanan and Sasisekharan's calculation of conceivable conformations.[12] All experimental data and theoretical results considered together support our opinion that the anti conformation predominates in aqueous solution, but that the equilibrium may shift to favor the syn conformation under certain conditions. Finally, a brief summary of our study involving the interactions of these nucleosides with actinomycin D is included. Only nucleosides that give "anti"-like CD curves are found to

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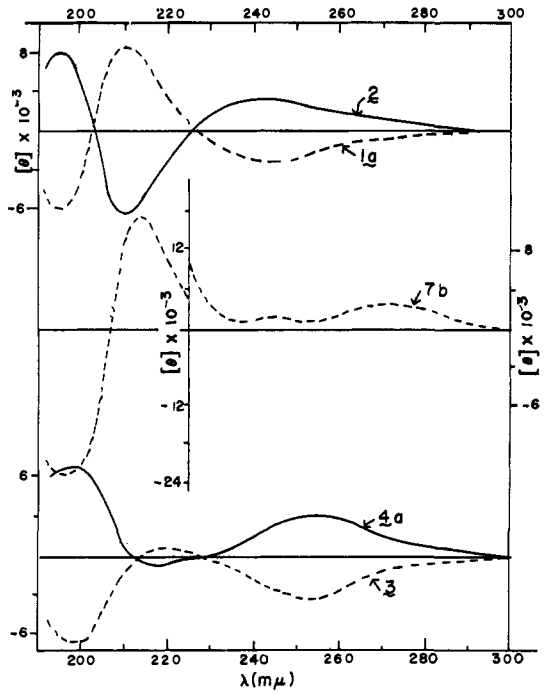

Figure 2. (Top) Circular dichroism spectra of 2′-deoxyguanosine (**1a**) and 2-amino-9-(2-deoxy-β-L-*erythro*-pentofuranosyl)purin-6-one (**2**) at pH 7. (Middle) Circular dichroism spectrum of 8-(2-hydroxy-2-isopropyl)-2′-deoxyguanosine (**7b**) at pH 7. (Bottom) Circular dichroism spectra of 2-amino-9-(2-deoxy-α-L-*erythro*-pentofuranosyl)purin-6-one (**3**) and 2-amino-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purin-6-one (**4a**) at pH 7.

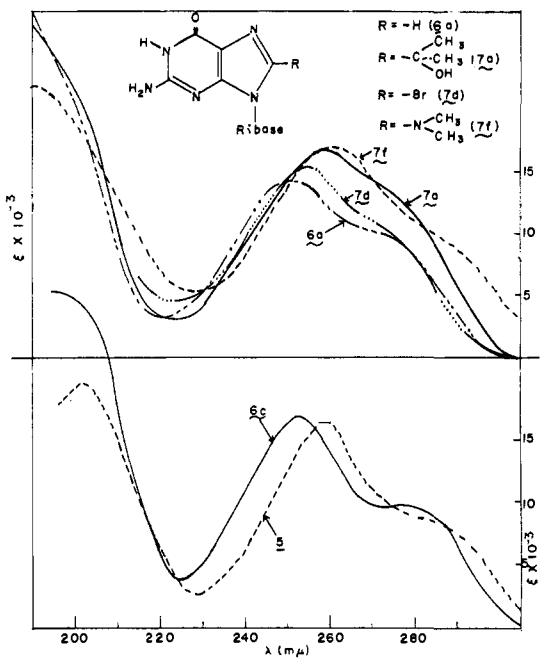

Figure 3. The absorption spectra at pH 7 of guanosine (**6a**), 8-bromoguanosine (**7d**), 8-dimethylaminoguanosine (**7f**), 8-(2-hydroxy-2-isopropyl)guanosine (**7a**), 2-dimethylaminoguanosine (**5**), and 2-monomethylaminoguanosine (**6c**).

provoke a characteristic change in the CD spectra of actinomycin.

Experimental Section

MCD and CD measurements were made using the Cary 60 spectropolarimeter at very low scan speeds when necessary. In regions

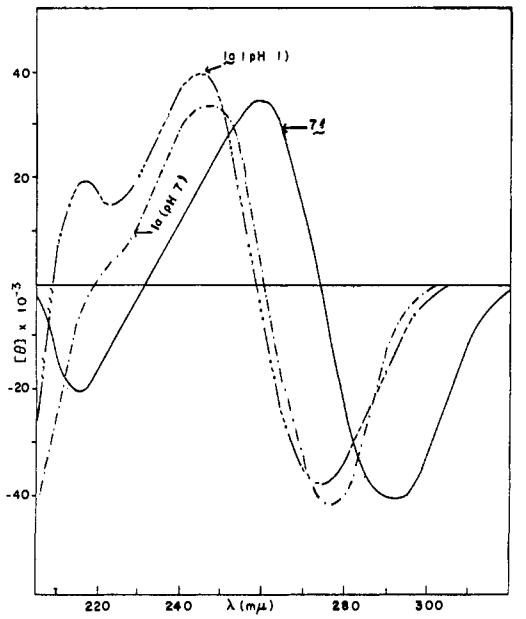

Figure 4. The MCD spectra of 2′-deoxyguanosine (**1a**) at pH 7 and pH 1 and the MCD spectra of 8-dimethylaminoguanosine (**7f**) at pH 7.

