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THE ELECTRONIC STRUCTURE AND SPECTRUM OF VITAMIN B_{12}

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Several contributors to this Symposium have emphasized the difficulties which beset detailed calculations of the electronic structure of small inorganic molecules such as $CuCl_4^{2-}$ when a nattempt is made to choose quantum mechanically realistic assumptions while retaining reasonable simplicity in the model. It might therefore appear rather audacious to attempt calculations of the electronic properties of such complicated biological coordination compounds as the corrins (Fig. 1). However, there are several good reasons, first why one ought to attempt such calculations and, second, for having some grounds for optimism that they might succeed. The first and most obvious reason for trying to calculate the

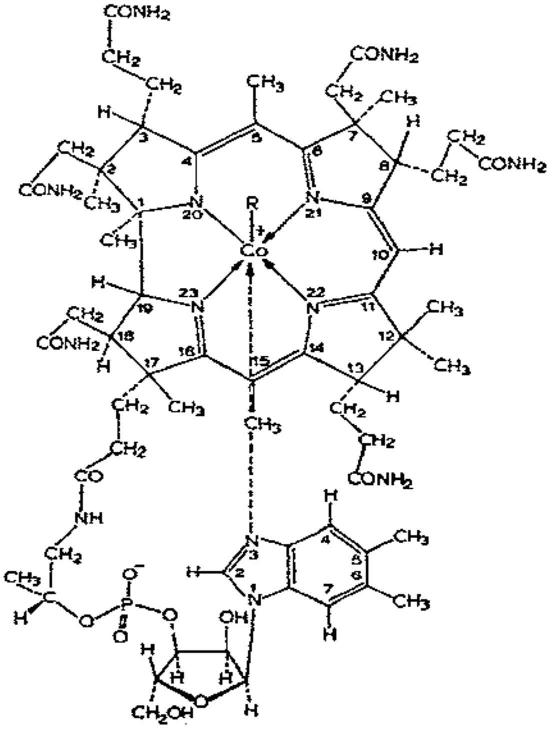


Fig. 1. The formula of a cobalamin. Compounds which do not contain the nucleotide side-chain are called cobinamides and the prefix (*e.g. cyano*cobalamin) identifies the group R.

electronic properties of biological molecules must be the hope that one may thereby obtain some insight into their reactivity and adaptation to their particular biological functions. It should be said at once that this hope lies very far from fulfilment at the present time. Even the most elaborate calculations, on, for example, the iron porphyrins, have so far failed to show up the electronic origins of those differences in oxidation potential that characterize the various cytochromes, our understanding of which is still more suitably couched in conventionally empirical chemical language.

A second and more compelling reason from the viewpoint of the coordination chemist for studying this type of molecule is the strange complexity of electronic structure and chemical behaviour found in them, not matched by many of the simpler coordination compounds whose properties have been so exhaustively studied in the last few years. Thus, for example, the molecular structure of vitamin B_{12} is so extraordinary and provides us with a unique example of such a variety of different electronic effects, that it is well worth studying in its own right aside from any relevance these studies might have to its biological reactivity. With what appears to be a single exception (complexes of 2-pyridinecarboxaldehyde 2-pyridylhydrazone (PAPY) described by Lions[1]) the corrins are the only known metal complexes which have a cyanine (odd atom conjugated chain ion) as a ligand. Furthermore, they are the only examples of long cyanine chains whose bond lengths have ever been accurately determined and thus they are of great interest in connection with the problem of bond length alternation in conjugated chains. As will appear later they also provide a unique example of a ligand whose electronic structure can be profoundly modified by the presence of other ligands attached to the metal atom. Thus while the cytochromes which have different ligands attached to the iron atom in the axial position all have rather similar electronic spectra[2], the spectrum of the corrin chromophore is so greatly modified by the axial ligands as to be almost unrecognizable. The two extremes of spectral behaviour in the corrin series are exemplified by vitamin B_{12} and the coenzyme, so that when the latter was first isolated there was serious doubt for some time whether the two compounds contained the same chromophore or the metal atom in the same oxidation state, and it was only the solution of the crystal structure[3] which showed that the difference merely consisted of replacing the cyanide axial ligand by C_5'-deoxyadenosyl carbanion (Fig. 1). If the reasons for this spectroscopic difference could be understood it would be possible to use the electronic spectra diagnostically and deduce the nature of the axial ligands in corrinoid compounds of unknown constitution, for example, the holoenzyme.

