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Kinetic and thermodynamic studies of the ligand substitution reactions of the cobalamins

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CHAPTER 1

INTRODUCTION

1.1 Vitamin B_{12} and its Structure

Vitamin B_{12} (cyanocobalamin) has attracted much attention since its discovery as the anti-pernicious anaemia factor. 1948 marked the beginning of the chemistry of vitamin B_{12} as a result of its isolation in the form of dark-red crystals by Smith[1,2] and Parker[2] as well as Folkers and co-workers[3]. Dorothy Crowfoot Hodgkin and her co-workers used X-ray crystallography to elucidate the unique chemical structure of cyanocobalamin in the years 1950–1961.[4-8] Chemists and biochemists have found the B_{12} structure, along with the chemistry surrounding its coenzyme, quite intriguing and much emphasis has been placed on the total synthesis of the cobalt (III) corrin.

Vitamin B_{12} belongs to a group of cofactors known as the uroporphinoid family, shown in Figure 1.1.[9] Nature has selected this variety of incredible structures by varying a single structural theme, the uroporphyrinogen, for which two modes of variation are available – the centrally coordinated metal and the ligand sphere.[9] Amongst them a diversity of biological functions are carried out in both the plant and animal kingdoms. Iron porphyrins are essential for the process of respiration, where as haemoglobin and myoglobin are responsible for oxygen carrying and storage, respectively. Iron is also involved in biological-electron transport systems, in which the cytochromes are key components. In the plant kingdom magnesium porphyrins are required for photosynthesis that provides energy to all living systems. However, corrins are found only in the animal kingdom.

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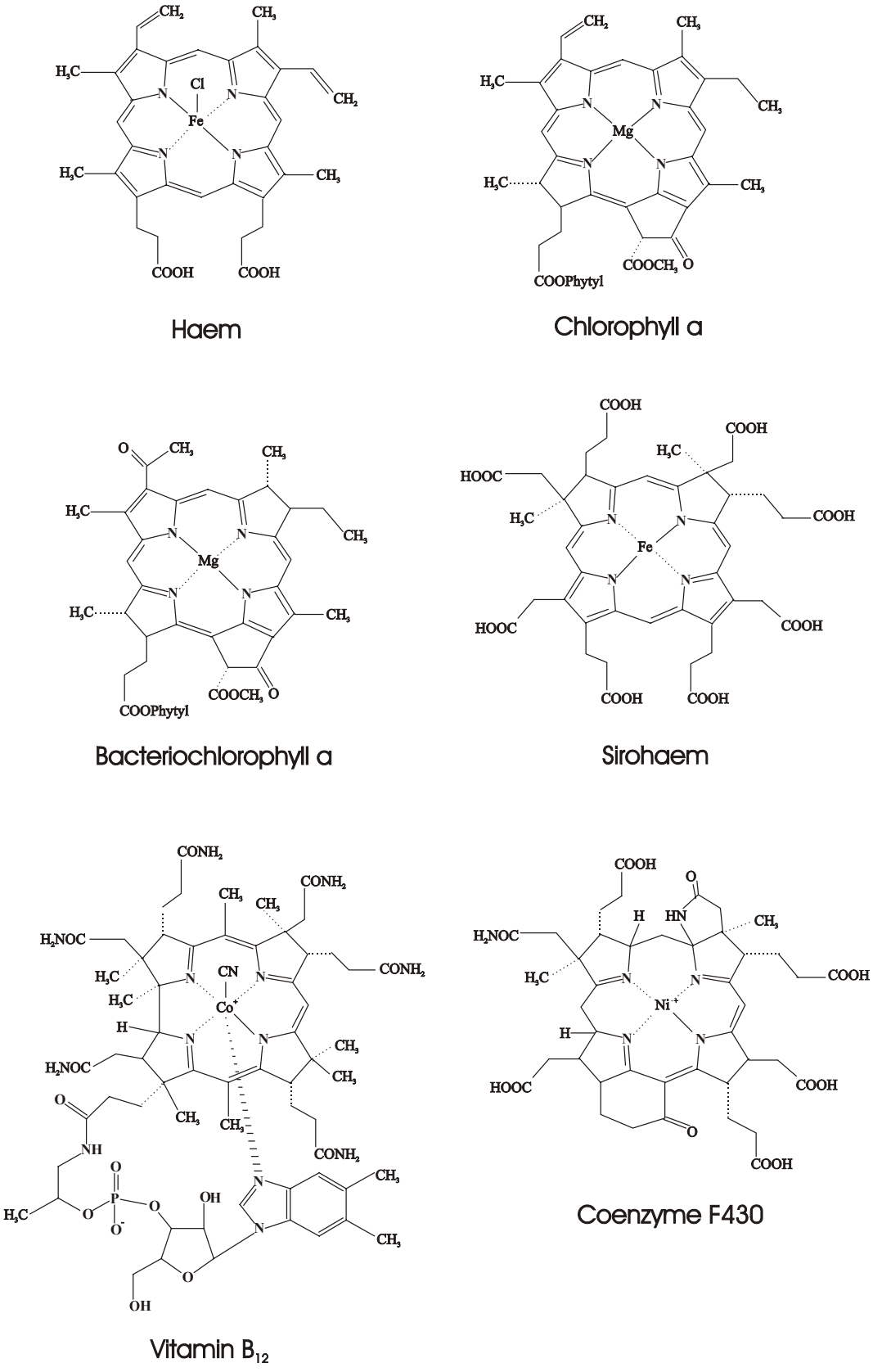

Figure 1.1 Family of uroporphinoid cofactors.[9]

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The structure of vitamin B_{12} involves two heterocyclic systems: a benzimidazole and a modified porphyrin nucleus, termed corrin. The four reduced pyrrole rings are joined into a macrocyclic ring by links between their α positions. Methine bridges form three of these links and a direct Cα–Cα bond forms the other. This is where the corrin structure differs from the porphyrin macrocycle – the porphyrin has this methine bridge between all of the pyrrole rings i.e. between rings D and A as well. The numbering is the same as that of the porphyrin nucleus and hence number 20 is missing from the ring system to preserve the identity (C20 is one of the methyl substituents).

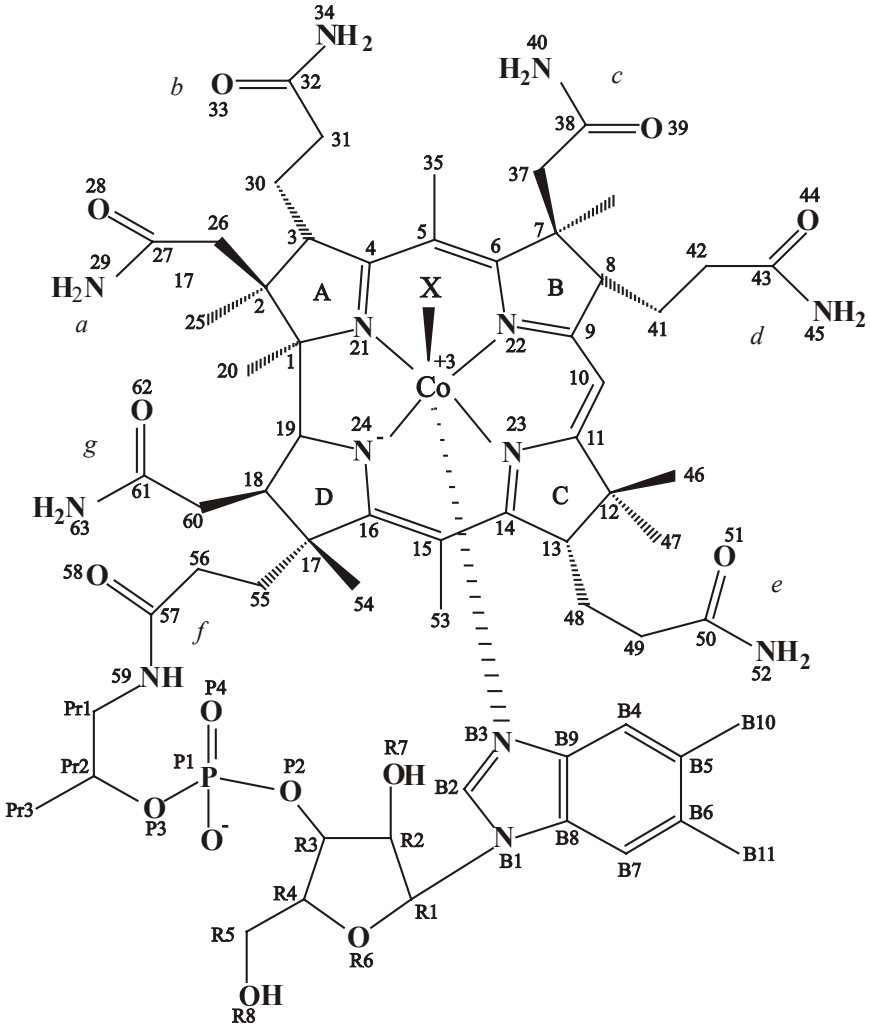

Figure 1.2 The structure and numbering scheme of the cobalamins where X = CN^- for cyanocobalamin and H_2O for aquacobalamin.

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A cobalt atom in the +3 oxidation state sits in the middle of the corrin and is octahedrally coordinated to the nitrogens of the pyrrole rings as well as the axial ligands. 5,6-Dimethylbenzimidazole is attached by a glycosyl link from its B1 N atom to R1 of the ribose and additionally linked by a bond between B3 and the cobalt (in the lower or α position). The ligand in the upper (or β position) is cyanide in the case of cyanocobalamin and H_2O in the case of aquacobalamin (Figure 1.2).

The cobalamins also have side chains, which occur at the β positions of the pyrrole rings. However, C12 does not possess a side chain. There are three acetamide chains, *a*, *c* and *g* which occur above the plane and four propionamide chains, *b*, *d*, *e* and *f* which occur below the plane of the corrin macrocycle. The *f* side chain is further linked to the propanolamine-phosphate-ribose-dimethylbenzimidazole group in the complete corrinoids.

The cobinamides have a water molecule attached in the α position instead of a 5,6-dimethylbenzimidazole group. The most well known of the cobinamides is cyanoaquacobinamide (better known as Factor B), which was first isolated from calf rumen.[10] Some of the naturally occurring nucleotide analogues of vitamin B_{12} are listed in Table 1.1.

