# The Chemistry of Vitamin $B_{12}$. Part 21.[1] Ethynylaquocobinamide: Novel Reaction of Diaquocobinamide with Acetylene catalysed by Copper Ions

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Copper ions catalyse the reaction of diaquocobinamide with acetylene in aqueous solution at room temperature to give ethynylaquocobinamide as a mixture of the two isomers which differ in the relative orientation of the axial ligands. This leads to the first reported value for an equilibrium constant (axial ligands only given) involving the substitution of co-ordinated $H_2O$ by $HC_2^-$ of $K = [HC_2\text{-Co-}OH_2]/([H_2O\text{-Co-}OH_2][HC_2^-]) \geqslant 10^{23}$ dm$^3$ mol$^{-1}$. Equilibrium constants have been determined for the substitution of co-ordinated $H_2O$ in ethynylaquocobinamide by $OH^-$ ($pK = 13.0 \pm 0.1$), $CN^-$ ($\log_{10}K \geqslant 6.8$), and imidazole ($\log_{10}K = 3.4$), which confirm the position of $HC_2^-$ between cyanide and vinyl in the *trans*-effect series for $Co^{III}$ corrinoids.

Acetylene can undergo a wide range of reactions with complexes of transition metals (see, for example, the products formed with iron carbonyls [2]) and of Group 1B and 2B metals (*e.g.* hydration to $CH_3CHO$ catalysed by $Hg^{II}$ in acid,[3] and the formation of insoluble products such as $Cu_2C_2$ [3]), yet surprisingly little is known about complexes containing the acetylide ($HC{\equiv}C^-$) ligand. Complexes containing $HC_2^-$ are difficult to prepare and usually require reaction with $Na/KC_2H$ in non-aqueous media,[2,3] although ethynylcobalamin has been prepared (together with the Co-CH=CHBr complex as a by-product) by reacting the $Co^I$ complex vitamin $B_{12s}$ with $BrC{\equiv}CH$ in aqueous solution.[4] However, no isolable complex containing the co-ordinated acetylide ion has yet been prepared from acetylene itself, whether in aqueous or non-aqueous solution. We have been interested in extending our knowledge of the preparation and properties of $Co^{III}$ corrinoids (especially the cobinamide †) which possess the $HC_2^-$ ligand, since this ligand occupies the key position in the *trans*-effect series $CN^- < HC_2^- < CH_2{=}CH^- < CH_3^- < CH_3CH_2^-$ [5,6a,7] linking the co-ordination chemist's cyanide with ligands more characteristic of organometallic chemistry, and we have discovered a novel and simple method of preparing ethynylaquocobinamide.

While studying [1] the kinetics of co-ordination of cyanide by dac‡ we detected the spectrum of an intermediate which probably contains HCN as a ligand; this prompted us to look for a weakly co-ordinated, π-bonded acetylene complex. We observed instead, however, the unreproducible formation of the ethynyl (*i.e.* σ-bonded acetylide) complex and established that the reaction, which can be represented by equation (1) (axial ligands only given), is catalysed by copper ions. This not only represents the first preparation of a mononuclear ethynyl or acetylide ($HC_2^-$) complex using gaseous acetylene as the reagent, but also provides the opportunity for calculating the first (minimum) reported value for the equilibrium constant for the substitution of co-ordinated $H_2O$ by $HC_2^-$ in any complex.

$$HO\text{-Co-}OH_2 + C_2H_2 \longrightarrow HC_2\text{-Co-}OH_2 + H_2O \quad (1)$$

† The cobinamides lack the nucleotide side-chain present in the cobalamins such as vitamin $B_{12}$ (cyanocobalamin) itself.

‡ Abbreviations: dac = diaquocobinamide, even when partly or wholly present as aquohydroxo- or dihydroxo-cobinamide ($pK_1 = 5.9$, $pK_2 = 10.3$);[1] $P^1$ and $P^2$ are the faster- and slower-moving (on t.l.c.) products respectively from the copper-catalysed reaction of dac with $C_2H_2$; cm, deae, and pei cellulose = carboxymethyl, diethylaminoethyl, and polyethyleneimine cellulose.

