The Chemistry of Vitamin B_{12}. Part 24.[1] Evidence for Hydride Complexes of Cobalt(III) Corrinoids

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The u.v.–visible absorption spectra of solutions produced by the reduction of aquocobalamin (B_{12a}) or cyanoaquocobinamide (Factor B) in glacial acetic acid with zinc dust show the presence of an unstable yellow complex, which has a spectrum similar to those of alkylcobinamides and is therefore identified as a five-co-ordinate Co^{III} corrinoid with hydride as the axial ligand. The reported ability of such solutions to react with unactivated olefins such as ethylene and propylene has been confirmed by the isolation and identification of ethylcobalamin and isopropylcobinamide as products. The equilibrium constant for the co-ordination of the hydride ion in aqueous solution is calculated to be $\log_{10}(K/dm^3\ mol^{-1}) \sim 41$ at 25 °C, where $K = [Co{-}H]/[H_2O{-}Co{-}OH_2][H^-]$ (axial ligands only given).

The lack of firm evidence regarding the postulated hydride complexes of cobalt(III) corrinoids constitutes a serious gap in our knowledge of the co-ordination chemistry of vitamin B_{12}. Other Co^{III} hydride complexes are known and may form the related Co^{I} complex by the reversible loss of a proton;[2] the $[Co^{III}(CN)_5H]^{3-}$ ion,[3] for example, has $pK = 20$ in aqueous solution.[4,5]

In the early 1960's several workers (ref. 6 and refs. therein) suggested that the highly reduced B_{12s} † (in aqueous solution) was a cobalt hydride complex because of its ability to react with certain acetylenes (*e.g.*, $+C_2H_2 \longrightarrow Co{-}CH{=}CH_2$) and olefins (*e.g.*, $+CH_2{=}CHCO_2H \longrightarrow Co{-}CH_2CH_2CO_2H$) as well as alkylating agents such as halides (*e.g.*, $+MeI \longrightarrow Co{-}CH_3$). It was subsequently shown, however, that B_{12s} reacted with MeI at pH 8—10 to form the Co–Me bond without the liberation of a proton, *i.e.* that B_{12s} must be the simple Co^{I} complex.[7] In 1971 Schrauzer and Holland[6] reported that the reduction of aquocobalamin (B_{12a}) in $MeCO_2H$ (glacial) or $MeCO_2H$–MeOH (1 : 1) with zinc dust produced a green solution which, unlike aqueous solutions of B_{12s}, was able to react with unactivated olefins (ethylene, propene, cyclohexene, and norbornene) to give alkylcobalamins; the products were identified only from changes in the spectrum of the solution, and the rates of reaction were not mentioned (except that ethylene reacted ' rapidly '). Here again it was proposed, more plausibly, that the solution contained a ' hydridocobalamin ' and its presumed spectrum was reported. We show below, however, that the spectrum reported by Schrauzer and Holland[6] is actually that of the Co^{I} complex and that the ' hydride ' is a yellow complex with a totally different spectrum. A possibly analogous reaction is observed when a suspension in ethanol of the dimeric $[\{Co^{II}(Hdmg)_2(py)\}_2]$ (Hdmg = dimethylglyoximate monoanion, py = pyridine) is kept under a high pressure of hydrogen and propene for 12 h to give a low yield of the isopropyl complex;[8] however, since the nature of the active species and the mechanism of reaction have not been established and there is no obvious explanation for the failure of ethylene and cyclohexene to react, this does not contribute to our understanding of the reactions of the corrinoids. In 1975 Lexa and Savéant[9] used electrochemical techniques to show that B_{12s} in aqueous solution may be reversibly protonated with $pK = 1$; they suggested that the protonated product was the hydride complex, but it was too unstable for further characterisation.

There are, therefore, strong indications that a protonated and very unstable form of B_{12s} with unusual activity may exist, but the complex has not yet been ' seen ' by any physical technique and the site of protonation has not been established. Cobalt(III) corrinoids dissolve in strong acid to give yellow complexes in which the corrin ring is almost certainly protonated,[10a] and acid catalyses H/D exchange at C^{10} (ref. 10*b*) and epimerisation at C^{13} (ref. 11). The proton could, therefore, be added either to the cobalt atom (to give a Co^{III} hydride complex) or to an atom of the corrin ring (to give a Co^{I} complex with a modified corrin ring). The reaction with olefins could also involve the transfer of a hydrogen atom either from the cobalt or from a position on the corrin ring (with or without the simultaneous addition of the carbon atom to the cobalt); several reactions involving addition (*e.g.* of acetylenes and nitriles) across both the cobalt atom and the bridge position of a conjugated cyclic ligand are known.[12]

