# The Chemistry of Vitamin $B_{12}$. Part 17.[1] The Effect of Steric Distortion of the Cobalt–Carbon Bond on the p*K* Values and Spectra of Organocobalamins

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The spectra of the neutral and acid forms and the apparent p*K* values for protonation and displacement of the heterocyclic base have been determined for organocobalamins with various alkyl and cycloalkyl ligands possessing no functional group. Increasing the degree of substitution or distortion on either $C_\alpha$ (in the series methyl < ethyl < isopropyl and cyclo-propyl < -butyl < -pentyl ~ -hexyl) or $C_\beta$ (ethyl ~ n-propyl < isobutyl < neopentyl) leads to parallel changes in the spectra and in the p*K* values with no fundamental difference between primary and secondary groups (methyl ≡ cyclopropyl, ethyl ≡ cyclobutyl, isopropyl ≡ cyclo-pentyl and -hexyl) or between substitution on $C_\alpha$ and $C_\beta$ (neopentyl ≡ cyclopentyl). It is shown that these effects must be steric, rather than electronic, in origin and are probably due to repulsion between the bonding electron pairs on $C_\alpha$, on the one hand, and the lone pairs on Co and the equatorial N atoms on the other. It is suggested that the main variable, as seen by the Co ion and the rest of the complex, is the Co–$C_\alpha$ bond length.

THE coenzyme forms of the cobalt corrinoids which are present in the isomerase and ribonucleotide reductase enzymes possess the ligand 5′-deoxy-5′-adenosyl (see Figure 1), the Co being attached to a primary carbon

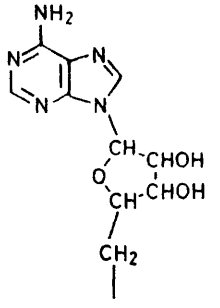

FIGURE 1 The 5′-deoxy-5′-adenosyl ligand ($R_{dbc}$) present in the vitamin $B_{12}$ coenzymes

atom; experimental evidence shows that the Co–C bond undergoes reversible, probably homolytic, fission during the enzymatic reaction.[2] Since functionally active enzymes can be formed with synthetic analogues of the coenzyme in which the –O– has been replaced by $-CH_2-$, or either of the –CHOH– groups by $-CH_2-$ or an acetal group,[3,4] it appears that the mechanism of cleavage does not require the presence of a heteroatom, at least not closer than the N atom situated five atoms away from the Co. Since bonds between Co and a primary alkyl group without any functional group are usually very stable,[5] the labilisation of the Co–C bond remains one of the major problems in understanding the mechanism of the isomerase reaction.

In 1969 we suggested in general terms that the Co–C bond of the coenzyme might be labilised by a steric misfit between the binding of the adenosine by the Co atom and by the protein.[6] We have demonstrated that the greater steric requirements of secondary alkyl ligands (such as isopropyl) compared to primary alkyl ligands can induce lability towards several types of reaction [6-8] as well as markedly increasing $pK_{obs.}$ for the protonation of dbzm (dbzm = 5,6-dimethylbenzimidazole, present in the cobalamin side chain);[5,7] *cf.* also the results and ideas of Brodie.[9] We subsequently made the more specific suggestion that the protein may (by a conformation change on binding the substrate) distort the coordination sphere of the Co (most likely by distorting the Co–$C_\alpha$–$C_\beta$ bond angle) and thereby displace the potential equilibrium between Co–R (the organocorrinoid) and $Co^{II}$ + the radical R· in favour of the latter.[10] The feasibility of such a mechanism might be demonstrated by studying the effects of steric distortion around $C_\alpha$ in protein-free organocorrinoids, and we have therefore been involved in a systematic study of the effects of steric distortion in alkyl ligands containing no heteroatoms on the spectra, equilibria, and reactivity of organocorrinoids. Our recent report [11] that cyclobutylcobalamin is far more stable than other secondary alkylcobalamins and that cyclopropylmethyl- and but-2-enyl-cobalamin are more labile than typical primary alkylcobalamins demonstrates the existence of a diversity of steric (and perhaps other) effects involving the $C_\alpha$ and $C_\beta$ atoms in relatively simple alkyl ligands.

Since the various equilibria shown by organocobalamins may involve changes in the number and nature of the axial ligands, in the position of the Co atom with respect to the equatorial plane, in the conformation of the corrin ring, and in various intramolecular interactions, a knowledge of the nature of the observed equilibria and of the interplay between these different changes is an essential prerequisite to any study of the steric and electronic effects of organo-ligands. In the accompanying paper [12] we present experimental evidence for the existence of two types of ' base-off ' organocobalamins in neutral solution and summarise and assess the available evidence for the nature of all the equilibria exhibited by organocobalamins in aqueous solution and for the interplay between the various types of change mentioned. In this paper we are interested in examining the effect of varying the structure of the alkyl ligand on the rest of the complex, as seen by changes in (i) $pK_{obs.}$ for the

reversible protonation and displacement of dbzm from co-ordination and (ii) the spectra of the yellow five-co-ordinate ' base-off ' (E) forms in acid and the red six-co-ordinate ' base-on ' (A) forms in neutral solution (A—E denote the various types of complex formed by organocobalamins in aqueous solution as described in ref. 12).