We describe here the reaction conditions and the identification of the two products as the two isomers (inversion of the axial ligands) of ethynylaquocobinamide, and also report equilibrium constants for the substitution of co-ordinated $H_2O$ by $OH^-$, $CN^-$, and imidazole, which serve to confirm the position of $HC_2^-$ in the *trans*-effect series of the $Co^{III}$ corrinoids.

## Experimental

*Materials.*—Samples of vitamins $B_{12}$ and $B_{12a}$ were kindly given by Mr. A. P. Domleo of Glaxo-Allenbury (Pty) Ltd., South Africa. Cyanoaquocobinamide (Factor B),[8] dac,[1] and vinylcobinamide [8] were prepared as previously described. Acetylcobinamide was prepared analogously to acetylcobalamin, namely by reduction of dac with zinc dust in 10% aqueous acetic acid to the $Co^I$ complex, followed by reaction with acetyl chloride and purification by phenol–chloroform extraction.[9] AnalaR reagents were used wherever possible (*e.g.* $CuSO_4$, NaCN, NaOH, and $HClO_4$). Acetylene (Afrox, Germiston, South Africa), phenylacetylene (Merck, C.P.), acetyl chloride (Merck, C.P.), and $NaBH_4$ (Hopkin and Williams) were all used as received. Imidazole (Aldrich, 99%) was recrystallised three times from benzene.

*U.v.–Visible Spectra.*—These were recorded with a JASCO UVIDEC-1 spectrophotometer in 1-cm cells and, unless otherwise stated, at 25 °C.

*pH Determinations.*—These were made with a Metrohm EA 147 microglass electrode.

*Chromatography.*—Thin-layer chromatography was carried out at room temperature on cellulose (Merck, 0.1 mm, pre-coated) and pei cellulose (Merck, pei, 0.1 mm, pre-coated) using $Bu^sOH$–0.88 ammonia–water (9.5 : 0.675 : 4) (solvent III of refs. 8 and 10); cm and deae cellulose (both Whatman) were used for column chromatography.‡

## Results

*Standard Conditions for the Preparation of Ethynylaquocobinamide by the Copper-catalysed Reaction of dac with Acetylene.*—The standard procedure finally adopted was to pass gaseous acetylene through 20 cm$^3$ of an unbuffered aqueous (pH *ca.* 5) solution of $10^{-4}$ mol dm$^{-3}$ dac and $10^{-3}$ mol dm$^{-3}$ $CuSO_4$ or $Cu[NO_3]_2$ for 30 min at room temperature, then seal the container and allow it to stand in the dark for 48 h.

Sealing such a solution in a spectrophotometer cell enabled the changes in spectrum to be followed. The initial spectrum, which showed a band at 350 nm, changed to a new spectrum with the maximum at 353 nm and a prominent shoulder at *ca.* 340 nm; the reaction followed first-order kinetics with $t_{\frac{1}{2}}$ *ca.* 5 h and gave reasonable isosbestic points. T.l.c. of the reaction mixture on pei cellulose after 48 h showed the presence of two red products [faster moving ($P^1$) and slower moving ($P^2$)] in approximately equal amounts, together with a small amount (<5%) of unreacted dac, while copper remained at the origin as a blue spot. The following $R_{B_{12}}$ values ($R_f$ values relative to that of $B_{12}$ as marker) [8,10] were observed: dac, 0.50; $P^2$, 0.99; $P^1$, 1.30.

