# Magnetic Circular Dichroism and Circular Dichroism of Riboflavin and Its Analogs*

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ABSTRACT: Magnetic circular dichroism and circular dichroism spectra of various riboflavin analogs have been measured. For the neutral, fully oxidized species, the results demonstrate that previously unobserved electronic energy levels exist on the short-wavelength sides of the 375- and 265-nm absorption bands, in agreement with molecular orbital calculations. The possibility that the longest wavelength transition (450 nm) also contains more than one electronic state is indicated.

The magnetic dichroism permits a number of conclusions to be drawn concerning the relative polarizations of the transitions. These are also in agreement with calculations. Solvent variations are seen to have a marked affect on the conformation of the riboflavin molecule, as evidenced by changes in the circular dichroism spectra which reflect the nature of the isoalloxazine ring–ribityl side-chain interaction. The longest wavelength absorption bands for both the riboflavin cation and the cation radical are shown to consist of two differently polarized transitions. A comparison of the magnetic circular dichroism spectra of the flavin and the 6,7-dimethylquinoxaline cations provides support for the assignment of the position of protonation of the flavins to the nitrogen atom in position one of the pyrimidine ring.

Circular dichroism in an absorption band of a molecule is defined as unequal absorptivity for right and left circularly polarized light, *i.e.*, $\epsilon_l - \epsilon_r \neq 0$. This inequality may be caused by either a permanent asymmetry in the electronic distribution which results from an optical transition to an excited state (intrinsically asymmetric chromophore) or by an environmentally induced asymmetry in the distribution (intrinsically symmetric chromophore). In quantum mechanical terms, this is equivalent to the statement that the asymmetry causes the electronic transition to have components of the electric and magnetic transition moments which are parallel or antiparallel (Mason, 1963). If the moments are parallel, the dichroism is positive; if the moments are antiparallel, it is negative.

In magnetic circular dichroism the inequality can be caused by an orbital Zeeman effect in which the magnetic field splits an orbitally degenerate ground or excited state into its differently polarized components (Buckingham and Stephens, 1966). It may also be the result of magnetic field-induced mixing of nondegenerate excited states of different electric dipole polarization.

It is thus evident that circular dichroism and magnetic circular dichroism spectra are capable of providing information concerning the electronic structures of molecules. One of the principal spectroscopic advantages of these techniques is the ability to see transitions which are "hidden" in the ordinary optical spectrum, either because of low transition probability or because of overlapping bands. Additionally, magnetic circular dichroism allows one to deduce relative polarizations of optical transitions. It is mainly with these facts in mind that we have carried out an investigation of the optical dichroism of riboflavin and its analogs. We were also interested in obtaining information about variations in the isoalloxazine ring–ribityl side-chain interaction as a function of solvent (through circular dichroism) and about the position of protonation of the isoalloxazine ring in acid solution (through magnetic circular dichroism).

The chromophoric group in the flavins (the isoalloxazine ring) is optically inactive and thus any natural circular dichroism must result from environmental perturbations, undoubtedly due to the optically active (but nonabsorbing in the visible and near-ultraviolet regions) ribityl side chains.[1] Also, the isoalloxazine ring has only twofold symmetry and thus cannot have orbitally degenerate ground or excited states. Thus, the magnetic circular dichroism must result from field-induced mixing of electronic states.

## Experimental Section

Riboflavin, FAD,[2] and FMN were obtained from Calbiochem and were used without further purification. 9-Methylisoalloxazine was synthesized as previously described (Guzzo and Tollin, 1964). 6,7-Dimethyl-

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[1] This is supported by our observation that no natural circular dichroism is present in isoalloxazine derivatives with a methyl side chain.

[2] Abbreviations are as listed in *Biochemistry 5*, 1445 (1966).