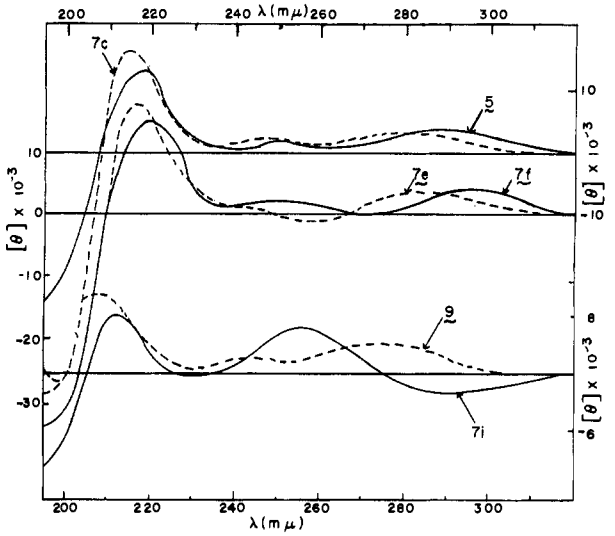

Figure 5. (Top) Circular dichroism spectra of 2-dimethylaminoguanosine (**5**) and 8-bromo-2′-deoxyguanosine (**7c**) at pH 7. (Middle) Circular dichroism spectra of 8-iodoguanosine (**7e**) and 8-dimethylaminoguanosine (**7f**) at pH 7. (Bottom) Circular dichroism spectra of 2′,3′,5′-triacetylguanosine (**9**) and 8-monomethylaminoguanosine (**7i**) at pH 7.

of low signal-to-noise ratio the pen response time was increased to a maximum. Ultraviolet absorption spectra were recorded on a Cary 14 spectrophotometer. All spectroscopic measurements were made at least three times in aqueous solution at optical densities of 1.5, 2.0, and 3.0.

Structures of compounds discussed in the text are given in Figure 1, along with the numbering scheme used throughout the paper. References describing the preparation and characterization of these nucleosides or their commercial sources are given in Figure 1 as well. CD and MCD data have not been tabulated because the figures include all representative curves. Our MCD results are reported as molar ellipticities at 49.5 kG (using the same sign convention adopted by other workers in this field),[18,19] thereby facili-

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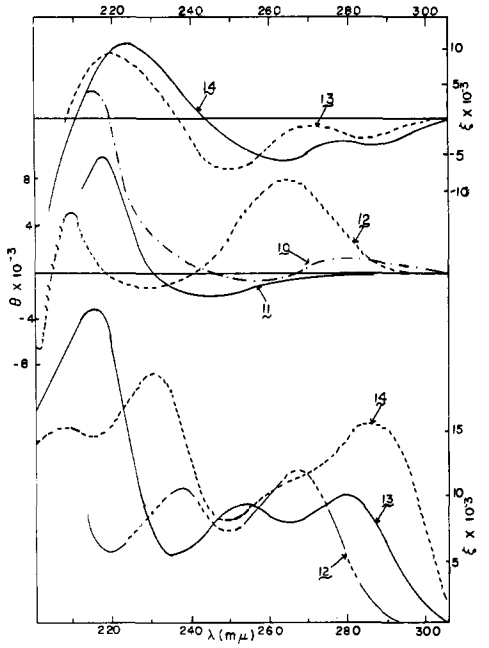

Figure 6. (Top) Circular dichroism spectra of 2,6-diamino-9-(β-D-ribofuranosyl)purine (**13**) and 2-amino-6-dimethylamino-9-(β-D-ribofuranosyl)purine (**14**) at pH 7. (Middle) Circular dichroism spectra of xanthosine (**11**), 3,5′-cycloxanthosine (**12**), and 8-bromoxanthosine (**10**) at pH 7. (Bottom) Absorption spectra of 3,5′-cycloxanthosine (**12**), 2,6-diamino-9-(β-D-ribofuranosyl)purine (**13**), and 2-amino-6-dimethylamino-9-(β-D-ribofuranosyl)purine (**14**) at pH 7.

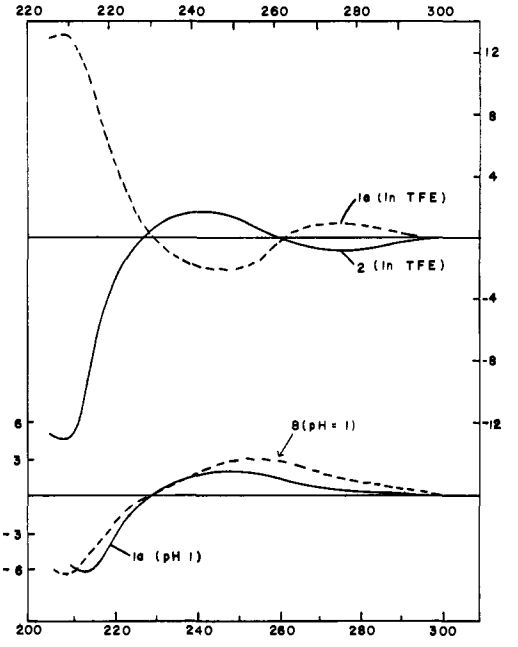

Figure 7. (Top) Circular dichroism spectra of 2′-deoxyguanosine (**1a**) and 2-amino-9-(2-deoxy-β-L-*erythro*-pentofuranosyl)purin-6-one (**2**) in trifluoroethanol (TFE). (Bottom) Circular dichroism spectra of 2′-deoxyguanosine (**1a**) and 2′,3′-*O*-isopropylideneguanosine (**8**) at pH 1.

tating the direct comparison of MCD and CD since both are expressed in the same units. MCD curves have been corrected for natural CD as determined with the same solutions but in the absence of the magnetic field.