The aims of this paper are, therefore, (*i*) to find conditions under which the hydride can be prepared and studied in more detail, (*ii*) to establish whether protonation occurs on the cobalt or on the corrin ring, and (*iii*) to confirm the reported reaction[6] with unactivated olefins with adequate characterisation of the products. We find that new reduced species (the cobalamin and cobinamide forms are here designated by X and X′ respectively) can be observed by u.v.–visible spectrophotometry in $MeCO_2H$ but are too unstable to be studied by techniques such as i.r. and n.m.r. spectroscopy. We have therefore attempted to identify the site of protonation from the u.v.–visible spectra as follows. Providing there is no marked solvent effect (as between H_2O and $MeCO_2H$) on the spectra, it would be expected that the spectrum of a Co^{III} hydride would resemble those of the analogous alkyl complexes, while the spectrum of a Co^{I} complex with a protonated ring might show similarities to those of other Co^{I} corrinoids where the conjugated ring has been interrupted. The recently reported structure of one of the so-called ' stable yellow corrinoids '[10c] shows that both C^5 and C^6 have been made tetrahedral by the addition of HO and a carboxylate group respectively.[13] We have therefore carried out qualitative tests to identify the Co^{I} complex of a stable yellow corrinoid and to

† Abbreviations: B_{12} = cyanocobalamin; B_{12a} = aquo- and hydroxo-cobalamin; B_{12r} is the Co^{II} derivative of B_{12a}, whether the base, 5,6-dimethylbenzimidazole (dbzm), is co-ordinated or free and protonated; B_{12s} is the Co^{I} derivative of B_{12a}, in which the base is never co-ordinated, but may be neutral or protonated; Factor B is cyanoaquo- and cyanohydroxo-cobinamide; X and X′ are the products to be identified as hydrido-cobalamin and -cobinamide respectively. Cobinamides lack the nucleotide side-chain terminating in dbzm, which is present in the cobalamins.

record its spectrum. In order to assess the importance of any solvent effect, we have also compared the spectra of several known corrinoids in $MeCO_2H$ and in aqueous solution.

Experimental

Materials.—Samples of B_{12} and B_{12a} were given by Mr. A. P. Domleo of Glaxo–Allenbury (Pty) Ltd. A sample of the stable yellow corrinoid, formed as a by-product in the oxidation of B_{12r} by air,[10c] was given by Dr. E. L. Smith and used without further characterisation except to confirm the presence of dbzm (from changes in the spectrum in the region 280—290 nm) [10d] and the absence of cyanide (i.r.). Factor B, methyl-, ethyl-, and isopropyl-cobalamin, and isopropylcobinamide were prepared by published methods.[14–16] Zinc dust (BDH and Hopkin and Williams, both AR grade) was dried at 145 °C for 24 h. Glacial acetic acid (Saarchem, Muldersdrift, Transvaal, and Merck), sodium acetate (Saarchem), ammonium chloride (Saarchem), sodium tetrahydroborate (Hopkin and Williams), nitrogen (Afrox, Germiston, Transvaal), ethylene (Afrox), propene (Afrox), methyl iodide (Merck), ethyl bromide (Hopkin and Williams), isopropyl bromide (Hopkin and Williams), acetic anhydride (Merck), and monochloroacetic acid (Hopkin and Williams) were all used without further purification.

Preparation and Reactions of Reduced Corrinoids.—Samples (2 cm^3) of (1.5—9.0) × 10^{-5} mol dm^{-3} solutions of reduced corrinoids were prepared inside a spectrophotometer cell closed with a rubber septum. The solutions were deoxygenated with a brisk stream of nitrogen (*via* syringe needles inserted through the septum) for 10—15 min. Solutions of B_{12r} were prepared (*a*) in unbuffered water by the photolysis of ethylcobalamin (up to 4 h required for complete decomposition of the initially formed B_{12s} to B_{12r}),[1] (*b*) in 1 mol dm^{-3} H_2SO_4 by acidification under nitrogen of the solution from (*a*), and (*c*) in $MeCO_2H$ by the addition of a small amount of $NaBH_4$. Zinc dust (30—40 mg) was used to reduce (*a*) B_{12a} and Factor B in $MeCO_2H$ (to X and X′ respectively), (*b*) B_{12a} in $MeCO_2H$ containing 14 mg of NaO_2CMe (to B_{12s}), (*c*) B_{12a} in 10% aqueous NH_4Cl (to B_{12s}),[10e] (*d*) the stable yellow corrinoid in 10% aqueous NH_4Cl (to the Co^I derivative), and (*e*) B_{12a} in $MeCO_2H$ (1.5 cm^3) and acetic anhydride (0.5 cm^3) to give acetylcobalamin (see Results section). The product from (*e*) is stable to air and the solution was filtered before examining the spectrum. In the other cases the spectra were examined without filtering off the zinc dust; most of the zinc settled out rapidly on the bottom of the cell after the flow of nitrogen was stopped and allowed spectra of reasonable quality to be recorded.