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Table 1.1 Some naturally occurring nucleotide analogues of vitamin B_{12}. (Data from Ref. 13).

Trivial Name	**Systematic Name**	**Base of Nucleotide**
Pseudovitamin B_{12}	α-Adenylcobamide cyanide	Adenine
Factor A (vitamin B_{12m}; pseudovitamin)	2-Methyl-α-adenylcobamide cyanide	2-Methyladenine
Factor C (Nocardia factor 1)	Guanylcobamide cyanide	Guanine
Factor F	2-Methylthio-α-adenylcobamide cyanide	2-Methylthioadenine
Factor G	α-Hypoxanthylcobamide cyanide	Hypoxanthine
Factor H	2-Methyl-α-hypoxanthylcobamide cyanide	2-Methylhypoxanthine
Factor I (vitamin B_{12111})	5-Hydroxy-α-benzimidazolycobamide cyanide	5-Hydroxy-benzimidazole

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1.2 Adenosylcobalamin (Coenzyme B_{12})

Derivatives of vitamin B_{12} are coenzymes in certain enzymatic reactions. The structure of the adenosylcobalamin cofactor (coenzyme B_{12}) was first elucidated by X-ray diffraction structural analysis in 1961.[11] The structure is the same as that of cyano- and aquacobalamin seen in Figure 1.1, but with an adenosyl group (Figure 1.3) occurring at the X position. Adenosylcobalamin catalyses various enzymatic reactions of which a representation can be seen in Figure 1.4.[12] The reactions involve a 1,2-interchange of a hydrogen atom and another group (e.g. OH, NH_2, COSCoA, CCH_2COOH and $CH(NH_2)COOH$) on adjacent carbon atoms.[14] It is well known that the first step of coenzyme B_{12}-catalysed reactions involves the homolysis of the Co–C bond to produce Co(II) (vitamin B_{12r}) and a 5′-deoxyadenosyl radical,[14,15]. Halpern[14] has suggested that one can view the role of coenzyme B_{12} as a 'reversible free radical carrier'.

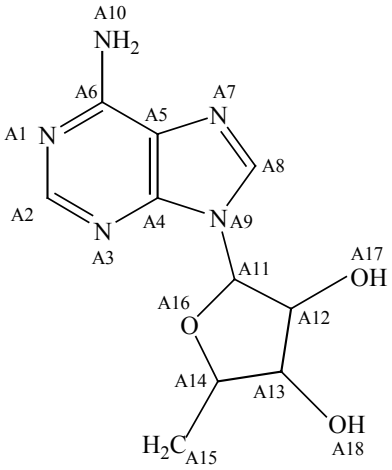

Figure 1.3 Structure and numbering scheme of the adenosyl group, which forms the compound adenosylcobalamin when it is the X ligand in Figure 1.2. Attachment to the Co atom occurs through A15.

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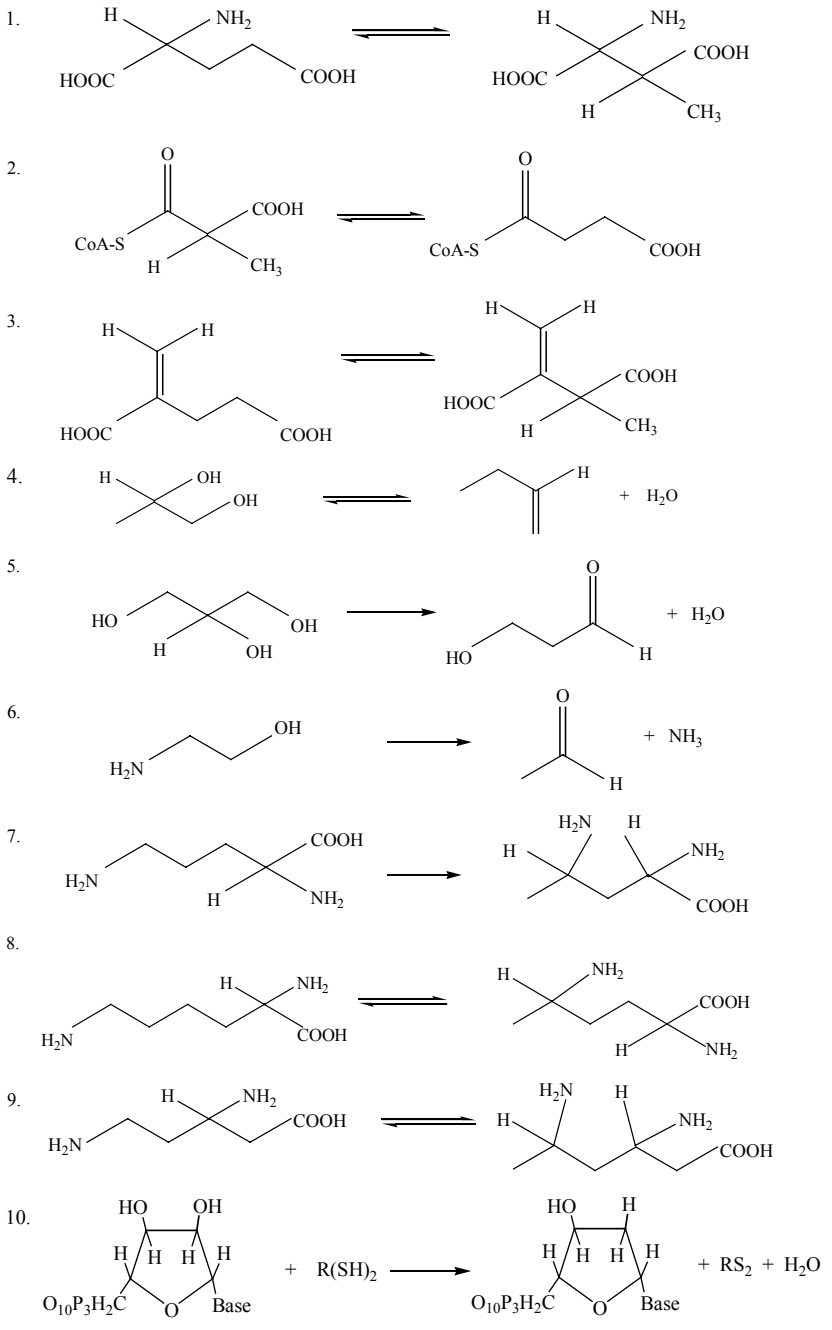

Figure 1.4 Coenzyme B_{12}-dependent rearrangements.[12]

The cobalt corrinoids differ from most types of complexes studied by coordination chemists due to the large amount of distortion, steric repulsion and flexibility observed within the complex. X-Ray analysis shows a surprising variation in the degree and kind of non-planarity of the corrin ring.[16] It is this flexibility that has led Halpern to speculate that the weakening of the cobalt-carbon bond is induced by a conformational distortion of the corrin ring towards the 5′-deoxyadenosyl group.[14] However, the mechanism by which coenzyme B_{12} achieves its efficient catalysis is not well understood and has led to many studies and much speculation. Halpern[14] found that although the bond dissociation energy was weak in comparison with covalent bonds of organic models, considerable further weakening of the Co–C bond was needed to achieve dissociation rates that matched the enzymatic rates. He also looked at some model compounds including [R-Co$(DH)_2$L] and [R-Co(saloph)L] and found that the Co–C bond dissociation energies differed by ~7 kcal mol^{-1} which led to the deduction that steric factors are involved. Abeles and Dolphin[12] proposed that it is a result of a distortion of the corrin ring that arises from interactions of the amide groups of the coenzyme with groups of the enzyme protein. They noted that the hydrolysis of one of the amide groups results in the loss of the coenzyme's activity. Brown and co-workers[17,18] undertook studies with neopentylcobalamin analogues as well as neopentylcobalamin analogues complexed to the vitamin B_{12} binding protein, haptocorrin, in order to investigate the contribution of the *c* side chain rotational motions to the thermal Co–C homolysis. They found that a steric restriction of the rotation of the *c* side chain of the analogues contributes significantly to the activation entropy for thermal Co–C bond homolysis. The studies with haptocorrin supported this theory, where neopentylcobalamin and its analogues were stabilised 380-fold toward homolysis on being complexed to haptocorrin. They believe that this entropic stabilization results from hydrogen-bonding interaction of the acetamide side chains with the protein pocket. Brown and co-workers have suggested that 30–40% of the catalytic ability of the adenosylcobalamin could come from this restricted motion but do not believe that this is the only mechanism in operation. Perry *et al.*[19] have suggested that the transfer of electron density onto the Co(III) centre and hence

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the conferring of some Co(II)-like character onto it, along with the further modulation of this by the binding of the protein, may activate the Co–C bond towards homolysis. This hypothesis is based on the findings that for adenosylcobalamin the homolysis of the Co–C bond is catalysed by 10^{10}-fold and higher[20,21,22] and that of methylcobalamin, the other naturally occurring alkylcobalamin, by a rate of $>10^{15}$.[23] These are just a few of the proposals that have been made; the exact mechanism, however, remains unresolved (see Section 1.5.2 for further discussion).

1.3 Cobaloximes

In an attempt to solve the problem of the mechanism of the adenosylcobalamin-dependent rearrangement reactions, studies have been extended to model compounds of coenzyme B_{12}. The interest in model compounds has arisen as a result of the complexity of the coenzyme B_{12} reactions and also due to the difficulty in determining certain aspects of the mechanism.[24] The model of choice for coenzyme B_{12} is cobaloxime or bis(dimethylglyoximato)cobalt,[26] represented in Figure 1.5.

