The Polarized Fluorescence Spectra of Some Naturally Occurring Corrins[1a]

A. J. Thomson[1b]

Contribution from the Department of Biophysics, Michigan State University, East Lansing, Michigan 48823. *Received September 3, 1968*

Abstract: The fluorescence spectrum, at room temperature and 77°K, has been detected from a naturally occurring red metal-free corrin compound. The low-temperature fluorescence spectrum has a highly distorted envelope suggesting the production in the excited state of a new species. The polarization ratios of the excitation spectrum of fluorescence give, for the first time, the relative directions of the four lowest energy transitions of the corrin chromophore. Good agreement is found with theoretical models. Comparison of the polarization ratios with the circular dichroism spectrum, reported here, shows that the relative signs of the circular dichroism bands are determined by the directions of the *electric* dipole transition moments. The fluorescence spectra from two yellow derivatives, formed on treatment of the red corrin with base, are recorded. The vibrational envelopes of the spectra and the polarization ratios of excitation suggest that yellow product I, formed at pH 11, is an unsymmetrical cyanine and that yellow product II, formed at pH 13, is a polyene-like pigment.

Despite wide investigations of the spectral properties of vitamin B_{12} and its derivatives[2] no exhaustive measurements have been made of the polarizations of the many bands in the electronic spectrum of the corrin ring. Although such information would be helpful in testing theoretical descriptions of the chromophore the data have proved difficult to obtain. Thus Kuhn[3] embedded vitamin B_{12} in a plastic matrix which was then stretched to produce a partially oriented specimen. A small dichroism was seen in the absorption spectrum. The absolute polarizations of the spectrum of a synthetic corrin derivative have been determined by polarized single-crystal reflection spectroscopy.[4] Recent measurements of the magnetic circular dichroism (MCD) spectrum of some synthetic corrin compounds have led to the suggestion that the signs of the bands in the MCD spectrum indicate the relative polarizations of the electronic transitions.[5] However, with the discovery[6] of a naturally occurring metal-free corrin compound the possibility arose of detecting fluorescence from the metal-free chromophore and of carrying out emission polarization studies to determine the *relative* polarizations of the absorption bands.

No emission has been detected from the corrin ring when it contains cobalt. But by analogy with the porphyrins and metalloporphyrins[7] it is expected that the metal-free corrin ring will yield a readily detectable emission. This report presents the emission spectrum of the naturally occurring metal-free corrin and the relative polarizations of the excitation spectrum.

It was shown by Toohey[6] that the metal-free corrin compound, which will be called red corrin, has an unusual pH-dependent spectrum. Raising the pH to 11 or treating with acid or light converts red corrin to a yellow product, designated yellow product I (YPI). Further treatment with base results in a third derivative, called yellow product II (YPII). The fluorescence spectra of YPI and II have been detected and the relative polarizations of the excitation spectra determined in an attempt to clarify the nature of these species and to aid in the interpretation of their spectra.

The circular dichroism spectrum of red corrin is reported. A comparison is made with the extensive circular dichroism measurements on vitamin B_{12} and its derivatives.[2]

Experimental Section

Materials. Metal-free red corrin (450 μg) was generously supplied by Dr. John I. Toohey of the University of California at Berkeley. All manipulations with this material were carried out under dim red light and it was stored as a concentrated aqueous solution at −20°. YPI was obtained either by photolysis in daylight or by raising the pH to 11 with addition of 1 *N* NaOH. Further addition of NaOH produced YPII.[8] For emission studies at 77°K good glasses could be produced by the addition to 50% by volume of glycerol (Matheson Spectroquality).

Measurements. Initial emission studies were carried out with an Aminco-Bowman spectrofluorimeter fitted with a 1P28 or a RCA 7102 red-sensitive photomultiplier. Subsequent measurements have been made with a sensitive fluorimeter constructed in this laboratory. A high intensity of monochromatic exciting radiation is supplied by a 500-W xenon arc and a 500-mm Bausch and Lomb monochromator fitted with a large grating (102 × 102 mm) blazed at 0.3 μ. Emission is detected by a 750-mm Engis spectrometer and a selected photomultiplier (EMI 9558Q). The spectrometer has curved slits 50 mm high and a large grating (102 × 102 mm) blazed at 0.5 μ. Samples were contained in a silica capillary that could be immersed in liquid nitrogen.

