Magnetic Circular Dichroism Studies. VI.[1] Investigation of Some Purines, Pyrimidines, and Nucleosides[2]

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Abstract: Magnetic circular dichroism spectra (MCD) are reported for a number of purine and pyrimidine nucleosides and bases in aqueous solution from 300 to 200 nm. The utility of MCD as a means of resolving overlapping absorption bands is emphasized. The information provided by MCD and CD is compared. The distinct and characteristic difference in the MCD spectra of purine and pyrimidine nucleosides is of analytical value when small amounts of material are available.

The resurgence of interest in magnetic optical activity (the Faraday effect), evident from the recent activity[4,4a] in this field, is due to recent advances in instrumentation and in theory. The commercial availability of sensitive spectropolarimeters, circular dichrographs, and superconducting magnets has enabled measurements of magnetic optical rotatory dispersion (MORD) and magnetic circular dichroism (MCD) to be made with ease through the visible and ultraviolet regions of the spectrum. The recent theoretical treatment of the Faraday effect[5] has been of particular value because of the derivation of quantitative expressions for the frequency dependence of MCD and MORD through absorption bands. Since magnetic optical activity is a universal property of all matter, the information provided by MCD and MORD measurements is of interest to organic and inorganic chemists as well as to theoretical chemists.

Although magnetic optical activity and natural optical activity have separate physical origins,[5,6] MCD and MORD can also be used in the study of optically active materials and provide information of theoretical as well as practical value. In a recent communication,[7] we investigated the MCD and CD (circular dichroism) of seven optically active metal-free chlorin derivatives and demonstrated that, at least in this series, MCD is considerably more sensitive to the position and nature of the external substituents on the chlorin skeleton than is either CD or absorption spectroscopy. This situation arises from the fact that, in this series, the amplitudes of certain MCD bands are some 20–30 times the amplitude of the corresponding CD bands and that the magnitudes of these MCD bands are very sensitive to the nature of the external substituent.

In the present communication we report our investigation of the MCD of another series of optically active molecules, namely, the purine and pyrimidine nucleosides which are constituents of the biologically important nucleic acids. This series was of particular interest to us for several reasons. First, there must be considered the potential analytical application of MCD as a means of detecting the very small quantities of these compounds which are isolated in the laboratory. Second, it is anticipated that the information provided by MCD may be of practical as well as theoretical value in the study of the electronic transitions of nucleic acids and their constituents. Finally, the availability of the component purine and pyrimidine bases as well as of several anomers afforded the opportunity of comparing and contrasting more closely the kind of information provided by magnetic and by natural circular dichroism. In particular it can be shown that while MCD measurements of optically active compounds do provide useful analytical and spectroscopic information, they do not, in general, offer information about the conformation of optically active molecules. Our investigation shows that MCD is a useful spectroscopic tool for distinguishing between purine and pyrimidine nucleosides and for resolving overlapping absorption bands.

Experimental Section

A Japan Spectroscopic Co. spectropolarimeter (Durrum-JASCO Model ORD-UV-5) modified to allow CD measurements and to accept a superconducting magnet built by Lockheed Palo Alto Research Laboratories (Model OSCM-103)[8] was used for both CD and MCD measurements. Absorption spectra were measured on a Cary Model 14 spectrophotometer. Measurements of pH were made on a Metrohm pH meter using a type X electrode. All measurements were made in aqueous solution and at concentrations and path lengths such that the optical density was about 2. For more detailed discussions of the instrumentation involved in our MCD measurements reference should be made to previous communications from this laboratory.[1,7]

Absorption, CD, and MCD data are collected in Table I. Representative curves are displayed in Figures 1–11. Since we were especially interested in a comparison of MCD and CD, our MCD results are not normalized with respect to the magnetic field but rather are reported as molar ellipticities at 49.5 kG (using the same sign convention previously adopted[7,9]). This enables a direct comparison to be made between MCD and CD since both are expressed with the same units. Unless otherwise noted (Figure 6) MCD curves and data have been corrected for natural CD as determined with the same solution but in the absence of the magnetic field. The error in ellipticity values has been estimated and is indicated in each figure with a vertical line.

(1) Paper V: B. Briat, D. A. Schooley, R. Records, E. Bunnenberg, C. Djerassi, and E. Vogel, *J. Amer. Chem. Soc.*, **90**, 4691 (1968).

(2) We are indebted to the National Science Foundation (Grant No. GP-7432) and to the National Institutes of Health (Grant No. GM-12173) for financial aid.

(3) Recipient of a NATO postdoctoral fellowship (1967–1968).

(4) B. Briat and C. Djerassi, *Nature*, **217**, 918 (1968).

(4a) NOTE ADDED IN PROOF. One of the referees called our attention to a similar, but as yet unpublished, investigation of the MCD of some purine and pyrimidine bases (M. E. McCarville, Ph.D. Thesis, Iowa State University, 1967).

(5) A. D. Buckingham and P. J. Stephens, *Ann. Rev. Phys. Chem.*, **17**, 399 (1966).

(6) A. Moscowitz, *Proc. Roy. Soc.* (London), **A297**, 16 (1967).

(7) B. Briat, D. A. Schooley, R. Records, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem. Soc.*, **89**, 6170 (1967).

(8) S. R. Hawkins and J. H. Harshman, *Rev. Sci. Instr.*, **38**, 50 (1967).

(9) P. N. Schatz, A. J. McCafferty, W. Suëtaka, G. N. Henning, A. B. Ritchie, and P. J. Stephens, *J. Chem. Phys.*, **45**, 722 (1966).