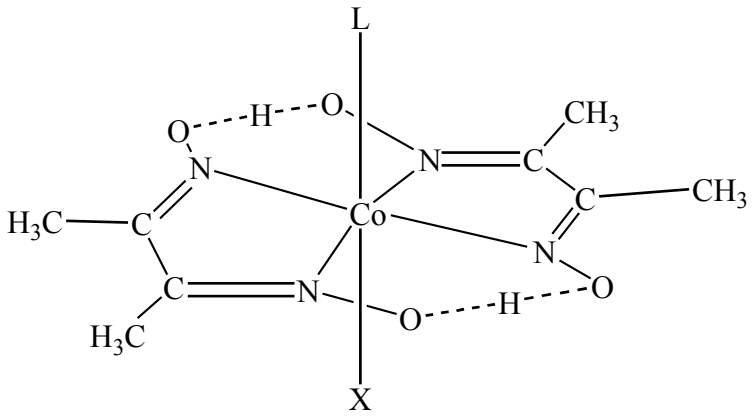

Figure 1.5 Cobaloxime, a model compound for coenzyme B_{12} where L and X can be a variety of ligands.[25]

Elliot and co- workers[24] used cobaloximes to compare the electrochemistry with that of coenzyme B_{12}. Their view was that the models were useful because large amounts of pure, characterised material were available and the need for ^1H NMR spectroscopy was also better accomplished by the use of these model compounds. However, the increased sensitivity of modern NMR spectrometers has made the direct NMR observations of cobalamins possible (see Chapter 10). Another advantage of using the cobaloximes is that they are soluble in noncoordinating solvents.[25] Elliot and co-workers[24] have stated that although the models have yielded important insights into the system and have contributed greatly to B_{12} and inorganic chemistry, there are numerous shortcomings. These include: an extremely strong Co–C bond, an incorrect overall charge, different electrochemical properties and axial base binding constants that are too high. Balt and co-workers[27] compared the solvent effects and kinetics of axial ligand substitution in both aquacobalamin and the model compound aquamethylcobaloxime. They found that the solvent effects did not differ but that the kinetics of B_{12} is clearly influenced by hydrogen bonding and steric factors from the acetamide chains that are absent in the model compound. They concluded that this is not a good model since steric effects and hydrogen bonding play an important role in these biological reactions.

1.4 Porphyrin vs. Corrin

The question has been raised as to why the porphyrin ligand, which is used in so many other biological systems, is not used in vitamin B_{12} and its coenzymes. Geno and Halpern[28] suggested that the porphyrins are unsuitable ligands for coenzyme B_{12} because of their inflexibility. However, they were unable to directly compare the steric and electronic influences with different axial ligands for organocobalt porphyrin complexes with the corresponding corrin complexes because the latter has a limited tendency to bind axial ligands. Instead they looked at a series of benzylcobaltoctaethylporphyrin complexes [$PhCH_2$–Co(OEP)L] and the corresponding benzylcobalt complexes with the flexible equatorial ligand, dimethylglyoxime. With these they compared the influence that

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varying the electronic and steric properties of the axial ligand has on the Co–C bond dissociation energy. Their results indicated that the porphyrin ligand is not flexible enough in this role. However, other researchers have reported on the extreme flexibility of the porphyrin ring.[29,30] Eschenmoser[9] has suggested that the reason for the occurrence of the corrin structure, rather than the porphyrin, in B_{12} is that the corrins preceded porphyrins in the evolutionary scale. Anaerobic bacteria exist that produce corrinoids but no porphyrins.[31,32]

1.5 Coordination Chemistry of the Cobalamins

The study of the coordination chemistry of vitamin B_{12} and its derivatives has been invaluable because it has led to the dismissal of certain misconceptions, such as the importance of crystal field theory in correlating chemical properties, the kinetic inertness of cobalt (III) complexes and the instability of the bond between a transition metal and an alkyl group.[16] It is well established that Co(III) in the cobalamins is low spin with six valence electrons occupying the d_{xz}, d_{yz} and d_{xy} orbitals. However, the kinetic inertness associated with this electronic arrangement is not seen in the corrinoids. This lability is due to the breakdown of the cobalt (III) oxidation state formalism.[33,34] Hague and Halpern[35] performed studies of SCN^- substitution with $Co(DH)_2(NO_2)OH_2$ and $Co(DH)_2(I)OH_2$ where DH^- is the dimethylglyoximate ion. The kinetics of the reactions of aquacobalamin were similar to those of the anation reactions of these two compounds but with k_1 approximately 10^7 times larger in aquacobalamin. Fleischer, Jacobs and Mestichelli[33] studied the reaction of the ligands cyanide and thiocyanate with Co(III), Mn(II) and Fe(III) hematoporphyrins in order to better understand the lability of the Co(III) ion towards substitution reactions. They compared their results with experiments done by Randall and Alberty[36,37] under similar conditions for aquacobalamin with azide and thiocyanate, and found that the reactions for B_{12a} were faster (k_{II} for B_{12a}[37] with N_3^- = 790 M^{-1} s^{-1} and SCN^- = 5000 M^{-1} s^{-1} compared to k_{II} for $Co^{III}HP$ of 140 and 1850 M^{-1} s^{-1}, respectively). They concluded that this result was due to the labilising effect of the nonaqua ligand in the fifth coordination position of Co(III).

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Fleischer and Krishnamurthy[38] also undertook an investigation into this phenomenon. They proposed that the labilisation of cobalt was based either on the occurrence of an internal redox reaction leading to Co(II) or a ligand system that interacted with the cobalt ion in such a way that it loses its d^6 character. They looked into d^3 Cr(III) complexes, which are well known to be very inert towards substitution, and are also more difficult to reduce to Cr(II) than Co(III) to Co(II). They studied the chromium salt of the water-soluble tetra(p-sulfonatophenyl)porphine (Cr(III)-TPPS) with fluoride, pyridine and cyanide. The anation reactions were 10^3–10^4 times faster than 'normal' chromium complexes. The same reactions for cobalt were 10^6 times faster. The Cr(III)-TPPS is not easily reduced and so an internal redox mechanism is unlikely. Thus, it seems that the porphyrin ligand labilises Cr(III) and Co(III) such that they lose their d^3 and d^6 character, respectively. They rationalised this by arguing that the metalloporphyrin has a delocalised electronic structure that strongly mixes the ligand and metal-ion orbitals.

1.5.1 *cis*- and *trans*-Effects of the Cobalamins

The corrinoids are also useful to coordination chemists as they provide an excellent opportunity for studying the effect of one ligand on another where the interaction is through the central metal ion. The effect that a ligand has on another in the *cis* and *trans* positions is referred to as the *cis*- and *trans*-effects, respectively. Chernyaev[39], who investigated square-planar platinum(II) complexes, introduced these studies. He was also the first to recognise the existence of a specific order for the *trans*-effect amongst the ligands. Pratt and Thorp[40] investigated these same effects in the cobalt(III) complexes and found that the order differs slightly from that of the platinum(II) complexes. Pratt and Thorp divided their studies of the *cis*- and *trans*-effects into three levels:[41,42]

i) Ground state effects such as bond lengths and force constants (*cis* and *trans* effects);
ii) Chemical equilibria (thermodynamic effects); and

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iii) Rates of reaction (kinetic effects).

They also showed that a parallel exists between *trans*- and *cis*-effects. The effect of the axial ligand on the electronic spectrum of the ring corrin system (*cis*-effect) and on the equilibrium between water and benzimidazole (*trans*-effect) shows a general similarity with the position of the ligand in the nephelauxetic series. This is representative of the amount of negative charge that the ligand donates to the metal ion through the σ-bond. This general order was first identified by Pratt and Thorp[41] as well as Firth and co-workers[42] and is as follows: N, O, Cl, Br and C in CN^- < S, Se, I and C in the organo-ligands; and CN^- < ethinyl < vinyl < ethyl for the carbanions.[16] Hayward *et al*.[43] list the following reasons for using the corrinoids in the study of the thermodynamic *trans*-effect:

i) They are octahedral which prevents the uncertainty of the binding of further ligands that may occur with square-planar complexes;
ii) The corrin ring prevents *cis*-*trans* isomerisation;
iii) In general the equilibria are rapidly established; and
iv) It is possible to study a wider range of ligands than in simpler cobalt(III) complexes.

The kinetic *cis*-effect is very prominent in the corrin structure and Table 1.2 reflects some second-order rate constants for the substitution of water by various ligands in a few Co(III) complexes (each containing four N-donor ligands in the equatorial plane).

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Table 1.2 Rate constants for the ligand substitution of water in various $Co^{III}(N_4)$ complexes.

X	Complex	L	k_2 /$M^{-1}s^{-1}$	Reference
OH^-	corrin	N_3^-	1.6×10^5	45
OH^-	porphyrin[a]	N_3^-	7.2×10^2	46
H_2O	corrin	I^-	2.2×10^3	47
H_2O	porphyrin[a]	I^-	1.62	48
I^-	corrin	SCN^-	1.5×10^2	47
I^-	cobaloxime[b]	SCN^-	1.2×10^{-3}	35
H_2O	corrin	SCN^-	8.2×10^2	47
H_2O	$(NH_3)_4$	SCN^-	8.6×10^{-7}	49

[a] Tetrakis(4-*N*-methylpyridyl)porphinato.

[b] Bis(dimethylglyoximato).

The results show that the rate constants for the substitution of water in the corrin, porphyrin, cobaloxime and amine complexes are in the approximate ratio of 10^9:10^6:10^4:1. Poon[44] showed that the rate constants for the hydrolysis of $Co^{III}(N_4)$ compounds increase with the extent of unsaturation in the ratio 1:36:270 as the N_4 system is changed from 1,4,8-11-tetraaza-*cyclo*-tetradecane, to 1,4,8,11-tetraazacyclotetradeca-1,7-diene, to cobaloxime.

Hill and co-workers[50] also showed the occurrence of a *cis*-effect from a study of the proton magnetic resonance spectra of some cobalamins. They found that the chemical shift of the methane hydrogen at the C10 position of the corrin ring is dependent on the ligand that is attached to the cobalamin. Establishing the resonance that belongs to the hydrogen at the C10 position by comparing it with the spectrum of the chlorinated derivative where the corresponding resonance was absent, enabled these measurements to be made.

Further studies revealed that keeping benzimidazole as the fixed ligand resulted in the shifts in the resonance of the C10 hydrogen towards high field due to the

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varying axial ligand being in the order: $H_2O < OH^- < C_2H_3^- < CH_3^-$. When water was the fixed ligand, the order became: $CH_3^- \sim C_2H_5^- < CN^-$.[50] They pointed out that their results with benzimidazole are comparable with those expected when the cobalt atom experiences an increase in electron density, and in line with an increase of electron density at the C10 position. They also found a correlation between the chemical shifts at this position and the energies of the α and β bands (Table 1.3). They noted that as the charge density at the C10 position increases, the shielding of the hydrogen at this position increases and the ^{1}H NMR resonance occurs at a higher field. This correlation ($R^2 = 0.97$) is clearly seen in Figure 1.6 which is a plot of the β band wavelength against the τ value of H10.