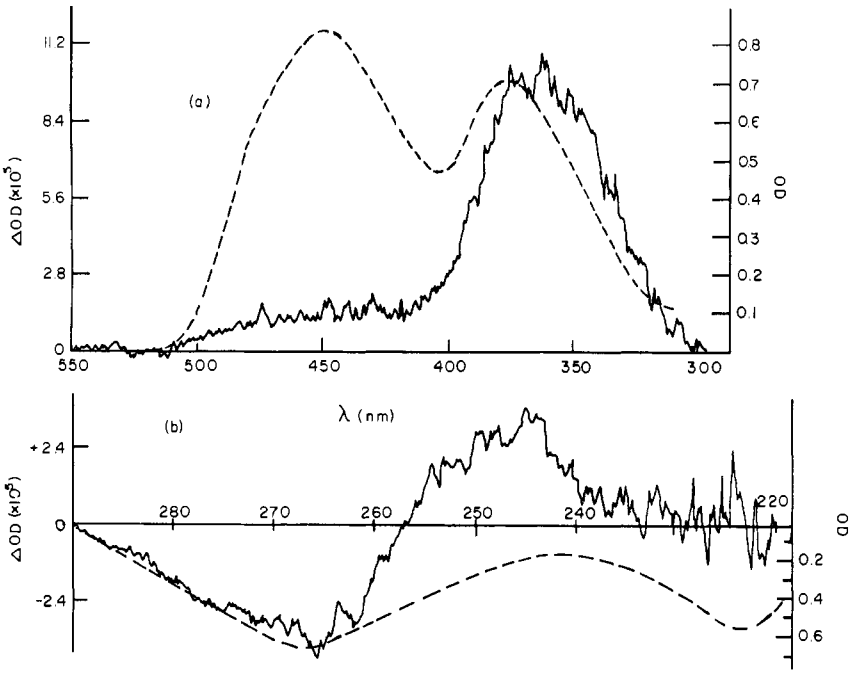



FIGURE 1: Magnetic circular dichroism spectra of FMN in 0.1 M phosphate buffer (pH 7.0); concentration in part a is $8.1 \times 10^{-5}$ M; concentration in part b is $2.5 \times 10^{-5}$ M. Dashed curve is optical absorption spectrum. Note that the optical spectrum has been inverted in part b. Magnetic field strength: 11.25 kgauss; 24 scans, 256 sec/scan in part a and 200 scans, 32 sec/scan in part b.

quinoxaline was from Aldrich and was twice recrystallized from water-ethanol. All solvents were reagent grade.

Circular dichroism and magnetic circular dichroism spectra were obtained using a spectrometer designed and built by Dratz (1966). This utilizes a xenon arc light source, a Cary Model 14 monochromator, Pockels cell modulation, phase-sensitive detection, and a solenoid electromagnet (for magnetic circular dichroism) with a maximum field strength of 11.25 kgauss (in one experiment, a magnet providing a field strength of 14.5 kgauss was used). Computer averaging is used to improve the signal-to-noise ratio (Nuclear Data ND 800 Enhancetron). A 1-cm path-length cell was used for all measurements. A positive dichroism corresponds to $\epsilon_l > \epsilon_r$. The number of scans used and the time per scan are indicated in the figure legends.

Optical absorption spectra were measured with a Cary Model 14 spectrophotometer. Flavin concentrations were determined from these spectra. In those figures in which optical spectra appear, the optical density ordinate is given on the right-hand side. In some cases, the optical spectra have been inverted so as to make comparisons with the circular dichroism or magnetic circular dichroism spectra more convenient.

## Results and Discussion

In Figure 1 is shown the magnetic circular dichroism spectrum of FMN in neutral solution. Four bands are evident, three of which correspond to easily observable transitions in the optical absorption spectrum. The positive magnetic circular dichroism band centered at about 245 nm does not have a clearly defined counterpart in the optical spectrum. However, self-consistent field calculations (Fox *et al.*, 1967) predict the existence of weak transitions in this region, which suggests that the band is due to a previously unobserved electronic energy level. Little or no magnetic optical activity is associated with the 222-nm band.

If we assume that only six states (the ground state and excited states at 450, 375, 265, 245, and 222 nm) are involved in producing the observed magnetic dichroism (*i.e.*, that no excited states with energies greater than 200 nm contribute), certain conclusions about the relative polarizations of the transitions can be drawn. For nondegenerate ground and excited states, the magnetic circular dichroism is determined by a triple product of integrals

$$\langle 1|\vec{u}|2\rangle \cdot \langle 0|\vec{r}|1\rangle \cdot \langle 0|\vec{r}|2\rangle$$

where 0, 1, and 2 are the wave functions for the ground state, the first excited state, and the excited state which mixes with 1 in the magnetic field, respectively; $\vec{u}$ and $\vec{r}$ are the magnetic and electric dipole moment operators. The numerical value of this triple product will be large when the 0→1 and 0→2 transitions are strong and have perpendicular polarizations (*i.e.*, the value will be proportional to $f_1 f_2 \cos\theta$, where $f_1$ and $f_2$ are the oscillator strengths for the 0→1 and 0→2 transitions and $\theta$ is the angle between the transition electric dipoles). Under these conditions, the dichroisms in the 0→1 and 0→2 bands will be large and *will have opposite signs*. In the case of the flavins, the relative signs of the observed magnetic circular dichroism bands suggests that the origin of the dichroism involves mixing of the 265-