In the standard procedure the corrinoids are separated from the copper, but not from each other, by passing the reaction mixture through a short (6 cm) column of deae cellulose which retains the copper as a blue-green band at the top of the column. In one experiment, in which the reaction mixture contained equal concentrations of dac and $CuSO_4$, the copper was eluted from the column with HCl (3 mol $dm^{-3}$); analysis of the copper in the eluate by atomic absorption showed that over 95% of the copper in the reaction mixture was accounted for, *i.e.* that $P^1$ and $P^2$ do not themselves contain any copper (*e.g.* as a binuclear Co–C≡C–Cu complex). Separation of the corrinoids from copper can also be achieved by phenol–chloroform extraction, but this is less convenient. Separation of $P^1$ and $P^2$ from each other can be done with cm cellulose, but it also hydrolyses them to the acetyl complexes (see below). When required, separation and purification of $P^1$ and $P^2$ can be accomplished by the use of preparative scale t.l.c. using pei cellulose; ordinary cellulose hydrolyses the products to the acetyl complexes. For most purposes (*e.g.* determining equilibrium constants) the solution from the deae column, which contained both products (and <5% dac), was used. This solution (and solutions of purified $P^1$ and $P^2$) can be concentrated to dryness by rotary evaporation without decomposition. The purity of the samples can be checked by t.l.c. No decomposition of either product was detected either on storage as a solid for over a year or in neutral aqueous solution kept in the dark for over one month.

*Further Observations concerning the Mechanism of Reaction.*—A slow and unreproducible reaction occurs even in the absence of added copper salts, which we ascribe to the presence of traces of copper and/or other metal ions; however, this could not be tested by, for example, suppressing the catalysis through the addition of ethylenediaminetetra-acetate (edta), since edta itself was found to react slowly (perhaps in conjunction with trace metal ions) to give so-called ' stable yellow ' corrinoids.[6b,11] The reaction with added $CuSO_4$ can be observed at least as high as pH 12.5 (but accompanied by considerable hydrolysis of the side-chains), but not at pH 2 or 3 ($10^{-2}$ and $10^{-3}$ mol $dm^{-3}$ $HClO_4$).

In an experiment to provide data for determining the binding constant of $HC_2^-$ (see Discussion section) using ' standard ' conditions, the pH was determined as 5.0, and the amount of unreacted dac determined quantitatively by dissolving the dac spot from the t.l.c. plate in aqueous cyanide solution and determining its concentration as the dicyanide complex. The amounts of unreacted dac corresponded to 2.3 and 2.5% in duplicate experiments; allowing for error, this is treated as ≤5%.

To show that the role of copper is catalytic, and not stoicheiometric, the dac concentration in the standard mixture was increased to $3.3 \times 10^{-2}$ mol $dm^{-3}$ to give a Co : Cu ratio of 10 : 1. Under these conditions the reaction showed $t_{\frac{1}{2}}$ *ca.* 5 h (as with the ratio of 1 : 10) and gave *ca.* 95% conversion over the 48 h period.

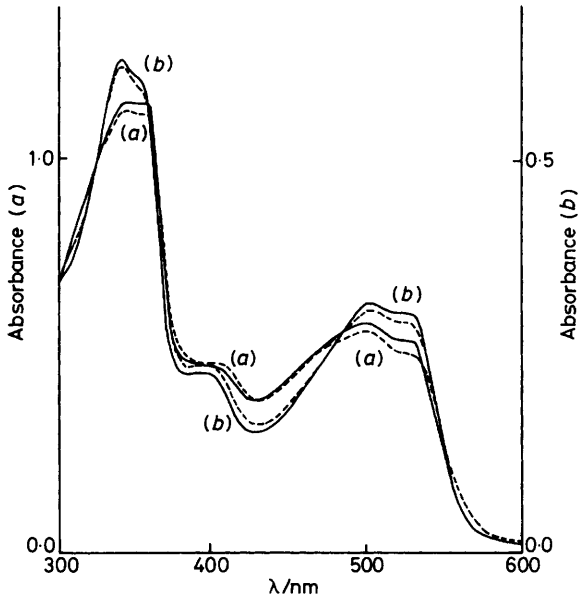


**Figure 1.** Spectra of the two isomers of ethynylaquocobinamide in neutral, unbuffered aqueous solution: (*a*) $P^1$ ($7.4 \times 10^{-5}$ mol $dm^{-3}$) and (*b*) $P^2$ ($3.5 \times 10^{-5}$ mol $dm^{-3}$) at 25 °C (——) and 65 °C (— — —)