For studying the reaction of X and X′ with olefins a more concentrated solution of B_{12a} (50 mg) or Factor B (5 mg) in $MeCO_2H$ (2 cm^3) was treated with zinc dust (*ca.* 200 mg for B_{12a} solutions, 77 mg for the Factor B), deoxygenated with a brisk stream of N_2 for 10 min, treated with a stream of ethylene or propene for 20—30 min while protected from light, and the zinc then filtered off in air in the dark. The filtrate from the reaction involving B_{12a} and propylene was then examined in the spectrophotometer without further purification. The filtrates from the reactions of the cobalamin with ethylene and the cobinamide with propene were then diluted with water and purified by extraction into phenol–1,1,1-trichloroethane (1 : 2) * and then back into water; the former was freeze-dried to give a red solid, while the latter was kept as a concentrated yellow solution. The corrinoids were identified by t.l.c. using cellulose (Merck) plates with s-butyl alcohol–water (95 : 40) as solvent and R_f values determined relative to that of B_{12}, *i.e.* as $R_{B_{12}}$ values.[17]

* 1,1,1-Trichloroethane used as a substitute for the usual potentially carcinogenic chloroform.[10f]

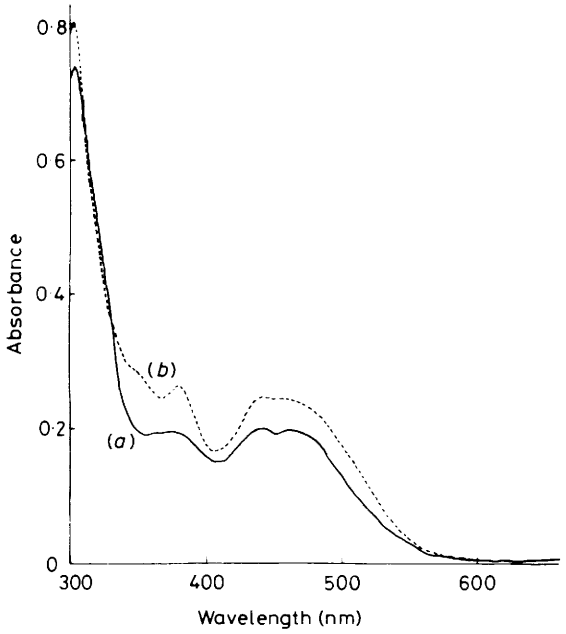

Figure 1. Comparison of the spectra of (*a*) *ca.* 3 × 10^{-5} mol dm^{-3} solution of the product X (*i.e.* hydridocobalamin) in $MeCO_2H$ (——) and (*b*) 3.1 × 10^{-5} mol dm^{-3} solution of ethylcobalamin in 0.05 mol dm^{-3} H_2SO_4 (*i.e.* as the protonated 'base-off' form) (— — —)

U.v.-Visible Spectra.—These were recorded with a JASCO Uvidec-1 or a Cary 219 spectrophotometer in 1-cm cells at 25 °C.