Polarized excitation and emission spectra were measured with two polarizing filters, Polacoat 105 UV film (Polacoat Incorporated, Cincinnati) deposited on spectrosil silica. One filter was placed between the exciting light and sample, the other immediately before the analyzing monochromator. The method of Azumi and McGlynn,[9] involving the measurement of four spectra with all the possible parallel and perpendicular alignments of the two polarizers,

---

(1) (a) This work has been supported by Grant NB-04145 from the National Institute of Neurological Diseases and Blindness of the Public Health Service. (b) Address correspondence to School of Chemical Sciences, University of East Anglia, Norwich, Norfolk, England.

(2) R. A. Firth, H. A. O. Hill, J. M. Pratt, R. J. P. Williams, and W. R. Jackson, *Biochemistry*, **6**, 2178 (1967).

(3) H. Kuhn and R. Eckhardt, *Z. Elektrochem.*, **64**, 356 (1960).

(4) B. G. Anex and G. J. Eckhardt, Symposium on Molecular Structure and Spectroscopy, Columbus, Ohio, 1966.

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

(6) (a) J. I. Toohey, *Proc. Nat. Acad. Sci. U. S.*, **54**, 934 (1965); (b) J. I. Toohey, *Fed. Proc.*, **25**, 1628 (1966).

(7) J. B. Allison and R. S. Becker, *J. Chem. Phys.*, **33**, 1410 (1960).

(8) Toohey[6b] has reported that cobalt-free red corrin placed directly into 0.1 *N* NaOH results in an absorption spectrum with a maximum at 25.97 kK, whereas a stepwise change in pH leads to a derivative with an absorption maximum at 27 kK, YPII. The latter is the derivative investigated here.

(9) T. Azumi and S. P. McGlynn, *J. Chem. Phys.*, **37**, 2413 (1962).

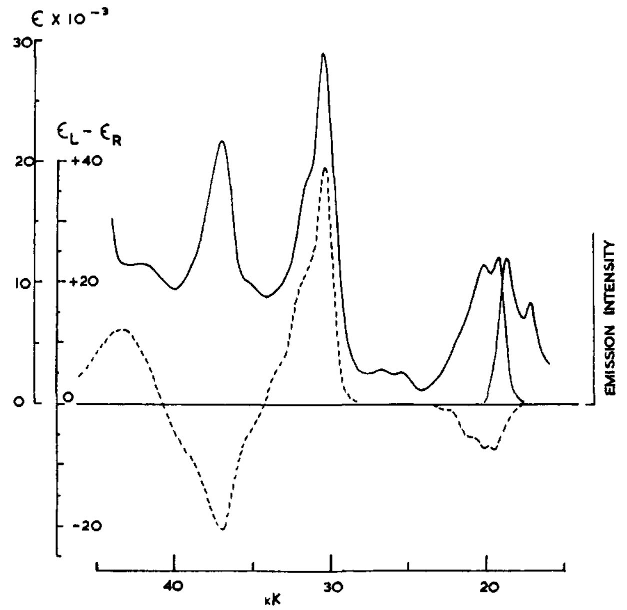


Figure 1. Room temperature absorption and emission (right-hand side) spectrum of red corrin, in 50% aqueous glycerol (——). Extinction values taken from ref 6 are expressed in units of cm^2 mol^{-1}. The emission spectrum is corrected for the response of the photomultiplier–monochromator combination and expressed in units of relative quanta per unit frequency band width. Concentration of red corrin 1.6×10^{-5} *M*. Room temperature circular dichroism spectrum of red corrin (- - -). Concentration 2.32×10^{-5} *M* in 50% aqueous glycerol.