Table I. Absorption, Magnetic Circular Dichroism, and Circular Dichroism Data

Compound	pH	Absorption,[a] λ, nm ($10^{-3}\epsilon$)			MCD,[a,b] λ, nm ($10^{-3}[\theta]_M$)			CD,[a,b] λ, nm ($10^{-3}[\theta]$)		
		B_{2u}	B_{1u}	E_{1u}	B_{2u}	B_{1u}	E_{1u}	B_{2u}	B_{1u}	E_{1u}
Xanthosine	7	258 (8.7)	241 (8.5)		267 (−22.0)	233 (+21.5)	(NEG)	245 (−2.5)		217 (+11.8)
	2	263 (9.9)	234 (9.0)		263 (−25.5)	233 (+27.2)	(NEG)	235 (−2.4)		211 (+15.0)
Xanthine	7	266 (8.9)	225 (3.7)[s]		265 (−10.2)	231 (+6.6)	(NEG)			
	2	266 (8.7)	225 (3.8)[s]		265 (−10.7)	230 (+7.5)	(NEG)			
Guanosine	7	275 (8.4)[s]	253 (12.7)		278 (−40.0)	248 (+35.8)	(NEG)	245 (−2.3)		215 (+7.9)
	2	275 (8.6)[s]	255 (12.5)		278 (−36.4)	250 (+30.9)	(NEG)	240 (−?)		220 (+2.3)
Guanine	2	273 (6.9)	248 (10.6)		275 (−24.2)	246 (+23.2)	(NEG)			
Inosine	7	248 (12.2)			263 (−35.0)	242 (+38.7)	210 (−43.3)	245 (−3.3)		(POS)
	2	248 (12.5)			262 (−35.2)	241 (+34.5)	206 (−44.2)	245 (−2.2)		(POS)
Hypoxanthine	7	248 (9.8)			263 (−27.5)	243 (+24.2)	(NEG)			
	2	248 (10.2)			263 (−27.4)	240 (+26.9)	(NEG)			
Adenosine	7	260 (14.4)		206 (21.2)	272 (−34.0)	253 (+29.7)	215 (−46.0)	265 (−2.5)		225 (+2.0)
	2	265 (12.9)[s]	257 (14.8)	203 (21.1)	273 (−40.1)	250 (+37.8)	215 (−38.8)	250 (−2.2)		225 (+2.7)
Adenine[c]	7	268 (9.9)[s]	260 (12.6)	205 (21.8)	270 (−27.5)	252 (+20.5)	210 (−65.1)			
	2	262 (13.3)		202 (19.5)	271 (−33.5)	249 (+28.0)	211 (−49.5)			
Uridine	7	261 (9.6)		205 (9.2)	260 (−9.0)		217 (+6.0)	268 (+9.7)	234 (−4.8)	217 (−4.8)
	2	261 (10.1)		204 (9.1)	260 (−8.9)		210 (+7.1)	269 (+8.5)	235 (−4.5)	218 (−4.6)
Uracil	7	258 (8.8)		203 (9.2)	255 (−8.5)		212 (+6.7)			
	2	258 (8.3)		203 (9.5)	255 (−9.0)		213 (+6.5)			
Thymidine	7	267 (8.0)		205 (9.6)	262 (−10.0)		220 (+8.5)	273 (+4.3)	235 (−3.3)	218 (−4.6)
	2	266 (10.3)		208 (10.2)	265 (−10.9)		216 (+10.8)	275 (+4.6)	240 (−3.4)	216 (−5.3)
Thymine	7	265 (7.8)		204 (9.7)	263 (−8.7)		215 (+8.7)			
	2	264 (8.0)		205 (10.0)	262 (−10.7)		214 (+8.2)			
α-TFU[d]	7	261 (10.0)		203 (11.1)	255 (−7.8)		215 (+6.0)	270 (+10.5)	239 (−4.5)	215 (+?)
β-TFU[d]	7	262 (8.9)		204 (9.6)	257 (−8.3)		213 (+7.8)	265 (−15.0)	238 (+2.0)	(NEG)
Cytidine[e]	7	270 (10.0)			270 (−4.9)			272 (+12.8)		220 (−11.0)
	2	280 (14.3)		211 (11.2)	280 (−8.1)		220 (+7.7)	283 (+9.2)		218 (−10.3)
Cytosine[f]	7	266 (6.8)			270 (−4.5)					
	2	275 (10.8)		209 (9.7)	270 (−9.5)		214 (+5.8)			

[a] Absorption, MCD, and CD measurements are reported from 200 to 300 nm. The letter s implies a shoulder. Other bands observed are given in subsequent footnotes. [b] MCD $[\theta]_M$ (related to a 49.5-kG magnetic field) and CD $[\theta]$ values are expressed in deg mol^{-1} cm^2. [c] MCD: 265 (−20.0)[s], see Figure 5. [d] α and β anomers of 2′-deoxy-5-(trifluoromethyl)uridine. [e] At pH 7, cytidine shows an absorption band, 229 (8.1), and a MCD band, 237 (−7.8), which can be attributed to an n–π* transition. [f] At pH 7, cytosine shows an absorption band, 225 (7.5)[s], and a MCD band, 235 (−5.7), of n–π* origin.

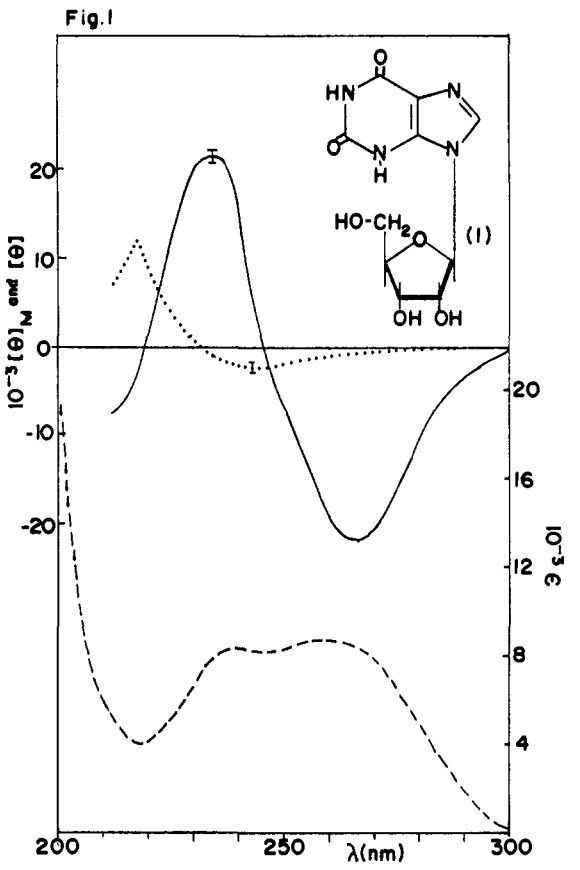


Figure 1. Absorption (- - -), MCD (——), and CD (· · ·) spectra of xanthosine (I) at pH 7.