Results

Reduction of B_{12a} *and Factor B in* $MeCO_2H$ *with Zinc Dust.*—The reduction of a pink solution of B_{12a} in $MeCO_2H$ with zinc dust (see Experimental section) first produces the yellow B_{12r} (see below) and then more slowly (up to 1 h) another yellow species (X), whose spectrum [see Figure 1(*a*)] shows peaks at 303, 384, 442, and *ca.* 470 nm with no bands detectable between 600 and 875 nm. An increase in gas pressure within the cell indicated that H_2 was being evolved, but the surfaces did not become obscured by bubbles of gas. The intensity of the peaks at 384 and *ca.* 470 nm was somewhat variable, probably due to the presence of traces of B_{12s} ($\lambda_{max.}$ at 391 nm, see below) and B_{12r} (*ca.* 470 nm, see below). Similar results were obtained with two different samples of $MeCO_2H$ and two different samples of zinc dust, *i.e.* the product X does not appear to be formed by reaction with impurities. Zinc amalgam and $NaBH_4$ (see below) only reduced B_{12a} to the Co^{II} level; granular zinc appeared to be ineffective. The reduction of Factor B gave a species (X′) with a virtually identical spectrum (bands at 303, 384, 442, and 470 nm). The admission of a small amount of O_2, either by piercing the septum with a syringe needle or brief flushing of the cell with N_2 (which presumably contained a trace of O_2), caused conversion of X and X′ to the Co^{II} complexes, which were then reduced back again to X and X′. Filtration of a turbid solution (containing zinc dust) of X under N_2 in a glove-bag gave a clear solution whose spectrum showed mainly

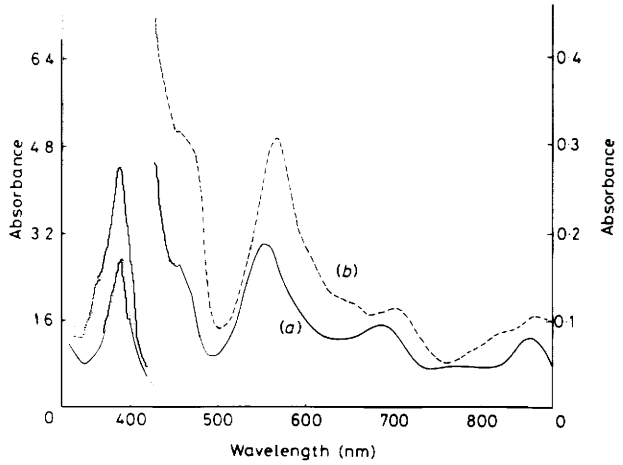

Figure 2. Comparison of the spectra of the Co^{I} cobalamins prepared by reducing B_{12a} with zinc dust in (*a*) 10% aqueous NH_4Cl (*ca.* 0.9×10^{-4} mol dm^{-3} Co) (——) and (*b*) $MeCO_2H$ containing NaO_2CMe (*ca.* 1.4×10^{-4} mol dm^{-3} Co) (— — —)

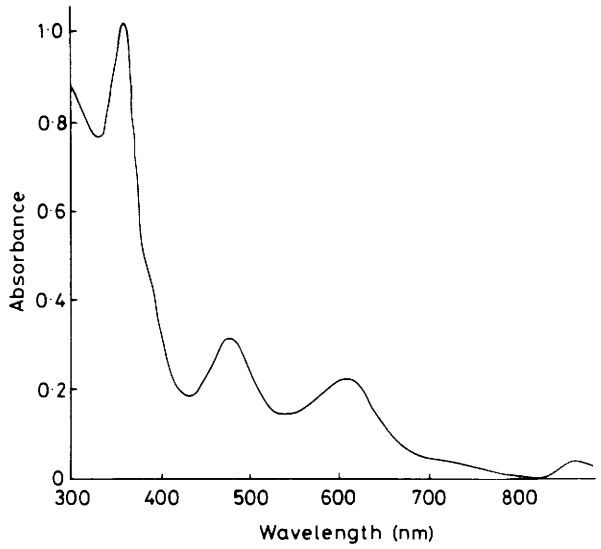

Figure 3. Spectrum of the product obtained by reducing the stable yellow corrinoid (concentration unknown) with zinc dust in 10% aqueous NH_4Cl

Co^{II} and filtration in air a mixture of Co^{II} and Co^{III} complexes. Attempts to obtain a solution of X in the absence of reducing agent were unsuccessful.

If B_{12a} is reduced by zinc dust in $MeCO_2H$ which contains some sodium acetate (*i.e.* the conjugate base of the solvent) to raise the effective pH of the solution, then reduction (see Experimental section) again proceeds *via* the intermediate formation of the yellow B_{12r} to give a green solution, whose spectrum [see Figure 2(*b*)] exhibits bands at 391, *ca.* 460, 568, 705, and 870 nm, *i.e.* throughout the visible region. This spectrum is similar, but not identical, to the spectrum of B_{12s} in aqueous solution [see Figure 2(*a*)] which has bands at 388, 460, 554, 687, and 850 nm (*cf.* ref. 10*g*).