Table 1.3 Correlation between absorption and NMR spectra of cobalamins and cobinamides (data from Ref. 50).

Compound	**α Band / 10^{-3} cm^{-1}**	**β Band / 10^{-3} cm^{-1}**	**C10 hydrogen chemical shift /τ**
Dicyanocobinamide	17.2	18.5	4.13
Ethylcobalamin	18.1	19.0	4.12
Methylcobalamin	18.2	19.1	4.12
Vinylcobalamin	18.2	19.2	4.05
Hydroxocobalamin	18.6	19.35	3.92
Aquacobalamin	19.0	20.05	3.72
Methylcobinamide	21.7	21.7	3.20

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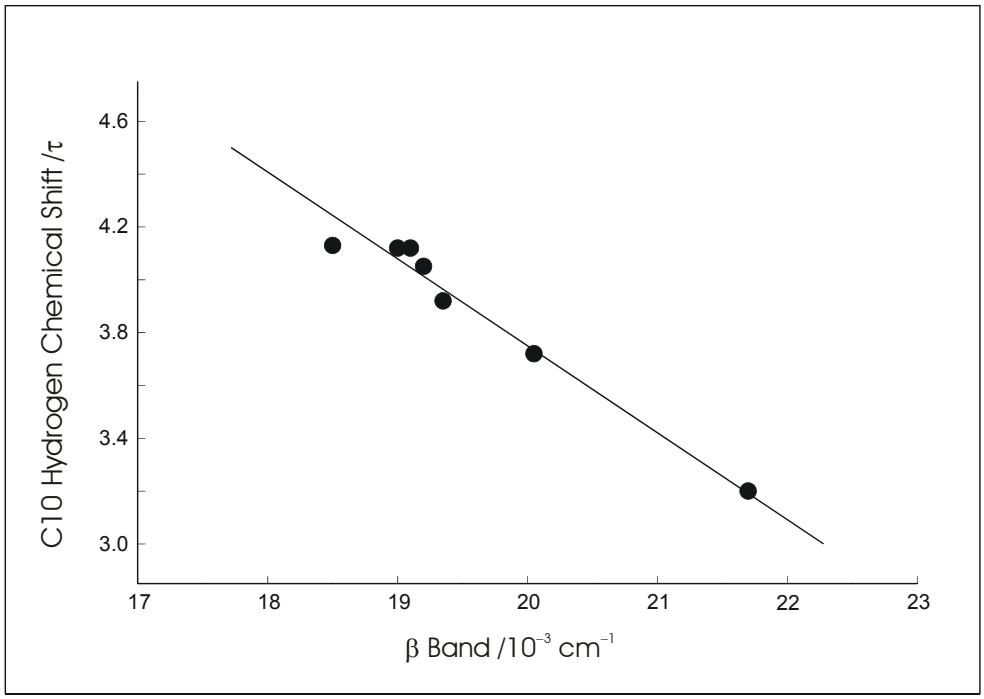

Figure 1.6 Correlation of the β band with the C10 hydrogen chemical shift (R^2 = 0.97). The data for the plot were obtained from Table 1.3.

Chemaly[51] has investigated various phosphite ligands with vitamin B_{12a} and found that the β band wavelength and the δ (or τ) values of H10 for the phosphitocobalamins with the ligands $P(OCH_2)_3CCH_2CH_3$, $P(OCH_3)_2O^-$, $P(OCH_2CH_3)_2O^-$ and $P[OCH(CH_3)_2]_2O^-$ all fall on this same line. She also found that this *cis*-effect correlated with the thermodynamic *trans*-effect of the phosphitocobalamins and suggested that this is further evidence for a correlation between the *cis*- and *trans*-effects of the cobalamins.

The effects of varying a ligand X on the Co–N bond lengths in both the *cis* and *trans* positions for three cobalt complexes can be seen in Table 1.4. Pratt[52] points out that these data show that varying the ligand mostly affects the *trans* position and only methyl and dimethylphosphite cause *cis* lengthening. The order of the *trans*-effect is the same for all three complexes i.e. ammonia < sulfite < methyl,

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Table 1.4 Ground-state *cis*- and *trans*-effects on the bond lengths of some Co(III) complexes.

Co–N bond lengths (in Å) in:							
X	$X–Co(NH_3)_5$		$X–Co(dmgH)_2NH_3$		X–Co(corrin)(dmbzim)		Ref.
	Co–N(*cis*)	Co–N(*trans*)	Co–N(*cis*)	Co–N(*trans*)	Co–N(*cis*)	Co–N(*trans*)	
H_2O					1.88–1.90	1.925	52[a]
NH_3	1.966	1.966	1.893	1.960			52[a]
HO^-						1.98	52[a]
CN^-					1.88–1.92	2.01	52[a]
$-CF_3$					1.87–1.95	2.05	52[a]
SO_3^{2-}	1.966	2.055		2.030		2.17	52[a]
$-CH_3$	1.973	2.105		2.053	1.88–1.97	2.19	52[a]
$PF(OMe)O^-$					1.88–1.97	2.09	52[a]
$P(OMe)O^-$					1.91–1.96	2.20	52[a]
NO_2^-					1.87–1.92	1.99	53
					1.89–1.92	2.01	20
$SeCN^-$					1.89–1.92	2.02	53
					1.89–1.93	2.10	20
SCN^-					1.89–1.92	1.99	53
					1.87–1.92	1.96	20
$S_2O_3^{2-}$					1.88–1.92	2.08	20
isoamyl					1.86–1.91	2.27	54
$i\text{-}Pr_2PO_3$					1.88–1.92	2.19	55
Cl^-					1.88–1.92	1.99	56
$SC(NH_2)_2$					1.85–1.91	2.01	57

[a] Data reproduced from Ref. 52.

which correlates with what has previously been mentioned pertaining to the nephelauxetic effect.

Firth and co-workers[42] undertook a study on the variation of an axial ligand on the stretching frequency of cyanide, which was coordinated in the *trans* position. Pratt[52] has shown that cyanide is a convenient ligand, with minimal steric requirements, for the study of these *cis*- and *trans*-effects of the cobalamins. Table 1.5 shows the outcome of this investigation. The frequency drops as X becomes more polarisable,[42] which indicates that the negative charge donated from the ligand to the cobalt atom through the σ-bond is the dominant contribution to the *cis*- and *trans*-effects. In the Pt(II) complexes both the σ-donor and π-acceptor capacities are involved in the *trans*-effect.[52]

Table 1.5 Ground-state *cis*- and *trans*-effects on CN stretching frequencies in Co(III) complexes (data from Ref. 52).

	ν_{CN}/cm^{-1} in:		
X	X–Co(corrin)CN	X–Co$(CN)_5$	X–Co$(dmgH)_2$CN
H_2O	2.133	2.128	
DMB	2.132		
-OH	2.131		
-CN	2.119	2.134	2.130
-C≡CH	2.110		~2.130
$-CH_2SO_3^-$	2.109	2.113	
$-CH=CH_2$	2.093		2.118
-Ado	2.091		
$-CH_2CO_2^-$	2.090	2.106	
-H		2.098	
-Me	2.088	2.094	2.112
-Et	2.082	2.094	2.109
(free CN^-)	(2.079)	(2.079)	(2.079)

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Perry[58] investigated the correlation between the mean positions of the Gaussian functions that comprise the αβ and γ bands (a measure of the *cis* influence) and the Co–NB3 bond length (a measure of the *trans* influence). As the polarisability of the β ligand increases all the bands shift to lower energies and the Co–NB3 bond lengths increase. He found a good correlation (R^2 = 0.90) between the αβ bands and the Co–NB3 bond length but only a weak correlation (R^2 = 0.30) with the γ bands.

1.5.2 Density Functional Theory (DFT) Calculations

Many authors have used DFT calculations to interpret the electronic properties of the cobalamins. These calculations have mostly been performed to shed more light on the Co–C bond activation in the B_{12} cofactors. De Ridder *et al.*[59] performed these calculations on the cobaloximes and noticed that the distances between Co–L and Co–X (see Figure 1.5) lengthen or shorten simultaneously but to different degrees. They called this an 'inverse' *trans*-effect which is opposite to the normal *trans*-effect where one bond shortens while the other lengthens. Zou and Brown[60] have also noticed this 'inverse' *trans*-effect with the compounds cyanocobalamin, β-trifluoromethylcobalamin, adenosylcobalamin and methylcobalamin. A plot of the Co–C bond length against the Co–X bond length gave them a fit of R^2 = 0.995. De Ridder and co-workers[59] believe that it is this 'inverse' *trans*-effect, which plays a vital role in the mechanism of the Co–C bond cleavage, creating strain simultaneously on both the Co–N and Co–C bonds in order to destabilise the Co–C bond and stabilise the cobalt(II) intermediate. Andruniow *et al.*[61] found, also from DFT calculations, that the donation of electrons leads to the simultaneous lengthening of the Co–N and Co–C bonds along with a conformational change of the corrin ring. However, the withdrawal of electrons affects the donor-acceptor character of the Co–N bond with no effect on the Co–C bond. This 'inverse' *trans*-effect was also observed by Randaccio *et al.*[62] for the ligands CF_3, Me and CMe_3, but not for the sulfur ligands HSO_3^-, SMe and $SC(NH_2)_2$, where a 'regular' *trans* influence was observed. They have suggested that since the increase of the σ donation from the ligand in the 'regular'

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trans influence is reflected by a decrease in positive charge on the corrin it must be electronic in nature, whereas for the 'inverse' *trans*-effect the metal charge increases with the σ-donating power of the ligand, making it a steric (due to the greater size of the σ-donor group) rather than an electronic effect. Hence, they believe that the 'inverse' *trans*-effect is not the 'general rule' with the cobalamins.

