CHAPTER 10

AQUA-10-NITROSOCOBALAMIN

10.1 Introduction

The effect of replacing the H atom at the C10 position of the corrin with a Cl atom, that could potentially act as either an electron donor towards the delocalised π electron system of the corrin ring or as an inductively electron withdrawing substituent, was explored in Chapters 8 and 9. It is already clear that the axial coordination site and the corrin are in direct electronic communication since changes in either lead to differences in the electronic spectra of the cobalamins. The formation constants with a group of anions are higher for aqua-10-chlorocobalamin than they are for aquacobalamin but the neutral ligands have

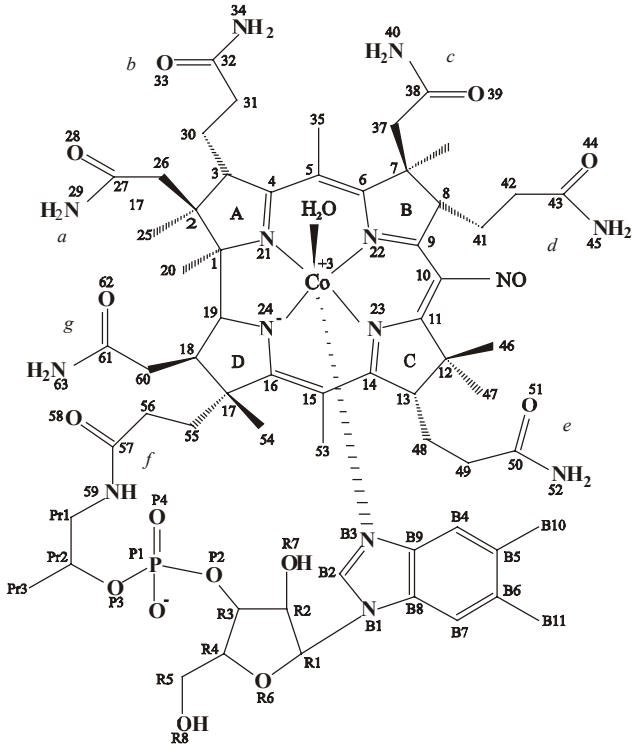


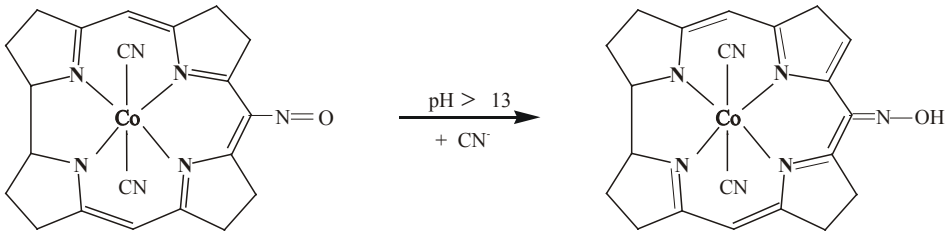
Figure 10.1 Aqua-10-nitrosocobalamin, where the H atom in the 10 position has been replaced with a NO group.

References on page 198

lower formation constants for aqua-10-chlorocobalamin than aquacobalamin. The studies with aqua-10-chlorocobalamin have led to a greater understanding of these trends. In order to further understand the *cis*-labilising effect it will be interesting to see what effect the substitution of other groups at the C10 position will have on the corrin ring. With this in mind the compound aqua-10-nitrosocobalamin was synthesised (Figure 10.1).

10.2 Results and Discussion

The compound aqua-10-nitrosocobalamin was synthesised from a modification of the procedure used by Wagner[1] to synthesise cyano-10-nitrosocobalamin from cyanocobalamin (see Experimental Section 2.8). Figure 10.2 shows the spectrum of aqua-10-nitrosocobalamin and the addition of both one equivalent, and excess cyanide to this compound. When cyanide is added to dicyano-10-nitrosocobalamin at pH > 13, the compound is converted into the isonitroso derivative (Scheme 10.1).[1]



Scheme 10.1. The formation of the isonitroso derivative from dicyano-10-nitrosocobalamin.

A comparison of the band peaks obtained here and by Wagner can be seen in Table 10.1. From the data for dicyano-10-nitrosocobalamin and dicyano-10-isonitrosocobalamin it is clear that the same compound has been synthesised as that reported by Wagner.

