Magnetic Circular Dichroism Studies. VIII.[1]
Investigation of Some Purine Cyclonucleosides[2]

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Abstract: The ultraviolet magnetic circular dichroism spectra (MCD) of some 3,5′-purine cyclonucleosides and related bases in aqueous solution are reported and correlated with the MCD spectra of ordinary purine nucleosides and bases. The complementarity of MCD and circular dichroism (CD) is emphasized and attention is drawn to the analytical utility of combined MCD and CD measurements of optically active compounds.

In a recent communication[4] we reported the magnetic circular dichroism (MCD) spectra of some purine and pyrimidine nucleosides and related bases and demonstrated the utility of MCD as a spectroscopic technique in nucleoside chemistry. In particular, it was shown that MCD provides the most unequivocal experimental evidence yet available of an accidental degeneracy in the 260-nm absorption band of adenine (I).

In the present communication we report the results of our MCD investigation in a closely related series of compounds, namely some purine cyclonucleosides in which the sugar moiety is attached to the purine ring at N-3. Purine cyclonucleosides, which have been proposed as possible intermediates in the transformation of ribonucleosides to deoxyribonucleosides,[5,6] have been the subject of several recent optical rotatory dispersion (ORD) and circular dichroism (CD) studies. Several investigators, following the successes in the pyrimidine nucleoside and cyclonucleoside series,[7] have attempted to utilize the conformationally rigid N-3 and C-8 cyclonucleosides as models for determining the solution conformation of purine nucleosides by means of ORD or CD.[8-10] Other investigators[11,12] have used the ORD and CD spectra of purine cyclonucleosides as a means of assessing the relative importance of base–sugar and base–base interactions and to effect thereby further refinements in the theories[13] on which the interpretation of the ORD and CD curves[14] of nucleic acids and polynucleotides is based.

This series of cyclonucleosides was of particular interest for several reasons. First, it was pertinent to ascertain whether the generalizations observed in the MCD spectra of ordinary nucleosides[4] would be applicable in the cyclonucleoside series as well since in some of these compounds rather severe perturbations of the purine chromophore are encountered. Thus it was anticipated that MCD might provide information about the electronic transitions of these compounds which is either less clearly revealed or which cannot readily be obtained by other spectroscopic techniques. Second, although magnetic optical activity is a universal property of all matter and therefore can be applied to optically active as well as to optically inactive substances, most of the MCD studies to date have dealt with optically inactive molecules. In two recent communications[4,15] from this laboratory we have drawn attention to some of the advantages to be gained from the application of MCD to compounds which are naturally optically active. The rigid cyclonucleosides which exhibit much stronger CD bands than the corresponding ordinary nucleosides constitute an excellent series in which to assess further the complementarity of CD and MCD. A similar approach has recently been illustrated in a series of riboflavin analogs.[16] Finally, we believe that, at the present stage of development in the application of the Faraday effect to chemical problems,[17] attention should be directed toward the investigation of a suitable but closely related series of compounds. Factors such as the relative simplicity of their MCD spectra, the availability of a wide variety of derivatives, the recent theoretical interest, and the potential importance of these compounds in a number of life processes suggests that the purine and related chromophores constitute a particularly suitable series for investigation.

Experimental Section

MCD and CD measurements were made using a Japan Spectroscopic Co. spectropolarimeter (Durrum-JASCO Model ORD-UV-5) which had been modified[15,18] to accept a Lockheed Palo Alto Research Laboratories superconducting magnet (Model OSCM-103).[19] Absorption spectra were measured on a Cary Model 14

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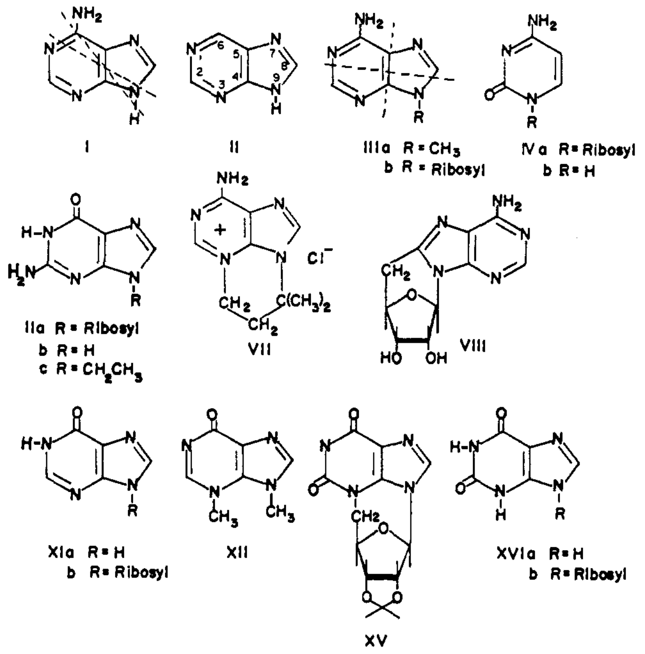

Figure 1. Structures of compounds discussed in the text. The calculated polarization directions for adenine (I) are taken from ref 34. The experimental polarization directions for 9-methyladenine (IIIa) are taken from ref 24b.

spectrophotometer. A Metrohm pH meter, equipped with a type X electrode, was used for pH measurements. All spectroscopic measurements were made in aqueous solution and at concentrations and path lengths such that the optical density did not exceed 2.