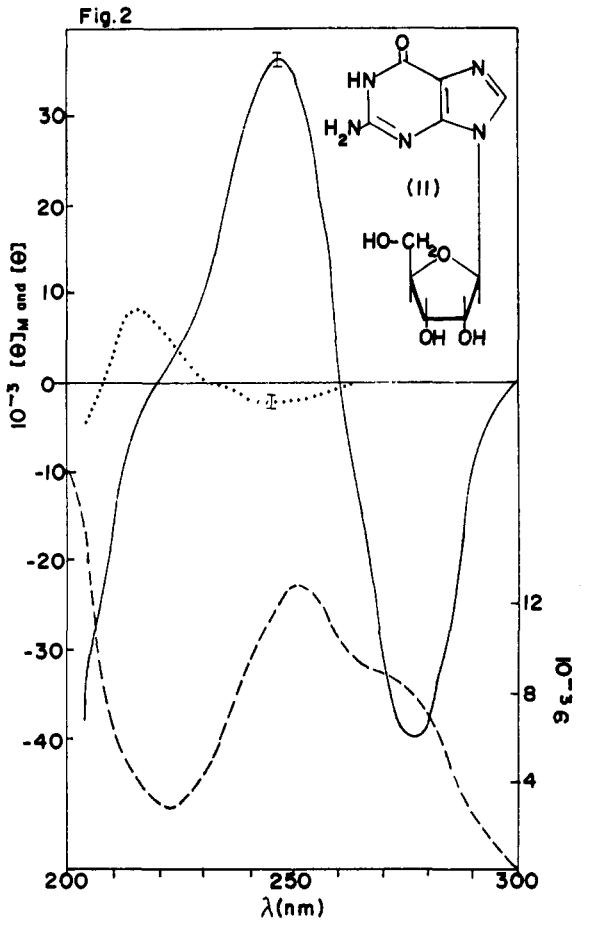


Figure 2. Absorption (- - -), MCD (——), and CD (· · ·) spectra of guanosine (II) at pH 7.

The nucleosides and bases were commercially available samples of high purity and were used without purification. The α and β anomers of 2′-deoxy-5-(trifluoromethyl)uridine were generously provided by Dr. L. Goodman.[10]

Results and Discussion

The biological importance of nucleic acids and the consequent necessity of understanding their secondary structure has motivated many investigations of the optical properties of their constituent components. These studies of the absorption spectra,[11,12] ORD,[13–15] and CD[15] of nucleosides and nucleotides as well as absorption studies of related purine and pyrimidine bases[16–19] can be expected to provide information of value in interpreting the spectral differences found in polynucleotides and their monomeric residues.[20]

The ultraviolet absorption spectra of the purine and pyrimidine chromophores of nucleic acids in aqueous solution,[11,16] acetonitrile,[12] methylcyclohexane,[12,17] trimethyl phosphate,[17] as well as in the vapor phase[18] show several absorption bands in the 185–300-nm region. Clark and Tinoco[17] have related the electronic transitions of these bases to those of benzene. The fact that these $\pi \rightarrow \pi^*$ transitions frequently overlap has, however, made their exact location uncertain. This situation has been greatly clarified, however, by the CD spectra of some pyrimidine nucleosides and purine cyclonucleosides recently published by Miles, Robins, and Eyring.[15] The increased resolution afforded by CD arises from the fact that, in most cases, the Cotton effects of adjacent absorption bands are of opposite sign.

The correlations derived from these studies can be summarized briefly as follows. The bands found in the 200-nm region are associated with the doubly degenerate $A_{1g} \rightarrow E_{1u}$ benzene transition. Because of the lower symmetry of these bases this transition is split and appears as two absorption bands. The $A_{1g} \rightarrow B_{1u}$ transition, which in benzene is found at about 203 nm, appears at about 240 nm in pyrimidine derivatives and at about 260 nm in purine derivatives. The 260-nm benzene band, the $A_{1g} \rightarrow B_{2u}$ transition, is found at about 260–270 and 260–280 nm in substituted pyrimidines and purines, respectively. Although the B_{2u} and B_{1u} bands are resolved in the guanine and xanthine derivatives, they overlap in the other members of both series and appear as a single unresolved band in the 250–280-nm region. The nature of this band is rendered more complex by the $n \rightarrow \pi^*$ transitions which are

(10) K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **31**, 1181 (1966).
(11) D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers*, **1**, 193 (1963).
(12) E. Charney and M. Gellert, *Biopolym., Symp.*, **1**, 469 (1964).
(13) T. L. V. Ulbricht, T. R. Emerson, and R. J. Swan, *Tetrahedron Lett.*, 1561 (1966).
(14) M. Ikehara, M. Kaneko, K. Muneyama, and H. Tanaka, *ibid.*, 3977 (1967).
(15) (a) D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U.S.*, **57**, 1138 (1967); (b) *J. Phys. Chem.*, **71**, 3931 (1967).
(16) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).
(17) L. B. Clark and I. Tinoco, *J. Amer. Chem. Soc.*, **87**, 11 (1965).
(18) L. B. Clark, G. G. Peschel, and I. Tinoco, *J. Phys. Chem.*, **69**, 3615 (1965).
(19) R. F. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255 (1963).
(20) I. Tinoco, *J. Amer. Chem. Soc.*, **82**, 4785 (1960).

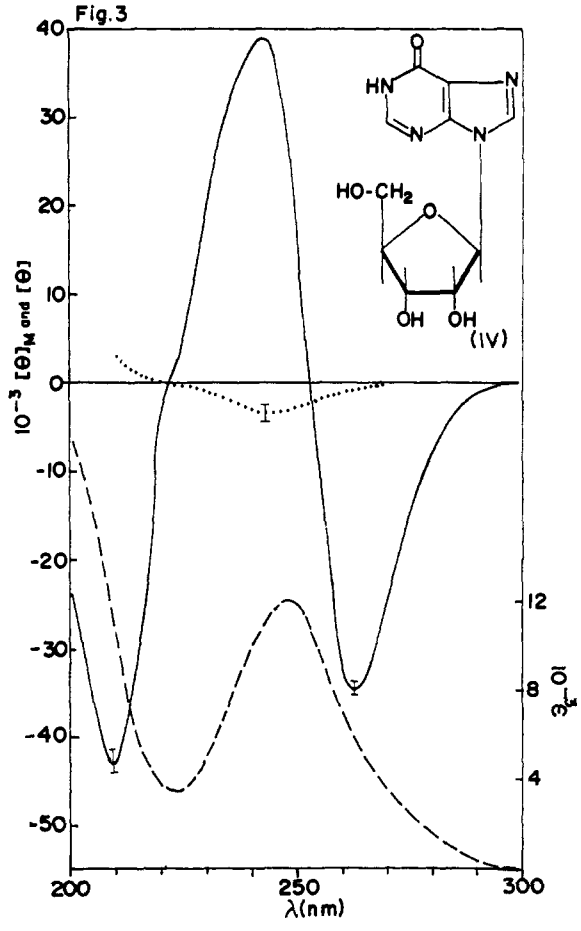


Figure 3. Absorption (- - -), MCD (——), and CD (· · ·) spectra of inosine (IV) at pH 7.