References on page 198

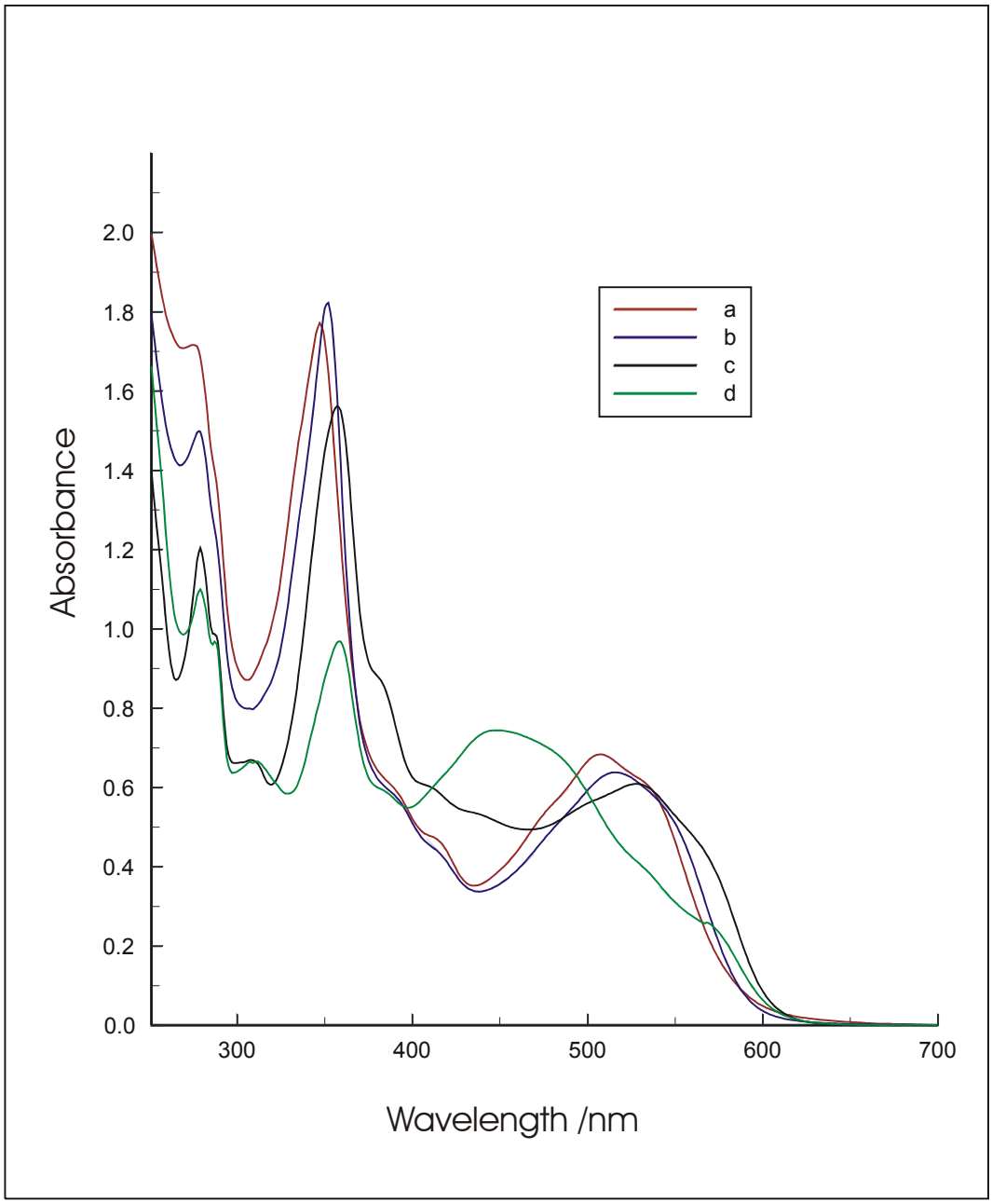


Figure 10.2 The UV-vis spectra of:

(a) 119 μM solution of aqua-10-nitrosocobalamin, pH 7, in aqueous solution;

(b) Addition of one equivalent of CN^- leads to the slow conversion to cyano-10-nitrosocobalamin;

(c) Excess CN^- results in the formation of dicyano-10-nitrosocobalamin; and

(d) At pH > 13 this compound is converted into the isonitroso derivative.[1]

References on page 198

Table 10.1 A comparison of the band peaks obtained for some of the nitroso derivatives.