Dölker *et al*.[63,64] have performed DFT calculations on various model compounds to determine the effect of the *trans* axial ligand on both the homolytic and heterolytic cleavage of the Co–C bond. The methyltransferases, for example methionine synthase, undergo heterolytic cleavage involving methylcobalamin as a cofactor.[63] Their calculations showed that both the thermodynamics and kinetics of the homolytic bond cleavage are not dependent on the position of the axial benzimidazole ligand and also cannot be attributed to strain placed on the benzimidazole ligand by the protein. They believe that the axial ligand may still play a role by, for example, coordination of the cofactor to the enzyme and perhaps prevention of competing reactions. It has been proposed that the role of the axial ligand in enzymes that carry out homolysis is actually the prevention of heterolysis.[65-67] This led Dölker and co-workers[64] to study the effect of the heterolytic cleavage on the Co–C bond. They found that the distance between the axial ligand and the Co metal centre can be crucial in preventing heterolytic cleavage of the Co–C bond – it must be a relatively long distance away to prevent heterolysis. Their calculations show that the thermodynamics of the heterolysis depends largely on the distance of the axial ligand from the cobalt centre – approaching the cobalt centre when the methyl must be attached and moving away from the centre in order to release the methyl group.

The DFT calculations have also led to further speculation as to nature's choice of the Co/corrin over the Fe/porphyrin.[68] The DFT calculations of Stich *et al.*[68] show that the frontier MOs of methylcobalamin possess significant Co 3d orbital character from the σ donation of electron density from the alkyl ligand. They suggest that other metals would probably possess d orbitals that are too high or low in energy, thus forming too weak or too strong an organometallic bond. They

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also believe that if the porphyrin was in place then contributions from the Co $3d_{z^2}$ and the methyl C $2p_z$ orbitals to the HOMO would be greatly reduced or absent, since the highest-energy occupied MOs are raised in energy relative to the corrin.

1.6 Absorption Spectra of the Cobalamins

The *cis*- and *trans*-effects can be seen in the UV-vis spectra of the cobalt corrins. Therefore, it is extremely important to have a good understanding of the general spectrum of the corrinoids. The main bands of the spectra of the cobalt(III) corrinoids, above 300 nm, are mostly the result of spin-allowed π-π^* transitions within the corrin ring. This has been supported by calculations performed by Day.[69] The band with the greatest intensity occurs in the region of 350–370 nm and is known as the γ band. The bands with the second largest intensity are known as the $\alpha\beta$ bands and these occur in the region of 420–600 nm. The δ bands are found in the 300–330 nm region and finally the D and E bands of lowest intensity occur in the 390–420 nm region. A labelled spectrum of B_{12a} can be seen in Figure 1.7. Kuhn *et al.*[70] performed studies on the absorption of B_{12a} and their results can also be seen in Figure 1.7. The lowest energy levels, i.e. from 1 to 7, of the π electron system are filled and the transition from $7 \rightarrow 8$ corresponds to the $\alpha\beta$ bands, transition $6 \rightarrow 8$ to the DE bands and finally transition $7 \rightarrow 9$ to the γ band. There is no agreement as to the nature of the next transition (δ bands).[16]

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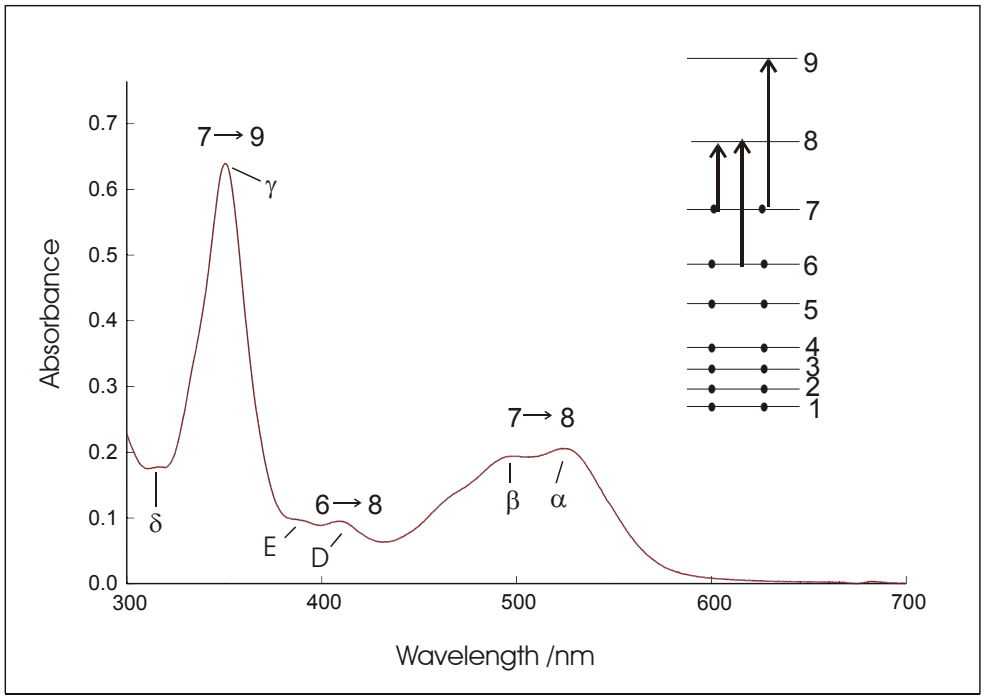

Figure 1.7 Energy levels of the π electron system and their corresponding bands in the absorption spectrum of aquacobalamin.[70]

Pratt[16] states that although charge-transfer bands are expected from transitions between the cobalt and the axial ligands or between the cobalt and the corrin ring, they are difficult to identify and thus far only one charge-transfer transition between cobalt and the phenolato axial ligand has been noted. Ochiai *et al.*[71] performed a Hückel molecular orbital calculation, on a model compound viz. [RCo^{III}(CR)Br]Y, that is in agreement with this statement. The energy scheme that they produced can be seen in Figure 1.8 where the $2a_1$ level is the σ-bonding orbital of the cobalt centre and the $3a_1$ level is the σ-antibonding orbital of the alkyl carbon atom. Their calculations indicate that the transition between these two levels is a charge-transfer band that is most likely hidden behind more intense π-π* bands in the same region.[72]

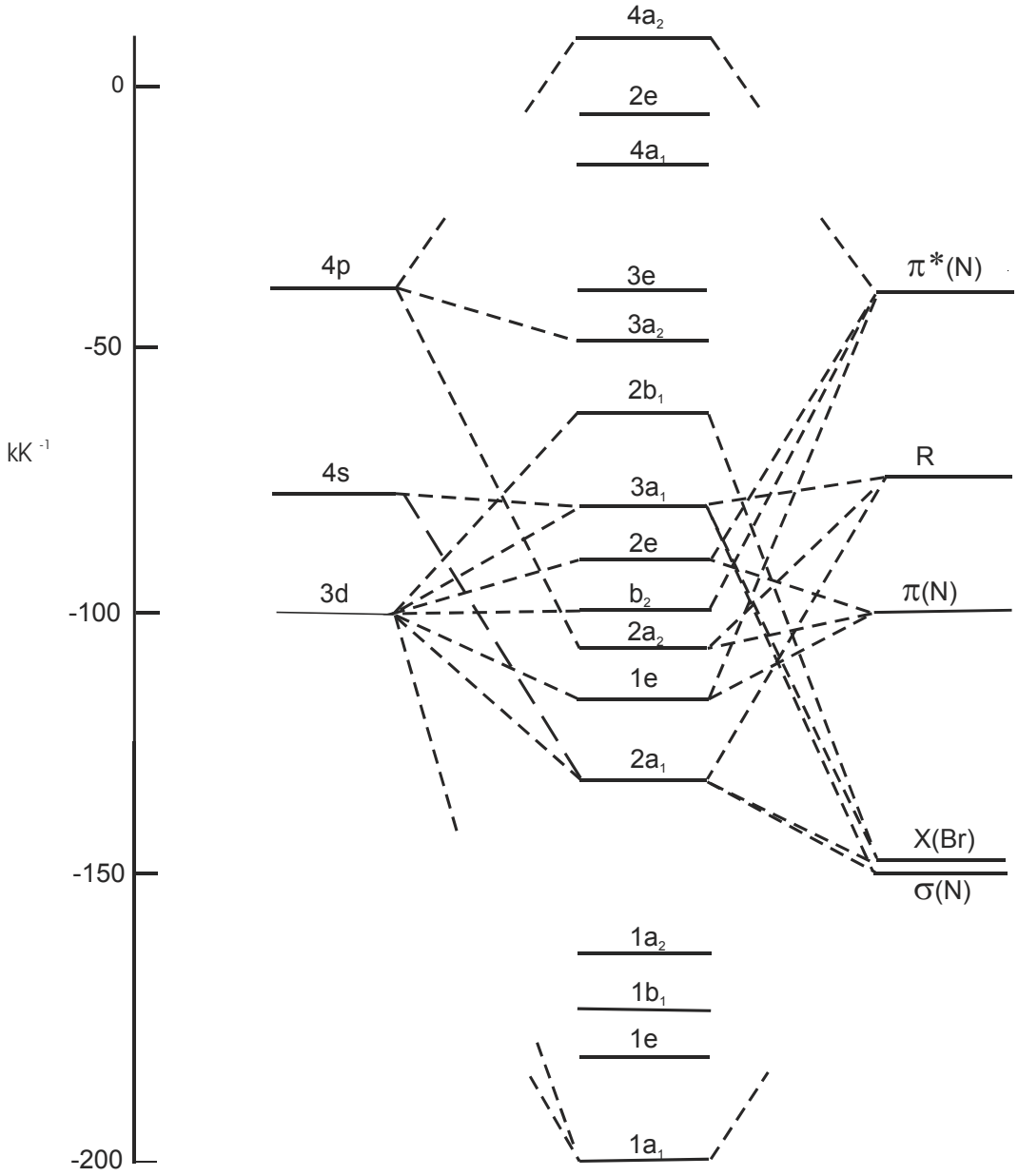

Figure 1.8 Molecular orbital diagram for $[CH_3Co^{III}\text{-}(CR)Br]PF_6$.[71]

The main bands of the cobalamins are very similar to those of the metal porphyrins, with the Soret or γ band at about 400 nm and the αβ bands around 550nm. The band positions of vitamin B_{12} are at shorter wavelengths because there are less conjugated double bonds.[73] The spectra of the cobalamins described

above and seen in Figure 1.7 is a 'typical' spectrum, but not all ligands exhibit this type of spectrum. An 'atypical' spectrum is seen with the alkylcobalamins such as methylcobalamin and vinylcobalamin, and the cobalamins containing the ligands I^-, $NCSe^-$ and $S_2O_3^{2-}$ have spectra that are very similar to 'atypical' types (Figure 1.9). In 'atypical' spectra, the γ band occurs at a longer wavelength (~370 nm) and is considerably reduced in intensity with the occurrence of other bands, of greater intensity, in the region of 300–350 nm. From these observations it is noted that the γ band moves to shorter wavelengths for the more electronegative ligand atoms (N, O, Cl, Br and C in CN^-) and to longer wavelengths for the less electronegative ligand atoms (S, Se, I and C in the organo-ligands).[16] This is also directly correlated to the effect of these ligands on the *cis*- and *trans*-effect where the more electronegative ligand atoms exert a weak *cis*- and *trans*-effect and the less electronegative ligand atoms a strong one.