*X*-Ray analysis [13] has shown that the Co–$C_\alpha$–$C_\beta$ angle in the six-co-ordinate coenzyme dbc (dbc = the co-enzyme form of vitamin $B_{12}$, dimethylbenzimidazolyl-cobamide coenzyme) is 125°, even though $C_\alpha$ is formally tetrahedral. This high value has been ascribed to repulsion between $C_\beta$ and N(24),[13] and to the need to maximise the overlap between the relevant hybridised orbitals of Co and $C_\alpha$ and to minimise the various repulsions between the bonding pairs on $C_\alpha$, on the one hand, and the lone pairs on Co and the ligand N atoms on the other; [5] it is probably close to the angle which should be considered ' normal ' for a primary alkyl ligand such as ethyl. The Co–$C_\alpha$–$C_\beta$ bond angle can be progressively decreased as the ligand is varied in the series Et > $Pr^i$ > $Bu^t$ (but no complex can be isolated) [14] and $C_3$ > $C_4$ > $C_5 \sim C_6$; even though no C–C bond is present, Me may be considered as the first member of the first series ($C_3$, $C_4$, $C_5$, and $C_6$ denote cyclo-propyl, -butyl, -pentyl, and -hexyl respectively). A ligand such as neopentyl (np) ($-CH_2CMe_3$) will obviously experience considerable steric compression against the acetamide side chains on the corrin ring; the Co–$C_\alpha$–$C_\beta$ bond angle may therefore be progressively increased in the series Et ⩽ Pr < $Bu^i$ < np. Both types of steric compression will tend to be less in the five-co-ordinate organocorrinoids, where Co has probably been displaced from the mean plane of the equatorial ligand atoms towards $C_\alpha$ with an increase in the N–Co–$C_\alpha$ angle.[12]

We present here the results * of a systematic study of the effects of steric compression around $C_\alpha$ in organocobalamins possessing the above mentioned ligands (except $Bu^t$) as seen by changes in the u.v.–visible spectra of both the six-co-ordinate ' base-on ' (A) forms and the five-co-ordinate ' base-off ' (E) forms and in $pK_{obs.}$ for the displacement of dbzm from co-ordination, with particular reference to answering the following questions. (i) Can the electronic and steric effects of organo-ligands be distinguished? (ii) Is there any parallel between the order of ligands obtained from their effect on the spectra and on the p*K* values? (iii) Are there fundamental differences in electronic (as distinct from steric) effects between primary and secondary alkyl ligands? (iv) Are there fundamental differences between the effects of the two main modes of distortion investigated, *viz.*, increasing and decreasing Co–$C_\alpha$–$C_\beta$ from the ' normal ' value of *ca.* 125°? We shall present our results on the effects of steric compression on reactivity in a subsequent paper.[15]

Grate and Schrauzer [14] have recently examined a similar series of organocobalamins. We note, however, that their main interests [*viz.*, the decomposition of secondary alkylcobalamins to give the cob(I)alamin] and some of their experimental results are different from ours, while their conclusions regarding the origin of the observed effects are totally dissimilar.

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## EXPERIMENTAL

*Materials.*—Samples of vitamin $B_{12}$ and $B_{12a}$ were kindly given by Mr. A. P. Domleo of Glaxo–Allenbury (Pty) Limited, South Africa and a sample of dbc by Dr. L. Mervyn of Glaxo Laboratories, England. Aquocyanocobinamide was prepared as previously described.[7] The compounds MeI (Merck, 99%), cyclopropyl bromide (Merck, for synthesis, 98%), cyclobutyl bromide (Fluka, purum, ⩾98%), and neopentyl bromide (Fluka, purum, ⩾97%) were used as received. The compounds EtBr and $Pr^iI$ (both Hopkin and Williams, GPR), $Pr^iBr$ and cyclopentyl bromide (both Schuchardt), $Bu^iBr$ (B.D.H., laboratory reagent), and cyclohexyl bromide (Emanuel, 95%) were all redistilled before use. Imidazole (Aldrich, 99%) was recrystallised three times from benzene.

*Preparation of Organocorrinoids.*—Three different methods were used for preparing organocobalamins, depending on the stability of the product. The cobalamins of $Pr^i$, $C_5$, and $C_6$ were found to decompose above their p*K* values (see Table 1) at a pH-independent rate even under $N_2$ (*cf.* ref. 14) and were therefore prepared *in situ* according to method (*a*) in the 1-cm spectrophotometer cell to be used for further experiments; this also enabled the course of the reduction, alkylation, and decomposition to be monitored. Neopentylcobalamin was found to be stable under $N_2$, but decomposed in air above its p*K* of *ca.* 4.7 (see Table 1); this permitted certain purification steps to be carried out and it was therefore prepared according to method (*b*). The other cobalamins were stable enough to be isolated as the pure solids and were prepared according to (*c*). All steps were carried out in subdued light.

The rate of reduction of $B_{12a}$ was found to vary considerably at the low concentrations used in methods (*a*) and (*b*), presumably due to varying concentrations of trace metals in the different samples of $B_{12a}$,[5] $Na[BH_4]$, and perhaps even ' deionised ' water. No attempt was made to study this systematically and the effect, if any, of the trace metals on the properties of the cobalamins remains unknown. The compound $Co[NO_3]_2$ was routinely added to catalyse the reduction by tetrahydroborate [16] in method (*a*) where the degree of formation of the product represents a steady state between the rates of formation and decomposition, and in method (*c*), where the rate of alkylation is rather slow. Additional, much slower preparations were also carried out without any added cobalt salt in order to confirm that the latter had no effect on the products of method (*a*).