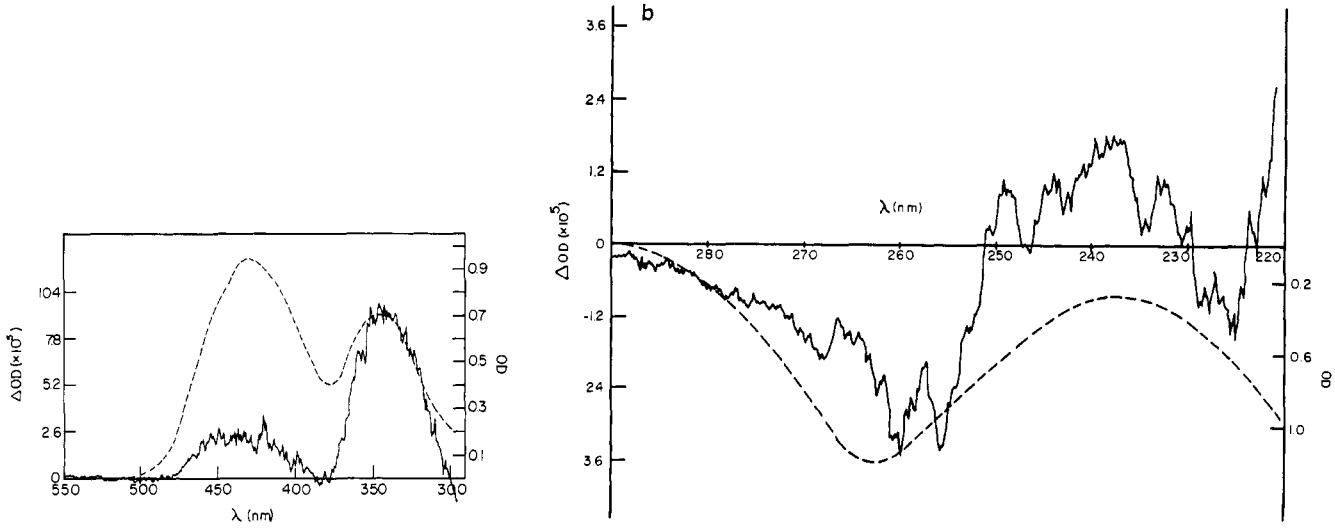



FIGURE 2: Magnetic circular dichroism spectrum of 9-methylisoalloxazine in water; concentration in part a is $1.4 \times 10^{-4}$ M; concentration in part b is $4.6 \times 10^{-5}$ M. Dashed curve is optical absorption spectrum. Note that the optical spectrum has been inverted in part b. Magnetic field strength: 11.25 kgauss; 20 scans, 256 sec/scan in part a and 80 scans, 32 sec/scan in part b.

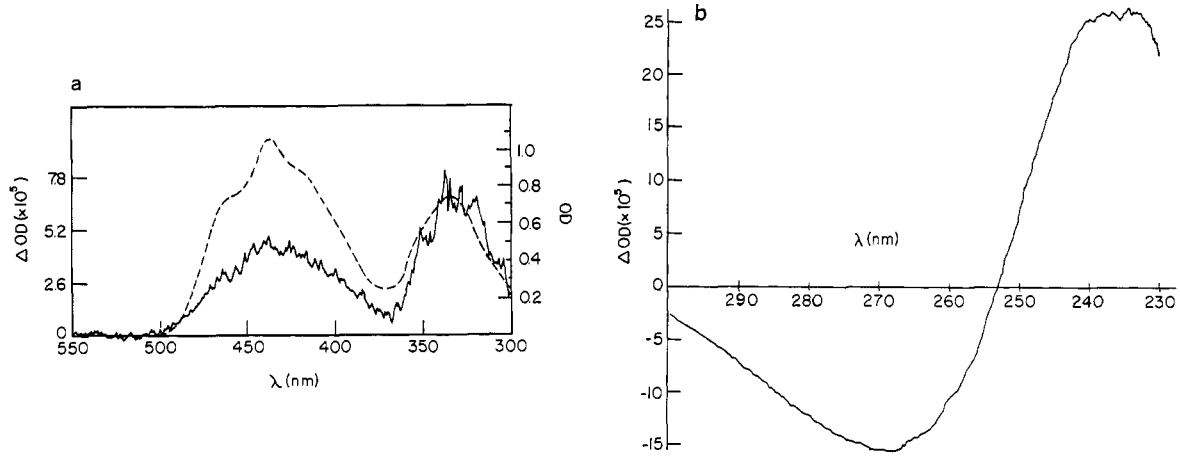



FIGURE 3: Magnetic circular dichroism spectrum of 9-methylisoalloxazine in chloroform; concentration in part a is $1 \times 10^{-4}$ M; concentration in part b is $4 \times 10^{-5}$ M. Dashed curve is optical absorption spectrum. Magnetic field strength: 11.25 kgauss in part a and 14.5 kgauss in part b; 20 scans, 256 sec/scan in part a and 100 scans, 32 sec/scan in part b.

nm level with the remaining states in the magnetic field (ignoring ground-state mixing). This implies that the transition dipoles are at an angle which is greater than zero. However, it does not necessarily follow from this that the transition moments of the two long-wavelength bands and the 245-nm band are all parallel to one another. This is because of the following: (a) it is possible for the moments to be nonparallel and yet not give rise to a dichroism because of a small value of the magnetic dipole matrix element; (b) because of the low symmetry of the flavin molecule, one cannot predict the precise direction of the transition moments with respect to a particular set of $X$ and $Y$ axes. This precludes a definitive statement concerning the relative polarizations of the 245-nm and long-wavelength bands, inasmuch as it is possible for them to be nonparallel to each other and still not parallel to the polarization of the 265-nm band.

The shoulders which appear on the 265-nm dichroism band (these show up even more clearly in some of the other spectra, *e.g.*, Figure 2a) are probably vibrational in origin. This is suggested by the energy separation of the bands.