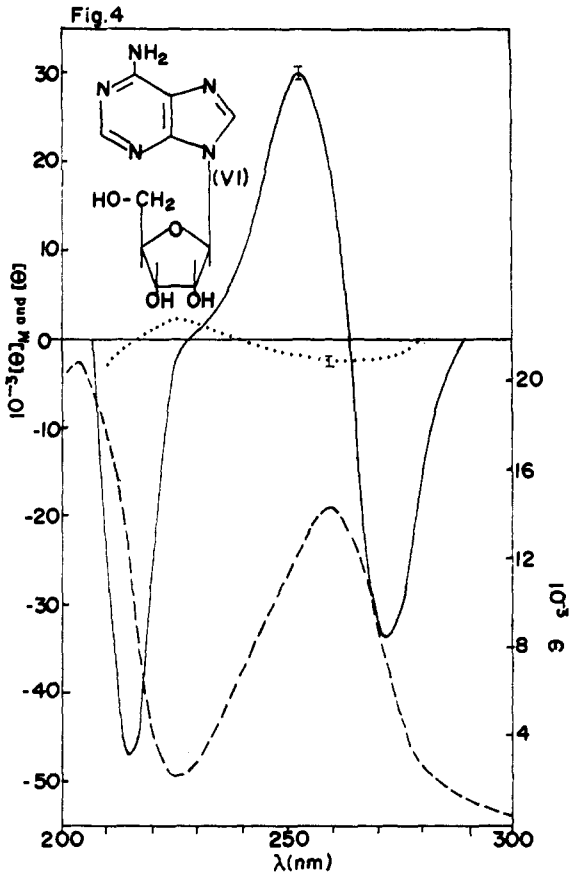


Figure 4. Absorption (- - -), MCD (——), and CD (· · ·) spectra of adenosine (VI) at pH 7.

also found in this region in some derivatives. It has been suggested[17] that the 235-nm absorption band found in cytosine derivatives in aqueous solution at pH 7 might be an n → π* transition. Recent ORD and CD measurements[15] of cytidine in aqueous solution at pH 7 and pH 1 lead to a similar conclusion. The removal of this band from the spectrum in acid solution is even more strikingly revealed by MCD (*vide infra*).

In their theoretical treatment of the Faraday effect, Buckingham and Stephens[5] derive quantitative expressions for the frequency dependence of MCD through absorption bands. Molar magnetic ellipticity ($[\theta]_M$) can be conveniently expressed in a single equation[9] as the summation of three separate contributions

$$[\theta]_M = -21.3458\frac{\nu^3\Gamma}{[\nu_0^2 - \nu^2]^2 + \nu^2\Gamma^2}A + \frac{4\nu_0\nu^3(\nu_0^2 - \nu^2)\Gamma}{h[(\nu_0^2 - \nu^2)^2 + \nu^2\Gamma^2]^2}\left(B + \frac{C}{kT}\right)$$

where ν stands for the frequency of the light, ν_0 for the frequency of maximum absorption, and Γ for the width at half-maximum absorption; ν, ν_0 and Γ are expressed in cm^{-1} and h is Planck's constant. Because of the different frequency-dependent coefficients for A on the one hand and for B and C on the other hand, two types of MCD curves may be observed for an isolated absorption band. Type A curves are S-shaped (like the familiar ORD Cotton effect) whereas type B (and C) are bell-shaped (like the Cotton effect observed in CD). Type B and C curves can be differentiated by the temperature dependence of the latter. The type of curve which will be observed, for an isolated absorption band, depends primarily on the degeneracy of the ground and excited states. The more complex features found in experimental curves, due to mixing of the two dispersion terms,[21] have been discussed in previous communications[7,22] from this laboratory. For molecules possessing nondegenerate ground and excited states one can expect to observe only the characteristic bell-shaped curve (*i.e.*, *B* terms). However, since *B* terms are due to magnetic coupling between excited states, adjacent *B* terms of opposite sign may result in a curve which superficially resembles the type A curve. Tinoco and Bush[23] have predicted that large magnetic Cotton effects will be observed in cases in which there are two perpendicular $\pi \rightarrow \pi^*$ transitions. The sign and magnitudes of the magnetic Cotton effects then depend on the relative polarizations of these transitions and the frequency separation between the transitions, and whether more than two excited states are coupled by the magnetic field.[1]

Purine Nucleosides and Bases. The absorption, CD, and MCD spectra, measured from 200 to 300 nm, of some purine nucleosides are presented in Figures

(21) P. J. Stephens, W. Suëtaka, and P. N. Schatz, *J. Chem. Phys.*, 44, 4592 (1966).

(22) B. Briat, D. A. Schooley, R. Records, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem. Soc.*, 89, 7062 (1967).

(23) I. Tinoco and C. A. Bush, *Biopolym., Symp.*, 1, 235 (1964). For discussion of MCD spectra of carbocyclic aromatic systems, see J. G. Foss and M. E. McCarville, *J. Amer. Chem. Soc.*, 89, 30 (1967).