Structures of compounds discussed in the text and not presented elsewhere are given in Figure 1. Our MCD results *are not normalized to 1 G but rather are reported as molar ellipticities at 49.5* kG (using the same sign convention previously adopted[15,20]) thereby facilitating the direct comparison of MCD and CD since both are expressed in the same units. MCD curves and data have been corrected for natural CD as determined with the same solution but in the absence of the magnetic field. In each figure signal-to-noise ratios are indicated by vertical bars.

2′,3′-Isopropylidene-3,5′-adenosine cyclonucleoside mesylate was a gift from Drs. J. P. H. Verheyden and J. G. Moffatt.[21a] 2′,3′-Isopropylidene-3,5′-guanosine cyclonucleoside and 2′,3′-isopropylidene-3,5′-inosine cyclonucleoside *p*-tolysulfonate were gifts from Professor R. K. Robins.[21b] In the latter compound the tosylate anion was exchanged for acetate by means of an acetate resin (BIO-RAD Laboratories AG 1-X2). 3-Methylhypoxanthine (grade 1) was purchased from Cyclo Chemical Corp. and was used without purification. 3,9-Dimethylxanthine was a gift from Professor F. Bergmann.

Results and Discussion

Purines exhibit two types of electronic transitions in the 180–300-nm region. The π–π^* transitions are polarized in the plane of the base and can be identified by their intensity and by their relative insensitivity to solvent and pH effects. Mason[22] labeled the bands appearing in the 230–300-nm region as the X bands and those below 220 nm as the Y bands. In some of these bases, for example, in purine (II), a band of weaker intensity, labeled X_2, can be observed on the blue side (at about 240 nm in hydrocarbon solvents) of the stronger X_1 band at about 260 nm. Clark and Tinoco,[23] by analogy with the symmetry classification of the benzene absorption bands, have designated these bands as B_{2u} (X_1), B_{1u} (X_2), and E_{1u} (Y). In this communication we again adopt this notation in order to facilitate the comparison of the MCD and CD spectra obtained in the cyclonucleoside series with similar spectra obtained in the ordinary nucleoside series[4] as well as with the ORD and CD results of other investigators.[11,12] Although the B_{2u} and B_{1u} bands are clearly resolved in the absorption spectra of purine (II) and many of its derivatives, purines which bear substituents at C-6 such as adenine (I) show only a single absorption band in the 220–300-nm region. Although polarized absorption spectra of single crystals of 9-methyladenine (III)[24] as well as the polarized fluorescence spectrum of adenine[25] suggest the presence of a weak π–π^* transition on the blue side of the main (260 nm) absorption band of adenine (I), the most unambiguous experimental evidence of an accidental degeneracy in this band has been obtained from MCD measurements (see Figures 4 and 5 in ref 4). The second type of electronic transition found in purine derivatives are the n–π^* transitions due to the nonbonding electron pairs of the nitrogen and oxygen atoms. These transitions are polarized in a direction normal to the plane of the base and may be distinguished from π–π^* transitions by their low intensity and by their sensitivity to solvent and pH effects.[26] Although n–π^* transitions have been observed in the spectra of purine[23,27,28] (II), in the anion of adenine (I),[29] and in 9-methyladenine (III),[24] they are usually hidden under the more intense nearby π–π^* transitions. McCaffery, *et al.*,[30] have demonstrated experimentally that the magnetic Cotton effect of the n–π^* carbonyl transition is of such low intensity as to be observable for only very few ketones. On the other hand, Tinoco and Bush[31] have suggested that the n–π^* transitions in heteroaromatic systems may give quite strong magnetic Cotton effects. McCarville[32] attributed the long wavelength tail in the MCD spectrum of purine to an n–π^* transition. In our previous communication[4] concerning the MCD of ordinary purine and pyrimidine nucleosides clear evidence of an n–π^* transition was found only in the case of the nucleoside cytosine (IVa) and its component base cytidine (IVb) (see Figures 10 and 11 in ref 4). In the case of the ordinary purine nucleosides and bases, evidence for the presence of n–π^* transitions by MCD was more speculative in nature. Thus, the increase in intensity of the B_{2u} and B_{1u} magnetic Cotton effects (except guanosine, Va) at pH 2, relative to measurement at pH 7, could be interpreted as revealing the presence of hidden n–π^* transitions. In the present communication we present

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additional experimental results in support of this interpretation (*vide infra*).

On the basis of substitutional perturbation agruments, Mason[22] proposed that the strong (B_{2u}) and weak (B_{1u}) purine transitions are longitudinally and transversely polarized, respectively. Polarized absorption measurements on single crystals of 9-methyladenine (IIIa) suggest, however, that the strong transition is approximately transversely polarized (Figure 1, structure IIIa).[24b] Recent calculations place the B_{2u} transition moment of adenine (Figure 1, structure I) in a direction roughly paralleling a line drawn through N-1 and N-3 with the angle between the polarization directions of the B_{2u} and B_{1u} transitions at about 30° [33,34] or perhaps, even less.[35] The magnitude of the magnetic Cotton effects associated with the B_{2u} and B_{1u} transitions in adenine (Figure 5 of ref 4) provides intuitive support for the larger angular estimate.