It should be noted that the magnetic circular dichroism associated with the 375-nm band has its maximum shifted to shorter wavelengths than the optical absorption maximum. This could be due to one of two causes: (a) the 375-nm absorption is actually composed of two electronic transitions and the higher energy state gives rise to a more intense magnetic circular dichroism than does the lower energy state (perhaps because its polarization is more nearly orthogonal to that of the 265-nm transition); (b) the higher energy vibrational states of the 375-nm level cause distortions of the molecular framework which enhance the intensity of the magnetic circular dichroism. It is not possible to decide between these alternatives on the basis of the magnetic circular dichroism spectra. However, as we will see below, the circular dichroism spectra provide

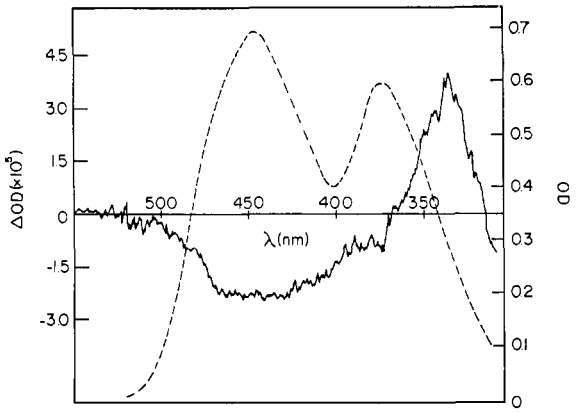



FIGURE 4: Circular dichroism spectrum of FMN ($6 \times 10^{-5}$ M) in 0.1 M phosphate buffer (pH 7.0). Ten scans, 256 sec/scan. Dashed curve is optical absorption spectrum.

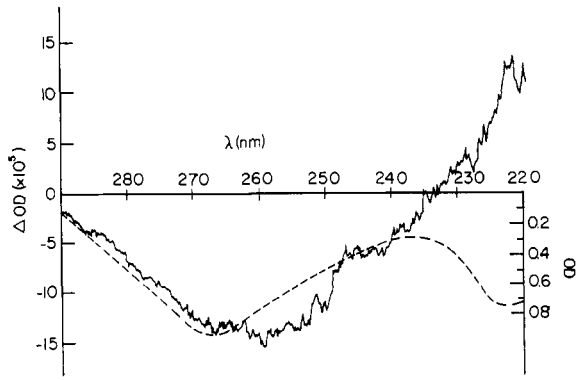



FIGURE 5: Circular dichroism spectrum of riboflavin ($3 \times 10^{-5}$ M) in 0.1 M phosphate buffer (pH 7.0). Fifty scans, 32 sec/scan. Dashed curve is optical absorption spectrum; note that this has been inverted.

additional information which allows a decision to be made.

It is of interest to compare these results with the predictions made by molecular orbital theory. SCF calculations show that a weak transition exists on the short-wavelength side of the 375-nm band (Fox *et al.*, 1967). This provides support for an electronic interpretation of the shift in the maximum of the magnetic circular dichroism spectrum in that region. Furthermore, the predicted angle between the transition dipoles is 12° for the 265-nm and the strong 375-nm absorption bands, whereas the angle is 94° between the dipoles of the 265-nm absorption band and the weak higher energy transition in the 375-nm region (L. S. Forster, unpublished results).[3] This is also consistent with such an interpretation, as one would expect a more intense magnetic circular dichroism absorption when the angle between the dipoles is large. The calculations also show that the angle between the two longest wavelength transition dipoles is 27° (L. S. Forster).[3] This is in good agreement with fluorescence polarization data (Weber, 1966) which indicate a 30° angle between

[3] The methods used in the calculation of these angles are described in Fox *et al.* (1967).

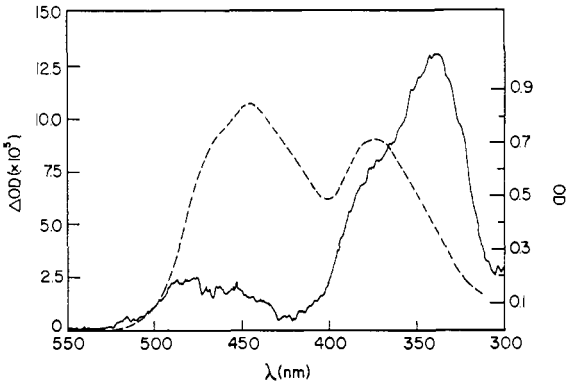



FIGURE 6: Circular dichroism spectrum of riboflavin ($6 \times 10^{-5}$ M) in water. Twelve scans, 256 sec/scan. Dashed curve is optical absorption spectrum.

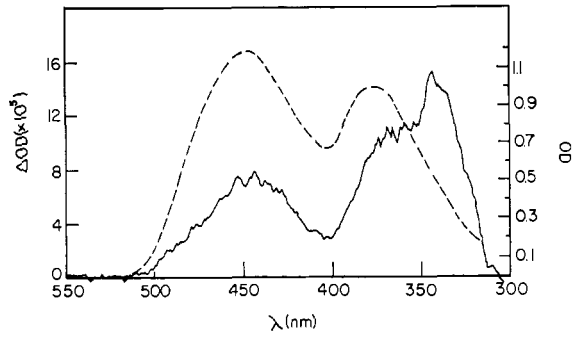



FIGURE 7: Circular dichroism spectrum of FMN ($1.1 \times 10^{-4}$ M) in Tris buffer (0.05 M) (pH 8.0). Four scans, 256 sec/scan. Buffer contains $10^{-3}$ M mercaptoethanol. Dashed curve is optical absorption spectrum.

these moments. They also suggest that the lack of an observable magnetic circular dichroism absorption due to mixing of these two states in the magnetic field is a consequence of a small magnetic dipole matrix element. The angle which is predicted between the transition moments of the 245- and the 265-nm absorptions is 65°. This accounts very nicely for the observed dichroism.