Results and Discussion

The representative curves, measured from 190 to 320 mμ, of the guanosine nucleosides are presented in Figures 2–9. CD curves for the protonated forms of the base guanosine are included for some of the molecules. The uv absorption spectra of all compounds considered are strikingly similar except for minor intensity changes and the anticipated red shift induced by ring substituents. In Figure 3 the absorption spectra of typical 8-substituted guanine nucleosides are given to illustrate the type of changes induced by a series of substituents with progressively increasing perturbing powers. A shoulder and two peaks (designated hereafter as B_{2u}, B_{1u}, and E_{1u}) are found in the uv spectra of most molecules included in the present study. The B_{2u} and B_{1u} CD bands (compare Figures 2 and 3, for example) generally match the absorption bands even when measurements are performed in acidic solutions. The correspondence of CD bands with absorption bands and the absence of significant shifts in the CD spectra measured at pH 1 suggest that the B_{2u} and B_{1u} transitions are generally responsible for the Cotton effects observed. Two oppositely signed CD bands span the E_{1u} spectral region—a characteristic feature in the CD spectra of most purine and pyrimidine nucleosides. The MCD spectra of the reference compound **1a** at pH 1 and 7 and its most perturbed derivative, **7f**, are presented in Figure 4. Visual inspection of the spectra shows the remarkable spectral similarities of the perturbed derivative with **1a**. MCD spectra are known to

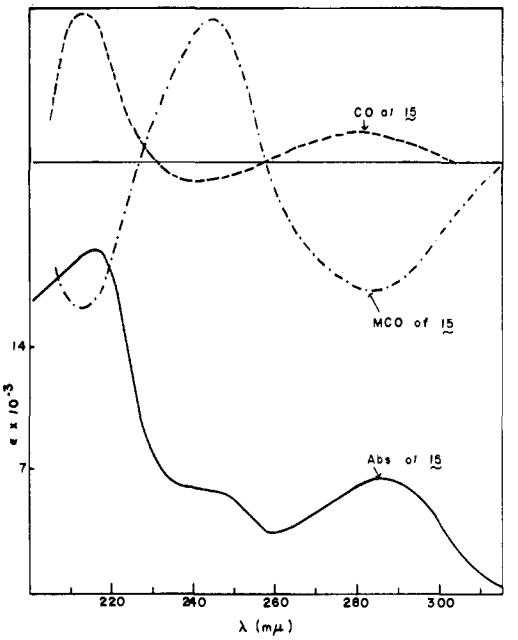

Figure 8. The absorption (———), MCD (-·-·-), and CD (- - - - -) spectra of 7-β-D-ribofuranosylguanine (**15**) at pH 7.

be extremely sensitive to changes in relative polarizations of $\pi-\pi^*$ bands.[19] The similar spectral features of these compounds indicate that polarization directions are not particularly sensitive to substitution at the 8 position of the purine ring. The above statement applies only to substituents included in this study which require no change in the formal structure of the chromophore. With the exception of wavelength shifts, the computed spectral features of all guanosine derivatives included in this study are nearly the same. The computed absolute changes in polarization directions are

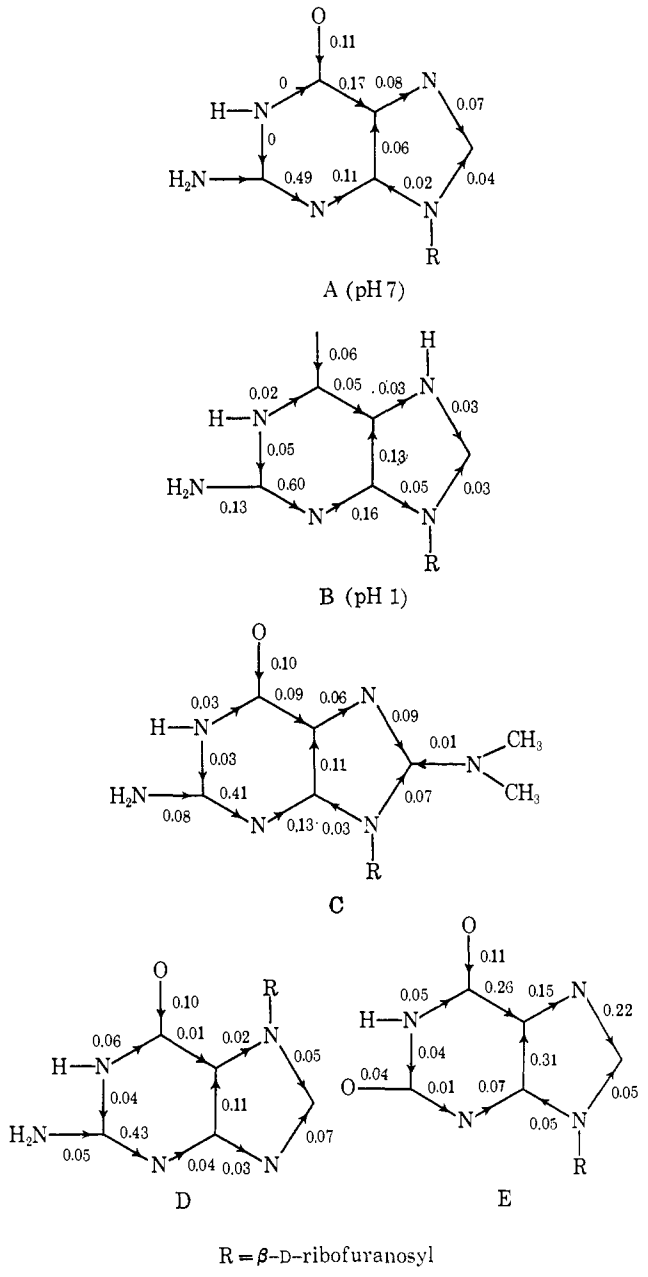

Figure 9. The calculated transitional bond orders (P_{rs}) obtained for guanosine (A), protonated guanosine (B), 8-dimethylaminoguanosine (C), 7-β-D-ribofuranosylguanine (D), and xanthosine (E).

small and not reported in this study; instead we prefer to emphasize the computed changes in the localized nature of the transitions as indicated by the transitional bond orders, P_{rs}, defined in the Theoretical Analysis section.