An analogous reaction of dac ($10^{-4}$ mol $dm^{-3}$) and $CuSO_4$ ($10^{-3}$ mol $dm^{-3}$) with phenylacetylene ($4.5 \times 10^{-2}$ mol $dm^{-3}$) was carried out in unbuffered 50% aqueous methanol (freshly distilled from $K[MnO_4]$) in order to increase the solubility of the organic reagent; this reaction also gave two products (total yield of 60—70% after 48 h at room temperature) with $R_{B_{12}}$ values of 1.54 and 1.84 on pei cellulose. The spectrum of the mixed solution showed bands at *ca.* 328, 352, 500, and 531 nm (*cf.* those of $P^1$ and $P^2$ in Figure 1). This shows that the reaction is not restricted to acetylene itself. Tests for the reaction of acetylene with $4 \times 10^{-5}$ mol $dm^{-3}$ aquocobalamin ($B_{12a}$) for over 4 days in the presence of $10^{-3}$ mol $dm^{-3}$ $CuSO_4$ at pH *ca.* 6 were negative.

*Spectra and Reactions of the Products* $P^1$ *and* $P^2$.—The i.r. spectra (KBr discs) of pure $P^1$ and $P^2$ both showed a prominent band at 1 999 ± 3 $cm^{-1}$, *i.e.* in the region expected for co-ordinated acetylide (see Discussion section).

The wavelengths of the main bands (nm) in the u.v.–visible spectra (see Figure 1) with their absorption coefficients ($\varepsilon_{molar}$ $10^4$ $dm^3$ $mol^{-1}$ $cm^{-1}$) in parentheses were determined as follows: $P^1$, 340 (*ca.* 2), 356 (2.0), 499 (0.94), 526 (0.91); $P^2$, 339 (1.8), 355 (1.7), 499 (0.90), 525 (0.87). Molar absorption coefficients were calculated by conversion to the dicyanide (see below) with $\lambda_{max.} = 367$ nm and $\varepsilon_{molar} = 3.04 \times 10^4$ $dm^3$ $mol^{-1}$ $cm^{-1}$.[6c] Varying the temperature causes similar small and reversible changes in the spectra of both compounds (see Figure 1), which involve a relative movement of intensity from the lower to the higher energy bands within both the αβ and γ regions as the temperature is raised. Prolonged heating of a neutral solution of the mixture (*e.g.* for 1 h at 94 °C) had no detectable effect on the shape of the spectrum but did deposit a small amount (*ca.* 5% of the total) as a very insoluble red film on the glass walls.

An aqueous solution of the mixture showed only a single ' instantaneous ' change in spectrum over the range of pH 2—14, namely a reversible change at pH *ca.* 13 which can be ascribed to ionisation of the co-ordinated $H_2O$ (see below). A slow change is observed on allowing the mixed solution to

**Table 1.** Equilibrium constants for the substitution of co-ordinated $H_2O$ in ethynylcobinamide

| Ligand | $OH^-$ | $CN^-$ | Imidazole [a] |
|---|---|---|---|
| (*a*) Conditions of experiment | | | |
| [Co]/mol $dm^{-3}$ | $1.1 \times 10^{-4}$ | $3.5 \times 10^{-6}$ | $1.0 \times 10^{-5}$ |
| pH | 10.9—14.0 [b] | 11.1 (bicarbonate) | 8.05 (phosphate) |
| $I$/mol $dm^{-3}$ | variable ($\geqslant$1.0) | 0.2 | 0.2 |
| $\lambda$/nm | 560 | 372 | 560 |
| (*b*) Main bands [c] in spectrum of product | | | |
| $\gamma$ region (nm) | *340*, 353 (sh) | 372 | 335 (sh), *364* |
| $\alpha\beta$ region (nm) | *523*, 560 (sh) | 542, 586 | 527, *556* |
| (*c*) Results | | | |
| ligands per Co | 0.92 ± 0.04 | 1.0 | 1.0 |
| $K$ [d]/$dm^3$ $mol^{-1}$ | | | (2.67 ± 0.01) $\times 10^3$ |
| p$K$ | 13.0 ± 0.1 | | |
| $\log_{10}K$ [d] | 1.0 ± 0.1 | $\geqslant$6.8 | 3.43 |