There is no doubt whatever that the semi-empirical parameterization of the self-consistent field equations for molecules, first written down in analytical form by Roothaan[4], has been accomplished most successfully to date for the π-electron systems of conjugated organic molecules. It is this fact which gives us reason to hope that by carrying out calculations for such molecules when acting as ligands we may obtain useful information about the metal–ligand interaction[5]. The electronic spectra of metal complexes of conjugated ligands fall into three limiting categories: transitions largely localized on the metal atoms, for example d-d, f-f or s-p: transitions localized on the ligand, π-π^* or n-π^*, or charge transfer transitions from metal to ligand or *vice-versa*. That the visible and near ultraviolet spectrum of vitamin B_{12} consists entirely of ligand transitions is demonstrated by the fact that a recently isolated metal-free corrin[6] had absorption bands showing a one to one correspondence with those of the vitamin up to an energy of at least 45 kK. The exact relationship between the conjugated π-system of the metal-free and metal-containing compounds has not yet conclusively been established but the overall charge on the molecule determined by electrophoresis[6] suggests strongly that there are two protons attached to the nitrogen atoms of the conjugated chain. If the latter were attached to those nitrogens at the ends of the chain (N-20 and N-23 in Fig. 1) the chromophore would have the same overall symmetry (C_{2v}) in both metal-free and metal-containing compounds. This would agree with the extreme similarity between their spectra and contrasts with a recent discussion by Schrauzer[7] who assumed that a single proton was attached to one of the terminal nitrogens, in which case the chromophore would have the character of a polyene rather than a cyanine. Apart from Schrauzer's there have been several calculations on the corrin chromophore of which the earliest was Kuhn's[8], using the free electron model. This model is not well adapted to dealing with heteroatoms in a conjugated chain of carbons for which the LCAO-MO model is altogether more suitable. Some simple Hückel calculations have also been briefly described[9] as well as an extended Hückel calculation[10], whose major aim was to obtain the energies of the highest filled and lowest empty orbitals. When attempting to interpret electronic transition energies, however, such effectively one-electron calculations are not reliable and the effects of electron repulsion must be taken into account. Some preliminary calculations using the Pariser–Parr–Pople (PPP) formalism have already been used to interpret the absorption and circular dichroism spectra of corrins but as these results will be published in full elsewhere[11] only a brief summary will be given here. As shown in Fig. 1, the π-electron conjugation of the corrin ligand extends over a bent chain of thirteen atoms and if, as is reasonable from its diamagnetism, the cobalt atom is assumed to be trivalent, the ligand carries a formal charge of minus one and its π-orbitals accommodate fourteen electrons. In the PPP approximation, the matrix elements in Roothaan's equation

$$\sum_{\nu} c_\nu (F_{\mu\nu} - ES_{\mu\nu}) = 0 \tag{1}$$

are approximated by

$$F_{\mu\nu} = \beta_{\mu\nu} - \tfrac{1}{2} P_{\mu\nu} \gamma_{\mu\nu} \tag{2}$$

and

$$F_{\mu\mu} = I_\mu + \tfrac{1}{2} P_{\mu\mu} \gamma_{\mu\mu} + \sum_{\rho \neq \mu} (P_{\rho\rho} - z_\rho) \gamma_{\mu\rho} \tag{3}$$

where the $P_{\mu\nu}$ are the elements of the charge-bond order matrix, $\beta_{\mu\nu}$ is the resonance integral between atoms μ and ν, I_μ is the valence state ionization potential of atom μ, z_ρ the core charge on another atom ρ and the $\gamma_{\mu\nu}$ are electron repulsion integrals $\langle \phi_\mu \phi_\nu \mid e^2/r_n \mid \phi_\mu \phi_\nu \rangle$. The customary procedure[12] when calculating excitation energies is to assume some form of molecular orbital basis functions, the coefficients of which are substituted into equations (2) and (3) to calculate the diagonal and off-diagonal matrix elements. The energies of various one-electron promotions are then calculated from these through the equation