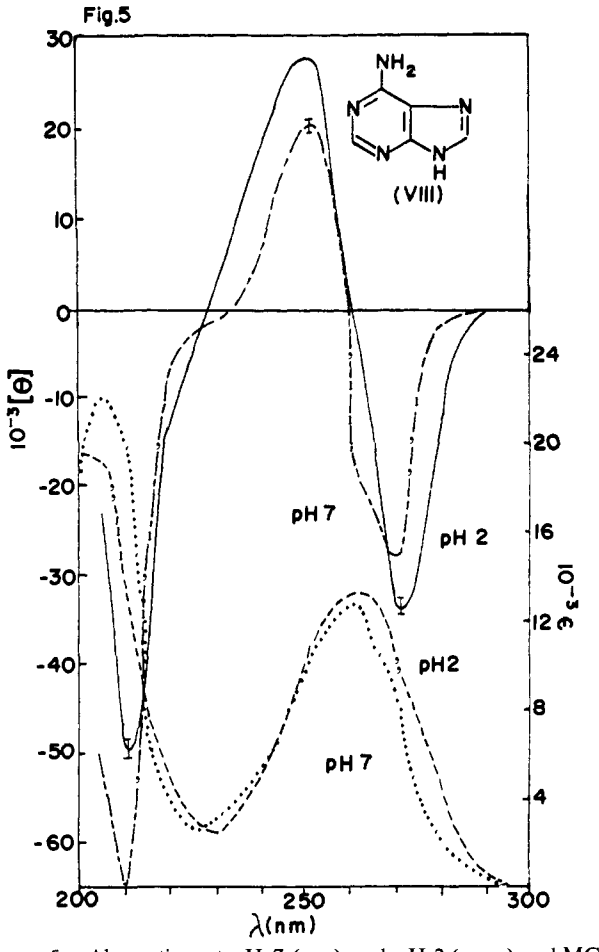


Figure 5. Absorption at pH 7 (· · ·) and pH 2 (– – –) and MCD spectra at pH 7 (— – —) and pH 2 (——) of adenine (VIII).

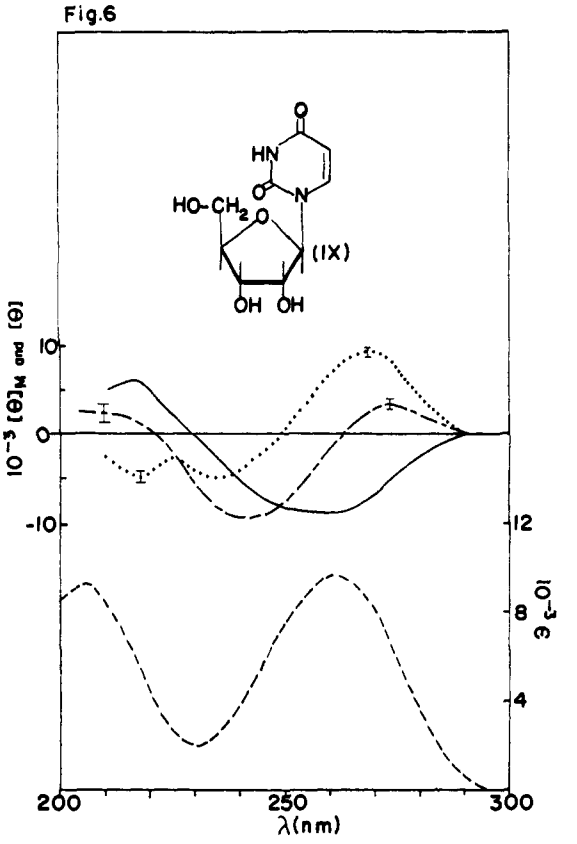


Figure 6. Absorption (– – –), *observed* MCD (– – –), MCD (——), and CD (· · ·) spectra of uridine (IX) at pH 7.

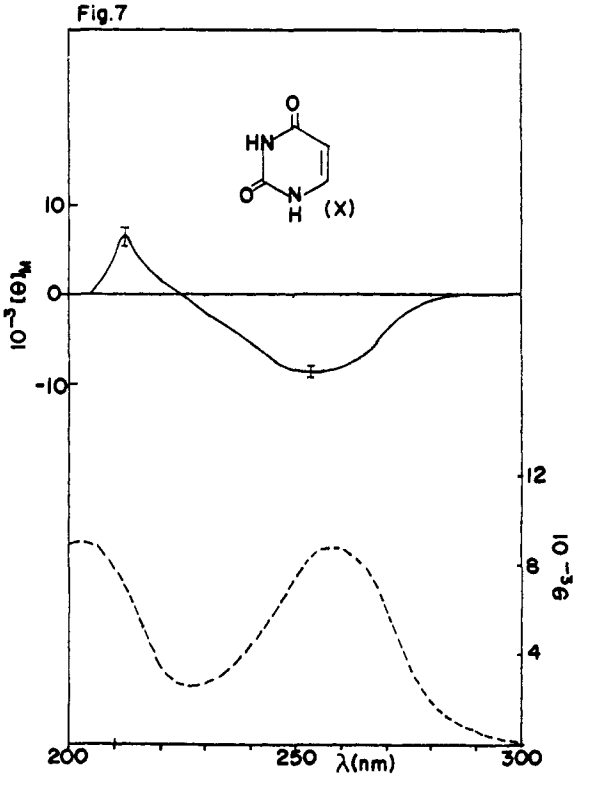


Figure 7. Absorption (– – –) and MCD (——) spectra of uracil (X) at pH 7.

1–4. The absorption and MCD spectra of the neutral and protonated forms of the base adenine are given in Figure 5. Relevant spectral data for the other purine bases are presented in Table I.

The absorption, CD, and MCD spectra of xanthosine (I) in water at pH 7 are given in Figure 1 and relevant spectral data at pH 2 are presented in Table I. The absorption curve of I shows two resolved bands at 258 and 241 nm. The failure of these bands to undergo a significant wavelength shift in acid solution suggests that they are of the π–π^* type and are probably derived from the benzene B_{2u} and B_{1u} transitions, respectively. The MCD curve exhibits a negative band (but positive value) (see ref 7 for nomenclature) at 267 nm and a positive band (negative value) at 233 nm. Although the MCD curve becomes negative below 219 nm, a negative maximum was not found above 200 nm. The correspondence of the MCD bands with the absorption bands and the absence of significant shifts in the MCD spectrum at pH 2 suggests that the B_{2u} and B_{1u} transitions are responsible for the magnetic Cotton effects observed. Furthermore the near-equality of the magnitudes, but opposite signs, of the two MCD bands suggest that they arise from the coupling of only two excited states.