Theoretical treatments of magnetic optical activity[36] predict, for an isolated electronic transition, the observation of one or more of three types of MCD curves. The A and C terms, which arise from Zeeman splitting in the ground or excited states, will be observed only in molecules having a principal axis of symmetry of order three or higher. The low symmetry of the vast majority of molecules precludes the existence of electronic degeneracies in their ground and excited states and, consequently, most MCD spectra will contain only the B term magnetic Cotton effects which arise from the mixing of the ground and two or more excited states by the magnetic field. In the three-state case, *i.e.*, for a ground state and two excited states which are coupled by the magnetic field, the magnitude of the observed magnetic Cotton effects will depend on the scalar triple product of the magnetic dipole transition moment between the two excited states and the electric dipole transition moments between the ground and the two excited states as well as on the energy separation between the two excited states. Therefore, one would expect to observe two MCD bands of opposite sign and of nearly equal magnitude with the intensity of the two bands being determined by the numerical value of the three integrals and by the extent to which these transition moments are orthogonally directed. A satisfactory explanation for the relative signs of these two bands, observed for example in the MCD spectra of substituted benzenes[37] and in some purine derivatives,[32] is not yet available. In some cases in which more than two excited states are coupled by the magnetic field a more complex pattern of MCD bands may result. A particularly impressive example is found in the MCD spectrum (Figure 3) of 3,5′-cycloguanosine (IX).

The absorption, CD and MCD spectra of 2′,3′-isopropylidene-3,5′-adenosine cyclonucleoside mesylate (VI) are presented in Figure 2. The absorption spectra of VI and adenosine (IIIb) are similar in that both nucleosides show only two resolved bands in the 200–300-nm region. They differ, however, in the position of the first band. In the absorption spectrum of adenosine[4] this band is found at 260 nm whereas in the absorption spectra of VI, its iodide salt,[38] and in the structurally similar alkaloid derivative pyrotriacanthine chloride[39] (VII) this band appears at 272 nm.

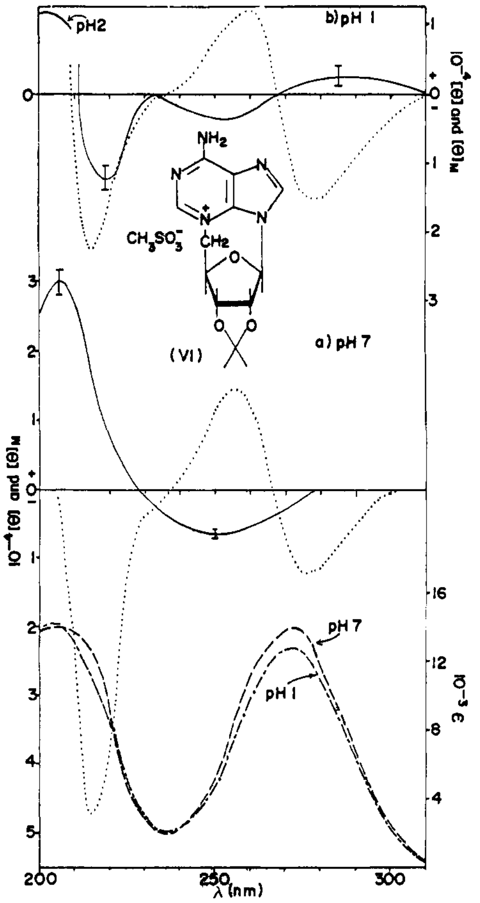

Figure 2. Absorption (– – –, pH 7; —·—, pH 1), MCD (···) and CD (——) spectra of 2′3′-isopropylidene-3,5′-adenosine cyclonucleoside mesylate (VI) at (a) pH 7 and (b) pH 1.

In their investigation of the ORD of purine nucleosides, Emerson and coworkers[8] found a positive ORD Cotton effect for the iodide salt of VI and a negative ORD Cotton effect for 8,5′-adenosine cyclonucleoside (VIII). Since the formation of the third ring necessarily ensures the *syn* conformation for VI and the *anti* conformation for VIII and since the common purine nucleosides generally show negative Cotton effects in this region,[40] it was proposed that ordinary purine nucleosides favor the *anti* conformation in solution. Subsequently, other investigators[10–12] have reported, and we herewith confirm (Figure 2a), that VI exhibits *at pH 7* a negative rather than a positive Cotton effect in the 230–280-nm region. This discrepancy is due to the labile nature of this compound in aqueous solution and, because of this instability, we were careful to ensure that our spectral measurements were completed in less than 0.5 hr after preparation of the solutions. Significant changes, especially in the MCD spectra, were noted after 1 hr and after 24 hr the sign of the long wavelength

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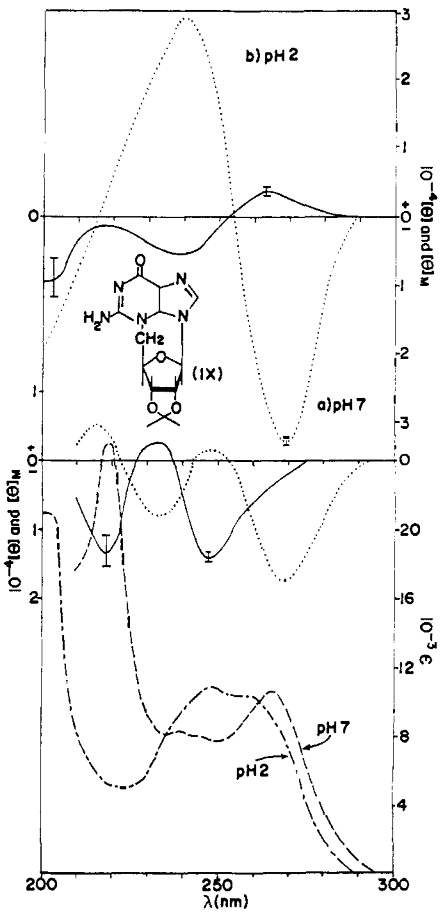

Figure 3. Absorption (- - -, pH 7; —·—, pH 2), MCD (···) and CD (——) spectra of 2′,3′-isopropylidene-3,5′-guanosine cyclonucleoside (IX) at (a) pH 7 and (b) pH 2.