*Method* (*a*). An aqueous solution of vitamin $B_{12a}$ (1—2 $cm^3$) containing *ca.* $10^{-4}$ mol $dm^{-3}$ $Co[NO_3]_2$ in a 1-cm spectrophotometer cell was treated under $N_2$ with a small quantity of solid $Na[BH_4]$, followed by the addition of the alkyl halide and decomposition of the unreacted tetrahydroborate with 3.6N $H_2SO_4$, phosphate buffer pH 5.5, or phosphate buffer followed by 2N Na[OH], to give 2.5 $cm^3$ of a $3 \times 10^{-5}$ mol $dm^{-3}$ solution of the cobalamin at pH 1, 6—7, or 12 respectively. The $Co[NO_3]_2$ forms specks of insoluble black cobalt boride, which is presumably the catalyst (*cf.* ref. 17). The $Co_2B$ usually settles out on the bottom of the cell fairly readily and does not then interfere with the spectrum; it also redissolves on acidification.

*Method* (*b*). A $10^{-3}$ mol $dm^{-3}$ aqueous solution of vitamin

$B_{12a}$ containing *ca.* $10^{-4}$ mol $dm^{-3}$ $Co[NO_3]_2$ was treated under $N_2$ with solid $Na[BH_4]$, neopentyl bromide and, after complete formation of the red product, with 0.2 mol $dm^{-3}$ $K[H_2PO_4]$ to destroy the unreacted tetrahydroborate; otherwise the evolution of gas would promote the formation of an emulsion and hinder the separation of the phases. The cobalamin was then extracted into phenol–chloroform in air and back into 0.001N $H_2SO_4$ to form a 'stock' solution, and the pH then adjusted as required. The spectrum of the product around 350 and 520 nm always revealed the presence of a small amount (<5%) of vitamin $B_{12a}$.

*Method* (*c*). Preparations were performed according to standard methods.[7,16] Thin-layer chromatography on Merck cellulose plates using $Bu^tOH$–$H_2O$ (95 : 40) (and sometimes other additional solvents) showed the presence of less than 1% impurity in each case.

Methylcobinamide was prepared, as required, from aquocyanocobinamide as previously described,[7] but without separation of the solid; the purity was checked from the shape of the spectrum at 350 nm, which clearly shows the presence of any unreacted vitamin $B_{12a}$. Analogous methods were used for neopentylcobinamide.

*Ultraviolet–Visible Spectra.*—These were recorded with a Unicam SP8000 or a JASCO UVIDEC-1 spectrophotometer, both fitted with a variable-temperature unit. All spectra were recorded in 1-cm cells and, unless stated otherwise, at 25 °C.

*pH Measurements.*—These were made with a Metrohm Microelectrode on solutions inside a 1-cm spectrophotometer cell.

## RESULTS

1. *pH-Dependent Equilibria* ($pK_{obs.}$).—The u.v.–visible spectra (300—600 nm) of all the cobalamins prepared were examined in aqueous solution at room temperature over the range of pH 0—12 for evidence of reversible equilibria or irreversible reactions. All the cobalamins showed a single reversible pH-dependent equilibrium in the region pH 2—5 between yellow complexes in acid and pink to orange complexes in neutral solution. All the cobalamins were stable at room temperature below their p*K* values, but in neutral and alkaline solution the neopentyl complex reacted readily with traces of $O_2$, while the complexes of $Pr^i$, $C_5$, and $C_6$ decomposed even under nitrogen.

Varying the temperature from 25 °C up to the onset of decomposition (*e.g.* ⩾90 °C for Me, *ca.* 50 °C for $C_5$) had no significant effect on the spectra (300—600 nm) of any of the yellow complexes at pH 1 [see also 3(*a*) below]. We conclude that in all these cases there is only one form (⩾95%) present below the p*K*, *viz.*, the five-co-ordinate 'base-off' (E) form. Above the p*K* value, however, all the organocobalamins studied here exist as a mixture of forms A and C (which includes the variant $C^{dbzm}$).[12] For these cobalamins the observed p*K* therefore corresponds to equation (1). Values of $pK_{obs.}$ determined by us at 25 °C are listed

$$K_{obs.} = [H^+]([A] + [C])/[E] \quad (1)$$

in Table 1, together with comparable (*i.e.* spectrophotometrically determined) values published for certain other ligands.

For the stable cyclopropyl, cyclobutyl, and isobutyl cobalamins $pK_{obs.}$ was determined by spectrophotometric titration of (3—5) × $10^{-5}$ mol $dm^{-3}$ solutions in 0.1 mol $dm^{-3}$ $H_2SO_4$ with 0.1 and 2 mol $dm^{-3}$ Na[OH]; the ionic strength was not held constant. The pH at each point was determined with a hydrogen electrode. Isosbestic points were observed in each case, and evaluation of the changes in optical density (at 440, 510, and 439 for $C_3$, $C_4$, and $Bu^i$ respectively) in terms of two species corresponded to equilibria involving one proton. Figure 2 shows the experimental plots for the cobalamins of $C_3$ and $C_4$; that for the isobutylcobalamin would virtually coincide with the latter.