Spectra of other Corrinoids in $MeCO_2H$.—In order to assess the possible ' solvent effect ' of $MeCO_2H$ on the spectra we studied the following corrinoids in $MeCO_2H$ and compared their spectra with those of analogous species in aqueous solution. The spectra in the region 280—290 nm can be used to distinguish whether the heterocyclic base is free and protonated (sharp band at 285 nm) or still co-ordinated (poorly resolved shoulder at *ca.* 288 nm).[10d] Methylcobalamin gives a yellow solution in $MeCO_2H$ of the ' base-off ' form in which the base is protonated (band at 285 nm). The wavelengths of the absorption bands (nm) and their relative intensities (in parentheses) are virtually identical (*a*) in $MeCO_2H$ [303 (2.53), 376 (1.02), 460 (1.00)] and (*b*) in 0.05 mol dm^{-3} H_2SO_4 (also protonated ' base-off ' form) [303 (2.4), 374 (0.97), 460 (1.00)]. Vitamin B_{12r} in $MeCO_2H$, prepared by the reduction of B_{12a} with $NaBH_4$ (see Experimental section), also exists in the protonated ' base-off ' form (sharp band at 285 nm); *cf.* the spectra of B_{12r} (*a*) in $MeCO_2H$ [313 (2.55), 404 (0.66), 473 (1.00)], (*b*) in 1 mol dm^{-3} H_2SO_4 (protonated ' base-off ') [315 (2.09), *ca.* 405 (0.59), 470 (1.00)], and (*c*) in water (' base-on ' form) [311 (2.51), 403 (0.74), 473 nm (1.00)]. Vitamin B_{12a} dissolves in $MeCO_2H$ to give a pink solution, in which the base remains co-ordinated to the cobalt (shoulder at 288 nm). The spectrum shows bands at 356 (γ-band, 2.15), 416 (0.35), 514 (0.91), and 540 nm (1.00); *cf.* the position of the γ-band in aqueous solutions of cobalamins where the axial ligand is H_2O (350), $MeCO_2^-$ (352), and OH^- (356 nm).[10h]

The Cobalt(I) *Derivative of the Stable Yellow Corrinoid.*—This corrinoid dissolves in water to give a yellow solution with its first absorption band at 458 nm (*cf.* ref. 10*c*) which, after deoxygenation with a stream of nitrogen, is readily reduced by the addition of a small amount of solid $NaBH_4$ to a blue solution with an intense band at 358 nm and a series of bands extending through the visible region. The blue solution reacts immediately with methyl iodide to give a yellow solution showing a band at 448 nm which is stable to air in the dark and over a wide range of pH but is decomposed by light back to the starting material. The parallel with the known cycle of reactions and complexes starting from aquocobalamin fairly conclusively identifies the blue complex as the Co^{I} derivative of the stable yellow corrinoid. In order to obtain a good spectrum of the blue solution free from interference from gas bubbles (evolved from $NaBH_4$) reduction was effected with zinc dust; the absorption bands (see Figure 3) are located at 359 (very intense), *ca.* 390, 479, 607, and 865 nm.

Reaction of X *and* X′ *with Olefins.*—The reactions of the cobalamin X with (*a*) ethylene and (*b*) propene, and the cobinamide X′ with (*c*) propene were studied by passing the gas through a solution of X or X′ in $MeCO_2H$ at room temperature for 20—30 min (for details see Experimental section). The resulting solutions were all yellow. The products from (*a*) and (*c*) were characterised after purification [by extraction through phenol–1,1,1-trichloroethane (1 : 2)] by both t.l.c. and u.v.–visible spectrophotometry. T.l.c. of the product from (*a*) gave a single red spot with $R_{B_{12}} = 1.50$ (*cf.* ethylcobalamin, 1.49), while t.l.c. of the product from (*c*) gave a yellow spot with $R_{B_{12}} = 1.32$ (*cf.* isopropylcobinamide, 1.32) and a trace of diaquocobinamide. Their spectra (300—600 nm) in aqueous solution were shown to be identical to those of genuine ethylcobalamin and isopropylcobinamide in both neutral and acid solution (for details of the spectra see ref. 16). Isopropylcobalamin slowly decomposes in solution unless the base is kept protonated and hence not co-ordinated; it cannot easily be purified and identified by t.l.c.[16] The spectrum (300—600 nm) of the reaction mixture from (*b*) after filtering off the zinc was shown to be identical to those [16] of isopropylcobinamide and acidified isopropylcobalamin in aqueous solution. The products from (*a*)—(*c*) are therefore identified as ethylcobalamin, isopropylcobalamin, and isopropylcobinamide respectively. The rates of reaction have not been

studied quantitatively, but in all three cases 20—30 min at room temperature under *ca.* 1 atm (10^5 N m^{-2}) of olefin is sufficient to ensure virtually complete reaction.