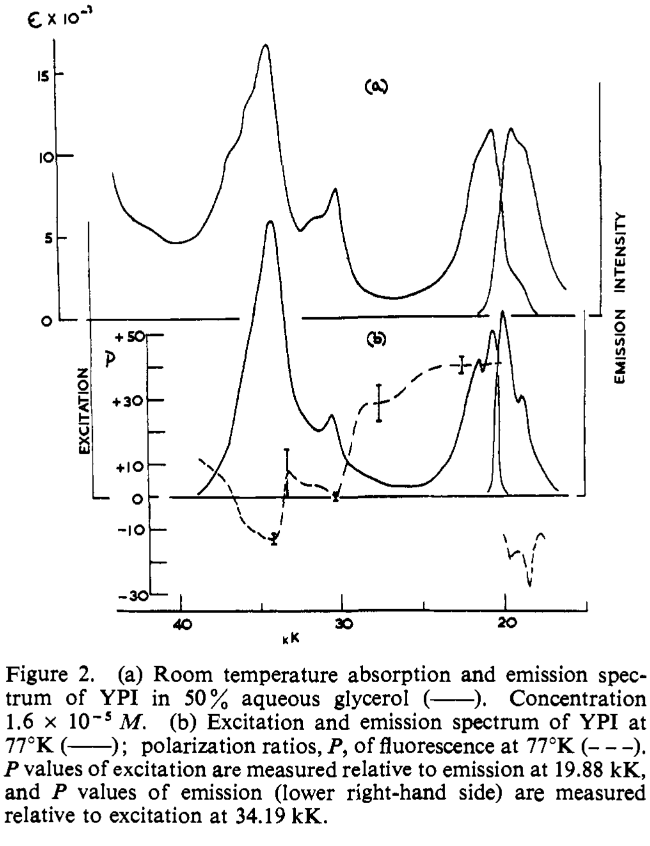


Figure 2. (a) Room temperature absorption and emission spectrum of YPI in 50% aqueous glycerol (——). Concentration 1.6×10^{-5} *M*. (b) Excitation and emission spectrum of YPI at 77°K (——); polarization ratios, *P*, of fluorescence at 77°K (- - -). *P* values of excitation are measured relative to emission at 19.88 kK, and *P* values of emission (lower right-hand side) are measured relative to excitation at 34.19 kK.

was used to compensate for the inherent polarization properties of the emission monochromator and photomultiplier.

Excitation spectra are corrected for the output characteristics of the lamp and monochromator combination, and emission spectra are corrected for the sensitivity of the detection system. Thus the

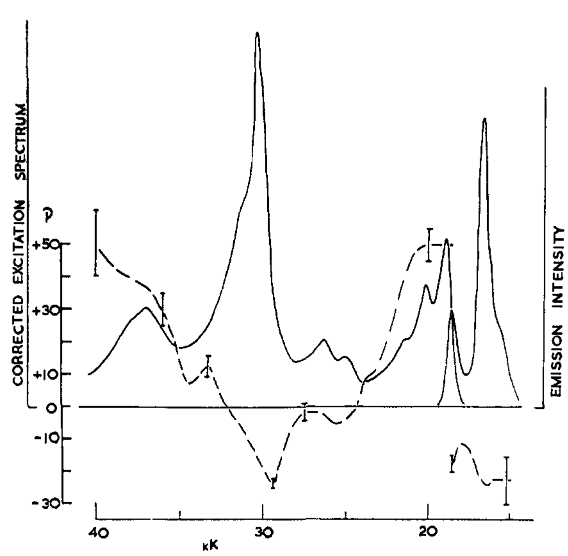


Figure 3. Corrected excitation and emission spectrum of red corrin at 77°K in 50% aqueous glycerol (——); polarization ratio, *P*, expressed as a percentage, at 77°K (- - -). *P* values of excitation are measured relative to emission at 16.7 kK and *P* values of emission (see lower right-hand side) are measured relative to excitation at 30.3 kK.

emission spectra are plotted on the basis of the *relative* number of quanta per unit energy band width. The correction factors were determined in two ways. First, rhodamine B was used as a quantum counter[10] and secondly an Eppley thermopile was employed.[11] Satisfactory agreement between the two sets of factors was obtained.

Room temperature absorption spectra were measured with a Cary 15 spectrophotometer. Circular dichroism spectra were obtained with a Durrum-Jasco ORD/UV-5 instrument made available by the Department of Biochemistry, Michigan State University.

Results

The room temperature absorption spectra of red corrin and YPI in 50% aqueous glycerol are shown in Figures 1 and 2a. The extinction coefficients have been taken from the work of Toohey,[6a] in which a molecular weight of 1143 was assumed for the red compound. The room temperature emission spectra of red corrin and YPI are also given in Figures 1 and 2a. The peak heights are normalized to provide a convenient comparison with the α band[12a] of the absorption spectrum.

No absorption spectra have been measured at 77°K owing to the scarcity of available material, but emission spectra of red corrin and YPI were obtained at this temperature in good glasses formed from 50% aqueous glycerol (see Figures 2b and 3). As a result the excitation spectra of emission have been obtained. They are plotted in Figures 2b and 3 corrected for the photon output of the xenon lamp and excitation monochromator combination. There is a large uncertainty in the value of the correction factor at energies greater than 33 kK[12b] since

---

(10) W. H. Melhuish, *J. Opt. Soc. Amer.*, **52**, 1256 (1962).

(11) C. A. Parker and W. T. Rees, *Analyst*, **85**, 587 (1960).