The magnetic circular dichroism spectrum of riboflavin is essentially identical with that of FMN. FAD has a magnetic circular dichroism spectrum similar to these in the visible region (except that the displacement of the 375-nm band is not as marked); the spectrum in the ultraviolet region is approximately the sum of the FMN and AMP spectra.

The possibility exists that the 245-nm band, which appears in the magnetic circular dichroism spectrum but not in the optical spectrum, is an $n-\pi^*$ transition. Such transitions are known to have small absorptivities. To provide evidence on this point, the magnetic circular dichroism spectra of 9-methylisoalloxazine in water and chloroform were compared. These are shown in Figures 2 and 3 (note the marked structure in the 260-nm band and the 240-nm band in water and the lack of such structure in the chloroform spectrum). It is seen that no appreciable solvent shift occurs in the 245-nm band, as would be expected if it were due to an $n-\pi^*$ transition. Thus it is probably not of this type. It should

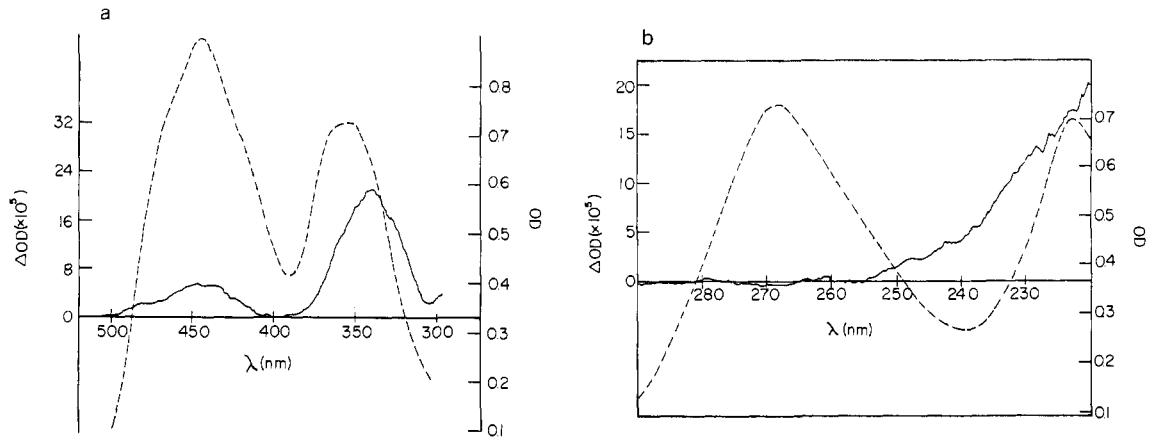



FIGURE 8: Circular dichroism spectrum of riboflavin in methanol; concentration in part a is $6 \times 10^{-5}$ M; concentration in part b is $1.7 \times 10^{-5}$ M. Dashed curve is optical absorption spectrum. Four scans, 256 sec/scan in part a and 50 scans, 32 sec/scan in part b.

also be noted that, as with FAD, no appreciable displacement of the second visible band occurs. We will come back to this point later.

In Figure 4 is shown the circular dichroism spectrum of FMN in 0.1 M phosphate buffer (pH 7.0) (riboflavin gives an essentially identical spectrum). The longest wavelength band is seen to give rise to a negative dichroism. The dichroism in the 300–400-nm region can best be interpreted in terms of two absorptions, a negative band superimposed upon a positive one (at shorter wavelengths).

For strong absorptions in which the vibronic contribution to intensity is small, one expects on theoretical grounds (Moffitt and Moscowitz, 1959) that the optical and circular dichroism spectra will have the same shape. This is not necessarily so for weak transitions (Moffitt and Moscowitz, 1959; Weigang, 1965). Thus, if we assume that the rather strong ($\epsilon \sim 10^4$) 375-nm band in the flavins has little or no vibronically induced intensity, we must conclude that two distinct electronic states are contributing to the dichroism in the 300–400-nm region. This would be consistent with the magnetic circular dichroism results and with the SCF calculations (see above). One can rationalize the lack of such displacement in the FAD and 9-methylisoalloxazine magnetic circular dichroism spectra in terms of a shifting of energy levels in this region so that they more nearly coincide. This would effectively mask the displacement.

The circular dichroism spectrum of riboflavin in the ultraviolet region is shown in Figure 5 (FMN gives an essentially identical spectrum). Four bands can be distinguished: three negative dichroisms at aproximately 265, 255, and 240 nm, and a positive band which is probably associated with the 222-nm absorption (although the data in this region are not particularly reliable). The two middle bands are undoubtedly related to the absorption seen in the magnetic circular dichroism spectrum in this region. It is noteworthy that the SCF calculations (Fox *et al.*, 1967) predict the existence of three weak transitions in this spectral region. Thus, these dichroism bands are probably electronic in nature. Also, they are too widely separated in energy to be vibronic overtones of the 265-nm band.