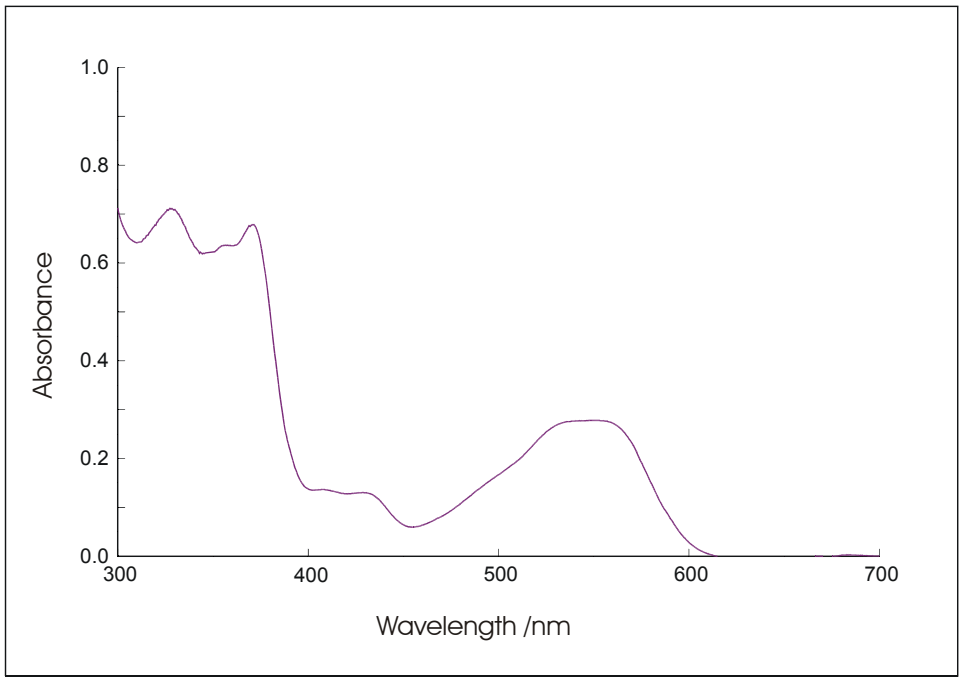

Figure 1.9 The 'atypical' spectrum of SeCNcobalamin with its reduced γ band occurring at 371 nm along with other bands, of greater intensity, in the region of 300–350 nm.

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Perry and Marques[74] undertook a Gaussian analysis on the electronic spectra of 11 cobalamin complexes and concluded that the model on which Kuhn *et al*.[70] based their study, that produced Figure 1.7, is an over-simplification. They fitted the γ band with three Guassian functions, which is in agreement with DFT calculations performed by Stich *et al.*[68] that suggests the γ band corresponds to at least three electronic transitions. Perry and Marques also believe that there is no difference between the 'typical' and 'atypical' spectra of the cobalamins and that the differences arise from the components of the γ band moving apart as a result of an increase in electron density from the axial ligand field.

In addition to the types of spectra mentioned above there exists another group of compounds with a unique set of spectra. These compounds are yellow or brown in colour and the first set of main bands occurs between 400 and 500 nm. These are termed stable yellow corrinoids because unlike some of the other yellow corrinoids such as cobalamin-cobalt-(II) and ethylcobinamide, they cannot be converted into the usual corrinoids by oxygen, cyanide, hydroxide or light.[16] The stable yellow corrinoids often occur when free radicals are present and they seem to be a result of these radicals attacking the corrin structure.[16]

A wide range of spectra occur in the corrinoids which are explained in terms of changes in the conformation of the corrin ring brought about by changes in the cobalt-nitrogen bonds.[41] Hill *et al*[75] decided from circular dichroism studies along with the absorption spectra that electronic rather than steric properties of the axial ligands play the dominant role in determining the conformation. They also performed ESR studies on B_{12r}, which showed that changing one axial ligand can create a large change in the electronic structure of the cobalt ion in the compound.

Pratt[16] summarised the effect of the axial ligands on the spectra as follows:

i) The axial ligands have a great effect on the intensities of the absorption bands;

ii) The σ-donor strength of the ligand is its most important property;

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iii) The sum of the donor strengths of the two axial ligands increases as the γ band moves to longer wavelengths; and

iv) The intensity of the γ band drops as the difference between the donor strengths of the axial ligands increase.

The absorption spectra of the cobalamins provide information on both the electronic and steric effects of the interaction of the ligand with the cobalt atom. Table 1.6[73] shows wavelengths of the *γ* band for a variety of ligands.

Table 1.6 The position of the γ band with the replacement of the water ligand by various ligands indicating the steric effect that the ligands have on the cobalt atom. (Adapted from Ref. 73).

LIGAND	**γ BAND**
H_2O	350
Pyridine	360
α-picoline	356
β-picoline	361
γ-picoline	361
Imidazole	358
Benzimidazole	354

The shift is least for the replacement of water by benzimidazole and α-picoline, which are the two ligands expected to exhibit the greatest steric hindrance.

In the cobaloximes, the interaction between the a_{2u} HOMO π_7 of the corrin and the π_{x_2}-type orbital of the axial ligand is absent.[76] The π-π* transitions are also much higher in energy (240 nm) than they are for the corrin system due to the reduced conjugation of the cobaloximes.

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1.7 Ligand Substitution Reactions

The study these *cis*- and *trans*-effects requires a good understanding of the ligand substitution reactions of this class of compound. These reactions involving coordination compounds have become an important part of inorganic chemistry. The general equation (Equation 1.1) for a ligand substitution reaction is:

$$[L_nMX] + Y \rightleftharpoons [L_nMY] + X \qquad (1.1)$$

The simplest type of replacement is the exchange of a coordinated ligand by an identical free ligand, an important example of which arises when the ligand is a solvent molecule.[41] It is suggested that there are few, if any, reactions in aqueous solution where X is not first replaced by H_2O, and then only does the other ligand, Y, enter the complex by displacing H_2O.[77]

Three simple pathways can be distinguished:

i) A dissociative path (D) in which the leaving ligand is lost in the first step;

ii) An associative path (A) in which the entering ligand adds in the first step, producing an intermediate of increased coordination number; and

iii) A concerted path called interchange (I), since the leaving ligand is moving from the inner to the outer coordination sphere as the entering group is moving from outer to inner.[78]

In a dissociative mechanism the activation energy is determined mainly by the energy required to break the bond of the leaving group whereas bond making is the main factor in determining the size of the activation energy for an associative mechanism. The three paths can be illustrated as follows (Equations 1.2–1.4):

D: $$[L_nMX] \underset{+X}{\overset{-X}{\rightleftharpoons}} [L_nM] \underset{-Y}{\overset{+Y}{\rightleftharpoons}} [L_nMY] \qquad (1.2)$$

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A: $$[L_nMX] \underset{-Y}{\overset{+Y}{\rightleftharpoons}} [L_nMXY] \underset{+X}{\overset{-X}{\rightleftharpoons}} [L_nMY] \quad (1.3)$$

I: $$[L_nMX] + Y \longrightarrow [L_nMX] \cdots Y \longrightarrow$$
$$[L_nMY] \cdots X \longrightarrow [L_nMY] + X \quad (1.4)$$

1.7.1 Mechanism for the Ligand Substitution Reactions of the Cobalamins

Randall and Alberty[36] performed a study of the kinetics of ligand binding to aquacobalamin with the thiocyanate ion in order to determine a mechanism for the process. They interpreted the data in terms of an S_N2 and an S_N1 mechanism and found that both mechanisms fitted data they obtained. They also compared their results with rate constants obtained by Ingold *et al.*[79] for several octahedral cobalt(III) complexes and found these rate constants to be several orders of magnitude smaller than what they had obtained. Hence, Randall and Alberty[37] extended their investigation with the ligands azide, cyanate and imidazole. They also wanted to look into Basolo and Pearson's[80] theory that a water molecule coordinated to cobalt(III) in cobalt complexes is replaced by another ligand via a slow unimolecular dissociation of the water group; followed by a very fast dissociation of the incoming ligand (the S_N2 mechanism). Their study showed that none of the three ligands could be interpreted by this mechanism despite the results for thiocyanate showing it to be feasible. Thusius[81] repeated the study with a variety of anions as the incoming ligand and found the equilibrium constants to vary by about 2×10^4 whilst the substitution rates were of the same order of magnitude. However, he disagreed with Randall and Alberty[37] and believed that Basolo and Pearson[81] had been correct in their argument of a dissociative mechanism. This debate prompted Reenstra and Jencks[82] to carry out further studies on the reactions of cyanide with cobalamins. They also considered the results of Thusius[81] and those of Poon[44] in which the rate and equilibrium constants vary over ranges of 10^2 and 10^{11}, respectively, for a wider range of

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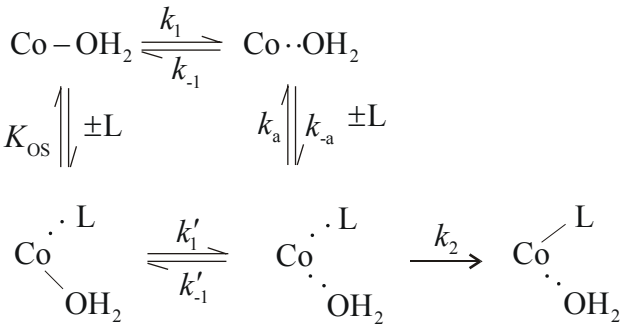
ligands and decided that these results were consistent with a dissociative interchange mechanism. They explained their theory in terms of Equation 1.5.