(12) (a) The absorption bands are labeled according to the proposals of Williams.[2,13] The α and β bands are the first two prominent components of the lowest energy band, D and E are the following low intensity bands, and γ and δ the next intense bands. (b) 1 kK = 1000 cm^{-1}.

(13) J. A. Hill, J. M. Pratt, and R. J. P. Williams, *J. Theoret. Biol.*, **3**, 423 (1962).

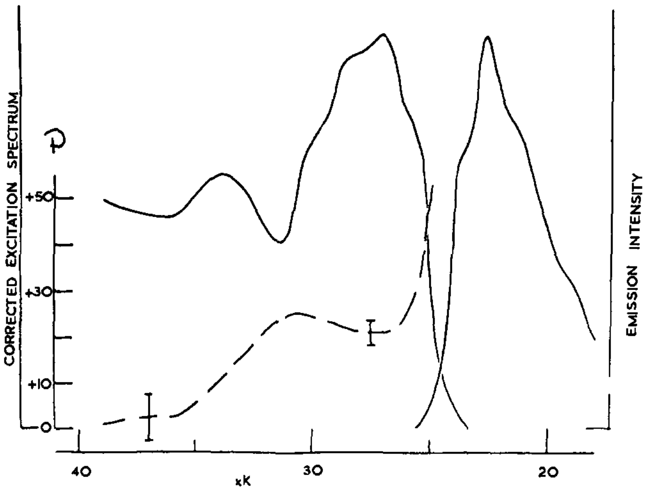


Figure 4. Excitation and emission spectrum of YPII at 77°K in 50% aqueous glycerol (——); polarization ratio, *P*, of fluorescence at 77°K (- - -). *P* values are measured relative to emission at 23.75 kK.

the output of the lamp falls rapidly beyond this. The uncertainty probably accounts for the apparent low intensity of the δ band of red corrin (see Figure 3) and the obvious loss of resolution in the γ band of YPI. However, the excitation and emission spectra sharpen considerably at 77°K.

It may be noted that in a mixture of red corrin and YPI it is possible to examine the excitation and, hence, absorption spectrum of YPI alone by photoselection, that is, by monitoring the emission intensity at 20 kK where no red corrin emission occurs. In this way it has been shown that the peak at 30.3 and the shoulder at 31.5 kK in Figure 2 are features of YPI spectrum and not due to the presence of a small amount of red corrin.

The emission spectrum at 77°K of red corrin, properly corrected, is shown in Figure 3. The unusual shape of this band has been carefully verified by determination of the spectrum with photomultipliers of three different response characteristics, namely, the 1P28 (S13), the RCA 7102 (S1), and the EMI 9558Q (S20). Good agreement is found between the measurements. The emission will not pass a rotating can shutter with a resolving time of 10^{-4} sec, and the emission persists in the form shown here in concentrations as low as 10^{-6} *M*. By contrast the emission spectrum of YPI at 77°K (see Figure 2b), has a normal vibrational envelope sharpened by cooling from room temperature.

The emission spectrum of YPII is difficult to detect at room temperature in 10^{-5} *M* solutions but it can readily be recorded in glasses of 50% aqueous glycerol at 77°K (see Figure 4). The corrected excitation spectrum is also shown in this figure. Since this spectrum has been obtained by observation of the emission intensity at 22.6 kK, well above the emission energies of both red corrin and YPI, the form of the absorption spectrum of YPII alone is now clearly seen, whereas it is not apparent from the pH-dependent spectra given by Toohey (see Figure 2B of ref 6).

No long-lived emission has been detected from any of the three corrin derivatives, although a search was made to 14 kK, the low-energy detection limit of the apparatus. The shutter employed has a resolving time of 10^{-4} sec.

Information about the relative polarization of different absorption bands has been obtained by measuring the polarization of the fluorescence as a function of the wavelength of exciting light when the latter is also polarized. The polarization ratio of fluorescence, *P*, is given by