Reaction of X *with Alkylating and Acylating Reagents.*—The reactions of X were studied with the following additional reagents, the products being identified only from their spectra in $MeCO_2H$ after filtering off the zinc. EtBr was selected in order to check whether, assuming that X is a hydride complex, the presence of the hydride ligand prevents direct alkylation of the Co atom by reagents of the type used with B_{12s}; it produced ethylcobalamin. Two reagents were studied in order to test the possibility that X was either the acetyl (Co–COMe) or the carboxymethyl ($Co-CH_2CO_2H$) complex. A solution of X in $MeCO_2H$ reacts with acetic anhydride (see Experimental section) to give a yellow product whose spectrum is the same in shape and band positions [*viz.* 300, *ca.* 355 (sh), and 447 nm] as that of the acidified ' base-off ' form of genuine acetylcobalamin in 0.05 mol dm^{-3} HCl [*viz.* 300, *ca.* 355 (sh), and 446 nm],[15] which is quite distinct from that of X. The reaction of X with $ClCH_2CO_2H$ gave only a mixture of B_{12r} and stable yellow products.

Discussion

We have shown that the reduction of B_{12a} in $MeCO_2H$ with zinc dust produces an unstable yellow corrinoid (X), whose spectrum [Figure 1(*a*)] shows bands at 303 (very intense), 384, 442, and *ca.* 470 nm (all of similar intensity) and that X (*i*) has a formal oxidation state less than Co^{II} (it can be oxidised to, and reduced from, B_{12r}), (*ii*) is probably related to B_{12s} by protonation (reduction gives B_{12s} or X depending on the presence or absence of NaO_2CMe as the conjugate base in $MeCO_2H$), and (*iii*) is a ' base-off ' cobalamin (the cobalamin X and analogous cobinamide X′ have identical spectra). Because of similarities in spectra (see below) we had to consider the possibility that X was an organocorrinoid containing a ligand derived either from acetic acid itself (Co–COMe or $Co-CH_2CO_2H$) or from some unknown impurity, even though the stability of most organocorrinoids (and instability of X) towards O_2 renders this unlikely. Acetylcobalamin was excluded because of differences in the spectrum (see Results section) and, provided carboxymethylcobalamin has the same spectrum in $MeCO_2H$ as in aqueous acid (as does methylcobalamin, see below), then this complex can also be excluded by reference to published spectra.[15] Reaction with impurities is rendered unlikely by observing the same reaction with different samples of zinc and of $MeCO_2H$.

We therefore tentatively conclude that X and X′ are both derived from the simple Co^I corrinoids by protonation of the chromophore (either on the cobalt or the ring), in addition to any protonation of dbzm in the case of the cobalamin. These complexes decompose in the absence of excess reducing agent and their occurrence appears to depend on a steady-state balance between formation and decomposition. We have been unable to prepare solutions of X in the absence of zinc dust for studying by i.r. and n.m.r. spectroscopy and have therefore used similarities in u.v.–visible spectra with other corrinoids as evidence for similarities in structure.

A comparison of the spectra of analogous corrinoids in $MeCO_2H$ and in H_2O shows that the ' solvent effect ' on the spectrum is negligible in the case of protonated ' base-off ' methylcobalamin; this corrinoid is present almost entirely as a five-co-ordinate complex[18] whose single axial ligand (methyl) does not interact with the solvent through hydrogen bonding. A ' solvent effect ' is, however, detectable in the case of aquocobalamin and of the protonated ' base-off ' form of B_{12r}, where the co-ordinated H_2O present in aqueous solution may be replaced by $MeCO_2^-$ or even $MeCO_2H$ and all the axial ligands can form hydrogen bonds to the solvent. The spectra of B_{12s} in aqueous solution [Figure 2(*a*)], and of the product of reducing B_{12a} in $MeCO_2H$ containing some NaO_2CMe [Figure 2(*b*)] show a sufficient degree of similarity to identify the latter as B_{12s}, although there is an increasingly obvious ' solvent effect ' at longer wavelength. The nature of these transitions has not been established, but they are likely to possess some charge-transfer character which could explain their sensitivity to the environment. We conclude that changing the solvent has a relatively minor effect on the spectrum, unless it entails a change in the nature of the axial ligands, and that a comparison of the spectrum of X in $MeCO_2H$ with those of other corrinoids in aqueous solution is valid.