(1.5)

In terms of this equation a D mechanism is when the Co–OH_2 bond is broken and the product is formed on addition of the ligand (k_a) through the k_2 process. A D mechanism is favoured over an I_d mechanism if the addition of water to the intermediate is slow compared with k_{-a}, but in order to be a second-order reaction, this addition of water must be fast relative to that of the ligand. They suggested that this was unlikely because water is not particularly reactive towards metals and hence an I_d mechanism is more probable. They also discussed their preference for an I_d mechanism in terms of liganding and nonliganding solvents. Nonliganding solvents favour a dissociative mechanism because the unstable intermediate has sufficient time for the binding of the new ligand. In liganding solvents, such as water, the concentration of the solvent is higher than that of the ligand and the short lifespan of the intermediate enforces an I_d mechanism.

Since 1988, Marques and co-workers have performed many studies on vitamin B_{12} with a variety of ligands, ultimately to determine the mechanism by which the substitution reaction operates.[83-90] The first study was a reinvestigation of Reenstra and Jencks's[82] work involving the reaction of cyanide with both aquacobalamin and aquacobalamin bound to a vitamin B_{12}-binding protein (haptocorrin), obtained from chicken serum. The reactions were performed as a function of pH and temperature. They found that the reaction occurs by the same mechanism for both compounds. Their studies yielded an activation enthalpy for HCN substitution of H_2O higher than that for the substitution by CN^-. Since CN^-

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is expected to be a far stronger nucleophile than HCN, the experiment provided good evidence for nucleophilic stabilisation of the transition state.[83] A decrease in $\Delta H^{\ddagger}$ is the result of the bond making and is therefore lowest when the extent of participation with the ligand is greatest.

Extending these studies led them to research various incoming ligands.[84] These ligands included imidazole and a number of its derivatives as well as histamine and histidine. The rate of substitution of the water by these ligands was found to depend linearly on the base strength of the ligand. This provides evidence that, for this range of ligands, there is nucleophilic participation of the incoming ligand in the transition state. Further evidence of nucleophilic participation was obtained from studies with SCN^{-}, $S_2O_3^{2-}$, NO^{2-}, SO_3^{2-} and HSO_2^{3-}.[85] These and previously determined activation parameters for CN^{-} demonstrated that only small differences exist between most small anionic and neutral ligands in their reactions with aquacobalamin. However, in at least two cases of small anionic ligands viz. CN^{-} and SO_3^{2-}, which have large log *K* values, they found evidence for their participation in the transition state of the substitution reactions of aquacobalamin, which is seen from the activation parameters ($\Delta H^{\ddagger}$ = 51.7 kJ mol^{-1} and $\Delta S^{\ddagger} = -25$ J K^{-1} mol^{-1} for CN^{-}; $\Delta H^{\ddagger}$ =79.9 kJ mol^{-1} and $\Delta S^{\ddagger}$ = 33 J K^{-1} mol^{-1} for SO_3^{2-} compared with $\Delta H^{\ddagger}$ = 63–67 kJ mol^{-1} and $\Delta S^{\ddagger}$ = 20–26 J K^{-1} mol^{-1} for $S_2O_3^{2-}$, NO^{2-} and HSO_2^{3-}). The low $\Delta H^{\ddagger}$ and very negative $\Delta S^{\ddagger}$ for the reaction with CN^{-} may be the result of the bond formation of Co and CN^{-} in the transition state compensating for the Co–O bond breaking.[85] The activation parameters for the reaction of SO_3^{2-} can be explained in terms of sulfite having a strong *trans*-labilising effect in the cobalamins.[16,91] This explains the large $\Delta H^{\ddagger}$ value, which arises from a weakening of the Co–N bond of the equatorially bound benzimidazole group as the Co–SO_3^{2-} bond is formed in the transition state.[85] Marques[86] also performed a study on a series of primary amines and found that $\Delta H^{\ddagger}$ varies between 80 and 50 kJ mol^{-1} whereas $\Delta S^{\ddagger}$ varies between 50 and –50 J K^{-1} mol^{-1}. The rate of substitution of the amines also varied linearly with their donor power. All these findings indicate that the incoming ligand participates in the transition state; thereby pointing to an I_d mechanism.

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An alternate means of determining the mechanism for a ligand substitution reaction is to determine the volume of activation.[92,93] The volume profile of the reaction is obtained by pressure-dependence studies where for a reaction (Equation 1.6), the reaction volume, $\Delta\overline{V}$, is defined by Equation 1.7 and the activation volume, $\Delta V^{\ddagger}$, by Equation 1.8. $\overline{V}$ is the partial molar volume of the reactant or product species.

$$A + B \rightleftharpoons [A \cdots B]^{\ddagger} \longrightarrow AB \tag{1.6}$$

$$\Delta\overline{V} = \overline{V}_{AB} - \overline{V}_{A} - \overline{V}_{B} \tag{1.7}$$

$$\Delta V^{\ddagger} = \overline{V}_{\ddagger} - \overline{V}_{A} - \overline{V}_{B} \tag{1.8}$$

A relationship between the pressure dependence, $\Delta V^{\ddagger}$ and $\Delta\overline{V}$ of the rate constants can then be derived from the thermodynamic Equation 1.9.

$$\left(\frac{\delta\mu_i}{\delta P}\right)_T = \overline{V}_i \tag{1.9}$$

For the mechanism of anation of aquacobalamin by cyanoferrates and azide, Stochel and co-workers[94] determined the volume of activation for both the D and I_d mechanisms and compared these with theoretically predicted values. They concluded that a D mechanism was in operation due to the agreement of the theoretically and experimentally determined values for this mechanism. Marques and co-workers[87] undertook this line of investigation and also investigated azide along with hydrazoic acid. They found the values of $\Delta H^{\ddagger}$, for the reaction of aquacobalamin with these two ligands, to be the same. This indicates that a D mechanism is in operation. However, when looking at the I_d mechanism, the experimentally determined values for the volume of activation were in good agreement with those determined theoretically. Thus, they concluded that in this case the mechanism of the reaction cannot be determined solely by considering

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the activation parameters. They also disputed the findings of Stochel *et al.*[94] because their calculations were based on a spherical aquacobalamin molecule with a net charge of +1. Marques *et al.*[87] pointed out that if the phosphate group is assumed to be too remote (10 Å from the metal) and the calculations were repeated with a +2 charge for aquacobalamin, then their predicted values were in good agreement with that determined experimentally for an I_d mechanism.

Stochel and van Eldik[95] undertook an investigation of the reaction of aquacobalamin with pyridine and found that a plot of k_{obs} versus [pyridine] produced a saturation curve. They felt this could be evidence for a D mechanism and compared this result with the plot obtained by Reenstra and Jencks[82] for the same reaction with cyanide. Reenstra and Jencks[82] interpreted this result as an I_d mechanism that was explained in terms of ion-pair formation. Stochel and van Eldik[95] felt that since pyridine is a neutral ligand the ion-pair formation is less important. They backed this up with a volume of activation study and concluded that a limiting D mechanism is in operation.

Now that a ligand exhibiting saturation at high ligand concentrations had been identified, Marques and co-workers[89] set about finding a range of ligands with saturating behaviour in order to finally settle the dispute over the mechanism by which incoming ligands substitute water in aquacobalamin. They studied the ligands: hydroxylamine, methyl glycinate, pyridine, 4-methylpyridine, imidazole and histamine. The results showed that the saturating rate constants and hence the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ differ for each of the ligands. This means that for this set of ligands an I_d mechanism is in operation since the activation parameters are dependent on the identity of L, which is not the case for a D mechanism since the saturating rate constant corresponds to the unimolecular release of water from the molecule. Meier and van Eldik[96] revisited the reactions by investigating thiourea and substituted thiourea. They concluded that they had erred in suggesting that a D mechanism was in operation for pyridine and the other ligands that their group had studied. They decided that the error with pyridine is the result of a very specific reaction of aquacobalamin with pyridine that may involve some π

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character of the pyridine. This resolved the controversy and it is now well established that the substitution reaction of aquacobalamin with any incoming ligand involves an I_d mechanism. This ligand substitution mechanism is shown in Equations 1.10 and 1.11 where the incoming ligand, L, includes various anions, pyridines, amines and imidazoles.

$$\text{dmbzim - Co - } H_2O + L \overset{K}{\rightleftharpoons} \{ H_2O \cdots \text{dmbzim - Co} \cdots L \} \qquad (1.10)$$

$$\{ H_2O \cdots \text{dmbzim - Co} \cdots L \} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{dmbzim - Co - L} + H_2O \qquad (1.11)$$

Further information on the mechanism is presented in the introduction to Chapter 4.

Many of the ligands that have been studied in substitution reactions with aquacobalamin, along with the corresponding references, are shown in Table 1.7.

Table 1.7 Ligands that have been used for ligand substitution reaction studies with aquacobalamin.