$$P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$$

where $I_{\parallel}$ is the intensity of emitted light which is polarized parallel to the electric vector of the exciting light and $I_{\perp}$ is the intensity which is polarized perpendicular to this. If there is an angle θ between the polarization vectors of absorption and emission it can be shown[14] that *P* is given by $(3 \cos^2 \theta - 1)/(\cos^2 \theta + 3)$ provided that the molecules do not change their orientation between excitation and emission and provided also that no transfer of excitation energy takes place. The limiting values of *P* are therefore $+{}^1/_2(\theta = 0)$ and $-{}^1/_3(\theta = 90°)$. These ideal limits are rarely found. Experimental difficulties can lead to some depolarization of the exciting and emitting light. It is advisable to trap the emitting molecule in rigid glass, both to enhance the intensity of emission and to limit rotational motion. The polarization ratios, *P*, of the excitation spectra have been determined for all the derivatives (see Figure 2–4). The errors are indicated on the figures where reliably known. Relatively large errors occur either when the signal level is low, that is, at the high-energy end of the spectrum, and in the troughs between peaks, or when the spectrum is changing rapidly with energy. Thus the polarization ratios at peak positions are generally the most accurate. The values plotted were obtained from an average of three separate runs. Runs which gave *P* values anomalously low or high were rejected. It was found that the state of the glycerol glass greatly affected the values. A crude annealing process of successive warming and cooling helped to improve the polarization ratios obtained. It was important to prevent condensation from forming on the Dewar since this effectively depolarized the exciting light. A stream of warm air readily overcame this problem.

The energy at which the emission intensity was monitored was chosen as far from the lowest energy absorption band as possible to avoid errors due to light scattered from the excitation beam into the emission monochromator.

In this way it has been possible to obtain consistently reliable polarization ratios. Thus the *P* values of the α and β bands of red corrin are 50%, within the experimental error, which is the theoretically expected value if the absorbing and emitting transition moments are parallel. Since the conditions were identical in the other experiments the low *P* values observed in the lowest energy band of YPII are considered reliable (see Figure 4).

The polarization ratios of the emission spectra relative to a fixed excitation energy have been determined for red corrin and YPI (see Figures 2 and 3). The energy of excitation was, wherever possible, chosen to be removed as far as possible from the energy at which the emission was being monitored in order to avoid errors due to light scattered from the excitation beam into the detecting system. Thus the emission polarization ratios of red corrin and YPI were measured relative to excitation in the

---

[14] A. Jablonski, *Z. Physik.*, **96**, 236 (1935).

γ band. No gross changes are seen across the emission band.

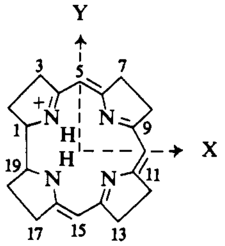
The room temperature circular dichroism spectrum of red corrin was measured in 50% aqueous glycerol. The spectrum is plotted in Figure 1. The $\Delta\varepsilon$ values were calculated using the ε values of Toohey's absorption spectrum. Although the largest $\Delta\varepsilon$ value found, 40 cm^2 mol^{-1}, is about twice the highest values obtained for vitamin B_{12} derivatives,[2] the significance of this remains uncertain until the molecular weight of red corrin has been accurately determined.

Discussion

Although the detailed structure of the cobalt-free red corrin extracted from *Chromatium* strain D is not yet known, the compound undoubtedly contains the intact corrin ring.[6] Red corrin will take up cobalt, becoming identical with a known cobamide, factor B. Several species of differing charge, but identical absorption spectra, have been isolated by Toohey. The compound investigated in this work remains uncharged between pH 3 and 9 and contains 1 mol of sugar phosphate per mol of corrin ring. The chromophore must therefore possess unit positive charge.

Recently Eschemoser and Fischli[15] have reported the synthesis and properties of a metal-free corrin compound having a cyano group substituted at position 15. The derivative has an absorption spectrum similar to the spectrum of red corrin in the relative intensities and energy spacing of the peaks, although the spectrum of red corrin is displaced to lower energy by 1.45 kK. The absorption spectrum of the synthetic corrin remains unchanged over the pH range 1–8. On the basis of nmr and infrared evidence the synthetic metal-free corrin is assigned a structure with two protons occupying the center of the corrin ring, thus conferring unit positive charge on the chromophore.[15]

This evidence strongly suggests structure I as the chromophore of red corrin. On raising the pH to 11, red corrin changes color to yellow. The pK_a for this change is estimated by Toohey to be 9.6. The synthetic derivative loses a proton with a pK_a of 8.6. Most plausibly this process corresponds to the loss of one proton from the center of the ring system. Toohey has observed that an identical spectral change is brought about by the action of light on red corrin or by 0.1 *N* HCl over 12 hr at room temperature. It is not known to what structural changes these spectral shifts correspond. However, it is noteworthy that synthetic metal-free corrin is not photosensitive in this way.