In order to see whether variations in solvent affect the conformation of the flavin molecule, circular dichroism spectra were obtained in water, Tris buffer, and methanol. These are shown in Figures 6–8. The circular dichroism spectrum of riboflavin in water (pH 5) (FMN gives an essentially identical spectrum) is quite different from that in phosphate buffer (Figure 4) in-

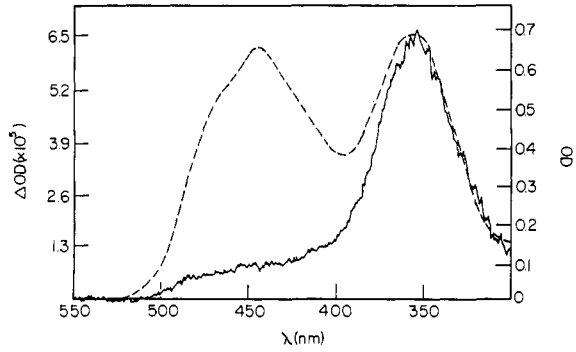



FIGURE 9: Magnetic circular dichroism spectrum of riboflavin ($6 \times 10^{-5}$ M) in 0.01 M sodium hydroxide. Dashed curve is optical absorption spectrum. Magnetic field strength: 11.25 kgauss; 20 scans, 256 sec/scan.

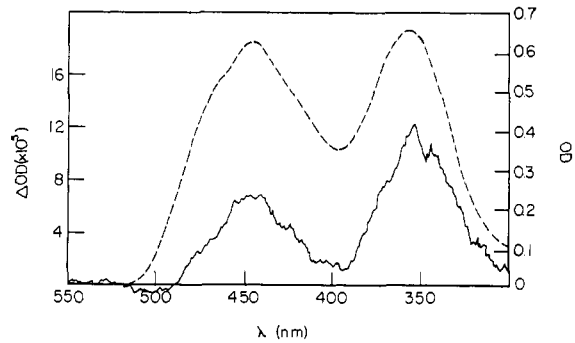



FIGURE 10: Circular dichroism spectrum of riboflavin ($6 \times 10^{-5}$ M) in 0.01 M sodium hydroxide. Four scans, 256 sec/scan. Dashed curve is optical absorption spectrum.

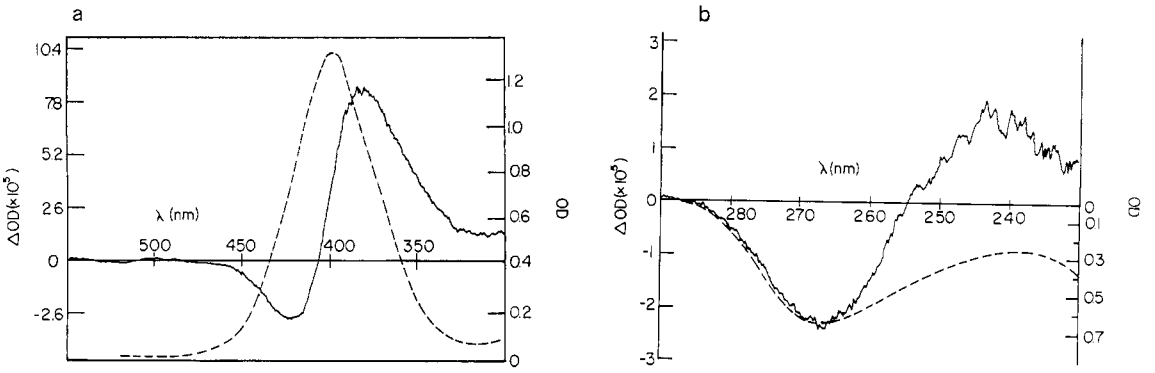



FIGURE 11: Magnetic circular dichroism spectrum of riboflavin in 6 M HCl. Concentration in part a is $7 \times 10^{-5}$ M; concentration in part b is $2.1 \times 10^{-5}$ M. Dashed curve is optical absorption spectrum. Note that the optical spectrum has been inverted in part b. Magnetic field strength: 11.25 kgauss; 20 scans, 256 sec/scan in part a and 200 scans, 32 sec/scan in part b.

TABLE I: Ellipticity Values for Flavin Analogs.[a]