[a] Value of p$K$ taken as 7.2 (see L. G. Sillén and A. E. Martell, 'Stability Constants of Metal-ion Complexes,' Supplement 1, Spec. Publ. No. 25, Chemical Society, London, 1971. [b] Ethynylaquocobinamide was dissolved in NaOH (1 mol $dm^{-3}$) and titrated with $HClO_4$; the values of pH, determined with an electrode, were corrected for the 'sodium error' at pH $\geqslant$ 13. The end-point corresponding to 100% hydroxo-complex was estimated by extrapolation of the plot of $A_{560}$ *versus* pH. [c] Most intense band in each region is italicised. [d] $K = [HC_2\text{-}Co\text{-}Z]/([HC_2\text{-}Co\text{-}OH_2][Z]$, where Z = $OH^-$, $CN^-$, and imidazole.

stand with acid (*e.g.* 2 mol $dm^{-3}$ HCl), leading to the appearance of new bands at 315 and 465 nm. Treating the mixture with HCl (2 mol $dm^{-3}$) for 1 h and then subjecting the reaction mixture to t.l.c. revealed two spots with the same $R_{B_{12}}$ values as the two spots given by a sample of acetylcobinamide (acc) both on cellulose (acc, 1.17 and 0.99; hydrolysed $P^1$ and $P^2$, 1.16 and 1.01) and on pei cellulose (acc, 1.23 and 0.85; hydrolysed $P^1$ and $P^2$, 1.21 and 0.85).

$P^1$ and $P^2$ are both remarkably stable to light, compared to most other organocorrinoids,[6d] and may safely be handled on the open bench.

*Determination of Equilibrium Constants.*—The products $P^1$ and $P^2$ are identified (see Discussion section) as the two isomers (differing in relative orientation of the axial ligands) of ethynylaquocobinamide. Equilibrium constants for the substitution of co-ordinated $H_2O$ by $OH^-$, $CN^-$, and imidazole were determined by spectrophotometric titration using the mixture of $P^1$ and $P^2$. The conditions, results, and main features of the spectra of the products are given in Table 1. With all three ligands the changes in spectrum were instantaneous and reversible and showed isosbestic points. No difference in equilibrium constant between $P^1$ and $P^2$ could be detected from the good data obtained for the co-ordination of imidazole (see Figure 2), where the points cover the range 19—98% formation of the imidazole complex.

The binding constant for cyanide has been calculated indirectly as $\log_{10}K = 6.7$.[12] To determine such a high value directly, one would normally reduce the pH below the p$K$ of HCN, which is here taken as 9.3.[13] Unfortunately, the complex was found to decompose in the presence of cyanide when the pH was reduced below 9. A spectrophotometric titration at pH 11.1 in a 10-cm cell confirmed that one cyanide per cobalt was involved but only provided a minimum value of $\log_{10}K \geqslant 6.8$. This cyanide complex is readily decomposed by

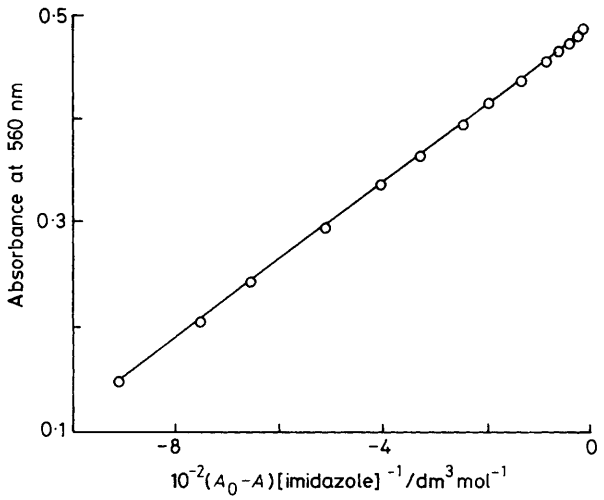


**Figure 2.** Evaluation of the experimental data for the equilibrium between ethynylcobinamide (mixed isomers) and imidazole; for details see Table 1