TABLE 1

Values of $pK_{obs.}$ for organocobalamins

| Ligand [a] | Present results [c] $pK_{obs.}$ [b] | Present results [c] No. of $H^+$ | Other results [d] $pK_{obs.}$ [b] | Other results [d] No. of $H^+$ | Other results [d] Ref. |
|---|---|---|---|---|---|
| 5′-Deoxy-5′-adenosyl ($R_{dbc}$) | | | 3.3—3.5 | 1 | 5 |
| Methyl | | | 2.5—2.7 | 1 | 5 |
| Ethyl | | | 3.87 | ? | 5 |
| i-Propyl | ⩾4.5 | ? | | | |
| Ethyl | | | 3.87 | ? | 5 |
| n-Propyl | | | 3.84 | ? | 5 |
| i-Butyl | 4.15 ± 0.05 | 1.0 | 4.20 | ? | 14 |
| Neopentyl | 4.7 ± 0.2 | ? | 4.55 | ? | 14 |
| Cyclopropyl | 2.82 ± 0.06 | 1.0 | 3.7 | ? | 14 |
| Cyclobutyl | 4.17 ± 0.04 | 1.0 | 3.8 | ? | 14 |
| Cyclopentyl | ⩾4.5 | ? | | | |
| Cyclohexyl | 4.7 ± 0.2 | ? | | | |

[a] Ligands are grouped according to three series of structural changes. [b] $K_{obs.} = [H^+]([A] + [C])/[E]$. [c] Conditions: 25 °C, ionic strength not held constant. [d] Conditions: various; see original papers.

2. *pH-Dependent Equilibria* ($pK_{obs.}$).—The u.v.–visible spectra (300—600 nm) of all the cobalamins prepared were examined in aqueous solution at room temperature over the range of pH 0—12 for evidence of reversible equilibria or irreversible reactions. All the cobalamins showed a single reversible pH-dependent equilibrium in the region pH 2—5 between yellow complexes in acid and pink to orange complexes in neutral solution. All the cobalamins were stable at room temperature below their p*K* values, but in neutral and alkaline solution the neopentyl complex reacted readily with traces of $O_2$, while the complexes of $Pr^i$, $C_5$, and $C_6$ decomposed even under nitrogen.

Varying the temperature from 25 °C up to the onset of decomposition (*e.g.* ⩾90 °C for Me, *ca.* 50 °C for $C_5$) had no significant effect on the spectra (300—600 nm) of any of the yellow complexes at pH 1 [see also 3(*a*) below]. We conclude that in all these cases there is only one form (⩾95%) present below the p*K*, *viz.*, the five-co-ordinate 'base-off' (E) form. Above the p*K* value, however, all the organocobalamins studied here exist as a mixture of forms A and C (which includes the variant $C^{dbzm}$).[12] For these cobalamins the observed p*K* therefore corresponds to equation (1). Values of $pK_{obs.}$ determined by us at 25 °C are listed

$$K_{obs.} = [H^+]([A] + [C])/[E] \quad (1)$$

in Table 1, together with comparable (*i.e.* spectrophotometrically determined) values published for certain other ligands.

For the stable cyclopropyl, cyclobutyl, and isobutyl cobalamins $pK_{obs.}$ was determined by spectrophotometric titration of (3—5) × $10^{-5}$ mol $dm^{-3}$ solutions in 0.1 mol $dm^{-3}$ $H_2SO_4$ with 0.1 and 2 mol $dm^{-3}$ Na[OH]; the ionic strength was not held constant. The pH at each point was determined with a hydrogen electrode. Isosbestic points were observed in each case, and evaluation of the changes in optical density (at 440, 510, and 439 for $C_3$, $C_4$, and $Bu^i$ respectively) in terms of two species corresponded to equilibria involving one proton. Figure 2 shows the experimental plots for the cobalamins of $C_3$ and $C_4$; that for the isobutylcobalamin would virtually coincide with the latter.

The determination of $pK_{obs.}$ for the cobalamins of np, $Pr^i$, $C_5$, and $C_6$ was made difficult by their instability (especially with $Pr^i$ and $C_5$) and by their much smaller change in optical density (especially with $C_6$). (We have previously suggested that dbzm was not co-ordinated in isopropylcobalamin;[5,7] in fact, it is *ca.* 25% 'base-on'.[12]) Each point on the titration curve was obtained separately by dilution of a 2 × $10^{-4}$ mol $dm^{-3}$ stock solution in $10^{-3}$ mol $dm^{-3}$ $H_2SO_4$ with the required amount of acetate buffer, phosphate buffer, $H_2SO_4$, or Na[OH] solution; no attempt was made to keep the ionic strength constant. All solutions containing the neopentyl complex were handled under nitrogen. For each of these complexes superposition of the spectra gave approximate isosbestic points. Plotting the changes in optical density against the pH gave the approximate values of $pK_{obs.}$ listed in Table 1 for the neopentyl (438 nm) and cyclohexyl (442 nm) cobalamins. The results were not sufficiently accurate to establish the number of

protons involved, but this is assumed to be one. In the case of the isopropyl and cyclopentyl complexes, which decompose fastest of all, the data could only provide minimum values for $pK_{obs.}$ (see Table 1).