We turn now to an examination of the CD spectra of guanosine nucleosides. The sign of each Cotton effect in these molecules is controlled by the disposition of the skeletal furanose ring relative to the base which in turn is controlled principally by configuration at C-1′ and the allowed torsion angle range(s). Inspection of Figure 2 shows that all four Cotton effects exhibited by configurationally related 2′-deoxyguanosine (**1a**) and 2-amino-9-(2- deoxy-α- L- *erythro*-pentofuranosyl)purin-6-one (**3**) have the same signs (both have the *R* configuration at C-1′). Magnitude changes and small wavelength displacements are noted below 220 mμ. The wavelength disparities are not serious since the observed and true positions of two closely spaced and oppositely signed CD Cotton effects will be considerably different and a function of the relative intensities of the overlapping bands. Inversion of configuration at C-1′ inverts the signs of the CD bands. Figure 2 shows the CD spectra of the enantiomeric pair, 2′-deoxyguanosine and 2-amino-9-(2-deoxy-β-L-*erythro*-pentofuranosyl)-purin-6-one (**2**). Figure 2 (bottom) gives the CD spectra of the enantiomeric pair **3** and **4a**. The β-(D–L) pair, **1a** and **2**, and the α-(D–L) pair, **3** and **4a**, represent the first purine 2′-deoxynucleoside enantiomorphs available for physical and biological investigation.[20] As expected, the two curves bear a mirror-image relationship to each other at neutral pH, where the predominant conformation is probably anti, and in acidic solution or in isopropyl alcohol, where the predominant conformation is probably syn (*vide infra*). Typical alcoholic and acidic curves are given in Figure 7. Incidentally, the MCD curves of enantiomorphs are identical after the natural CD has been substracted out.

A most interesting effect observed in this study deals with the conformational changes about the glycosidic bond induced by solvent, pH, and substituents. The hypothesis of a simple two-state equilibrium (anti $\rightleftarrows$ syn) was adopted quite early in this work and a number of experiments were designed to test this hypothesis. For example, model building studies using the Kendrew skeletal wire models and the Corey–Pauling–Kolton space-filling models show that close steric contacts are introduced by bulky substituents on carbon 8 which act to exclude the anti conformation. Numerous close contacts are observed when the sugar residue is rotated to the anti conformation, whereas few steric problems exist in the syn conformation. Thus for $\phi_{CN} = -10°$ (the anti range), the $O_1' \cdots Br$ back-contact distance in 8-bromoguanosine (**7d**) is approximately 3.0 Å. This is 0.4 Å closer than the expected van der Waals separation between these atoms and would result in a large repulsive term in the energy potential for this conformation. Similar analysis through the anti range made it appear safe to assume that close steric contacts introduced by bulky substituents such as bromo, dimethylamino, iodo, and certain γ alkyl groups act to exclude the entire anti range. Smaller substituents such as keto, amino, or monomethylamino sterically hinder torsion angle conformations in the lower anti ranges (0 to −30°) but not the higher anti range. Figure 2 shows that the CD spectra of **7b** which contains the bulky 8-isopropyl alcohol substituent is inverted in the B_{2u} spectral region and significantly modified in the E_{1u} spectral region. Compound **7b** and **1a** have identical configurations and nearly superimposable uv absorption and MCD spectra; therefore, changes in conformational equilibrium are implicated.

We adopt the hypothesis that changes in the CD spectra produced by the 8-isopropyl alcohol substituent reflect primarily a change from a predominately anti conformation to a syn conformation. The two-state model has been consistently upheld by calculations of the total potential energy as a function of the torsion angle.[10–12] For our purposes here, the recent work of

(20) M. J. Robins, T. A. Khwaja, and R. K. Robins, *J. Org. Chem.*, **35**, 636 (1970).

Lakshminarayanan and Sasisekharan[12] is particularly relevant since their study included the four major puckered conformations of the ribose ring. The essential utility of their results in the present work is to eliminate from consideration about 75% of the conceivable conformations, leaving the allowed ranges as ϕ_{CN} = 115–145° (syn) and 0 to −60° (anti). Since the conformational energy calculations show that the syn–anti ranges are virtually the only conformational states for guanosine, it seems reasonable to identify the two types of spectra with the syn and anti conformers. The CD spectra of **7b** may be assigned to the "pure" syn conformers on the basis of the model building studies.