Compound	γ /nm	β /nm	α /nm	γ /nm	β /nm	α /nm
	This work			Reported by Wagner[1]		
Cyano-10-nitroscobalamin	347	510	531	348	500	520
Dicyano-10-nitrosocobalamin	356	530	568	354	530	
Dicyano-10-isonitrosocobalamin	358	450		358	442	

Many attempts were made to obtain diffraction-quality crystals of aqua-10-nitrosocobalamin but the crystals obtained have been too small and of very poor quality. Since suitable crystals were unobtainable, NMR spectroscopic data was relied on for any deductions about the structure of aqua-10-nitrosocobalamin in solution. The significant differences in the ^{13}C NMR resonances from the effect of substitution of H by NO at the C10 position can be seen in Table 10.2 and Figure 10.3. Table 10.2 also indicates that the differences in the shifts of the ^{13}C NMR resonances are greater for the substitution of NO than they are for that of Cl. Appendix 1 contains a full comparison of the ^{13}C NMR resonances of aquacobalamin,[2] aqua-10-chlorocobalamin[3] and aqua-10-nitrosocobalamin. The greatest differences are seen in the eastern part of the molecule. The C10 resonance has shifted by 35.2 ppm and this decreases as one moves away from the C10 position, with even remote carbon atoms such as C3, C19 and C54 being affected. This is an indication that the substitution of NO for a H atom at the C10 position has a significant effect on the electronic properties of the corrin ring.

References on page 198

Table 10.2 The significant differences in the ^{13}C NMR resonances, in ppm, between aqua-10-nitrosocobalamin and aquacobalamin, and between aqua-10-chlorocobalamin and aquacobalamin.

Group	**H_2OCbl^+**	**H_2O-10-$NOCbl^+$**	**$\Delta\delta_1$**	**H_2O-10-$ClCbl^+$**	**$\Delta\delta_2$**
C10	97.58	132.77	35.19	106.84	9.26
C11	181.49	170.88	–10.61	177.75	–3.74
C9	177.24	171.28	–5.96		
C37	48.21	44.12	–4.09		
C8	60.02	56.77	–3.25		
C12	50.9	53.80	2.90	53.36	2.46
C15	106.85	109.65	2.80		
C46	34.84	32.20	–2.64	32.13	–2.71
C36	23.81	21.30	–2.51		
C5	110.63	113.06	2.43	112.15	1.52
C14	168.53	166.41	–2.12		
C13	56.42	58.05	1.63	57.92	1.50
C16	183.85	182.24	–1.61	182.51	–1.34
C19	77.73	76.25	–1.48		
C47	22.18	20.74	–1.44	24.63	2.45
C7	53.59	54.85	1.26		
C6	166.75	165.68	–1.07		
C26	45.95	44.90	–1.05		
C38	178.12	177.08	–1.04		

References on page 198

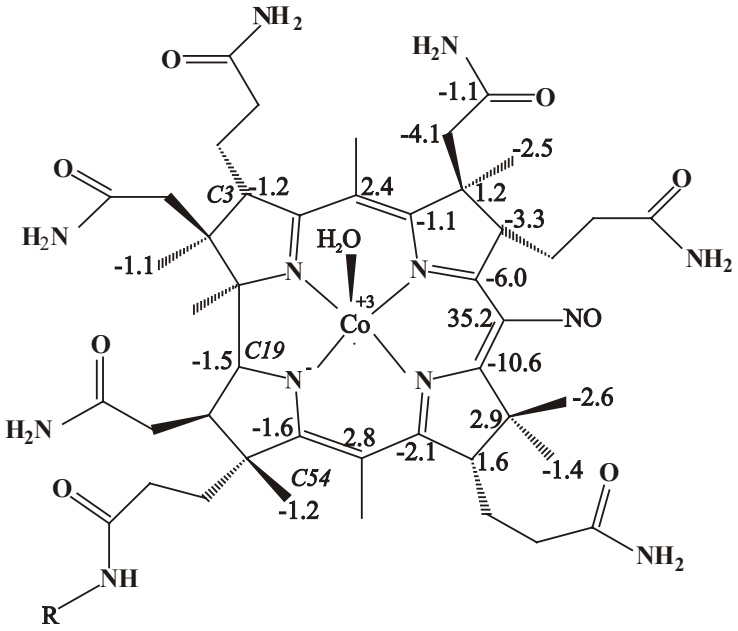


Figure 10.3 The significant differences between ^{13}C NMR resonances of aquacobalamin and aqua-10-chlorocobalamin where the values represent $\delta_{10\text{-NO-H}_2\text{OCbl}^+} - \delta_{\text{H}_2\text{OCbl}^+}$.