The spectrum recorded (but only over the range 400—1 000 nm) by Schrauzer and Holland[6] for the reduced green corrinoid in $MeCO_2H$ and attributed by them to hydridocobalamin is almost identical in general shape and band positions (*ca.* 460, 570, and 700, *ca.* 850 nm) to that of our greenish B_{12s} in $MeCO_2H$ containing some NaO_2CMe and completely different from that of the yellow hydride complex in $MeCO_2H$. This suggests that their solution had a higher effective pH than ours, perhaps due to a greater extent of liberation of H_2 or reduction of O_2.

Our test for the site of protonation in X is, therefore, to compare the spectrum of X in $MeCO_2H$ [Figure 1(*a*)] with those of analogous alkylcorrinoids (cobinamides and ' base-off ' cobalamins) and the Co^I derivative of the stable yellow corrinoid (see Figure 3), all in aqueous solution. These alkylcorrinoids usually possess the alkyl ligand in the upper (β) co-ordination site, although some isomeric forms are known, and are five-co-ordinate, although the six-co-ordinate aqua-derivatives may be observed below room temperature.[10i] All the reported normal isomers of the five-co-ordinate alkylcorrinoids are yellow and their spectra show an intense band at *ca.* 303 nm and bands of lower intensity at *ca.* 380 nm and at *ca.* 440 and/or 460 nm;[16] in the six-co-ordinate aquomethyl- and aquoethyl-cobinamides observed at low temperature the first band has moved out to 503 nm.[14] The spectra of the isomeric methyl- and ethyl-corrinoids show a first maximum in the region 470—490 nm,[16] but in this case the possible presence of five- as well as six-co-ordinate alkylcorrinoids has not been investigated. A comparison of the spectra in Figures 2 and 3 shows that the unusual feature of several absorption bands throughout the visible region, together with an intense band in the region 360—390 nm, is shared by both B_{12s} [Figure 2(*a*)] and the Co^I derivative of the stable yellow corrinoid (Figure 3); one might therefore expect similar features to be exhibited by a Co^I complex in which the corrin ring had been protonated.

The spectrum of X [Figure 1(*a*)] shows no resemblance to those of the Co^I complexes (Figures 2 and 3) but does show a remarkable similarity to that of a five-co-ordinate alkylcorrinoid such as an ethylcorrinoid [Figure 1(*b*)] in all three band positions (*ca.* 303, *ca.* 380, and 440—460 nm); the only noticeable difference is that the first band of the hydride occurs at *ca.* 470 nm, while all corrinoids with unsubstituted alkyl ligands have their first band at *ca.* 460 or 440 nm.[16] By analogy with the alkylcorrinoids we conclude that X is the five-co-ordinate, protonated, ' base-off ' hydridocobalamin and that X′ is the analogous cobinamide. Alkylation of Co^I corrinoids occurs predominantly in the upper (β) co-ordination site, apparently due to greater steric hindrance around the lower site.[10i] There is indirect evidence that the ratio of the upper to the lower isomer present at equilibrium in alkylcorrinoids falls from *ca.* 100 for the ethyl to *ca.* 10 for the methyl complex.[10i] Extrapolation would then suggest that the hydride complex might, like the cyanoaquo-[10j] and ethynyl-

aquo-cobinamides,[19] exist as a more equal mixture of the u- and l-isomers (u and l represent a given ligand in the upper or lower axial co-ordination site). This prevents any conclusions being drawn from the interesting fact that the spectrum of the hydride resembles that of the ethyl rather than the methyl complex.