The very weak Cotton effects observed in the CD spectra of ordinary purine nucleosides and the consequent uncertainty in the interpretation of ORD and CD measurements in this series are illustrated in Figure 1. The CD curve of xanthosine (I) in neutral solution shows a negative Cotton effect at about 245 nm. Below 230 nm the CD curve is positive and goes through a maximum at about 217 nm.

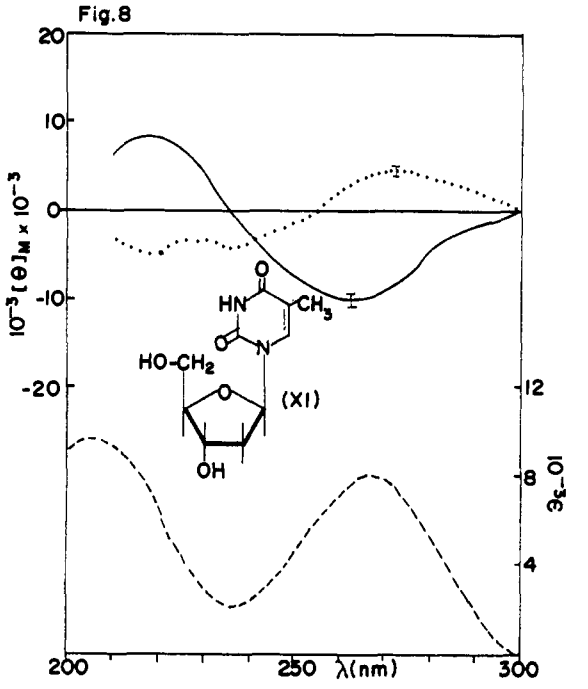


Figure 8. Absorption (– – –), MCD (——), and CD (· · ·) spectra of thymidine (XI) at pH 7.

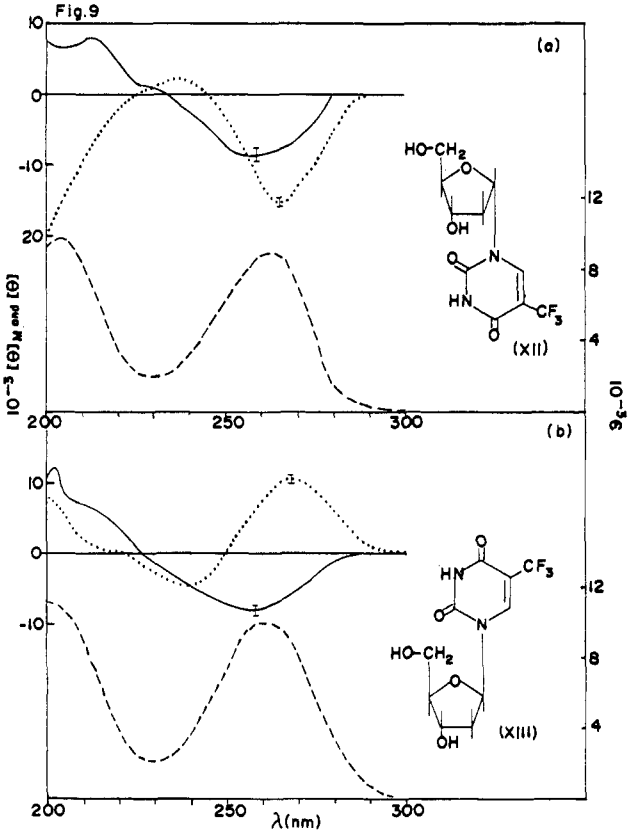


Figure 9. Absorption (– – –), MCD (——), and CD (· · ·) spectra of 2′-deoxy-5-(trifluoromethyl)uridine: a, α anomer (XII); b, β anomer (XIII); observed at pH 7.

The features observed in the absorption spectrum of the base xanthine (Table I) are rather different from those observed for xanthosine (I). The first absorption band is found at 266 nm and has approximately the same intensity (ϵ 8900) as the 258-nm band at xanthosine. The second band, however, now appears only as a shoulder at about 225 nm and is considerably diminished in intensity (ϵ 3700). These spectral changes are also reflected in the MCD spectrum of xanthine. The MCD of xanthine shows negative and positive maxima at 265 and 231 nm, respectively; however, the intensities as well as the distribution of intensities of these bands are considerably reduced.

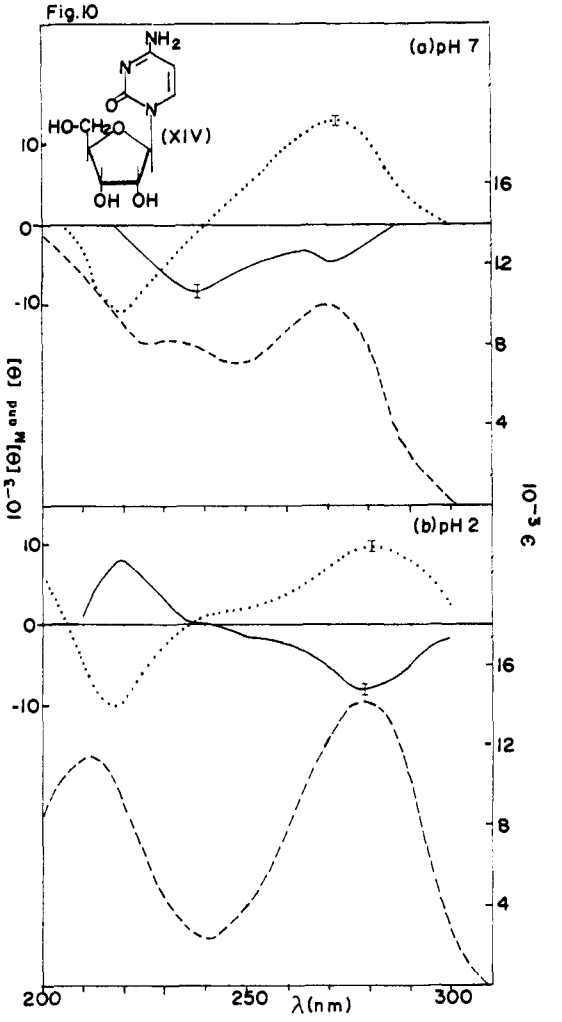
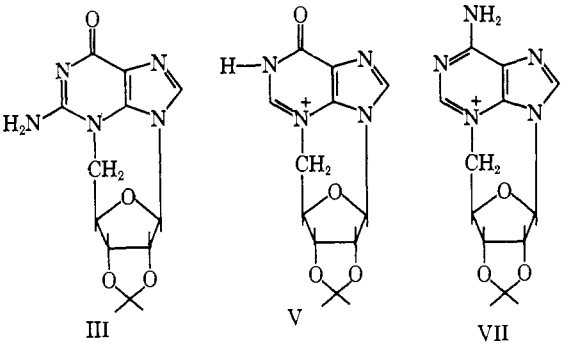


Figure 10. Absorption (– – –), MCD (——), and CD (· · ·) spectra of cytidine (XIV) at (a) pH 7 and (b) pH 2.