The potential energy calculations also predict the syn range to be narrow and deeply grooved. The overall qualitative correctness of Lakshminarayanan and Sasisekharan's results appears to be supported by the similarities discovered in the CD spectra of six guanosine nucleoside derivatives containing bulky 8 substituents, *i.e.*, compounds **7a–f**. Representative curves are displayed in Figure 5 as well as in Figure 2 discussed above. In addition, Figure 5 contains the CD curves for 2-dimethylaminoguanosine (**5**) and 2′,3′,5′-tri-*O*-acetylguanosine (**9**), respectively. The CD spectra of 2′,3′-*O*-isopropylideneguanosine (**8**) is virtually identical with that of compound **9** and is not shown. It is quite significant that **5** (which does not contain a bulky 8 substituent) and compounds **7a–f** all exhibit essentially identical CD spectra (after allowances are made for the known shifts in band position induced by the various substituents). The spectral similarities argue against an equilibrium composition involving the syn range and one or more intermediate ranges. This is because the diverse substituents are expected to produce noticeable shifts in the equilibrium composition, giving a rich variety of magnitudes and even sign changes. Moreover, theoretical optical rotation calculations presented below indicate that the molar ellipticity in the syn range is changing markedly with relatively small changes of the torsion angle. The solvent effect studies presented below support the concept of a single remaining allowed range once the anti range is sterically excluded. The CD spectra of **5** and **7a–f** (and **8** and **9**, which also appear to belong to this basic group) are taken as the characteristic spectra expected for the pure "syn" conformers of β-D-guanine nucleosides and related compounds.

All syn curves exhibit a positive CD band (θ_{max} 3000) in the B_{2u} spectral region, a large positive E_{1ua} band (θ_{max} 15,000–18,000), and a very large negative E_{1ub} CD band. No clear CD maximum is observed in the B_{1u} region where a very small positive or even negative molar ellipticity is measured.

The CD spectra of the pure anti conformer may be close to the spectrum of **1a** and related compounds. We have some preliminary temperature study results which support this assumption. The CD curves of **1b**, **1c**, **6a**, **6b**, **13**, and **14** and many other types of purine nucleosides give CD spectra with the same basic sign pattern observed in the spectrum of **1a**. For example, see the CD spectra of xanthosine (**11**), 2,6,-diaminopurine riboside (**13**), and compound **14** given in Figure 6 and the CD spectra of several purine nucleoside derivatives found in paper VI of this series.[21]

(21) D. W. Miles, M. J. Robins, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U. S.*, **62**, 22 (1969).

Solvent studies are in basic agreement with the two-state model. Owing to the low solubility of guanosine derivatives in organic solvents, only the low molecular weight alcohols are suitable for solvent studies. As in water, where the CD curves of the anti series of guanosine derivatives are quite similar, the CD curves determined in isopropyl alcohol are quite similar but now mimic the syn curves more than the anti curves. On the other hand, the syn series of guanosine derivatives are rather insensitive to solvent changes, although a definite positive CD band is now found in the B_{1u} spectral region. Typical curves in alcohol solvent, which show that the B_{2u} and E_{1ua} bands are decidely more syn-like, are given in Figure 7.

In the absence of overriding hydrogen-bonding effects, the syn conformation may be preferred over anti in guanosine derivatives, as suggested by two independent potential energy calculations.[11,12] The energy released by hydrogen-bond formation at N-3 might, for example, destabilize the otherwise energetically favored syn range. Intermolecular hydrogen bonding at this site is sterically feasible only when the conformation is anti, and even then certain appropriately placed substituents may preclude this association. We suggest the possibility that derivatives capable of accomodating a water molecule at N-3 are predominantly anti in this solvent. In weaker associating solvents, or when steric factors hinder hydrogen-bond formation, the equilibria may shift to favor the syn conformation. Thus in isopropyl alcohol all guanosine derivatives studied give CD curves intermediate between the anti and syn spectra of **1a** and **7a**, respectively. Similarly the syn conformations of **5**, **8**, and **9** arise from the absence of solvent association at N-3. The 2-dimethylamino ring substituent of **5** and the large sugar ring substituents of **8** and **9** appear to block close solvent association at this site.

In summary, the data considered to this point can be represented by the spectra of a syn or an anti conformer or an obvious linear combination of these two basic spectra. The data are certainly most economically understood in terms of a simple two-conformer hypothesis rather than a multiplicity of allowed states undergoing complex population redistribution.

A few anomalous CD curves, however, have been uncovered which do not fit neatly into the two-state hypothesis. The β-D-guanosine derivatives **7g–j** give a third characteristic CD curve that is essentially anti in the B_{2u} and E_{1u} spectral regions but anomalous in the B_{1u} region. A representative CD curve of this type is given in Figure 5 (bottom). It is noted that compounds **7g–j** carry an 8 substituent which sterically prohibits the lower anti ranges but not the higher anti ranges. The molar ellipticity of the B_{1u} band as a function of the torsion angle could have a nodal point within the anti range such that lower anti conformations give negative Cotton effects while higher anti conformations give positive Cotton effects. This interpretation is still basically in agreement with the simple two-range hypothesis provided the anti range is broad and flat and lower in energy than the syn range.