If the pH of a solution of the naturally occurring corrin is raised slowly to 13 a further change is brought about in the spectrum, a maximum developing at 27 kK. This reaction, resulting in the generation of yellow product II, has a pK_a of 11.2.[6]

Emission Spectra. All the emissions observed are attributed to a prompt fluorescence from the lowest excited electronic state. The vibrational progressions appearing in the emission spectra correspond to vibrations of the ground electronic state, whereas the progressions observed in the absorption bands arise from vibrational levels of the excited electronic state. Table I collects the positions of the absorption and emission peaks measured at both room temperature and 77°K. The 0–0 bands are clearly identifiable in red corrin and YPI, but since YPII displays emission and absorption spectra with Franck–Condon forbidden envelopes, the location of the 0–0 bands is not unambiguous. It is probable that the 0–0 bands in emission and absorption are located close to the energy of overlap of the two spectra. The vibrational assignments shown in Table I then follow, the 0–2 and 2–0 being the most intense components.

Presumably the vibrational frequencies are related to a stretching frequency of the chainlike chromophore which constitutes the corrin ring. It is clear that, for red corrin and YPI, a decrease in the vibrational frequency occurs upon excitation, whereas the opposite is found for YPII.

The unusual feature of these results is the marked change in the intensity distribution of the emission band of red corrin on cooling to 77°K. No comparable change is seen in the excitation spectrum at this temperature (Figure 3). Since the shape of the emission spectrum persists at concentrations of 10^{-6} *M* excimer and aggregation phenomena are excluded. The emission is completely cut off by a shutter of resolving time 10^{-4} sec. Thus it seems improbable that the emission originates from a state of different spin parity to the ground state. It might be supposed that the spectrum is an example of a Franck–Condon forbidden transition in which the peak intensity is found in an excited vibrational level. However, this explanation is untenable for two reasons. First, a Franck–Condon forbidden envelope, corresponding to an excitation involving a large change in equilibrium bond lengths, invariably contains a long progression of vibrational intervals. The spectrum of YPII (Figure 4) is a good example of this. Secondly, the excitation spectrum would also exhibit the characteristics of a Franck–Condon forbidden band.

The sharp peak at 16,700 cm^{-1} has the appearance of a second origin to the emission band, suggesting the emission arises from a species which is produced photolytically from red corrin and which lives long enough at 77°K, that is, greater than 10^{-8} sec, to emit. A proton transfer in the excited state is an example of a possible reaction. This suggestion receives some support from the fact that red corrin is photolabile. The emitting species could not, however, be YPI, the final product of photolysis of red corrin, since the first emission band of this derivative occurs at 20 kK.

Weller[16] has shown that heterocyclic bases commonly have an excited-state protolytic equilibrium constant 6 pH units higher than the ground-state constant. A study of the pH dependence of the relative intensities of the peaks of red corrin at 18.55 and 16.70 kK should test this hypothesis.

---

(15) A. Eschenmoser and A. Fischli, *Angew. Chem. Int. Ed. Engl.*, **6**, 866 (1967).

(16) A. Weller, *Progr. Reaction Kinetics*, **1**, 199 (1961).

Table I. Peak Positions of Absorption and Emission Spectra

Process	Transition	Room temperature		77°K	
		E,[a] cm^{-1}	ΔE, cm^{-1}	E, cm^{-1}	ΔE, cm^{-1}
Red corrin					
Absorption	0–2	Shoulder		21,500	1,400
	β 0–1	20,100	1,000	20,200	1,300
	α 0–0	19,100		18,900	
Emission	0–0	18,600	1,500	18,550	1,850
	1–0	17,100		16,700	
				15,500 sh	
YPI					
Absorption	0–1	Shoulder		21,400	800
	0–0	20,600		20,600	
Emission	0–0	19,400		20,000	1,200
	1–0	Shoulder		18,800	
YPII					
Absorption	0–4			30,000 sh	1,500
	0–3			28,500 sh	1,500
	0–2			27,000	1,000
	0–1			26,000 sh	1,500
Emission	0–0		Crossing point →	24,500	1,000
	1–0			23 500 sh	900
	2–0			22,600	1,000
	3–0			21,500 sh	

[a] E, energy.

Polarization Ratios. From the polarization ratios of red corrin plotted in Figure 3 it is evident that the transitions of the α, β, and δ bands are polarized parallel to one another and to the emitting transition. The γ band is polarized perpendicular to this direction. The data suggest that bands D and E are of mixed polarization although with the major component polarized in the same direction as the γ band. In view of the low intensity of the D and E bands it is possible that a vibronic mechanism makes an important contribution to their intensity.

The polarization data divide the spectrum into four distinct regions which evidently correspond to the first four electronic transitions and their vibrational overtones: thus the α and β bands are the 0–0 and 0–1 components of the lowest energy electronic transition; D and E the vibrational components of the next highest transition with the probability that the 0–0 band has little intensity and its polarization is therefore not seen; the γ band and at least one vibrational overtone; finally, the δ band from 35 to 40 kK.