**Table 2.** Comparison of the thermodynamic *trans* effect of $HC_2^-$ and other carbanions.[a] Unless otherwise stated all constants taken from ref. 6(*g*)

| | $OH^-$ | | $CN^-$ | Imidazole |
|---|---|---|---|---|
| X | p$K$ | $\log_{10}K$ | $\log_{10}K$ | $\log_{10}K$ |
| N≡C- | 11.0 | 3.0 | 8 | 4.7 |
| HC≡C- | 13.0 [b] | 1.0 | $\geqslant$6.8 [b] | 3.4 [b] |
| $CH_2$=CH- | 15—15.5 [b] | −1 to −1.5 | 2.7 | |
| $CH_3$- | >15 | <−1 | 2.1 | 1.0 |
| $CH_3$-$CH_2$- | >15 | <−1 | 0.6 [c] | |

[a] $K = [X\text{-}Co\text{-}Z]/([X\text{-}Co\text{-}OH_2] + [X\text{-}Co])[Z]$, where Z = $OH^-$, $CN^-$, and imidazole; only the axial ligands are given and X-Co is the five-co-ordinate form.[15] [b] This work. [c] Ref. 14.

light into the well known purple dicyanide complex with $\lambda_{max.} = 367$ nm.

In order to complete the comparison of ethynylaquo- with cyanoaquo- and vinyl-cobinamide (see Table 2), we have checked the p$K$ of vinylcobinamide. It has been reported that no immediate change in spectrum was observed in solutions containing up to 1 mol $dm^{-3}$ NaOH.[14] We now find that an immediate, distinct, and reversible (on dilution) change in the spectrum can be produced in the region of 1—10 mol $dm^{-3}$ NaOH. This change is characterised by a decrease in the main bands at 325 and 450 nm, a shift in the position of the shoulder from *ca.* 365 to 360, and an increase in absorption beyond 500 nm; this approximates to the changes in the $\alpha\beta$ and $\gamma$ regions expected [15] on conversion of the five-co-ordinate vinylcobinamide to a six-co-ordinate complex. It can be estimated that 10 mol $dm^{-3}$ NaOH induces 25—50% conversion to the hydroxide complex, *i.e.* $K = [CH_2{=}CH\text{-}Co\text{-}OH]/([CH_2{=}CH\text{-}Co][OH^-]) = 0.033$—0.1 $dm^3$ $mol^{-1}$, $\log_{10}K = -1$ to −1.5, and p$K$ = 15—15.5.

## Discussion

Our results show that copper ions catalyse the reaction between dac and acetylene in aqueous solution at room temperature to give two products ($P^1$ and $P^2$) in approximately equal amounts. The properties and reactions of these com-

pounds closely parallel those of the known ethynylcobalamin. In the cobalamin the C≡C stretching frequency occurs at 1 996 $cm^{-1}$; [4] both $P^1$ and $P^2$ show a band at *ca.* 1 999 $cm^{-1}$. The cobalamin is unusually stable to light, at least under nitrogen; [6d] $P^1$ and $P^2$ are remarkably stable to light, even in air. The cobalamin is decomposed by acid to acetylcobalamin; [16] $P^1$ and $P^2$ are hydrolysed by acid to two new products which are identical to the two forms (see below) of acetylcobinamide. Treating ethynylcobalamin with cyanide displaces the base from co-ordination to give the ethynyl cyanide complex with $\lambda_{max.} = 373$ nm; [17] the mixture of monocyanide complexes derived from $P^1$ and $P^2$ shows $\lambda_{max.} = 372$ nm. We conclude that $P^1$ and $P^2$ are both ethynylcorrinoids. The identity of the products of acid hydrolysis with the two forms of acetylcobinamide provides evidence that the side-chains have not been irreversibly changed during the copper-catalysed reaction.