The CD curves in acid do not conform exactly to either the syn or anti CD patterns. The reader may recall that **1a** gave an anti spectrum and **8** gave a syn spectrum at neutral pH but, as shown in Figure 7

(middle), both compounds exhibit quite similar CD behavior in the cationic states. The cationic curves shown in Figure 7 are quite typical of the spectra measured for other β-D derivatives, except for the 8-substituted derivatives, which present decomposition problems before protonation is achieved. The cationic CD curves resemble the syn spectra in the B_{2u} region but give large negative Cotton effects in the E_{1u} region. The E_{1ub} band is positive but its maximum could not be reached with current instrumentation. Thus the E_{1u} CD bands are inverted relative to *either* the anti or syn curve of the uncharged species. Protonation may well promote the syn conformation over the anti, but no compelling evidence can be offered to support this belief. However, it is quite conceivable that protonation could invert the energies of the E_{1u} transitions. In this event the positive sign of the B_{2u} Cotton effect indicates that guanosine is syn in acid solution as protonation does not induce a large chromophoric effect on the low-energy transition. The parameterization of protonated nitrogen has received only scant attention to date from molecular quantum chemists. Preliminary molecular orbital results using the Nishimoto scheme[22] for the protonated nitrogen and adjacent carbon show that while the low-energy transitions are localized essentially in the pyrimidine ring, higher energy transitions are delocalized over both the pyrimidine- and the imidazole-ring subsystems. Hence the higher energy E_{1u} bands are much more sensitive to protonation effects, which could conceivably shift the E_{1ua} band to the blue and the E_{1ub} band to the red (*i.e.*, interchange their respective spectral positions.)

Actinomycin–Nucleoside Complexes

Actinomycin C_1 (AM) forms weak complexes with certain purine nucleosides and nucleotides depending on the electron-donor capacity of the purine base. The electron-donor property of a molecule is the greater the smaller the value of the energy coefficient of its highest filled molecular orbital.[23] Electron-donating ring substituents are found to enhance complex formation, which is indicated in the CD spectrum by the appearance of a positive CD band at 440 mμ.[24] We have routinely measured the CD spectra of AM at 0.3×10^{-4} *M* concentration in saturated aqueous solutions of the derivatives of Figure 1. Only the β-D derivatives such as **1a–c**, **6a–c**, etc., which give anti CD curves in water produce the positive 440-mμ CD band. The β-D derivatives which produce syn CD curves in water do not cause any change in the AM spectrum, even though derivatives such as **7f** are stronger electron donors than the parent compound, guanosine. The data suggest that the anti conformation is necessary for complex formation.

Theoretical Analysis

While sector rules have been proposed to explain the optical activity of aromatic chromophores attached to far-uv absorbing groups, not until recently have computational procedures been attempted. Dipole–dipole coupling between the strong near-uv bands of the chromophore and the strong far-uv bands of the sugar has been demonstrated to produce a significant coupled oscillator contribution to the optical activity of aromatic chromophores. The coupled oscillator expression for the rotational strength, R_{0a}, is

$$R_{0a} = \sum_{j\neq i}\sum_{b\neq a}\frac{2}{ch}\frac{\nu_a\nu_b}{\nu_b^2 - \nu_a^2}V_{i0a;\,j0b}\vec{\mu}_{i0a} \times \vec{\mu}_{j0b}\cdot\vec{R}_{ij} \quad (1)$$

where the $\vec{\mu}$'s are group electric dipole transition moments, the ν's are their frequencies, and $V_{i0a;\,j0b}$ governs their interaction. The subscripts 0, a, and b designate the ground and two excited electronic states, i refers to the chromophore under consideration, and j refers to the vicinal groups. The distance vector, R_{ij}, is measured from group i to group j. In chromophores not fully aromatic the transition 0 → a may be essentially localized in the vicinity of only a few of the chromophoric bonds and the proper location of the vector

$$\vec{\mu}_{i0a} = \frac{\sqrt{2}\beta_m c}{\pi\nu_a}\langle 0|\vec{\nabla}|a\rangle \quad (2)$$

may be better approximated by breaking down this vector into bond transition moments and then utilizing a bond–bond oscillator scheme to get the rotational strength. Thus, if r and s designate the atoms of bond i, then for the transition 0 → a the component of $\vec{\mu}_{i0a}$ along bond i is

$$\vec{\mu}_{ia} = \frac{2\beta_m c}{\pi\nu_a}P_{rs}\vec{\nabla}_{rs} \quad (3)$$

and depends chiefly on the magnitude of $P_{rs} \equiv (C_rC_s^* - C_r^*C_s)$ called the transitional bond order. In the equations and discussion above, the symbol β_m is the Bohr magneton; C_r and C_r^* are the coefficients of the atomic orbital ϕ_r of the nuclear atom r in the molecular orbitals ψ_0 and ψ_a, respectively. The $\vec{\nabla}_{rs}$ integrals, defined and evaluated in ref 16, vary only slightly with the nature of the bond. The final expression for bond–bond rotational strength using the dipole–dipole approximation for the interaction $V_{i0a;\,j0b}$ is

$$R_s = \sum_i\sum_j \frac{2\pi}{hc}\frac{\nu_a\nu_b}{(\nu_b^2 - \nu_a^2)}\mu_{ia}^2\mu_{jb}^2 GF_{ij} \quad (4)$$

$$GF_{is} = \sum\left[\hat{e}_i\cdot\hat{e}_j - \frac{3(\hat{e}_i\cdot\vec{R}_{ij})(\hat{e}_j\cdot\vec{R}_{ij})}{R_{ij}^2}\right]\hat{e}_i \times \hat{e}_j\cdot\vec{R}_{ij}$$

where all symbols are as defined and discussed in ref 16. Equation 4 is only rigorously correct when the transition is localized along a single bond as, for example, is very nearly the case for uracil and guanine. We consider only the B_{2u} transitions, since the MO's are obtained from the Pariser–Parr–Pople program of Adams and Miller, which includes all single-electron excitations in the configuration interaction. The resulting P_{rs} values obtained for guanosine, 8-aminoguanosine, N-7 protonated guanosine, and **15** are presented along the bonds of the appropriate structure given in Figure 9. These results cover the reference compound guanosine, its most extensively perturbed derivatives, and xanthosine. The P_{rs} values enable us to monitor changes in the nature of the B_{2u} transition induced by the substituents. The vicinal group bond effective transition moments were those used in our previous papers.[16,17] The rotational strength calcula-