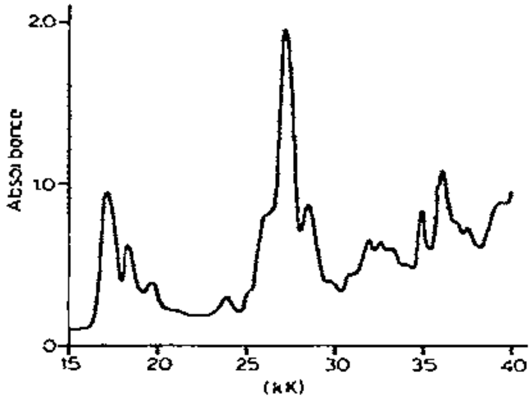
$$\begin{aligned}\langle {}^1\Psi_k{}^r \mid \mathscr{H} \mid {}^1\Psi_k{}^r \rangle - E_0 = \sum_\mu \sum_\nu \{ &(c_{r\mu} c_{r\nu} - c_{k\mu} c_{k\nu}) F_{\mu\nu} + \\ &+ (2 c_{k\mu} c_{r\mu} c_{k\nu} c_{r\nu} - c_{k\mu}{}^2 c_{r\nu}{}^2) \gamma_{\mu\nu} \}\end{aligned} \tag{4}$$

and the effect of electron repulsion in mixing the one-electron excited states thus obtained follows from the solution of a determinant in which the off-diagonal element is

$$\langle {}^1\Psi_k{}^r \mid \mathscr{H} \mid {}^1\Psi_l^s \rangle = \sum_\mu \sum_\nu \{ (2 c_{l\mu} c_{s\mu} c_{k\nu} c_{r\nu} - c_{l\mu} c_{k\mu} c_{s\nu} c_{r\nu}) \gamma_{\mu\nu} \} \tag{5}$$

In equations (4) and (5) ${}^1\Psi^r{}_k$ is the singlet wave function of an excited state obtained by promoting an electron from orbital k to orbital r. The simplest basis functions for carrying out such a procedure would be those of a conjugated chain of thirteen atoms, in which no allowance is made for the fact that four are nitrogen and the rest carbon. This function has a simple analytic form and the charge-bond matrix can be at once written down. It has been shown by several authors[13, 14] that π–π^* transition energies for nitrogen heterocycles such as pyridine and quinoline may be calculated with reasonable accuracy starting from the basis functions for benzene and naphthalene and including the electronegativity difference of the nitrogen atom by changing the appropriate $F_{\mu\mu}$ and $\gamma_{\mu\mu}$.

To obtain a "birds eye view" of the change in electronic structure to be expected when the axial ligand attached to the cobalt atom is changed, we presume that as the axial ligand becomes a progressively better σ-donor more charge is placed on the four nitrogen atoms of the corrin ligand. One may envisage a number of mechanisms for this, for example, the 4s orbital of the metal atom is σ-bonding to both the axial and equatorial ligands. Thus we have calculated the variation in the energies of the first four excited states of the corrin chromophore for a range of values of the valence state ionization potential ($I_\mu{}^N$) of the four nitrogen atoms. The result of such a simple calculation is extremely interesting and reveals an electronic structure quite unique among conjugated ligand molecules. The energy of the one-electron promotion from orbital 7 to orbital 9 varies quite steeply with $I_\mu{}^N$, while that from orbital 6 to orbital 8 scarcely varies at all. If the chromophore is assumed to have C_{2v} symmetry, these two excited states may be mixed together by the matrix element of equation (5) and thus for some values of $I_\mu{}^N$ the second excited state will consist largely of ${}^1\Psi_6{}^8$ while for other values it will be predominantly ${}^1\Psi_7{}^9$. The sign of the off-diagonal matrix element of electron repulsion which mixes these two configurations together is such that the lower energy combination is anti-symmetric, and thus for values of $I_\mu{}^N$ at which ${}^1\Psi_6{}^8$ and ${}^1\Psi_7{}^9$ are nearly degenerate, we expect to find that the second electronic transition is weak while the third is particularly intense. Such an effect is in complete contrast to the prediction of simple Hückel theory, for which the calculated transition dipoles of the first four one-electron promotions are all of similar magnitude.