The decrease in the binding constant for dbzm (*i.e.* increase in $pK_{obs.}$) is paralleled by a decrease in the binding constants for other ligands including imidazole when Me is replaced by Pr or $C_6$ (see Discussion section). To confirm that this

TABLE 2

Ultraviolet–visible spectra of organocobalamins

(*a*) Five-co-ordinate ' base off ' (E) forms in acid

| | αβ Region Maxima | | | γ Region Maximum | 303 nm |
|---|---|---|---|---|---|
| Ligand | λ/nm ($10^{-4}\varepsilon$/dm³ mol⁻¹ cm⁻¹) | | $\varepsilon_{440}/\varepsilon_{460}$ | λ/nm ($10^{-4}\varepsilon$) | Band $10^{-4}\varepsilon$ |
| $R_{dbc}$ | 458 (0.88) | | 0.89 | 376 (0.82) | |
| Me | 460 (0.93) | | 0.90 | 374 (0.90) | 2.2 |
| Et | 455 (0.82) | 440 (0.84) | 1.08 | 380 (0.91) | 3.0 |
| $Pr^i$ | | 442 (1.1) | 1.23 | 382 (1.2) | >3.2 |
| Et | 455 (0.82) | 440 (0.84) | 1.08 | 380 (0.91) | 3.0 |
| Pr | 455 (0.86) | 441 (0.88) | 1.05 | 381 (0.94) | 2.8 |
| $Bu^i$ | | 439 (0.85) | 1.12 | 384.5 (0.92) | 2.9 |
| np | | 438 (0.93) | 1.22 | 388.5 (1.0) | 3.0 |
| $C_3$ | 460 (0.83) | | 0.97 | 374 (0.83) | 2.0 |
| $C_4$ | | 438 (0.84) | 1.19 | 378 (0.86) | 2.7 |
| $C_5$ | | 440 (0.83) | >1.1 * | 382 (0.90) | 3.1 |
| $C_6$ | | 442 (0.93) | 1.27 | 384 (0.93) | >3.3 |

(*b*) Six-co-ordinate ' base-on ' (A) forms

| | Maxima: λ/nm ($10^{-4}\varepsilon$/dm³ mol⁻¹ cm⁻¹) | | | |
|---|---|---|---|---|
| Ligand | αβ Region | γ Region | Further u.v region | |
| $R_{dbc}$ | 522 (0.84) | 374 (1.1) | 340 (1.2) | 305 (1.3) |
| Me | 518 (0.87) | 372 (1.1) | 342 (1.3) | 310 (1.3) |
| Et | 506 (0.91) | 375 (0.97) | 342 (1.3) | 305 (1.7) |
| Pr | 510 (0.95) | 375 (0.97) | 343 (1.3) | 305 (1.6) |
| $C_3$ | 522 (0.87) | 375 (0.95) | 342 (1.4) | 305 (1.3) |

* Only a minimum value could be obtained due to the presence of some $B_{12r}$ which absorbs at 470 nm.

The combined data of Table 1 show that $pK_{obs.}$ rises in the order: Me (2.6) < $C_3$ (2.8) < $R_{dbc}$ (3.4) < Pr (3.8) ~ Et (3.9) < $Bu^i$ ~ $C_4$ (4.2) < $Pr^i$, np, $C_5$, and $C_6$ (all ⩾4.5)

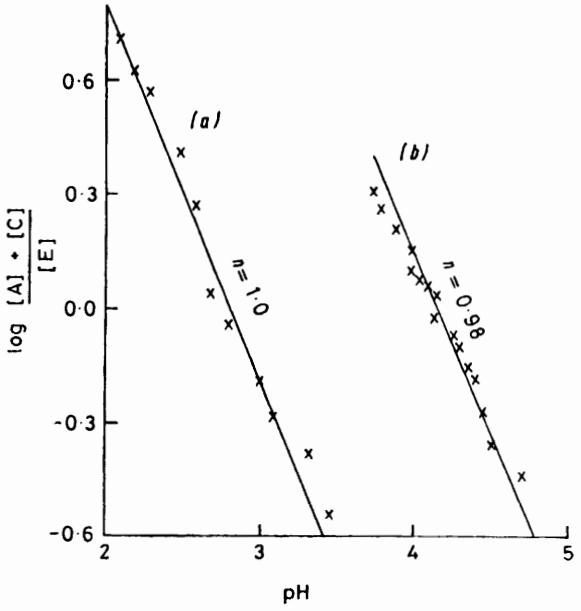


FIGURE 2 Experimental plots for determining the values of $pK_{obs.}$ and *n* (no. of protons) for (*a*) cyclopropylcobalamin and (*b*) cyclobutylcobalamin

($R_{dbc}$ = the alkyl ligand present in the coenzyme derivatives).

2. *Co-ordination of Imidazole by Neopentylcobinamide.*—The correlation also extends to the effects of substitution on $C_\beta$, we have studied the possible co-ordination of imidazole by neopentylcobinamide. Neopentylcobinamide alone in neutral solution is quite stable to air, but is slowly decomposed in the presence of imidazole, presumably through the intermediate formation of the imidazole complex. The rate of decomposition is not, however, fast enough to obscure any initial changes in the spectrum. Comparison of the spectra of neopentylcobinamide alone and in the presence of up to 3.3 mol $dm^{-3}$ imidazole (im) at pH 9 revealed no significant differences in spectra. Assuming that the imidazole adduct has the same spectrum in the 400—550 nm region as the six-co-ordinate (A) forms of most organocobalamins puts a limit of <10% formation for any adduct under these conditions. This gives a value of $K = [np\text{–}Co\text{–}im]/[np\text{–}Co][im] < 3 \times 10^{-2}$ $dm^3$ $mol^{-1}$ or $\log_{10}K < -1.5$.