(22) K. Nishimoto, K. Nakatsukasa, and R. Fujishino, *Theor. Chim. Acta*, **14**, 80 (1969).
(23) B. Pullman, "Molecular Biophysics," Academic Press, New York, N. Y., 1965, p 164.
(24) Y. Courtois, W. Guchlbauer, and P. Fromageo, *FEBS (Fed. Eur. Biochim. Soc.) Lett.*, **3**, 330 (1970).

tions were carried out for conformations throughout the range of the torsion angle by generating conformations from crystallographic data using a digital computer program. The coordinates given by Haschemeyer and Sobell in the deoxyguanosine–bromodeoxycytidine crystalline complex where chosen because deoxyguanosine has the 2′-endo ribose conformation in this crystal.[25] The purine nucleosides favor the 2′-endo ribose conformation in aqueous solution.[26]

The calculated rotational strengths of the B_{2u} band of **1a**, **7f**, and **11** as a function of the torsion angle is presented in Figure 10 in the form of a rotation–conformation (R–C) projection diagram. The diagram includes a Newman projection for handy reference along with the torsion angle ranges of several key compounds (indicated by arrows near the rim). The rotational strength and its sign are represented on the diagram as inward (negative) or outward (positive) projections on the rim. The allowed torsion angle ranges according to Lakshminarayanan and Sasisekharan's potential energy calculation[12] are marked on the diagram by a thick line. We predict guanosine and its derivatives to exhibit a positive Cotton effect when the conformation is predominantly syn and a negative Cotton effect for the anti conformation. Essentially the same R–C projection diagram is calculated for **7f**, the most perturbed 8-substituted derivative included in this study. This guanosine diagram should approximately describe the R–C behavior of all guanosine derivatives with the same Kekulé structure as guanosine. Tautomeric forms of guanosine and derivatives such as 3,5′-cycloguanosine which have different predominating Kekulé structures would only fortuitously conform to the R–C diagram of guanosine. The R–C diagrams of **1a** and **7f** have nodes dividing the circle into positive and negative Cotton effects at about 0 and 180°. Additional tests of the theory involve the xanthosine derivatives **10**, **11**, **12**, and **15**. The generally accepted Kekulé structure of **10–12** fixes the double bonds between C-4 and C-5 and between N-7 and C-8.

Figure 6 contains the CD curve of xanthosine, 8-bromoxanthosine, and cycloxanthosine, while the CD, MCD, and absorption curves of **15** are presented in Figure 9. The CD curves of xanthosine and 8-bromoxanthosine are quite comparable with those of analogous guanosine nucleosides in which the intramolecular interactions of the base with the various substituents of the ribose ring are expected to be similar. Cycloxanthosine is fixed in the syn conformation (ϕ_{CN} = 150°) by a $C_{5'}$–N_3 bond. The cyclicization process does not change the Kekulé structure of the xanthine ring nor do the absorption or MCD spectra deviate appreciably from that of the reference compound, xanthosine. According to the theoretical diagram for xanthosine, *syn*-xanthosine derivatives (**10** and **12**) are predicted to give positive Cotton effects to nearly the same magnitude as the syn guanosine derivatives. Visual inspection of the R–C diagram for xanthosine shows, however, some notable departures from the R–C diagram of guanosine. In

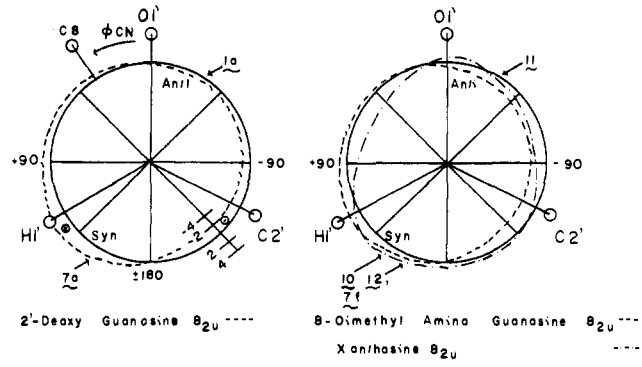

Figure 10. The R–C projection diagrams discussed in the text for the B_{2u} transition of 2′-deoxyguanosine, xanthosine, and 8-dimethylaminoguanosine.

particular, a node occurs in the anti range such that the anti conformation can be associated with either a positive or negative Cotton effect. An extremely small negative Cotton effect is measured in water.

Since the bond transition dipole moment, μ_{ia}, has a quadratic dependency in the rotational strength formula, the P_{rs} values given in Figure 9 should be squared before estimating the contribution of each bond to the rotational strength. Squaring the P_{rs} values listed for guanosine in Figure 9 leads to the suggestion that the B_{2u} Cotton effect arises almost exclusively from the coupling of the N_2–C_3 bond with the far-uv sugar transitions. Moreover, according to our P_{rs} values, the situation is not greatly changed upon portonation at N-7 or substitution at C-8 of the guanine ring. The MO results substantiate the idea that the sign reversals involving the B_{2u} Cotton effect are largely conformation effects and not due to changes in polarization directions of the B_{2u} transition. The P_{rs} values for the ketonic form of xanthine, in contrast to those of guanine, indicate that the B_{2u} transition is localized largely in the C_4–C_5–C_6 submolecular region. (The P_{rs} values for the 2-hydroxy-6-keto tautomer of xanthine are quite similar to those of guanosine. Thus the lactam and lactim forms of xanthosine would both give positive B_{2u} CD bands for syn conformation and probably negative B_{2u} CD bands for anti.)