| Compound | Solvent | λ (nm) | $[\theta]$ ($M^{-1}$ $cm^{-1}$) | $[\theta]$mag ($M^{-1}$ $cm^{-1}$ $gauss^{-1}$) |
|---|---|---|---|---|
| FMN | Phosphate (0.1 M), pH 7.0 | 450 | −1,380 | $5.1 \times 10^{-2}$ |
| FMN | Phosphate (0.1 M), pH 7.0 | 375 | −6,600 | $3.6 \times 10^{-1}$ |
| FMN | Phosphate (0.1 M), pH 7.0 | 265 | −1,430 | $-3.1 \times 10^{-2}$ |
| 9-Methylisoalloxazine | Water | 430 | | $5.0 \times 10^{-2}$ |
| 9-Methylisoalloxazine | Water | 345 | | $1.9 \times 10^{-1}$ |
| 9-Methylisoalloxazine | Water | 260 | | $-2.3 \times 10^{-1}$ |
| 9-Methylisoalloxazine | $CHCl_3$ | 440 | | $1.2 \times 10^{-2}$ |
| 9-Methylisoalloxazine | $CHCl_3$ | 335 | | $1.9 \times 10^{-2}$ |
| 9-Methylisoalloxazine | $CHCl_3$ | 268 | | $-9.1 \times 10^{-1}$ |
| Riboflavin | Water | 450 | 1,100 | |
| Riboflavin | Water | 375 | 4,400 | |
| FMN | Tris buffer (pH 8.0) | 450 | 2,250 | |
| FMN | Tris buffer (pH 8.0) | 375 | 3,000 | |
| Riboflavin | Methanol | 450 | 3,080 | |
| Riboflavin | Methanol | 355 | 8,800 | |
| Riboflavin | Methanol | 268 | 0 | |
| Riboflavin | NaOH (0.01 M) | 450 | 3,850 | $4.4 \times 10^{-2}$ |
| Riboflavin | NaOH (0.01 M) | 355 | 6,600 | $3.2 \times 10^{-1}$ |
| Riboflavin | HCl (6 N) | 425 | | $-1.3 \times 10^{-2}$ |
| Riboflavin | HCl (6 N) | 380 | | $3.6 \times 10^{-2}$ |
| Riboflavin | HCl (6 N) | 265 | | $-3.4 \times 10^{-2}$ |
| Riboflavin | HCl (6 N) | 395 | 20,900 | |
| 6,7-Dimethylquinoxaline | HCl (6 N) | 370 | | $-3.6 \times 10^{-2}$ |
| 6,7-Dimethylquinoxaline | HCl (6 N) | 335 | | $8.1 \times 10^{-2}$ |
| Riboflavin | HI (47%) | 545 | | $-1.5 \times 10^{-2}$ |
| Riboflavin | HI (47%) | 470 | | $9.8 \times 10^{-3}$ |

[a] $[\theta] = 3300\Delta\epsilon$; $[\theta]\text{mag} = 3300\Delta\epsilon/H$, where $H$ = magnetic field strength and $\Delta\epsilon = \epsilon_l - \epsilon_r = \Delta OD/Cl$.

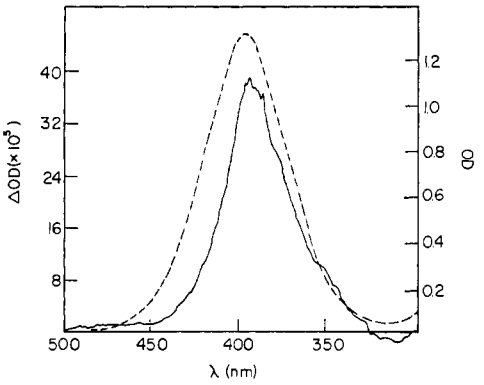



FIGURE 12: Circular dichroism spectrum of riboflavin ($6 \times 10^{-5}$ M) in 6 M HCl. Six scans, 256 sec/scan. Dashed curve is optical spectrum.

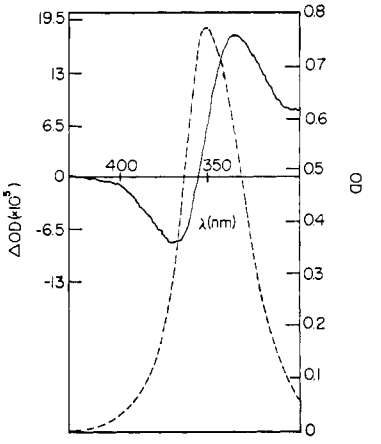



FIGURE 13: Magnetic circular dichroism spectrum of 6,7-dimethylquinoxaline ($6.5 \times 10^{-5}$ M) in 6 M HCl. Dashed curve is optical absorption spectrum. An identical magnetic circular dichroism spectrum is obtained in ethanolic HCl (absolute ethanol–12 N HCl, 1:1,). Magnetic field strength: 11.25 kgauss; 20 scans, 128 sec/scan.

dicating that the conformation of the flavin molecule is radically altered. The negative circular dichroism band in the 450-nm region is inverted in sign, as is the band around 375 nm. Note also the displacement of the maximum of the 450-nm circular dichroism band to longer wavelengths than the absorption (this is actually slightly apparent in the phosphate buffer spectrum). Inasmuch as the 450-nm absorption is intense ($\epsilon \sim 10^4$), this latter effect suggests the presence of a second electronic transition in this region. However, SCF calculations predict only a single energy level (Fox *et al.*, 1967). Thus, we must leave open the possibility that vibronic effects are important in this transition, even though it is strongly allowed. Perhaps circular dichroism measurements at low temperatures would be able to resolve the vibronic structure and thus provide a more definitive answer. This would be worthwhile inasmuch as the possibility of a second transition lying within the 450-nm band has important implications for the chemistry and photochemistry of the flavins.

The circular dichroism spectrum of FMN in Tris buffer (Figure 7) is quite similar to the water spectrum. The principal differences are the lack of displacement of the 450-nm band maximum and the relative intensities. Thus, the conformations are similar but not identical in these two solvents, and in both solvents the flavin molecular conformation is different than it is in phosphate buffer. One can rationalize the lack of displacement either in terms of a shifting of the energy levels or of an effect on the molecular vibrations.