We have shown that ethylene and propene react with solutions where the main species present is hydrido-cobalamin (X) or -cobinamide (X') and have identified the products as ethylcobalamin, isopropylcobinamide (both by u.v.–visible spectra and t.l.c.) and isopropylcobalamin (u.v.–visible spectrum only). Our results therefore fully support the original suggestion of Schrauzer and Holland [6] that the olefins react with a hydride complex, although it appears that their solutions contained only low concentrations of the active hydride complexes. Solutions of X also react with simple alkylating and acylating agents [*e.g.* EtBr and $O(COMe)_2$, see Results section], but it cannot be concluded that the reaction involves attack on the hydride rather than on the Co^{I} complex present in equilibrium at low concentration. It should be noted that most s-alkylcobalamins (including isopropyl) are too unstable to isolate and identify by t.l.c., though the analogous cobinamides are easy to handle. Our observation that Factor B can be reduced to give virtually complete formation of the hydride X' without the need for prior removal of the strongly bound cyanide simplifies the use and study of cobinamides in $MeCO_2H$.

We have also tried unsuccessfully to observe the spectrum of the hydride in aqueous solutions of B_{12s} acidified to pH < 1, but decomposition to B_{12r} occurred too rapidly. There seems no reason, however, to doubt Lexa and Savéant's conclusion [9] that the protonation of B_{12s} in aqueous solution with $pK = 1$ forms the hydride, presumably with the same five-co-ordinate structure as observed in $MeCO_2H$. We can then use published values of the redox potentials as follows to calculate an equilibrium constant for the co-ordination of the simple hydride ligand (H^-) according to reaction (1) (axial ligands only given).

$$[H_2O{-}Co^{III}{-}OH_2] + H^- \rightleftharpoons [Co^{III}H^-] + 2H_2O \quad (1)$$

The reversible, pH-independent redox potentials linking diaquocobinamide with its Co^{II} and Co^{I} derivatives have been determined [20] as +0.27 V for the Co^{III}/Co^{II} couple at pH 1.4—3.8 and −0.73 V for the Co^{II}/Co^{I} couple at pH 1.4—11; hence $(0.27 - 0.73)/2 = -0.23$ V *versus* s.c.e. (saturated calomel electrode) or +0.01 V *versus* s.h.e. (standard hydrogen electrode) for the Co^{III}/Co^{I} couple. The presence of the protonated dbzm in the side-chain has no effect on the potentials; *cf.* E^0 *versus* s.c.e. for the Co^{II}/Co^{I} couple for the 'base-off' cobalamins when the base is protonated (−0.740) and unprotonated (−0.742) and for the cobinamide (−0.73).[20,21] We can, therefore, assume that the Co^{I} cobinamide will, like B_{12s}, be protonated with $pK = 1$ and that the value of E^0 for the Co^{III}/Co^{I} couple will rise *ca.* 60 mV per pH unit below pH 1 to give $E^0 = +0.07$ V *versus* s.h.e. at pH 0, hence $\log_{10} K_{e1}$ (defined below) $= 2E^0/0.06 = +2.3$ (at 22 °C and in 1 mol dm^{-3} $HClO_4$).[21] Expressing the concentration of diaquocobinamide as $[H_2O{-}Co^{III}{-}OH_2]$, the equilibrium constant K for reaction (1) can be derived as in equation (2):

$$K = \frac{[Co{-}H]}{[H_2O{-}Co{-}OH_2][H^-]} = \left(\frac{[Co{-}H][H^+]}{[H_2O{-}Co{-}OH_2]p_{H_2}}\right)\left(\frac{p_{H_2}}{[H_2]}\right)\left(\frac{[H_2]}{[H^+][H^-]}\right) \quad (2)$$

i.e. $\log_{10}(K/dm^{-3}\ mol^{-1}) = \log_{10} K_{e1} - \log S + pK_{H_2} = +2.3 + 3.1 + 35.3 \simeq 41$, where S is the solubility of H_2 in 1 mol dm^{-3} $HClO_4$ at 22 °C and is taken to be the same as in 1 mol dm^{-3} HNO_3 at 25 °C (*viz.* 0.8×10^{-3} mol dm^{-3} or $\log S = -3.1$) [22] and pK_{H_2} is the pK of H_2 which has been estimated as 35.3 ± 0.2.[23]

This value represents the second equilibrium constant reported for the co-ordination of H^- in a transition metal complex; *cf.* the value of $\log K \geqslant 29$ for the substitution of H_2O by H^- in $[Co^{III}(CN)_5(OH_2)]^{2-}$.[24] Possible reasons for the unusual stability of $Co^{III}{-}H$ and σ $Co^{III}{-}C$ bonds, and hence for the choice of cobalt corrinoids as co-factors for organometallic chemistry in enzymes, have been discussed elsewhere.[18]

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