The effect of substitution at the C10 position can be seen in Figure 10.4. A resonance-donating substituent such as Cl causes the bands to shift to longer wavelengths whereas an electron-withdrawing group such as NO causes a shift to shorter wavelengths. Hisaeda has synthesised dicyano-10-NO_2cobalamin and also observed a shift to shorter wavelengths with the γ, β, and α bands equal to 361, 538 and 572 nm, respectively (reported by Pratt from a personal communication[4]). The bands for dicyanocobalamin are usually at 369, 545, and 586 nm for the γ, β, and α bands, respectively.[5] Hisaeda observed that the bands for dicyano-10-NO_2cobalamin have not shifted as much as those for cyano-10-nitrosocobalamin (see Table 10.1) i.e. the NO group is more electron-withdrawing than the NO_2 group. The NO_2 group results in the β band being more intense than the *α* band, which is a phenomenon observed with the replacement of H by CN at the C15 position in heptamethylcorrin.[4] This is also seen here for the nitroso derivatives (Figure 10.2).

References on page 198

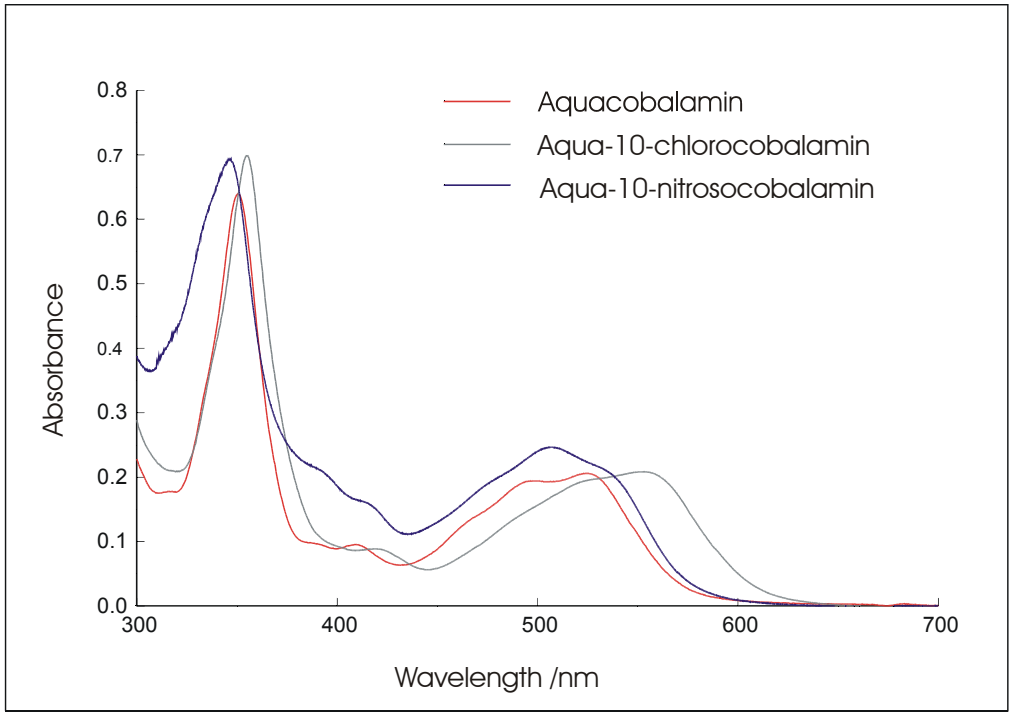


Figure 10.4 A comparison of the spectrum of aquacobalamin with those of aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin, where Cl and NO have substituted the H at the 10-position, respectively.