Cyanoaquocobinamide (Factor B) exists at equilibrium in solution as an approximately 1 : 1 mixture of the two isomers which differ in the relative orientation of the axial ligands ($CN^-$ and $H_2O$ or $OH^-$).[6e] We conclude that $P^1$ and $P^2$ are the two analogous isomers of ethynylaquocobinamide, and that acetylcobinamide also exists as two isomers. The absolute orientation of the $HC_2^-$ and $H_2O$ ligands in the two isomers can be deduced by the following two different methods. Firstly, there are significant differences in the spectra of $P^1$ and $P^2$ (see Figure 1). The spectrum of ethynylcobalamin in acid,[17] where the base has been displaced and protonated and where $HC_2^-$ must occupy the upper (or b) co-ordination site, strongly resembles that of $P^1$ but not $P^2$ (*cf.* two bands of equal intensity in the γ-region and a prominent shoulder at *ca.* 460 nm). Secondly, the isomer of Factor B with cyanide in the upper [6f] (or b) co-ordination position has been fairly conclusively identified as that having the higher $R_f$ value [6e] and in view of certain emerging patterns of t.l.c. behaviour,[1,10] it seems likely that the faster-moving product (*i.e.* $P^1$) also has the ethynyl ligand in the upper position. It is gratifying that both approaches give the same result. The isomers of Factor B are fairly readily interconverted in solution.[6e] By contrast, we have observed no changes in spectrum or t.l.c. behaviour of the pure isomers to indicate interconversion of the two isomers of ethynylaquocobinamide. The absence of any detectable difference in the binding constants towards imidazole (see Figure 2), as well as their formation in approximately equal amounts, strongly suggests that at true equilibrium the two isomers would be present in approximately equal concentrations.

The question as to whether ethynylcobinamide forms any detectable concentration of the five-co-ordinate complex (with loss of co-ordinated $H_2O$) cannot be satisfactorily resolved by reference only to the effect of temperature on the u.v.–visible spectrum (*cf.* ref. 15) and will be discussed later in connection with kinetic studies on the substitution of co-ordinated $H_2O$ by $CN^-$.

The equilibrium constants determined here are compared with those of other cobinamides in Table 2. The data further emphasise the position of $HC_2^-$ in the *trans*-effect series of $Co^{III}$ corrinoids between $CN^-$ and vinyl.

The mechanism of catalysis by copper ions of the reaction between dac and acetylene remains unknown, but a linear Co–C≡C–Cu intermediate can be excluded because dac also reacts with phenylacetylene. However, an intermediate in which a $Cu^I$ or $Cu^{II}$ ion is π-bonded to the triple bond of Co–C≡CR (R = H or Ph) remains a possibility. This appears to be the first example of the formation of co-ordinated $HC_2^-$ from acetylene itself and also the first example of catalysis in the formation of an acetylide complex. This novel reaction may open up new routes for the synthesis of other organometallic complexes.

The present catalysed reaction provides an opportunity for determining a minimum value for the equilibrium constant $K$ [equation (2)] for the substitution of co-ordinated $H_2O$ in dac by $HC_2^-$. If the p$K_a$ of acetylene is taken to be 25 (ref. 18)

$$K = \frac{[HC_2\text{–}Co\text{–}OH_2]}{[H_2O\text{–}Co\text{–}OH_2][HC_2^-]} = \frac{[HC_2\text{–}Co\text{–}OH_2][H^+]}{[H_2O\text{–}Co\text{–}OH_2]K_a[C_2H_2]} \quad (2)$$

and its solubility in water at 25 °C under $10^5$ Pa of acetylene to be $4.2 \times 10^{-2}$ mol $dm^{-3}$,[19] then the observation of ≥ 95% formation of the acetylide complex at pH 5.0 gives equation (3). This value must obviously be treated as approximate