CD Cotton effect became positive, as observed by Emerson.[8] It should be noted that our absorption and CD spectra for 3,5′-cycloadenosine (VI) at pH 7 and pH 1, and consequently our interpretation of the particular transitions responsible for the observed CD Cotton effects, are in conflict with the spectra reported recently by Miles, Robins, and Eyring.[11,12]

If one considers only the pH 7 data (Figure 2a), then a comparison of the absorption and CD spectra of VI suggests that the 272-nm absorption band contains two overlapping bands; that the intensity of the lower energy band (B_{2u}) is at least twice that of the higher energy band (B_{1u}); that the B_{1u} transition is primarily responsible for the observed CD Cotton effect; and that the sign of the Cotton effect due to the B_{2u} transition is negative. The features observed in the MCD spectrum of VI, in neutral solution, are qualitatively similar to those previously observed[4] for the ordinary nucleoside adenosine (IIIb).

A comparison of the dramatic differences in the CD spectra of VI at pH 7 (Figure 2a) and pH 1 (Figure 2b) suggests, however, that one or more n–π* transitions may be responsible for the rather unusual shape of the CD curve observed at pH 7. Prompt readjustment to pH 7 restored the original (Figure 2a) CD curve, but prolonged exposure in acid solution caused decomposition. Since four CD Cotton effects are found in acid solution whereas only two were observed in the same spectral region in neutral solution, it might be argued that two n–π* transitions are present whose energies and rotational strengths are such as to obscure the CD Cotton effects due to the B_{2u}, B_{1u}, and E_{1u} π–π* transitions. For this explanation to hold the n–π* CD Cotton effect in the 210–230-nm region must be strongly positive while the n–π* CD Cotton effect at longer wavelengths must be negative and much weaker. The changes in the absorption spectra are consistent with this interpretation. Comparison of the MCD spectra of VI at pH 7 and at pH 1 reveals that the negative MCD band at 215 nm is considerably more sensitive to pH effects than are either of the lower energy MCD bands. This behavior as well as the shoulder at about 232 nm (Figure 2a) can be interpretated as reflecting the presence of an n–π* transition in the 210–230-nm region. The evidence for an n–π* transition in this region is less tenuous in the MCD spectra of 3-methylhypoxanthine (XIII) shown in Figure 5. The lack of significant changes in the MCD bands at longer wavelengths suggests either the absence of an n–π* transition in this region or that, if present, it is not effectively coupled with the nearby B_{2u} and B_{1u} transitions. In the case of 3,5′-cycloguanosine (IX), MCD provides more convincing evidence for an n–π* transition in the 260–280-nm region.

If one accepts the former explanation, *i.e.*, that the broad 250-nm CD Cotton effect observed for VI at pH 7 is composed of two overlapping negative CD bands which are related to the B_{2u} and B_{1u} transitions, then it is necessary to invoke a rationale such as the one proposed by Miles, Robins, and Eyring[12] to explain the ORD sign changes attending substitution at C-8 in ordinary guanosine nucleosides in order to explain the CD spectrum of VI in acid solution.

The absorption, CD, and MCD spectra of 2′,3-isopropylidene-3,5′-guanosine cyclonucleoside (IX) are displayed in Figure 3. Comparison of Figures 3a and 3b reveals that the absorption, CD, and MCD spectra of IX are sensitive to pH whereas in the case of 3,5′-cycloadenosine (VI) protonation was most clearly reflected in its CD spectrum. The rather extensive perturbation in the guanine (Vb) chromophore attending bond formation at N-3 is evident on comparison of the pH 7 absorption spectrum of the ordinary nucleoside guanosine (Va) (Figure 2, ref 4) with that of its cyclo analog, IX. Guanosine exhibits two strong absorption bands (275 and 253 nm) in the 200–300-nm region. A recent calculation places the polarization directions of the first transition (B_{2u}) along a line approximately paralleling the N-3—C-4 bond, the second transition (B_{1u}) along a line drawn through positions 6 and 9, and the third transition (E_{1u}), at 196 nm,[41] in a direction perpendicular to that of the second transition.[34] Although these polarization directions are not expected to be applicable to IX, they will provide a basis for rationalizing the differences observed in the MCD spectra of IX in neutral and in acid solution. Whereas the absorption spectra of adenosine and 3,5′-cycloadenosine (VI) presented very similar features, the features observed in the absorption spectra of guanosine (Va) and 3,5′-cycloguanosine (IX) are quite dissimilar. The shape of the pH 7 absorption curve at intermediate wavelengths suggests the presence of one or more weaker π–π* transitions in the 230–250-nm region. Thus, the energy separation

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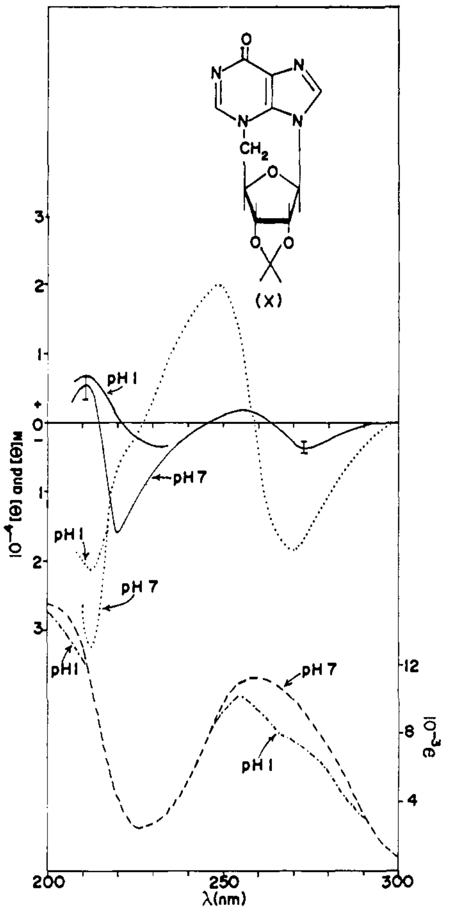