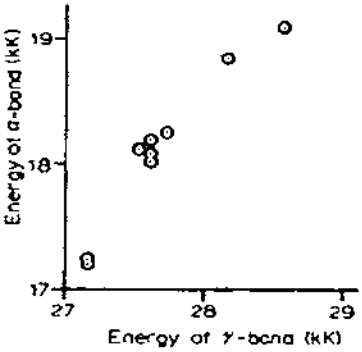


Fig. 2. The absorption spectrum of dicyanocobalamin at 77 °K in an ethanol–methanol–propanol glass. The very sharp bands between 35 and 37 kK are due to the benziminazole.

Fig. 3. The energies of the major absorption bands of some cobalamins and cobinamides. In order of increasing energy of the α-band, the axial ligands are: dicyanide, cyanide–methyl isocyanide, ammonia–cyanide, hydroxide–cyanide, azide–cyanide, iminazole–cyanide, benziminazole-cyanide(B_{12}), water–cyanide, water-benziminazole(B_{12a}). Data from P. George, D. H. Irvine and S. C. Glauser, *Ann. N.Y. Acad. Sci.*, 88 (1960) 393.

The absorption spectrum of dicyanocobalamin, measured at 77 °K (Fig. 2) clearly shows a very weak band at 23.7 kK following after the vibrational progression of the first electronic transition (the α-band) and before the very intense ultraviolet transition commonly called the γ-band. The energies and oscillator strengths of the first four transitions of dicyanocobinamide, which contains the same corrin chromophore as dicyanocobalamin are actually 18.1 kK ($f = 0.13$), 23.7 (0.01), 27.2 (0.27) and 32.5 (0.13). To explain this very marked intensity alternation Schrauzer[7], using the simple Hückel model, was forced to invoke Dale's rule[15] that overtone transitions in polyenes and cyanines only occur from the highest filled to successive empty orbitals and not from successively lower filled orbitals to the lowest empty. However, there is no reason to believe that this rule has any validity, as the transition dipole moments for the one-electron excitations show very clearly: 0.29 (7 → 8), 0.40 (6 → 8), 0.35 (7 → 9) and 0.18 (6 → 9).

The simple model just outlined also provides an explanation of some other features of corrinoid spectra. Thus the γ-band has been shown[16] to move to lower energies as the axial ligands are changed in a sequence which appears similar to the nephelauxetic series[17]. That the α-band moves in the same direction as the γ-band is also shown for a number of cobalamins and cobinamides in Fig. 3. This agrees with our predictions if it is assumed that the more "nephelauxetic" an axial ligand is, *i.e.* the greater the covalency of the metal ligand bond, the more charge is placed on the corrin nitrogen atoms. Other features of the π–π^* excited states which ought to be predictable include the effect of substituents within the conjugated chain and polarisations of the electronic transitions. A number of C-10 -substituted cobalamins were prepared by Wagner[18], who found the interesting effect that the α-band on substitution moves to lower energy very much more than the γ-band. This result follows at once from symmetry considerations since the one-electron MO's are alternately symmetric and anti-symmetric with respect to the mirror plane passing through C-10. Thus orbitals 6 and 8 have zero charge density on C-10 and transition 7 → 8 is accompanied by a large change in charge density at C-10, while a transition to an excited state consisting partly of 6 → 8 will be expected to have a much smaller change. There is but little information about the polarisations of corrinoid absorption bands. Our model predicts that the α-band should be polarised in the direction of atoms C-5 to 15, while the small band near 24 kK and the γ-band should be polarised at right angles to this, while the next band above the γ-band (δ-band) is again parallel to the α-band. The polarised reflection spectrum of a single crystal of nirrin chloride[19], the synthetic nickel analogue of the cobalt corrins, confirms the prediction for the α-band but further conclusions are made difficult by the fact that the α- and γ-bands are very much closer in energy than in the cobalt corrins. Also the nirrin derivative whose structure was determined by Dunitz[20] and used in the reflection work was unsymmetrically substituted, carrying a cyano-group on C-5 and thus the assumption of a mirror plane through C-10 may not be a good one in this case. The polarized fluorescence spectrum of metal-free corrin[21], however, provides very clear evidence for the polarisations of the α-, γ- and δ-bands in agreement with the theoretical predictions.