Kuhn's measurement[3] on vitamin B_{12} embedded in a plastic matrix showed the γ band to be oppositely polarized to the α and β bands. Polarized single-crystal data[4] on the synthetic nickel corrin, nirrin, demonstrated that the lowest energy band was polarized in the y direction and that the second two transition are oppositely polarized to the former band. Thus the data are consistent with findings on other corrin ring derivatives.

It is of interest to compare these results with the predictions of a recent theoretical model. Day[17] has described the chromophore as a 14-electron π system extending over 13 atoms, the symmetry of the π system belonging to the point group C_{2v} when a metal occupies the center of the ring. The lowest energy transition takes place between the highest filled and lowest empty levels, that is, between levels 7 → 8. Then follow two transitions of the same symmetry, 6 → 8 and 7 → 9, predicted to be of equal energy in a one-electron model but interacting under the influence of electron repulsion and splitting into two transitions whose relative intensities will depend upon the degree of interaction. The fourth transition will occur between levels 6 → 9. Symmetry arguments predict that these transitions are polarized y, x, x, and y, respectively.

If red corrin possesses two protons in the center of the ring system symmetrically disposed about the x axis it will belong to the point group C_{2v} and the predictions of Day must apply. The results given here are entirely consistent with this model, suggesting that bands (D + E) and γ are the interacting pair, with (α + β) and δ being the lowest and highest energy transitions of the four level model.

The recent determination by Briat[5] of the MCD spectrum of a synthetic dicyanide has led to the proposal that the sign of the MCD band, in the absence of degenerate ground and excited states, is related to the polarization of electronic or vibronic absorption bands. Although this hypothesis has not yet been thoroughly tested, the relative polarizations of the four lowest energy transitions of the synthetic cobalt-containing corrin are the same as those presented here for the metal-free corrin ring system on the basis of Briat's proposal.

The polarization ratios of YPI, given in Figure 2b, immediately suggest a relationship between the spectrum of YPI and red corrin. The lowest energy band is polarized parallel to the emission band; the low-intensity peak at 30.5 kK has mixed polarization with a major component polarized perpendicular to the emission band; finally the intense peak at 34.3 kK is also polarized perpendicular to the emission band. No counterpart of the δ band of red corrin has been detected. Thus the spectrum of YPI is closely similar to red corrin merely shifted to high energy.

All the theoretical models of the corrin chromophore agree in their treatment of it as a symmetrical cyanine

---

(17) P. Day, *Theor. Chim. Acta*, **7**, 328 (1967).

(18) B. H. Offenhartz, *Proc. Roy. Soc.* (London), **A288**, 350 (1965).

(19) H. Kuhn, K. H. Drexhage, and H. Martin, *ibid.*, **A288**, 348 (1965).

dye.[17–19] If YPI possesses only one proton in the center of the ring system it can be described as an unsymmetrical cyanine provided the proton is firmly attached to one nitrogen atom. It has long been known that an unsymmetrical cyanine absorbs at higher energy than its symmetrical counterpart.[20,21]

Comparison of the YPI results with the model due to Day[17] implies that the peaks at 30.5 and 34.3 kK arise from the pair of interacting states. Since, in YPI, the intensities of the pair are more nearly equal, the interaction between the states is less than in red corrin, possibly due to a lowering of the symmetry of the chromophore.

The polarization ratios of YPII, shown in Figure 4, were determined by monitoring the emission intensity as close as possible to the position of the 0–0 band of the emission envelope, that is, at 23.75 kK. It is seen that the lowest energy absorption band is of mixed polarization but becoming more nearly parallel to the 0–0 component of emission as the 0–0 component of the absorption band is approached. This points to the coexcitation of vibrations in the excited electronic state which are governing the polarization of the band. The peak at 34 kK is also of mixed polarization, but with a larger component polarized perpendicular to the emitting transition.

These results can usefully be compared with the emission spectra and polarizations of *cis*-polyenes. The fluorescence spectra of polyenes are very weak and exhibit a small overlap with the lowest absorption band.[22] These features have been successfully rationalized in terms of a gross geometry change of the polyene chain on excitation to the lowest singlet state,[23] leading to a vibrational envelope containing a long progression of vibrations often with the maximum intensity far removed from the origin. It has also been pointed out[23] that the intensity of the lowest energy absorption band of a polyene is two or even three times lower than the corresponding absorption band of a cyanine with an equal number of π electrons. Inspection of the nest of spectra given by Toohey[6] shows that the main absorption band of YPII is a factor of two less intense than the lowest energy band of red corrin.