It is instructive at this point to tabulate the contributions of each chromophoric bond to the rotational strength at intervals in the anti and syn ranges. The rotational strength values given in Table I are derived by assigning each bond a transition moment of 2.3 D and coupling the bond with the effective transitions along sugar ring bonds according to the procedure outlined in our previous papers. One simple use of this table arises from the MO results which suggest that the near-uv transitions of purine bases are localized to a large degree along one or another of the fixed double bonds of the proper Kekulé structure. The potential usefulness of this table can be illustrated by predicting the B_{2u} Cotton effect of **15**. In this compound the sugar ring is attached to N-7 instead of N-9 and the syn range is sterically prohibited by the 6-keto function. The absorption and MCD curves of **15** are somewhat red shifted to those of **1a**, but the general nature of the spectra of **1a** and **15** match quite well. Moreover, the MO treatment of **15** predicts the red shift

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Table I. The Rotational Strength Contributions (When the Sugar-Base Conformation is Syn or Anti, As Indicated) of a Transition (2.3 D in Magnitude) Localized on the Bond Indicated. Calculations Are Made with Eq 4 of Text

	Syn			Anti						
Bond[a]	140°	130°	150°	0°	−10°	−20°	−30°	−40°	−50°	−60°
1–2	1.94	1.26	2.29	0.94	1.00	0.91	0.71	0.46	0.21	0.03
2–3	2.60	2.98	2.30	−1.32	−1.36	−1.42	−1.45	−1.44	−1.40	−1.33
3–4	−1.67	−1.31	−2.35	10.16	9.60	8.58	7.28	5.79	4.20	2.26
4–5	6.11	5.24	3.26	−3.25	−2.89	−2.61	−2.37	−2.16	−1.96	−1.73
5–6	−0.25	−0.26	−0.52	2.27	1.98	1.62	1.20	0.73	0.22	−0.30
6–1	−0.23	−0.20	−0.30	2.60	2.70	2.61	2.37	2.00	1.54	1.01
2–11	−1.83	−0.44	−2.40	1.62	1.54	1.45	1.37	1.17	0.97	0.71
6–10	1.18	0.70	1.62	−1.08	−1.00	−0.92	−0.92	−0.74	−0.64	−0.50
5–7	1.12	−0.09	2.19	2.91	3.26	3.25	2.90	2.23	1.33	0.28
7–8	−2.88	−2.74	−2.91	5.78	5.81	5.47	4.74	3.65	2.25	0.65
8–9	11.58	8.94	12.76	−12.05	−10.77	−9.41	−8.11	−6.96	−5.98	−5.13
9–4	−4.29	−3.11	−5.39	12.28	10.60	8.54	6.18	3.64	1.05	−1.59

[a] The official IUPAC system for numbering the purine ring is used in this paper.

and shows the B_{2u} transition to remain localized in the vicinity of the C_2–N_3 bond (see Figure 9). From a purely geometrical point of view, the C_2–N_3 bond is to the anti conformer of **15** as the C_6–N_1 bond is to the anti conformer of **1a**. Referring to Table I, we find that the rotational strength contribution of the C_6–N_1 bond is positive in the anti range. Referring to Figure 6, which gives the CD curve of **15**, we note that the predicted and experiment results are in agreement.

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Chemical Evolution of a Nitrogenase Model. II. Molybdate–Cysteine and Related Catalysts in the Reduction of Acetylene to Olefins and Alkanes

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Abstract: The catalytic reduction of acetylene to ethylene by molybdenum complexes with sulfur-containing ligands (*e.g.*, cysteine or thioglycerol) and $NaBH_4$ or $Na_2S_2O_4$ as the reducing agent strikingly resembles the enzymatic reduction of acetylene by nitrogen-fixing enzymes. Under these conditions, molybdenum exhibits the highest catalytic activity of all metals. The catalytically active species are presumably Mo(IV)–thiol complexes in which two cis positions are available for the binding of acetylene in the side-on fashion. Under certain conditions acetylene is also reduced to ethane. In this case the reaction apparently involves binuclear catalytically active species. As in the enzymatic reaction the stereochemical course of the reduction is predominantly cis and is significantly stimulated by biological phosphorylating agents such as ATP. Higher alkynes, *e.g.*, propyne, propargyl alcohol, and 2-butyne are also reduced by the model systems. In the absence of substrate the molybdenum–thiol complexes catalyze hydrogen evolution which is stimulated by ATP, unaffected by CO, and inhibited by acetylene. On the basis of these analogies, we conclude that the reactions of acetylene with nitrogenase occur at a molybdenum-containing binding site of the Mo–Fe protein. Iron, the more abundant metal in nitrogenase, does not seem to participate directly in the binding and the reduction of acetylenic substrates, but may function as an electron-transfer catalyst.

The molybdenum–iron protein of *Azotobacter vinelandii* nitrogenase (N_2-ase) has recently been isolated in crystalline form[1] and was shown to consist of a protein of molecular weight between 270.000 and 300.000, containing molybdenum, iron, cysteine, and "labile sulfide" in the ratio of 1:20:20:15.

The protein, which was also isolated from various other sources, is essential for typical N_2-ase reactions, in combination with an additional iron protein which presumably serves as an electron-transfer system.[2–6]

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