Figure 2 displays the absorption, CD, and MCD curves of guanosine (II) in aqueous solution at pH 7. The absorption curve shows an intense band at 253 nm and a rather well-defined shoulder at about 275 nm. A third absorption band at 188 nm, probably of E_{1u} origin, has been reported.[11] With the exception of a small red shift of 2–3 nm, the absorption spectrum of II is unchanged in either 0.01 or 1 *N* HCl whereas the CD and absorption spectra of 2′,3′-O-isopropylidene-3,5′-guanosine cyclonucleoside (III) were found to be very sensitive to protonation in acid solution.[15] The negative and positive MCD bands at 277 and 248 nm, respectively, are also only slightly red shifted in acid solution. Although the MCD curve becomes negative at shorter wavelengths, a third band was not found above 200 nm. Again, the close correlation of the first two MCD bands with the first two bands in

the absorption spectrum as well as the lack of a significant pH effect suggest that these bands are related to the B_{2u} and B_{1u} benzene transitions.



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The features of the CD curve of guanosine (II), indicated in Figure 2, are similar to those observed for xanthosine (I).

The absorption, CD, and MCD spectra of inosine (IV) in water at pH 7 are presented in Figure 3. The data observed at pH 2, given in Table I, show that the spectra of inosine are not sensitive to pH effects. The B_{2u} and B_{1u} absorption bands are at least partially resolved in the spectra of xanthosine (I) and guanosine (II) whereas inosine (IV) shows only a single band at 248 nm. The CD curve of 2′,3′-O-isopropylidene-3,5′-inosine cyclonucleoside *p*-toluenesulfonate[15] (V) provides clear evidence of the composite nature of the 260-nm absorption band of this cyclonucleoside because of the resolution afforded by the oppositely signed CD Cotton effects at 276 and 257 nm. It is evident in Figure 3 that the CD curve does not provide this information in the case of the ordinary purine nucleoside, inosine (IV). However, the presence of a negative MCD band at 263 nm and a positive one at 242 nm indicates that the 248-nm absorption band of inosine (IV) is also composite, as expected. Furthermore, the symmetry of the CD Cotton effects relative to the 260-nm absorption band of the cyclonucleoside closely corresponds to the symmetry observed for the MCD bands relative to the 248-nm absorption band of inosine suggesting that the intensity distribution of the B_{2u} and B_{1u} transitions is the same in both compounds. The negative MCD band at 210 nm is probably of E_{1u} origin. A positive MCD band, of nearly equal magnitude, is expected in the 190–200-nm region.

The features of the absorption, CD, and MCD spectra of adenosine (VI) at pH 7, displayed in Figure 4, are qualitatively similar to those of inosine (Figure 3). The 260-nm absorption band, unresolved in neutral solution, is slightly broadened at pH 2 with a shoulder appearing at about 265 nm. These small band shifts are also reflected in the MCD spectrum (Table I). In contrast to the situation existing for the cycloinosine derivative (V) discussed above, the CD curve of 2′,3′-O-isopropylidene-3,5′-adenosine cyclonucleoside *p*-toluenesulfonate (VII)[15] shows only a single broad negative CD band in the region between 220 and 280 nm which suggests that the Cotton effects of the B_{2u} and B_{1u} absorption bands have the same sign. MCD is, however, much more effective in revealing the composite nature of the 260-nm absorption band. Again, MCD Cotton effects observed at 272, 253, and 215 nm are related to the B_{2u}, B_{1u}, and E_{1u} benzene transitions, respectively.

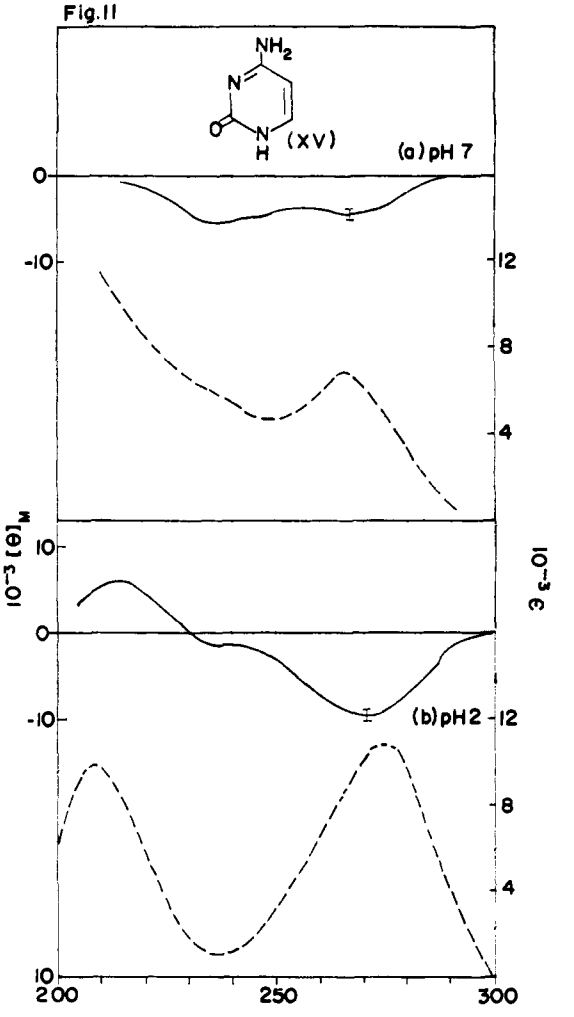

Figure 11. Absorption (– – –) and MCD (——) spectra of cytosine (XV) observed at (a) pH 7 and (b) pH 2.