Ligand	References
Br^-	81
I^-	81, 90, 100
SCN^-	36, 85, 90, 97, 108
NCO^-	37, 81
$S_2O_3^{2-}$	81, 85, 90, 105
N_3^-	37, 81, 87, 90, 94, 99, 109
SO_3^{2-}	81, 85
HSO_3^-	81, 85
NO_2^-	85, 90
CN^-	82, 83, 98
HCN	82, 83
HN_3	86, 87, 99
NH_2OH	86, 89
$NH_2(CH_2)_2OH$	86
$NH_2(CH_2)_3OH$	86
$NH_2CH_2CH(OH)CH_2OH$	86
NH_2Me	86
NH_2OMe	86
$NH_2CH_2CO_2Me$	86
NH_2Pr	86
Imidazole	37, 84, 89, 109
1-Methylimidazole	84
1,5-Dimethylimidazole	84
2-Methylimidazole	84
4(5)-Dimethylimidazole	84
Imidazole4(5)-lactic acid	84
N-Acetylimidazole	102
Histidine	84

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Table 1.7 *(continued)*

Ligand	References
Histamine	84, 89
Glycine	88, 109
Methyl glycinate	88, 89
Pyridine	89, 95
4-Methylpyridine	89, 101
2,4-Dimethylpyridine	101
4-Acetylpyridine	101
3-Acetylpyridine	101
1,2,4-Triazole	101, 102
Pyrazole	101, 102
$Fe^{II}(CN)_5NO^{2-}$	94
$Fe^{II}(CN)_6^{4-}$	94
$Fe^{III}(CN)_5H_2O^{2-}$	94
Thiourea	96, 106
Thioacetamide	107
Thiosemicarbazide	107
Thiocarbohydrazide	107
Methylthiourea	107
Dimethylthiourea	107
Tetramethylthiourea	107
Phenylthiourea	107
N,*N*′-dimethylthiourea	96
L-Cysteine	103, 104
N-Butanoyl-L-cysteine	104
N-Octanoyl-D,L-cysteine	104
N-Decanoyl-D,L-cysteine	104

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Many other investigations have been performed on the mechanism involved in the kinetics of the cobaloximes and other model compounds. Brown *et al.*[110] investigated the kinetics of 11 substituted alkylcobaloximes with pyridine and found that a dissociative mechanism is operational. However, the data, could not explain whether an I_d mechanism was occurring. Hamza *et al.*[111] studied the ligand substitution reactions of the model compound *trans*-$[Co^{III}(en)_2(Me)H_2O]^{2+}$ with the ligands cyanide and imidazole, using activation volumes, and found that the reactions follow a dissociative mechanism and that the nucleophile could be weakly bound in the transition state i.e. an I_d mechanism could be operational. A strictly D mechanism was found for the ligand substitution reaction of $[Co(NH_3)_5(CH_3)](NO_3)_2$ with ethylenedaiamine,[112] as well as for methylaquacobaloxime with a series of thiolate anions, 4-substituted pyridines and aliphatic amines,[113] and also for a series of alkylaquacobaloximes with dimethoxyethylamine.[114] The most interesting result was from a study of the displacement of adenosyl by cyanide in coenzyme B_{12} itself, where the mechanism was found to be associative.[115] It was found that the first cyanide attacks the β-adenosyl site (the rate-determining step) followed by a fast attack of a second cyanide at the α-dimethylbenzimidazole position. However, further work carried out in 92% DMF/8% D_2O shows that the rate-determining step is preceded by the fast addition of cyanide to the α-position.[116] A (β-5′-deoxyadenosyl)(α-cyano)cobalamin intermediate was identified by ^1H NMR spectroscopy. Brasch and Haupt[116] also suggested that a solvent molecule is involved in the transition state that precedes the rate-determining step.

In an attempt to further understand this mechanism Hamza *et al.*[117] performed kinetic studies on the reactions of alkylcobalamins with cyanide. They found that the reactions of β-CF_3CH_2Cbl and β-CF_3Cbl with CN^-, proceed through an I_d and a limiting D mechanism, respectively.[117] The 'mechanistic changeover' reported for coenzyme B_{12} was explained in terms of the ratio between the five- and six-coordinate species, shown in Figure 1.10. They believed this to be crucial in controlling the ligand substitution mechanism and that the base-off form of both adenosylcobalamin and ethylcobalamin is mainly the five-coordinate species (C in

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Figure 1.10) whereas β-CF_3CH_2Cbl exists in equilibrium between the five- and six-coordinate species and β-CF_3Cbl mainly exists as the six-coordinate species (A in Figure 1.10). Hence the 'mechanistic changeover' from R = adenosyl resulting in an associative mechanism, R = CH_2CF_3 in an I_d mechanism, and R = CF_3 in a limiting D mechanism. From various data, van Eldik and co-workers[118,119] listed a kinetic *trans*-effect for Xcobalamin where the order was found to decrease along the series Pr ≥ Ado ≥ Me ≥ CF_3CH_2 > CF_2H > $NCCH_2$ > CF_3 > CN^-. They concluded that the second-order rate constant that had previously been found for the reaction of adenosylcobalamin with CN^- [(7.4 ± 0.1) × 10^{-3} M^{-1} s^{-1}] is not in agreement with the kinetic *trans*-effect and must be the value for the subsequent slow step.[119] As a result, they reinvestigated the kinetics of the reaction of adenosylcobalamin with CN^- over a higher concentration range and saturation kinetics were now observed.[118]

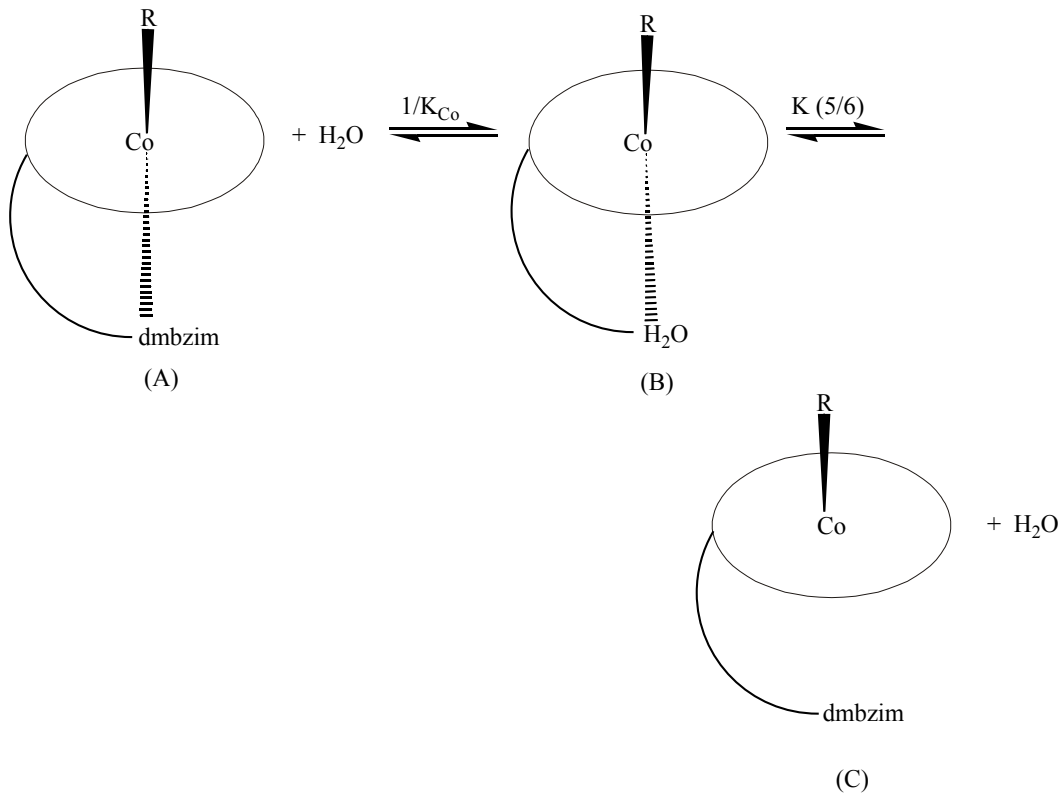

Figure 1.10 Organocobalamins with different R groups most probably occur in solution as a base-on form (A) and two base-off forms (B and C), which are either five- or six-coordinate.[117]

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Following studies to determine the involvement of the solvent in the heterolytic Co–C bond cleavage for the vitamin B_{12} derivatives,[120] Hamza and van Eldik[119] suggested that the negative volume of activation that was obtained from the reaction of adenosylcobalamin with cyanide was incorrectly interpreted as evidence for an associative mechanism. They believe that this large negative volume of activation is actually the result of equal negative contributions from the rapid formation of the intermediate and the solvent-assisted heterolysis reaction.

1.8 Aims of this Study

The main aim of this thesis is to further investigate the kinetics of aquacobalamin. From the introduction, it is clear that the mechanism for the ligand substitution reaction of aquacobalamin is well established as being that of a dissociative interchange mechanism. After this work was completed by Marques *et al.*[90], Prinsloo and co-workers[99] noted that the KCl, which was used as an ionic strength adjustor, was actually binding to aquacobalamin. The resultant chlorocobalamin compound is more inert to substitution than aquacobalamin and the limiting rate constant for the reaction of 4-methylpyridine in $NaClO_4$ was found to be higher than that in KCl by a factor of three. These results do not affect the conclusion about the mechanism of the substitution reactions of aquacobalamin but may affect other conclusions that have been drawn from the absolute values of the rate constants and their activation parameters. As a result it is necessary to reinvestigate the ligand substitution reaction of aquacobalamin with various ligands using a $NaNO_3$ medium instead of KCl.

A further aim is to determine whether the mechanism remains unchanged when the environment of aquacobalamin is altered in some manner. Hence, the substitution reaction of aquacobalamin with pyridine will be performed in both water and 70% ethanol. It will also be interesting to determine what happens to the mechanism when a bulkier ligand such as I^- replaces the axial water ligand. Will the mechanism remain that of I_d or will the bulky ligand prevent the incoming ligand from forming an outer-sphere complex with the metal centre?

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Hence the kinetics of the reactions of iodocobalamin with the ligands $S_2O_3^{2-}$, N_3^- and imidazole will be investigated.

A modification at the C10 position of the corrin ring will provide further insight into the *cis*-labilising effect of the cobalamins. Therefore, studies will be made on aquacobalamin that has been modified by three different groups at the C10 position, viz. aqua-10-chlorocobalamin, aqua-10-nitrosocobalamin and aqua-10-aminocobalamin. The acid dissociation constant, pK_a, will be determined for aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin in order to enable correction for the pH at which the experiment is undertaken. It will be interesting to determine the formation constants for aquacobalamin with a variety of ligands and compare them with those of aqua-10-chlorocobalamin to decide whether a change in the electronic properties of the corrin ring has any affect on them. Formation constants will thus be determined for both aquacobalamin and aqua-10-chlorocobalamin with the anions: nitrite, thiocyanate, azide, cyanate, thiosulfate and selenocyanate and the neutral ligands: methylamine, pyridine and imidazole.

The kinetics of both aqua-10-chlorocobalamin and aquacobalamin with the ligands pyridine and azide will be compared. It will also be of interest to see whether an I_d mechanism remains in place when the electronic arrangement of the corrin is altered in some way and whether the kinetics are faster or slower than that of aquacobalamin.

Finally, some of the spectra of aqua-10-nitrosocobalamin and aqua-10-aminocobalamin will be presented. The results will be discussed in each chapter and the main findings summarised at the end of the chapter. The final chapter, Chapter 12, will contain a summary of all the conclusions reached along with a discussion on this work's contribution to the *cis*-labilising effect of the cobalamins.

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