Flash photolysis studies of the photobleaching of flavins in alcoholic solvents (Green and Tollin, 1968) have indicated that the interaction between the ribityl side chain and the isoalloxazine ring is stronger in alcohols than in phosphate buffer. The circular dichroism spectrum of riboflavin in methanol (Figure 8) suggests that the interaction is at least different in methanol than it is in water. Thus, the rotatory strength is considerably greater in methanol (three to four times). Also, the conformations appear to be quite different. The sign of the 450-nm band is inverted in methanol (also note the lack of displacement); the 375-nm transition and the 268-nm transition are both optically inactive in methanol whereas they are active in phosphate buffer.

The magnetic circular dichroism and circular dichroism spectra of the riboflavin anion are shown in Figures 9 and 10. The magnetic circular dichroism spectrum is similar to that obtained with the neutral flavin species. The circular dichroism spectrum is somewhat different, particularly in the 300–400-nm region. This is probably a reflection of the fact that the energy levels in this range are significantly altered in going to the anion.

The magnetic circular dichroism spectrum of the riboflavin cation is shown in Figure 11. Of particular interest is the double-dichroism band observed for the longest wavelength transition. The optical absorption is quite symmetrical in this region. This suggests that two electronic transitions, of different polarization, are located within this band.[4] No reliable SCF calculations exist for this species.

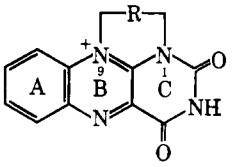
The magnetic circular dichroism spectrum in the ultraviolet region is quite similar to that of the neutral species, as is the optical spectrum. The circular dichroism spectrum of the flavin cation in the visible region (Figure 12) is consistent with the magnetic circular dichroism spectrum. Thus, one observes a displacement of the maximum to shorter wavelengths indicating that the higher energy transition is more optically active than the lower energy one. Little or no circular dichroism can be observed in the ultraviolet region.

The position of protonation of the isoalloxazine ring in solution is somewhat uncertain.[5] The best evidence

[4] It is also possible that the additional band represents a vibronic (*i.e.*, symmetry forbidden, but vibrationally allowed) component of the same electronic transition, although the high absorptivity of the optical absorption makes this somewhat unlikely.

[5] Recent X-ray diffraction studies of crystals of riboflavin hydrobromide (Tanaka *et al.*, 1967) indicate that protonation occurs at N-1.

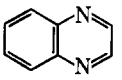
(Dudley *et al.*, 1964) comes from optical spectra of flavins in which an alkyl bridge is placed between N-1 and N-9 (I). These are similar to the flavin cation



I

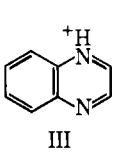
spectra, indicating that the protonation may occur at N-1.

The first two rings of the isoalloxazine structure (rings A and B) are analogous to quinoxaline (II).

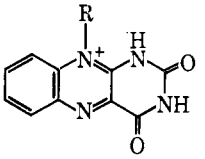


II

A protonated quinoxaline (III) would be electronically similar to the flavin cation, if the latter were protonated at N-1 (IV). Thus, the magnetic circular dichroism



III



IV

spectrum of 6,7-dimethylquinoxaline in HCl is of interest, and this is shown in Figure 13. Measurements of the optical absorption in neutral and acidic solutions were used to show the presence of the cationic species. The neutral molecule has an absorption maximum at about 323 nm and a shoulder at about 335 nm; the cation gives a single symmetrical absorption at about 350 nm. It is apparent that the magnetic circular dichroism spectrum of the quinoxaline cation is similar in shape to that observed for the flavin cation, although shifted to shorter wavelengths, thus confirming the assignment of the position of protonation. The magnetic circular dichroism in the ultraviolet region, although weak, is also like that of the flavin cation. The similarity in the optical spectra of the two cations is also striking.

In Figure 14 is shown the magnetic circular dichroism of the flavin cation radical. This species, like the cation, gives a double band in the long-wavelength transition, again indicating a degeneracy of the type mentioned above. Only a very weak circular dichroism spectrum could be obtained. In Table I, ellipticity values for

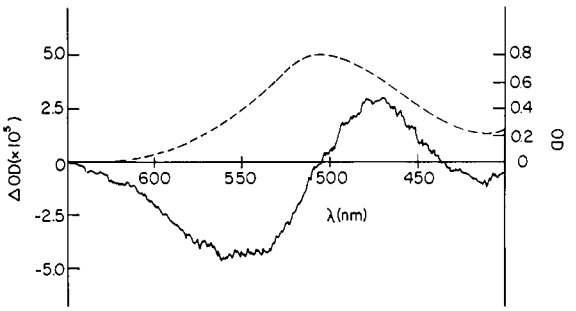



FIGURE 14: Magnetic circular dichroism spectrum of flavin cation radical ($9 \times 10^{-5}$ M). This was prepared by dissolving riboflavin in 47% HI (*cf.* Fleischman and Tollin, 1965). Dashed curve is optical absorption spectrum. Magnetic field strength: 11.25 kgauss; 12 scans, 256 sec/scan.

some of the flavin magnetic circular dichroism and circular dichroism transitions are given.

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