For comparison, the MCD and absorption spectra of the base adenine (VIII) in water at pH 7 and at pH 2 are presented in Figure 5. With the exception of changes in magnitude, the basic features of the MCD spectra of adenine are the same as those found for the nucleoside, adenosine (Figure 4). However, it should be noted that the shoulder observed at about 268 nm in the absorption spectrum in neutral solution is also reflected by the suggestion of a shoulder at about 265 nm in the MCD spectrum and that this shoulder disappears from the MCD curve measured at pH 2. Since polarized absorption studies[19] indicate that the 260-nm band contains an $n-\pi^*$ transition, in addition to the two $\pi-\pi^*$ transitions, it is tempting to attribute this change in the MCD spectra as further evidence of an $n-\pi^*$ transition. More convincing evidence of an $n-\pi^*$ transition is found in the MCD spectrum of cytidine (XIV) (Figure 10).

Pyrimidine Nucleosides and Bases. The characteristic features typical of the MCD spectra of all the pyrimidine nucleosides are illustrated in Figure 6 in terms of uridine (IX). Its absorption spectrum exhibits maxima at 261 and 205 nm and the composite nature of these bands is evident from the CD Cotton effects at 268, 234, and 217 nm which have been correlated with the B_{2u}, B_{1u}, and E_{1u} transitions, respectively.[15] The *observed* MCD curve has also been included in

Figure 6 to illustrate the relative magnitudes of the CD and MCD bands. The observed MCD curve is, of course, the summation of both natural Cotton effects and the Cotton effects induced by the magnetic field. The true MCD curve is then the difference between the observed MCD curve and the CD curve obtained at zero field. For comparison, the MCD curve of uracil (X) is presented in Figure 7. The MCD curve of uridine (IX) shows a broad negative band with a maximum at about 260 nm and a positive band at about 217 nm. The latter is probably related to the benzene E_{1u} transition, and it is interesting to note that this band is positive in the pyrimidine nucleosides and negative in the purine derivatives. Considering the band resolution afforded by CD, it seems evident that MCD Cotton effects due to the B_{2u} and B_{1u} transitions have the same sign with the major contribution resulting from the B_{2u} transition. Furthermore, the pattern of the MCD Cotton effects as well as their greatly diminished amplitudes (compared to the purine nucleosides and bases) indicates that more than two nondegenerate excited states are coupled by the magnetic field.

The CD spectra of thymidine (XI) (Figure 8) and uridine (IX) (Figure 6) are qualitatively rather similar, the principal difference being that the amplitude of the first positive Cotton effect is approximately half the amplitude of the corresponding Cotton effect in uridine. The even smaller difference between the MCD spectra of these two compounds, in the same region, reflects again the relative ability of these two techniques in detecting small perturbations of the same chromophore. The differences noted in the MCD spectra in the E_{1u} region are probably due to the increased uncertainty attending measurements in this region.

The absorption, CD, and MCD spectra of the α (XII) and β (XIII) anomers of 2′-deoxy-5-(trifluoromethyl)uridine are presented in Figure 9. These spectra are presented to emphasize the point that, although MCD measurements of optically active compounds often provide information of analytical value the presence of a high external magnetic field *does not* necessarily result in the enhancement of natural Cotton effects, but rather that the two phenomena are of entirely different physical origin. The MCD curves of the two anomers are essentially superimposable whereas the CD curves show the mirror image relationship expected.

The dramatic differences in the absorption, CD, and MCD spectra of cytidine (XIV) observed at pH 7 and at pH 2 are shown in Figure 10. The absorption curve, measured at pH 7, reveals a broad band at about 270 nm and a weaker one at 229 nm. At pH 2, the first band is shifted to 280 nm; the 229-nm band, at pH 7, disappears; and the 200-nm band[11] is shifted to 211 nm. The CD curve, in water, shows a positive maximum at 272 nm and a negative maximum at 220 nm. The asymmetry of the CD curve in the 230–260-nm region suggests the presence of unresolved positive and negative Cotton effects in the first and second bands, respectively. In acid solution, the first CD maximum is shifted to 283 nm and the long wavelength tail of the 220-nm band disappears. This behavior has led to the conclusion that the Cotton effects of the B_{2u} and B_{1u} transitions are both positive, in contrast to the other pyrimidine nucleosides, and that the 230-nm absorption band is an $n-\pi^*$ transition.[15]

The spectral changes produced on protonation of cytidine are even more strikingly revealed by MCD. The MCD curve of cytidine, at pH 7, exhibits negative maxima at 270 and 237 nm. On protonation, the maximum of the first band is shifted to 280 nm, a shoulder appears at 250–255 nm, and a positive band is found at 220 nm. The pattern of the MCD bands is now the same as the pattern observed for the other pyrimidine nucleosides. It seems reasonable to attribute the shoulder at 250–255 nm to the B_{1u} transition which is now partially resolved due to the pronounced red shift of the B_{2u} transition in acid solution.

The absorption and MCD spectra (Figure 11) of the base cytosine (XV) were measured at pH 7 and at pH 2 in order to confirm the shape of the MCD curve obtained for cytidine since some uncertainty existed in the subtraction of the observed MCD curve from the CD curve. The changes in the spectra of cytidine, at pH 7 and pH 2, are also found, as expected, in the spectra of cytosine.

Summary

The present communication delineates the utility as well as some of the limitations of MCD as a spectroscopic technique. The distinct and characteristic difference in the MCD spectra of purine (Figures 1–5) and pyrimidine (Figures 6–11) nucleosides and bases provides the nucleic acid chemist with a technique, more certain than either absorption or circular dichroism spectroscopy, for distinguishing between these two types of residues—especially when relatively small amounts of sample are available.

Furthermore, MCD can, in favorable cases, be used to resolve overlapping absorption bands. Thus, in the purine nucleosides, the strong oppositely signed MCD Cotton effects provided convincing evidence for the composite nature of the $\pi-\pi^*$ transitions in this series. Such information is not available from the CD spectra of the ordinary purine nucleosides. This information is available from the CD spectra of the rigid purine cyclonucleosides; adenosine cyclonucleoside (VII) is, however, a notable exception, but at the expense of some modification in the chromophore. As already discussed MCD is not of particular value in the resolution of the absorption bands in the pyrimidine series. MCD did, however, provide clear evidence for an $n-\pi^*$ transition in the case of cytidine (XIV). The results of MCD measurements of nucleotides and polynucleotides will be published subsequently.

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