Figure 4. Absorption (– – –, pH 7; -·-, pH 1), MCD (· · ·) and CD (——) spectra of 2′,3′-isopropylidene-3,5′-inosine cyclonucleoside.

between the B_{2u} and B_{1u} bands on the one hand and the E_{1u} transitions on the other appears to be considerably smaller in the case of the cyclonucleoside IX than in the ordinary nucleoside Va. A second aspect of the perturbation in the guanine chromophore accompanying formation of the third ring is revealed by the differences in the absorption spectra of these two compounds in acid solution. Whereas Va shows essentially identical absorption spectra in aqueous solution at pH 7 and pH 2,[4] rather remarkable changes can be noted in the absorption spectra of IX. The very strong higher energy band which is probably of E_{1u} origin is now found at about 202 nm whereas the B_{2u} and B_{1u} transitions appear coalesced under a common broad envelope with only a hint of resolution evident in the absorption curve.

Miles, Robins, Eyring[11] attributed the dramatic changes in the appearance of the CD spectra of 3,5′-cycloguanosine (IX) in neutral (Figure 3a) and in acid solution (Figure 3b) to an n–π^* transition and to wavelength shifts in the π–π^* transitions. The negative CD Cotton effects observed in neutral solution at 248 nm and at 218 nm were correlated with the B_{1u} and E_{1u} transitions, respectively. It was assumed that the tail on the red side (*i.e.*, at about 260 nm) of the B_{1u} CD Cotton effect was due to an n–π^* transition whose sign and magnitude (rotational strength) obscured the less intense B_{2u} CD Cotton effect which is also present in this region. The small positive CD Cotton effect at 233 nm was not assigned; however, on the basis of a qualitative analysis of the MCD spectra of IX it seems reasonable to attribute this band to an π–π^* transition (*vide infra*). Since on protonation in acid solution, n–π^* transitions are expected to appear at considerably higher energies, the positive CD Cotton effect associated with the B_{2u} transition can now be observed. Consequently, in Figure 3b, the positive CD Cotton effect at 264 nm and the negative CD Cotton effects at 237 nm and at 202 nm can be correlated with the B_{2u}, B_{1u}, and E_{1u} transitions, respectively. This rationalization[11] appears to be quite reasonable and receives additional support from MCD as well as from the similar changes in the CD spectra of 3,5′-cycloadenosine (VI) in neutral and in acid solution shown in Figures 2a and 2b.

In contrast to 3,5′-cycloadenosine (VI), the MCD spectra exhibited by 3,5′-cycloguanosine (IX) in Figures 3a and 3b are dramatically different in neutral and in acid solution. The positions of the lowest and the highest energy MCD bands (pH 7) are in reasonable correspondence with the absorption bands attributed to the B_{2u} (at 266 nm) and to the more intense one of the E_{1u} transitions (at 219 nm), respectively, whereas the positions of the 248 and 233 nm MCD bands are in exact correspondence with the CD Cotton effects attributed to the B_{1u} and to the weaker of the two E_{1u} (*vide infra*) transitions. A measure of the extent to which the guanine chromophore is perturbed by formation of the third ring at N-3 can be obtained from a comparison of the pH 7 MCD spectrum of IX with the pH 7 MCD spectrum of Va.[4] It is of particular interest to note that, in the case of Va, the B_{2u} and B_{1u} terms are of nearly equal magnitudes (−40,000 and +36,000) and that the sum of the intensities of these two bands (76,000) is greater than that observed for any other member of the ordinary or cyclopurine nucleoside series. This is in conformity with the nearly orthogonal orientation of the polarization directions of these two transitions discussed previously. The greatly diminished intensities of the MCD bands observed for IX in Figure 3a is particularly striking, and it is possible to rationalize the relatively greater intensity observed for the 268 nm MCD band as being the resultant of a significant contribution from the n–π^* transition which is presumably[11] also in this region. It would be difficult not to associate the MCD Cotton effects at 270 and 240 nm with the B_{2u} and B_{1u} transitions, respectively. It is of interest to note that now the MCD bands related to the B_{2u} and B_{1u} transitions are of comparable intensity and that the sum of the intensities of these two bands (62,000) is almost that (67,000) observed for guanosine (Va) at pH 2. Comparison of the changes in the absorption and MCD spectra in acid solution with those observed in neutral solution suggests that the negative MCD band at 233 nm as well as the positive MCD band at 215 nm in the pH 7 MCD curve (Figure 3a) can be correlated with the components of the degenerate E_{1u} transition. In acid solution only the first of these components, and then only in part, can be observed in the MCD curve (Figure 3b). The corresponding positive CD Cotton effect at 233 nm (Figure 3a) then correlates with the negative CD Cotton effect observed in Figure 3b at 202 nm, thus requiring that protonation causes an inversion in the sign of the E_{1u} CD Cotton effects. On the basis of CD measurements alone one would be inclined to attribute the 233-nm pH 7 Cotton effect to an n–π^* transition.