10.2.1 Ligand-Binding Properties of Aqua-10-nitrosocobalamin

In order to investigate the ligand-binding properties of aqua-10-nitrosocobalamin, solutions of pyridine (1.2 M) and azide (0.7 M) were added to a solution of aqua-10-nitrosocobalamin (*ca*. 120 μM, pH 7.0). The solutions were monitored by UV-vis spectroscopy for more than a week and no visible changes were observed in the spectra. Figure 10.5 shows the addition of pyridine (1.2 M) to aqua-10-nitrosocobalamin after 72 hours. It is usual with the cobalamin derivatives to observe as little as 20% complex formation from UV-vis spectra. log *K* values for the coordination of aquacobalamin and aqua-10-chlorocobalamin are 4.85 and 4.97, respectively, for N_3^- and 1.23 and 0.95, respectively, for pyridine. Thus, it appears that the electron-withdrawing NO group has markedly deactivated

References on page 198

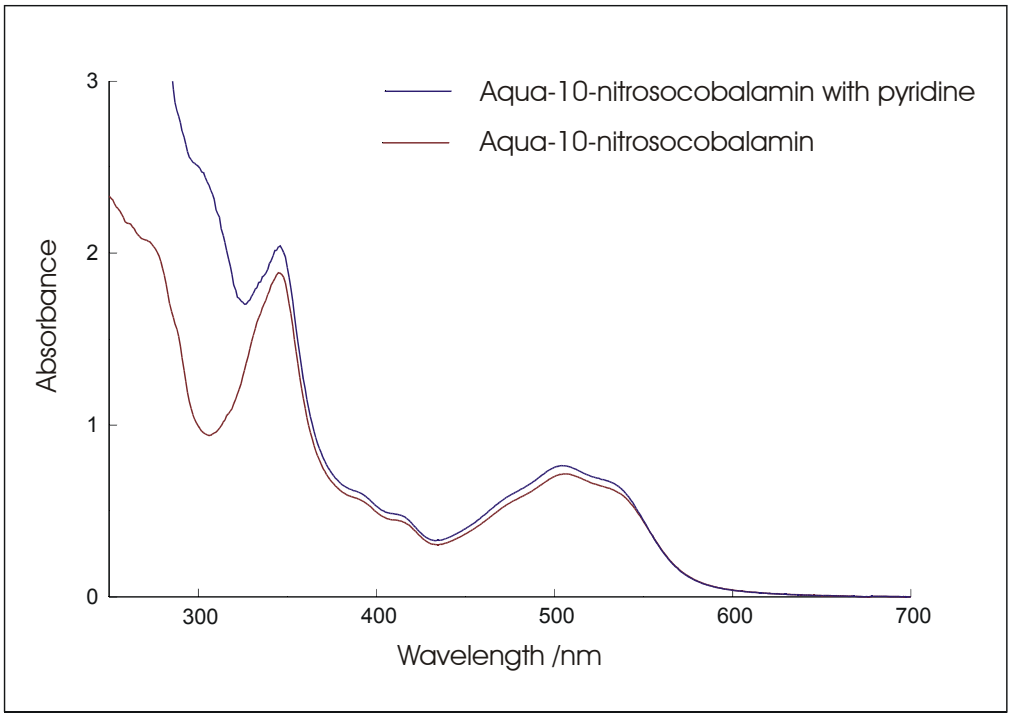


Figure 10.5 The addition of 1.2 M pyridine to aqua-10-nitrosocobalamin after 72 hours, indicating that no ligand substitution of H_2O has occurred.