3. *Spectra of the Five-co-ordinate ' Base-off ' (E) Forms in Acid.*—The spectra were recorded at pH 1.2 or 0 at concentrations of $3 \times 10^{-5}$ or $6 \times 10^{-5}$ mol $dm^{-3}$; no Beer's law plots were made. The wavelengths and molar absorption coefficients are given in Table 2(a). It is useful to discuss three regions (400—600, 325—400, and *ca.* 300 nm) separately. There is, unfortunately, no real understanding of the nature of the transitions involved in these yellow corrinoids, except that most or all of the observed bands represent $\pi$–$\pi^*$ transitions within the corrin ring.[5]

(*a*) 400—600 nm (αβ) *Region.* All the spectra show absorptions starting beyond 560 nm and rising to a maximum in the 440—460 region. Comparison of the spectra of the methyl, ethyl, and cyclohexyl complexes (see Figure 3) reveals an unusual feature of these spectra. Varying the ligand (*e.g.* $H_2O$, $NH_3$, or $CN^-$) in the ' typical ' six-co-ordinate cobalamins causes a progressive and systematic

change in the wavelengths of the α,β,γ, and other bands without any marked change in the overall shape.[5] Varying the organo-ligand, on the other hand, produces maxima in only one of two regions (*ca.* 440 and *ca.* 460 nm), has no significant effect on the wavelengths within these regions, but does have a marked influence on the ratio of their

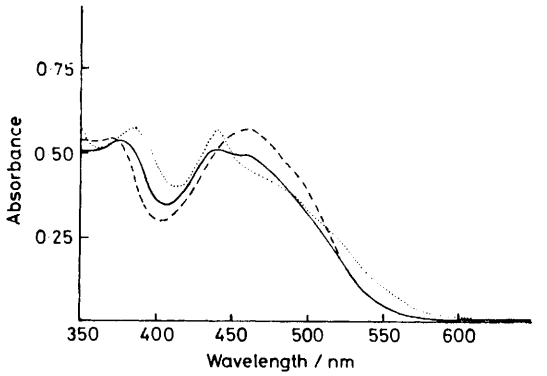


FIGURE 3 Spectra of $6 \times 10^{-5}$ mol dm$^{-3}$ solutions of the yellow, protonated 'base-off' (E) forms of methyl (— — —), ethyl (——), and cyclohexyl (· · · ·) cobalamins

absorption coefficients. The ratios of the optical densities at 440 and 460 nm are listed in Table 2(*a*) and give the order: Me (0.90) ~ $R_{dbc}$ (0.89) < $C_3$ (0.97) < Pr (1.05) ≤ Et (1.08) < $Bu^i$ (1.12) < $C_4$ (1.19) < np (1.22) ~ $Pr^i$ (1.23) ≤ $C_6$ (1.27). The first three show clear maxima at 458—462 nm, the last five at 438—442 nm, while the complexes of Et and Pr represent an intermediate case with bands of almost equal intensity at 440 and 455 nm. Similar spectra have been shown, but no wavelengths or absorption coefficients reported, for some of these complexes by other workers.[14]

This pattern of variation suggests the interesting possibility that these organocorrinoids may exist in two different forms, either in their ground or excited state. No significant changes in the relative intensity of these two bands were, however, observed on varying the temperature over the range 25—90 °C for the complexes of Me, Et, $C_3$, or $C_4$ or over the range 25—50 °C for those of $Pr^i$ and np or, in the case of the ethyl complex, on comparing the spectra at concentrations between $3 \times 10^{-4}$ and $3 \times 10^{-6}$ mol dm$^{-3}$. If any equilibrium does exist, $\Delta H$ for the interconversion must be very small. We are not, however, interested here in the origins of these variations, which may be difficult to establish conclusively, but in their use as an experimental parameter for establishing the order of ligands. The results show that all the ligands can be included in a single order, as measured by the relative intensities at 440 and 460 nm.

(*b*) 325—400 nm *Region*. The organocorrinoids show several overlapping bands, probably of diverse origins, in this region. Only the band at lowest energy, usually referred to as the γ band,[5] is listed in Table 2(*a*); the absorption coefficients and, to a lesser extent, the wavelengths will be affected by overlap with neighbouring bands. In spite of these complications, it is clear that the γ band shows a gradual shift to longer wavelengths (in contrast to the 'jump' to shorter wavelength found in the 440—460 nm region) as the ligand is varied in the order: Me ~ $C_3$ < $R_{dbc}$ < $C_4$ < Et ~ Pr ≤ $Pr^i$ ~ $C_5$ < $C_6$ ~ $Bu^i$ < np. This is *approximately* the order obtained (see Table 3) from other parameters, except for $Bu^i$ and np. For some reason the γ band is the only experimental parameter which distinguishes np from $Pr^i$ and $C_5$ and also $Bu^i$ from $C_4$, *i.e.* is more sensitive than the other parameters to substitution on $C_\beta$. The neopentyl complex shows the longest wavelength yet recorded (388.5 nm) for the γ band of any cobalt(III) corrinoid, even beyond that (386.5 nm) [5] of the main band of the cobalt(I) complex $B_{12s}$.

(*c*) 300 nm *Region*. All the complexes show an intense band at 303 nm, whose wavelength is unaffected by changing the ligand, *i.e.*, its behaviour is different again from that of the αβ and γ bands. It is more dangerous to draw conclusions from the intensities because of unknown contributions from neighbouring bands, but there does seem to be a regular and significant increase in the series from $C_3$ to $C_6$.

4. *Spectra of the Six-co-ordinate 'Base-on' (A) Forms.*— The existence of a pH-independent equilibrium between the A and C (or $C^{dbzm}$) forms [12] for many of the organocobalamins studied here and the difficulty of obtaining sufficiently good spectra for the A (or any other single) form limits discussion to those cobalamins listed in Table 2(*b*), which contain ≥85% of the A form.[12] Figure 4 compares the spectra of the methyl and ethyl cobalamins.