Although there is little information available about the polarization characteristics of polyene chains, recent studies[22] have revealed low polarization ratios for the typical Franck–Condon forbidden envelopes of some polyenes related to vitamin A. Also, it can be noted that ζ-carotene, a polyene chain containing seven double bonds, a 14-π-electron chain, has the lowest energy vibrational component of the first absorption band at 23.7 kK and the *cis* peak, which is predicted to be perpendicularly polarized to the first band, at 33.8 kK.[24]

This comparison shows that YPII can reasonably be described as a polyene, that is, a 14-π-electron system over 14 atoms. It is possible that the chain extends either to carbon 1 or to carbon 19.

Circular Dichroism. Although the $(\varepsilon_L - \varepsilon_R)$ values of Figure 1 are subject to the uncertainty in the molecular weight of red corrin, the derivative undoubtedly has a high rotational strength. This arises most plausibly from a helical configuration of the chromophore brought about by saturated carbon atoms at positions 1 and 19.[25] The effect of the helicity is to place a small component of the electric dipole transition moment in the z, or out-of-plane, direction parallel to the magnetic dipole transition moment. Rotational strength may also arise from any component of the magnetic dipole transition moment that is placed in the plane of the chromophore.

Comparison of the polarizations of the electric dipole transition moments of red corrin (see Figure 3), with the signs of the rotational strengths (see Figure 1) shows that the *relative* signs of the latter are determined by the directions of the electric dipole transition moments. This simple situation is in sharp contrast to that seen in the cobalt-containing derivatives where the signs of the rotational strengths are evidently much influenced by the cobalt ion presumably on account of the magnetic dipole transitions of the cobalt ion.[2]

Comparison with Cobalt-Containing Corrins. An examination of vitamin B_{12} and dicyanocobalamin has revealed no emission that can be attributed to the corrin ring. An emission, excited at 278 nm, has been observed at 303 nm[26] but is attributed to the benzimidazole moiety.[27] The presence of the cobalt ion may quench the fluorescence of the corrin ring in two ways. The cobalt nucleus will, owing to the effect of spin-orbit coupling, cause the rate of intersystem crossing from the singlet to the triplet manifold of the corrin chromophore to be increased, with a consequent lowering of the fluorescence intensity and an increase in the phosphorescence intensity. No phosphorescence has been detected down to 14 kK, the low-energy detection limit of the apparatus. Alternatively the cobalt ion may supply energy levels lying below even the lowest triplet levels of the corrin chromophore, thereby providing an efficient radiationless path to the ground state or emitting in the inaccessible near-infrared region. The detection of the phosphorescence of $Co(CN)_6^{3-}$ at 14.4 kK[28] indicates that the former mechanism is operating at least in dicyanocorrins. A search below 14 kK for long-lived emission of the metal-free corrin ring should be rewarding.

Acknowledgment. The writer thanks Dr. John I. Toohey for supplying materials and Dr. P. Day for discussions. The hospitality of the Department of Biophysics, Michigan State University, and of Professor Barnett Rosenberg is gratefully acknowledged.

---

(20) H. Kuhn, *J. Chem. Phys.*, 17, 1198 (1949).

(21) It is of interest that red corrin produces, on protonation, a yellow product.[6] The possibility then arises that addition of this third proton produces an unsymmetrical cyanine, with consequent blue shift of the spectrum.

(22) B. Rosenberg and A. J. Thomson, unpublished work.

(23) J. R. Platt, *J. Chem. Phys.*, 25, 80 (1956).

(24) L. Zechmeister, "Cis-trans Isomeric Carotenoids, Vitamins A, and Arylpolyenes," Springer-Verlag, Vienna, 1962.

(25) This assumption rests on the analogy with vitamin B_{12} and the structural determination of the latter by D. C. Hodgkin, J. Lindsey, R. A. Sparkes, K. N. Trueblood, and J. G. White, *Proc. Roy. Soc.* (London), **A266**, 494 (1962).

(26) D. E. Duggan, R. L. Bowman, B. B. Brodie, and S. Udenfriend, *Arch. Biochem. Biophys.*, **68**, 1 (1957).

(27) J. W. Longworth, R. O. Rahn, and R. G. Shulman, *J. Chem. Phys.*, **45**, 2930 (1966).

(28) M. Mingardi and G. B. Porter, *ibid.*, 44, 4354 (1966).