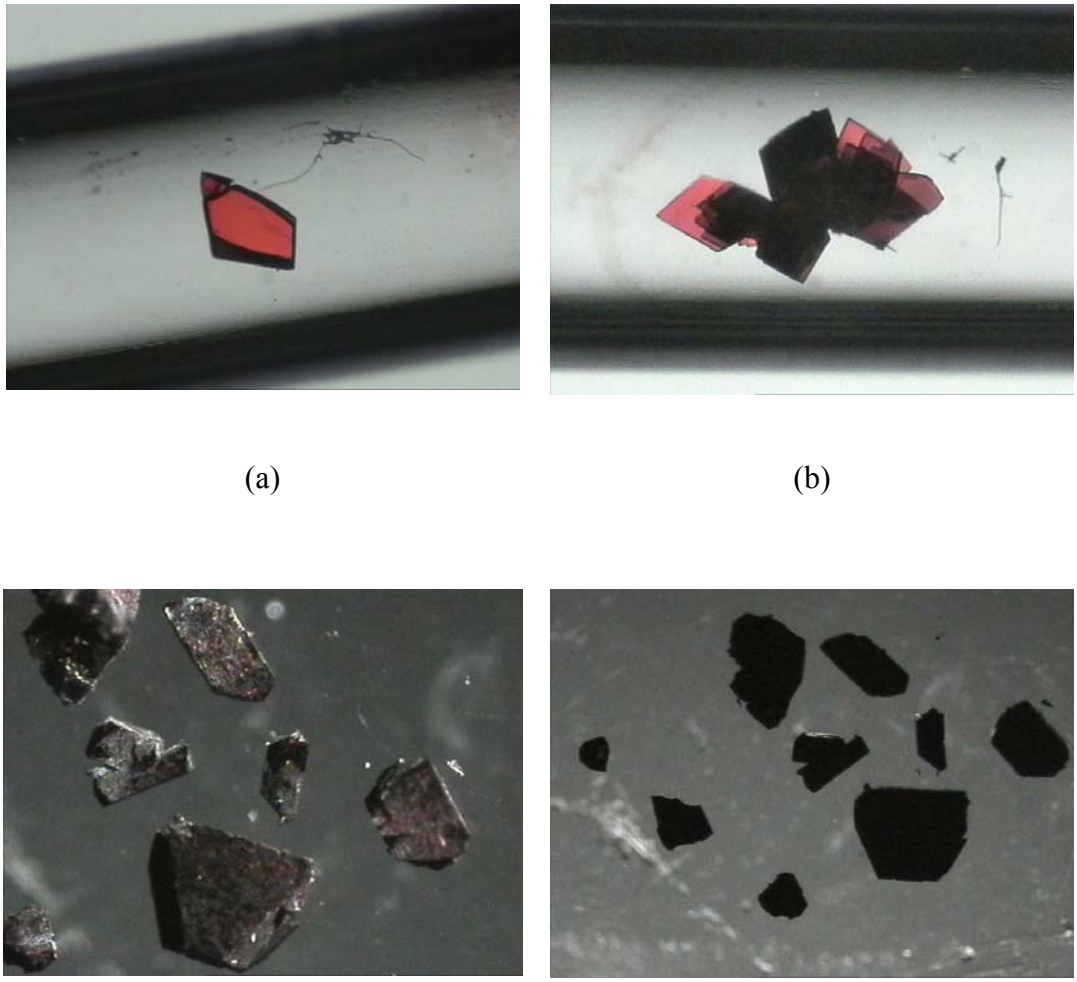
the metal ion towards substitution of the coordinated water group. In the case of N_3^-, the log *K* has decreased by at least four orders of magnitude. An attempt was then made to obtain kinetic data for the substitution of H_2O in aqua-10-nitrosocobalamin by the ligand CN^- because a shift in the spectrum is seen for this ligand (see Figure 10.2). Unfortunately, meaningful data could not be obtained because the compound was found to be unstable in acid and also decomposes in solution with time. Denitrosation may be occurring and the problem with kinetic studies is that one cannot be sure how much of the kinetics being measured is actually that of the binding of the CN^- ligand to aquacobalamin.

References on page 198

10.2.2 Crystals of Aqua-10-nitrosocobalamin

Numerous attempts were made to obtain diffraction-quality crystals of aqua-10-nitrosocobalamin. Crystals were obtained by using the hanging drop method of vapour diffusion as well as slow evaporation from a 50% acetonitrile/water mixture (see Section 2.10.3). However, the crystals that were grown were always very thin (plate-like, as can be seen in Figure 10.6a) and produced a very weak diffraction pattern that prevented data from being collected. The difference between the crystals of aquacobalamin and aqua-10-nitrosocobalamin can be seen in Figures 10.6a-d. The aquacobalamin crystals (Figure 10.6c and 10.6d) are prismic and dark red, which is normally the case irrespective of the ligand used to substitute the axial H_2O group.[6] The light orange-red crystals of aqua-10-nitrosocobalamin (Figure 10.6a and 10.6b) are visually very different from those of aquacobalamin. This may indicate that the structures of the two compounds are quite different and that the NO group has a significant effect on the crystal structure. To counter this, an attempt was made to minimise the effect of the NO group on the crystal structure by co-crystallising aquacobalamin and aqua-10-nitrosocobalamin. The crystals were grown by the hanging drop method from a 50:50 mixture of the two compounds. In addition, it was hoped that the aquacobalamin would have a templating effect on the crystal structure. Crystals suitable for a diffraction study experiment were obtained. Unfortunately the crystal structure that was determined from these crystals was found to be that of aquacobalamin.