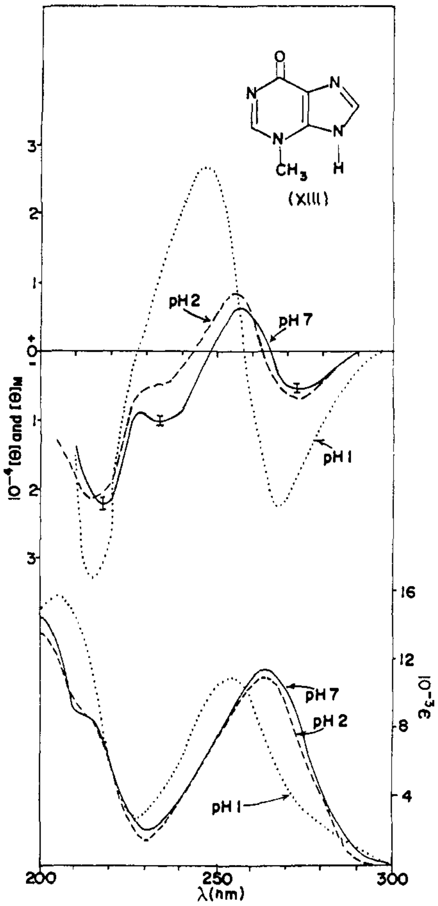

Figure 5. Absorption and MCD spectra of 3-methylhypoxanthine (XIII) (—, pH 7; - - -, pH 2; · · ·, pH 1).

The features observed in the MCD spectrum of IX at pH 7 (Figure 3a) and the dramatic changes associated with protonation at pH 2 (Figure 3b) can be interpreted as being primarily caused by the complex mixing of several nearby excited states by the magnetic field. Thus, the effectiveness of magnetic coupling between the B_{2u}, B_{1u}, and E_{1u} transitions depends, in part, on their directions of polarization, their energy separation and on the magnitudes of the magnetic dipole moments between their respective excited states. The absorption spectrum of IX at pH 7 suggests that the energy levels of these transitions are more closely spaced in neutral solution than they are at pH 2 and would thus favor complex mixing. In addition the perpendicular orientation of the electric dipole transition moments of nearby n-π^* transitions is such as to facilitate their mixing with several of the in plane π-π^* transitions. At pH 2 the increased energy separations between the various excited states, as well as probable reorientations in their polarization directions favors two state mixing and the MCD spectrum becomes "normal."

The absorption, CD, and MCD spectra of 2′,3′-isopropylidene-3,5′-inosine cyclonucleoside (X) are presented in Figure 4. The CD and MCD spectra of X measured at pH 1 differed significantly from the spectra observed at pH 7 only in the shorter wavelength region. The CD curves of X are qualitative agreement with the curves reported by Miles, Robins, and Eyring for the *p*-toluenesulfonate salt.[11,12] Although our sample of X was obtained as the *p*-toluenesulfonate salt, the tosylate anion was exchanged for acetate; this exchange of anions being dictated by the necessity, inherent in MCD, of eliminating substances which absorb light in the same spectral region as the chromophore of interest.

In a previous communication[4] we demonstrated, by means of MCD, that the 248-nm absorption band of inosine (XIb) consisted of two overlapping π-π^* transitions. The spectra presented in Figure 4 reveal that a similar situation also exists for 3,5′-cycloinosine (X). It is of interest to note that the sum of the intensities of the B_{2u} and B_{1u} MCD bands of XIb and guanosine (Va) are almost equal (74,000 and 76,000, respectively), intimating that the relative orientations of the polarization directions of the B_{2u} and B_{1u} transitions in the two compounds are comparable. As in the case of 3,5′-cycloadenosine (VI), the sum of the intensities of the B_{2u} and B_{1u} MCD bands of 3,5′-cycloinosine (X), 39,000, is only one-half that of its ordinary nucleoside counterpart XIb. A molecular orbital calculation of the polarization directions of these two transitions places their relative orientations at about 47° for X and at about 45° for VI.[12] In conformity with the behavior of 3,5′-cycloadenosine (VI), but in contrast to the behavior of 3,5′-cycloguanosine (IX), no increase in the intensities of these MCD bands was observed in acid solution. This may be reflective of differences in either the site of protonation or the pK_a's of the several bases as well as rather subtle electronic factors affecting the extent of the magnetic coupling of n-π^* and π-π^* transitions (compare the MCD pH sensitivity of VI and IX).

The CD bands at 273, 256, and 211 nm are relatively insensitive to pH effects and are correlated with the B_{2u}, B_{1u}, and E_{1u} transitions, respectively. It is of interest to note that the sign pattern of the CD bands associated with the B_{2u} and B_{1u} transitions is opposite to that observed for 3,5′-cycloadenosine (VI) (Figure 2b), 3,5′-cycloguanosine (IX) (Figure 3b), and 3,5′-cycloxanthosine (XIV) (Figure 2, ref 12). The changes observed in the CD spectra of X at pH 7 and at pH 1 have been attributed[11,12] to an n-π^* transition located in the 220–230-nm region. Although the MCD curve of X is not dramatically sensitive to pH, the diminution in the magnitude of the 212-nm MCD Cotton effect at pH 1 as well as the slight shoulder at about 223 nm observed at pH 7 are not inconsistent with this interpretation. In Figure 4 it will be noted that the CD Cotton effect, which is presumed to be of n-π^* origin, is not entirely removed from the spectrum at pH 1. Unfortunately, after exchange of the tosylate anion, insufficient sample remained for measurements at still lower pH's. In order to clarify this situation and as well to confirm the shape of the MCD curve presented in Figure 4 (another sample, purported to be 3,5′-cycloinosine, gave a completely different MCD curve), we searched for a structurally similar, but optically inactive, analog for comparison. Since the compound containing the necessary structural features, namely 3,9-dimethylhypoxanthine (XII), has not been synthesized,[42] 3-methylhypoxanthine (XIII) was chosen for comparison purposes.