$$K \geq \left(\frac{95}{5}\right) \cdot \frac{(10^{-5})}{(10^{-25})(4.2 \times 10^{-2})}$$
$$\geq 4.5 \times 10^{22} \text{ dm}^3 \text{ mol}^{-1}; \textit{ i.e. } \log_{10}K \geq 22.7 \quad (3)$$

because of the large uncertainty in the p$K_a$ of acetylene; however, the value is undoubtedly very high and serves to emphasise the ability of the $Co^{III}$ ion to form very stable Co–C bonds.[20] This appears to be the first value reported for the binding constant of the $HC_2^-$ ligand in any metal complex.

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## References

1 Part 20, D. A. Baldwin, E. A. Betterton, and J. M. Pratt, preceding paper.
2 P. L. Pauson, ' Organometallic Chemistry,' Edward Arnold Ltd., London, 1967.
3 G. E. Coates, ' Organometallic Compounds,' 2nd edn., Methuen, London, 1960.
4 A. W. Johnson, L. Mervyn, N. Shaw, and E. L. Smith, *J. Chem. Soc.*, 1963, 4146.
5 J. M. Pratt and R. G. Thorp, *Adv. Inorg. Chem. Radiochem.*, 1969, **12**, 375.
6 J. M. Pratt, ' Inorganic Chemistry of Vitamin $B_{12}$,' Academic Press, London, 1972, (*a*) chs. 5, 8, and 13; (*b*) ch. 15, sect. II; (*c*) p. 46; (*d*) ch. 14; (*e*) pp. 119—124; (*f*) pp. 25 and 118; (*g*) pp. 140—147.
7 D. A. Baldwin, E. A. Betterton, and J. M. Pratt, *S. Afr. J. Chem.*, 1982, **35**, 173.
8 R. A. Firth, H. A. O. Hill, J. M. Pratt, and R. G. Thorp, *J. Chem. Soc. A*, 1968, 453.
9 D. Dolphin in ' Methods in Enzymology,' eds. D. B. McCormack and L. D. Wright, Academic Press, New York, 1971, vol. 18, part *C*, p. 34.
10 R. A. Firth, H. A. O. Hill, J. M. Pratt, and R. G. Thorp, *Anal. Biochem.*, 1968, **23**, 429.
11 A. Gossauer, B. Grüning, L. Ernst, W. Becker, and W. S. Sheldrick, *Angew. Chem., Int. Ed. Engl.*, 1977, **16**, 481.
12 R. A. Firth, H. A. O. Hill, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *J. Chem. Soc. A*, 1969, 381.
13 L. G. Sillén and A. E. Martell, ' Stability Constants of Metal-ion Complexes,' Spec. Publ. No. 17, Chemical Society, London, 1964.
14 R. A. Firth, H. A. O. Hill, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *J. Chem. Soc. A*, 1968, 2428.

15 R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *J. Chem. Soc. A*, 1968, 2419.
16 G. C. Hayward, H. A. O. Hill, J. M. Pratt, N. J. Vanston, and R. J. P. Williams, *J. Chem. Soc.*, 1965, 6485.
17 H. A. O. Hill, J. M. Pratt, and R. J. P. Williams, *Proc. R. Soc. (London), Ser. A*, 1965, **288**, 352.
18 N. S. Wooding and W. C. E. Higginson, *J. Chem. Soc.*, 1952, 774; D. J. Cram, ' Fundamentals of Carbanion Chemistry,' Academic Press, New York, 1965, p. 19; A. Streitweiser and D. M. E. Reuben, *J. Am. Chem. Soc.*, 1971, **93**, 1794.
19 ' Chemical Engineers' Handbook,' 5th edn., eds. R. H. Perry and C. H. Chilton, McGraw-Hill, New York, 1973, pp. 3–96.
20 J. M. Pratt, in ' Vitamin $B_{12}$,' ed. D. Dolphin, John Wiley, New York, 1982, vol. 1, p. 325.

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