References on page 198



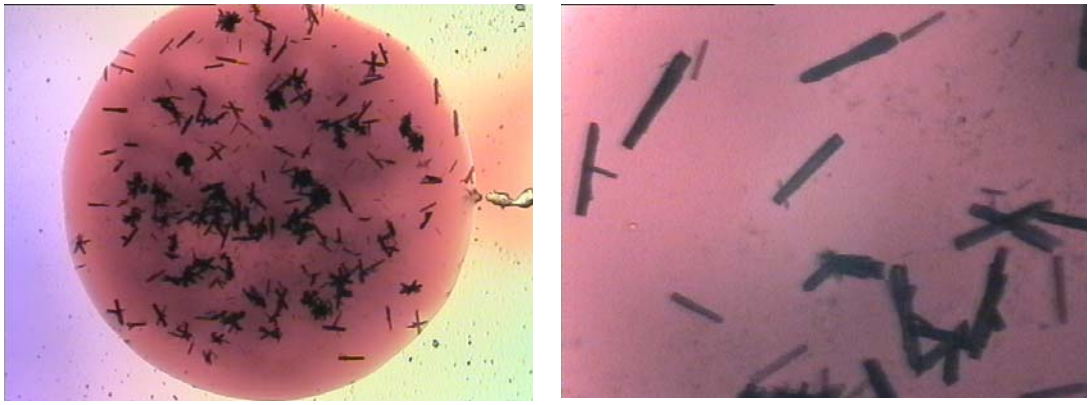
(a) (b)

(c) (d)

Figure 10.6 Photographs of aqua-10-nitrosocobalamin (a) and (b) and cobalamin[4] (c) and (d) crystals. These show that aqua-10-nitrosocobalamin crystals are much thinner and plate-like when compared with the prismic shaped cobalamin crystals. This has serious consequences on the diffracting ability of the aqua-10-nitrosocobalamin crystals, which makes X-ray diffraction studies by conventional methods extremely difficult.

References on page 198

The experiment was repeated with aquacobalamin making up only 5–10% of the mixture. The resulting crystals that were obtained are more needle-like in shape and less plate-like than before (Figure 10.7a and 10.7b), but unfortunately are also too small to be analysed by conventional diffraction methods. The templating effect does seem to be working and it is suggested that this be considered for a future study.



(a) (b)

Figure 10.7 Photographs of the crystals obtained from the mixture of aqua-10-nitrosocobalamin and 5–10% aquacobalamin using the hanging drop method.

A widely used method for growing crystals of aquacobalamin derivatives is vapour diffusion of acetone into an aqueous solution of the specific cobalamin.[3,7,8] This method was also attempted with aqua-10-nitrosocobalamin but was unsuitable because the acetone caused the compound to decompose.

References on page 198

10.3 Conclusion

The synthesis of aqua-10-nitrosocobalamin and its ligand studies provide further evidence that there is electronic communication between the Co(III) ion and the corrin ring in the cobalamins. UV-vis studies with aqua-10-nitrosocobalamin and the ligands N_3^- and pyridine showed no binding. This suggests that the substitution at the C10 position has withdrawn electron density resulting in deactivation of the corrin ring. The crystal habits of aqua-10-nitrosocobalamin and the aquacobalamin derivatives are markedly different, further indicating that the modified electronic structure has a significant effect on the shape of the crystals of aqua-10-nitrosocobalamin.

References on page 198

REFERENCES FOR CHAPTER 10

1. F. Wagner, *Proceedings of the Royal Society, Series A*, 1965, **288**, 344.
2. K. L. Brown, D. R. Evans, J. D. Zubkowski and E. J. Valente, *Inorganic Chemistry*, 1996, **35**, 415.
3. K. L. Brown, S. Cheng, X. Zou, J. D. Zubkowski, E. J. Valente, L. Knapton and H. M. Marques, *Inorganic Chemistry*, 1997, **36**, 3666.
4. J. M. Pratt, in *Chemistry and Biochemistry of B_{12}* (Ed.: R. Banerjee), Wiley and Sons, Inc., New York, 1999.
5. J. M. Pratt, *The Inorganic Chemistry of Vitamin B_{12}*, Academic Press, London, 1972, Chapter 5, p 50.
6. M. A. Fernandes, personal communication.
7. C. B. Perry, M. A. Fernandes and H. M. Marques, *Acta Crystallographica,* 2004, **C60**, m165.
8. G. Garau, S. Geremia, L. G. Marzilli, G. Nardin, L. Randaccio and G. Tauzher, *Acta Crystallographica,* 2003, **B59**, 51.