The maxima in the αβ region occur at considerably longer wavelength (506—522 nm) in the red A forms than in the yellow E forms (440—460 nm), the maxima are not so pronounced (*cf.* Figures 3 and 4), and there is a smaller shift

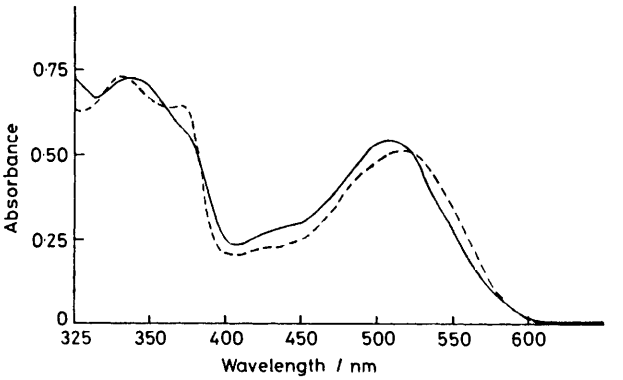


FIGURE 4 Spectra of $6 \times 10^{-5}$ mol dm$^{-3}$ solutions of the red 'base-on' (A) forms of methyl (— — —) and ethyl (——) cobalamins

to shorter wavelenth as the ligand is changed in the order: $R_{dbc}$ ~ $C_3$ (522 nm) ≥ Me (518 nm) > Pr (510 nm) ≥ Et (506 nm).

A large number of overlapping bands, probably of diverse origins, occur beyond the minimum at 400 nm. Although both the wavelength and intensity of the first band (γ band) may be affected by the presence of neighbouring bands, it appears that in dbc and in the methyl and cyclopropyl complexes the γ band is reasonably distinct and located at 372—375 nm. In the ethyl and propyl complexes the γ band has become a mere shoulder on the side of the next broad band, *i.e.* the true wavelength is probably ≥380 nm. Comparison with the data in Table 2(*a*) shows that the wavelength of the γ band probably varies by only ±2 nm as between the A and E forms, in marked contrast to the large variation in wavelength in the αβ region.

There is relatively little change, and so far no obvious pattern, in the wavelengths of the other bands between 300 and 370 nm, though the intensities of the band at *ca.* 305 nm are significantly lower for dbc and the methyl and cyclo-

propyl complexes and higher for the ethyl and propyl complexes, *i.e.* for this small sample there is a positive correlation with changes in wavelength in the αβ region.

## DISCUSSION

The data of Table 1 show that, even when the range of ligands is restricted to alkyl ligands in which the ligand atom is a tetrahedral carbon atom attached only to other C or H atoms, the values of $pK_{obs.}$ can increase by as much as two units from Me to np, *etc.*; in other words, the equilibrium constant for the co-ordination of unprotonated dbzm can decrease by as much as two logarithmic units. It will be shown in the following paper [12] that the increase in $pK_{obs.}$ is accompanied by a decrease in the proportion of the ' base-on ' (A) to ' base-off ' (C + $C^{dbzm}$) forms in neutral solution (though the ' base-on ' form is still observed with the highest $pK$ values) and that the highest values of $pK_{obs.}$ are consistent with the value of $pK = 5.0$, which has recently been obtained [18] for the pendant dbzm in dicyanocobalamin by a kinetic method. Table 1 shows that $pK_{obs.}$ rises with increasing substitution on either $C_\alpha$ (Me < Et < $Pr^i$) or $C_\beta$ (Et ~ Pr < $Bu^i$ < np) or with increasing size of the alicyclic ring ($C_3 < C_4 < C_5 \sim C_6$). The combined ligand order is given in Table 3.

Pailes and Hogenkamp [19] reported that methylcobinamide binds imidazole, $NH_3$, and $CN^-$ with equilibrium constants of 11, 0.1, and 230 $dm^3\ mol^{-1}$ respectively, while the equilibrium constant for the co-ordination of imidazole by propylcobinamide is only 0.1 $dm^3\ mol^{-1}$. Brodie [9] noted that methylcobinamide, but not cyclohexylcobinamide, will co-ordinate $CN^-$, $NH_3$, $NMeH_2$, $NEtH_2$, and pyridine; although he gave no quantitative data, it is obvious that replacing Me with $C_6$ leads to a large decrease in the equilibrium constants. We have obtained a maximum value of $<0.03$ $dm^3\ mol^{-1}$ for the equilibrium constant for the co-ordination of imidazole by neopentylcobinamide, *i.e.* the binding constants ($\log_{10}K$) for the co-ordination of imidazole by organocobinamides fall in the order: Me (+1.0) > Pr (−1.0) > np (< −1.5). It is clear that the decrease in the equilibrium constant for the co-ordination of unprotonated dbzm by the organocobalamin is accompanied by a decrease in the equilibrium constants for the co-ordination of various other ligands, including the related imidazole, by the corresponding organocobinamides; there is therefore nothing unique about dbzm as a ligand.