The absorption and MCD spectra of 3-methylhypoxanthine (XIII) are shown in Figure 5. In Figure 5 the structure shown for 3-methylhypoxanthine, XIII, is that of the tautomer having a hydrogen atom at position 9 although intramolecular hydrogen bonding would probably favor the 7-H tautomer.[22] The 7- as well as

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[42] Z. Neiman and F. Bergmann, *Israel J. Chem.*, 5, 243 (1967).

the 9-monoalkyl isomers of several purine bases are available and their MCD spectra will be compared in a future communication.

Although the features observed in the pH 7 absorption spectrum of XIII are very similar to those observed in the pH 7 absorption spectrum of 3,5′-cycloinosine (X) presented in Figure 4, differences will be noted in the positions and in the broadness of the higher and lower energy absorption bands. The higher energy absorption bands as well as the negative pH 7 MCD band at 218 nm are correlated with the E_{1u} transitions. A second, and positive, MCD band corresponding to the E_{1u} component of higher energy is predicted at about 190–200 nm. At pH 1, the long wavelength absorption band undergoes a hyposochromic shift of 10 nm and it appears that, in XIII, the B_{2u} and B_{1u} transitions have undergone a greater redistribution of intensities than they did in 3,5′-cycloinosine (X) (Figure 4) since in the latter compound the absorption band associated with the B_{2u} transition appears as a clearly defined shoulder, whereas this band apparently underlies the long wavelength tail in the pH 1 absorption curve of 3-methylhypoxanthine (XIII).

A comparison of the MCD spectra presented in Figures 4 and 5 reveals that each of the four MCD bands exhibited by XIII is sensitive to pH whereas in the case of X only the E_{1u} MCD band exhibited significant sensitivity to protonation effects. In the discussion of the spectra of X (Figure 4) it was noted that the slight shoulder at about 223 nm on the pH 7 MCD curve constituted the principal MCD evidence for the presence of an n–π^* transition in this conpound. The MCD spectra of XIII provide, however, substantial support for this assignment. The pronounced pH sensitivity exhibited by the negative MCD band at 234 nm (at pH 7) is especially striking. This MCD band, which has neither an obvious nor a conjecturially reasonable π–π^* counterpart in the absorption spectrum, experiences a 50% reduction in intensity at pH 2 whereas the intensities of the MCD Cotton effects associated with the B_{2u} and B_{1u} transitions (274 and 257 nm, respectively, at pH 7) exhibit significant, though smaller and opposite intensity changes. This behavior is most reasonably interpreted as reflecting the contribution of the n–π^* transition of a partially protonated species, specifically, the one which gives rise to the negative MCD band at 234 nm. The changes in the MCD spectra at lower pH's are consistent with this interpretation. A comparison of the pH 1 and pH 7 MCD spectra of XIII shows that the MCD bands associated with the B_{2u} and B_{1u} transitions have undergone a blue shift of about 8 nm as well as a four fold increase in total intensity (11,500–49,100) whereas the MCD band associated with the E_{1u} transition shows only an increase in intensity. These wavelength shifts of the MCD bands associated with the π–π^* transitions parallel the behavior of the corresponding bands in the absorption spectra. It is particularly noteworthy, however, that the MCD band (234 nm at pH 7) which is attributed to an n–π^* transition is no longer visible in the MCD spectrum at pH 1. The diminution of this band at pH 2 supports this assignment since, from a consideration of only the pH 7 and pH 1 MCD spectra, it might be argued that this band is only obscured by the more intense blue shifted B_{1u} magnetic Cotton effect.

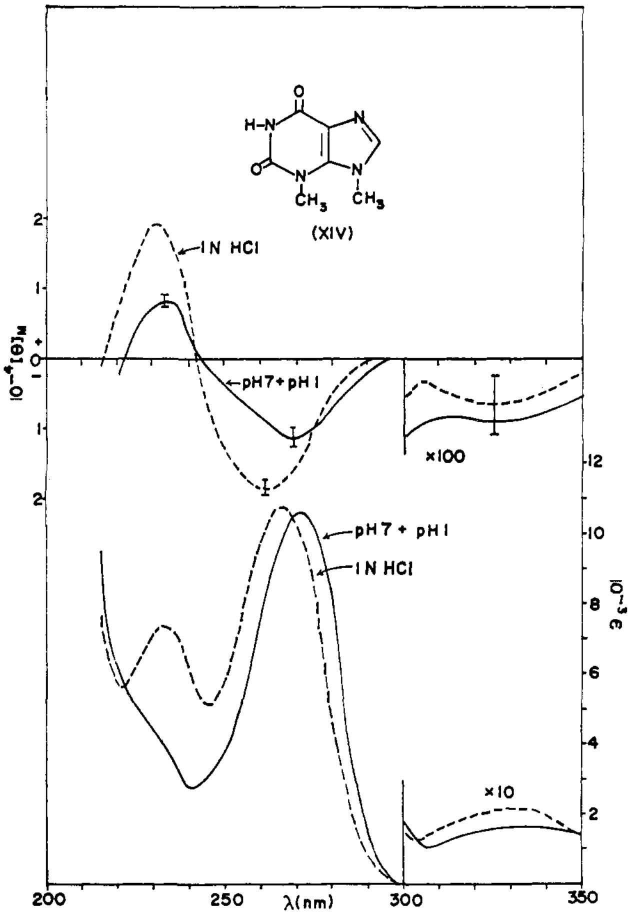

Figure 6. Absorption and MCD spectra of 3,9-dimethylxanthine (XIV) (—, pH 7 and pH 1; – – –, 1 *N* HCl).