Despite the success of our simple model, to assume that the basis functions are those of a conjugated hydrocarbon chain is a very drastic simplification. We have therefore carried out further calculations using as basis functions the Hückel orbitals of the conjugated chain with $\alpha_N = \alpha_C + 0.75\,\beta_{CC}$. The energy variation of the first five excited states with I_μ^N is shown in Fig. 4, where the dotted lines represent the energies of one-electron excitations before configuration interaction and the full lines represent the actual excited state energies. An additional feature of this calculation is that the transition 5 → 8 has been included since for all reasonable values of I_μ^N it lies quite close to 6 → 9, with which it can interact. A single calculation of the energy of transition 7 → 10, the next in increasing energy, using $I_\mu^N = -140$ kK showed that it lies above 50 kK and therefore need

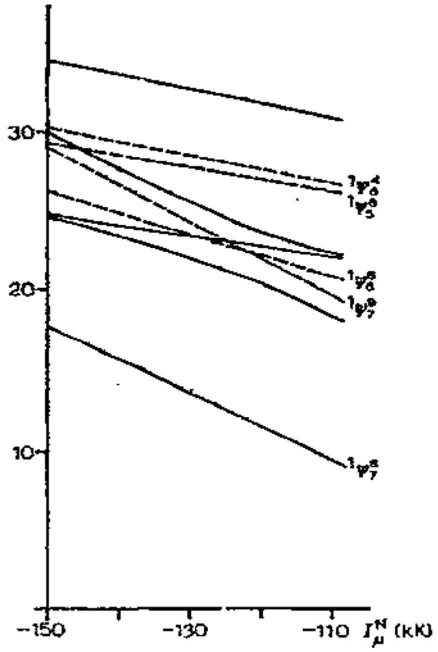


Fig. 4. The calculated energies of the lowest π–π^* singlet excited states of corrin as a function of I^N (see text).

not be considered. The effect of configuration interaction on the pair 5 → 8 and 6 → 9 is to introduce another very weak transition into the region just below the γ-band. This provides an explanation for the frequent occurrence of two small bands in this region, an otherwise rather puzzling feature of the spectra. For the parameter choice $I^C = -90$, $I^N = -140$, $\gamma_{11}{}^{CC} = 64$, $\gamma_{11}{}^{N} = 91$, $\gamma_{12}{}^{CC} = \gamma_{12}{}^{CN} = 11$ and $\beta_{\mu\nu} = -18$ kK we obtain a succession of singlet excited states 15.75 kK (.083), 23.47 (.004), 24.20 (.006), 26.93 (.248) and 33.55 (.074). The numbers in brackets are the oscillator strengths calculated according to the dipole velocity approximation. This, rather than the more usual dipole length formalism, was employed because calculations were also made of the magnetic dipole transition moments so that, using the observed rather than idealised geometry of the molecule, estimates could be made of the rotational strengths of the transitions. Bearing in mind that oscillator strengths calculated in this way using approximate wave functions are always smaller than those observed, in contrast to those calculated with the dipole length formalism, which are always much larger, the agreement between these energies and intensities and those experimental values quoted above for dicyanocobinamide is extremely satisfactory.

Both the theoretical calculations and experimental observations of corrin spectra at very low temperatures are being actively pursued in our Laboratory and further results, including calculations on circular dichroism spectra and the extent to which magnetic dipole strength may be induced in π–π^* spectra by internal metal transitions, will be published later. It is my hope that this work shows how in favourable circumstances a relatively simple electronic model can be applied to rationalize some of the properties of even such complicated biological metal complexes as the corrins. There remain, of course, many features which we do not yet understand, for example, the precise form of the intensity variation between cyano- and methyl-cobalamins, but the success so far achieved suggests that further refinement of the present model will provide a satisfactory answer to these problems.

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