The absorption and MCD spectra of 3,9-dimethylxanthine (XIV) at pH 7 and in 1 *N* HCl are shown in Figure 6. Except for the strain attending formation of the third ring, XIV is electronically similar to 3,5′-xanthosine cyclonucleoside (XV). This perturbation is reflected in the absorption spectra of the two compounds. The 2′,3′-isopropylidene derivative of XV exhibits absorption maxima at about 265 (ϵ ∽12,000), 237 (ϵ ∽11,000), and 197 nm (ϵ ∽25,000) in aqueous solution at pH 7 as well as at pH 1. These bands were correlated with the B_{2u}, B_{1u}, and E_{1u} benzene transitions, respectively.[11,12] The features observed in the absorption spectra of XIV at pH 7 and at pH 1, although very similar to those observed for the base xanthine (XVIa) (Table I, ref 4), are rather different from those reported for the cyclonucleoside analog XV. Although the position and intensity of the absorption band at 271 nm which is associated with the B_{2u} transition is in reasonable correspondence with the position and intensity reported for the B_{2u} band in XV, the intensity of the B_{1u} band is much weaker and appears only as a shoulder at about 230 nm. A maximum was not observed for the E_{1u} band but is expected to lie in the 190–200-nm region. In addition to these bands which, adopting the nomenclature of other investigators,[11,12] we have labeled B_{2u}, B_{1u}, and E_{1u}, XIV exhibits a very weak absorption band at about 335 nm (ϵ 160). From its behavior in acid solution (Figure 6) this band seems to be of π–π^* rather than n–π^* origin.

The MCD spectrum of XIV exhibits, in neutral as well as in acid solution, magnetic Cotton effects whose positions are in close correspondence with the positions of the three bands observed in the absorption spectra. It will be noted in Figure 6 that, although the maxima of the B_{2u} and B_{1u} MCD bands (at pH 7) are in close correspondence with their counterparts in the absorption spectra, the integrated intensities of these two MCD bands are quite different. A similar situation exists in the CD spectrum of XV.[11,12] In this respect, the pH 7 MCD spectrum of XIV is very similar to that of the base xanthine (XVI) (Table I, ref 4). This inequality in the intensities of these two bands suggests the mixing of other nearby states; a situation which is made more favorable by the energy separation between the B_{2u} and B_{1u} states in this compound. In the case of the ordinary nucleoside xanthosine (XVIb) the B_{2u} and B_{1u} states are less widely separated and the corresponding MCD bands are of more nearly equal intensities (Figure 1, ref 4). In the spectra of XIV, observed in 1 *N* HCl, it will be noted that the B_{2u} and B_{1u} absorption bands merge; that the position of the B_{2u} MCD band in particular reflects this wavelength shift; that the intensities of the B_{2u} and B_{1u} bands increase; and that the intensities of the two MCD bands are more nearly equal. Although the increase in intensity of the B_{1u} MCD band appears to reflect the increase in intensity of the corresponding band in the absorption spectrum, this behavior is not found in the B_{2u} band. This lack of a simple relationship between the intensities of absorption and MCD bands is even more clearly evident in the absorption and MCD spectra of chlorin derivaties.[15]

Summary

In the present communication we have extended our investigation of the MCD spectra of the purine chromophore to some 3,5′-purine cyclonucleosides and related bases anticipating that the results obtained may, in concert with recent theoretical treatments of the Faraday effect in polymers,[43] be of value for the interpretation of the MCD of polynucleotides. In addition, purine derivatives are a particularly suitable series for continuing investigation.

Although the features observed in the MCD spectra of the 3,5′-purine cyclonucleosides and the ordinary purine nucleosides are qualitatively similar in so far as the magnetic Cotton effects associated with $\pi-\pi^*$ transitions are concerned, the observation of $n-\pi^*$ transitions by means of MCD was unexpected. The, sometimes dramatic, change in the MCD spectra of these compounds in neutral and acid solution is interpreted as revealing the presence of $n-\pi^*$ transitions, remembering, however, that other factors such as reorientation of polarization directions must also be considered.

Another point meriting emphasis is that the present study represents another example of the analytical utility of MCD in optically active compounds,[15] since in many cases (Figures 2–5) the MCD spectra differ greatly from their CD counterparts. Consequently, measurement of the circular dichroism in the presence and absence of a magnetic field affords two diagnostically meaningful parameters which can be used for precise "finger printing" purposes. Since the intensity of the MCD signals is proportional to the magnitude of the magnetic field, considerably smaller quantities of material are often required to give meaningful MCD spectra as compared to the quantities necessary for their CD spectra. A good example is Figure 3b, where 1 μg/ml (path length 1 cm) of IX would still be sufficient for the 50-kG MCD spectrum, whereas such a concentration would not be suitable anymore for measurement of its natural optical circular dichroism.

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[43] R. A. Harris, *J. Chem. Phys.*, **46**, 3398, 4481 (1967).