It is known that the magnitude of $pK_{obs.}$ is very sensitive to the nature of the axial ligand and that for a wide range of ligands (where the donor atom may be C, N, O, S, Cl, or I) $pK_{obs.}$ tends to rise as the σ-donor power of the ligand increases.[5] The following data compare the effect of various alkyl ligands (R) on $pK_{obs.}$ of the cobalamins (data from ref. 5 and Table 1) and on $pK_a$ of the analogous substituted acetic acids $RCH_2COOH$ (values from ref. 20 except for ethinyl [21]).

| R | N≡C– | HC≡C– | $CH_2$=CH– | Me | Et | $Pr^i$ |
|---|---|---|---|---|---|---|
| $pK_a$ of $RCH_2COOH$ | +2.47 | 3.32 | 4.35 | 4.87 | 4.82 | 4.78 |
| $pK_{obs.}$ of cobalamin | +0.1 | 0.7 | 2.4 (1.9) | 2.6 | 3.9 | ⩾4.5 |
| Difference | 2.4 | 2.6 | 2.0 (2.5) | 2.3 | 0.9 | ⩽0.3 |

Direct comparisons cannot be made throughout the whole series of cobalamins because of the varying nature of the equilibria involved. For the cyanide and probably the ethinyl complexes $pK_{obs.}$ represents a simple equilibrium between the six-co-ordinate ' base-on ' (A) and protonated ' base-off ' (D) forms; but more complex equilibria are involved in the other cases.[5,7,12] It is clear, however, that there is a correlation between the cobalamins and substituted acetic acids up to at least vinyl; correction for the formation of 70% of the five-co-ordinate E in acid [7] yields $pK = 1.9$ (added above in parentheses) for the equilibrium between the six-co-ordinate A and D forms of vinylcobalamin, which gives an even better correlation with the acetic acids. The positive correlation is evidence that for these C⁻ ligands the electronic (*i.e.* inductive) effect of R plays the major role in determining the value of $pK_{obs.}$. There is no such correlation beyond Me; substitution of Me by other saturated alkyl and cycloalkyl groups can change $pK_{obs.}$ by ⩾2 in the cobalamins but $pK_a$ in substituted acetic acids by only ⩽0.1.[20,22,23] We conclude that this breakdown in correlation between the cobalamins and acetic acids provides presumptive evidence for the increasing importance of steric relative to electronic effects beyond Me. Further support is provided by a more detailed analysis of the ligand order between Me and $Pr^i$.

The experimentally observed orders of organo-ligands, which are summarised in Table 3, show two striking

TABLE 3

Experimentally observed orders of organo-ligands

| (*a*) | Observable parameter | Observed order of ligands |
|---|---|---|
| | I $pK_{obs.}$ | $Me \leqslant C_3 < R_{dbc} < Et \sim Pr < C_4 \sim Bu^i < Pr^i \sim np \sim C_5 \sim C_6$ |
| | II Spectra of E complexes in αβ region | $Me \sim R_{dbc} < C_3 < Pr \leqslant Et < Bu^i < C_4 < Pr^i \sim np\ (?C_5) \leqslant C_6$ |
| | III Spectra of E complexes in γ region | $Me \sim C_3 < R_{dbc} < C_4 < Et \sim Pr \leqslant Pr^i \sim C_5 < C_6 \sim Bu^i < np$ |
| | IV Spectra of A complexes in αβ region | $C_3 \sim R_{dbc} \leqslant Me < Pr \leqslant Et$ |
| (*b*) | Variable parameter of ligand structure | Observed order of ligands (in series I and II above) |
| | Substitution on $C_\alpha$ | $Me < Et < Pr^i$ |
| | Substitution on $C_\beta$ | $Et \sim Pr < Bu^i < np$ |
| | Size of alicyclic ring | $C_3 < C_4 < C_5 \sim C_6$ |
| | | $R_{dbc}$ |

features. Firstly, the data of part (*a*) show that there is a remarkable parallel between the ligand order obtained from the effect of the ligand on $pK_{obs.}$ (series I) and on the lowest energy bands in the spectrum of the yellow ' base-off ' (E) complexes (series II); even the complex ligand present in dbc occupies virtually the same position in all the ligand orders. The more limited data available (series IV) suggest that the same order also occurs in the six-co-ordinate ' base-on ' (A) forms. If the same order is observed in both the five-co-ordinate (E) and six-co-ordinate (A) complexes as is obtained from the difference in energy between these complexes (as represented by $pK_{obs.}$) and if we assume that this order depends on the degree of steric compression around $C_\alpha$, then this implies that the steric compression must be increased proportionately for all ligands on changing from five- to six-co-ordination. Since the Co atom is probably displaced out of the mean plane and towards $C_\alpha$ in the five-co-ordinate complexes and coplanar in the six-co-ordinate complexes,[12] the accompanying decrease in $C_\alpha$–Co–$N_{eq}$ provides a natural explanation for the proportionate increase in steric compression around $C_\alpha$ on going from the E to the A forms. This, in turn, provides additional support for the predominance of steric over electronic effects in the ligands being studied. Grate and Schrauzer [14] have discussed the effect of large alkyl ligands in raising $pK_{obs.}$ in terms of the known steric interaction between the co-ordinated dbzm and the C(5)–C(6) portion of the corrin ring, and have concluded that ' large alkyl substituents attached to cobalt should bend the corrin ring downwards, resulting in cleavage of the co-ordinative bond between the axial base and the corrin cobalt atom.' We merely note that such an explanation is at best inadequate, since it cannot apply to other ligands such as $NH_3$ and imidazole and cannot explain the observed effect of the alkyl ligands on the spectra of the ' base-off ' (E) complexes in acid.

Secondly, it is remarkable that all the ligands in series II can be placed in a single order dependent on one main parameter (the ratio of the intensities of the bands at *ca.* 440 and *ca.* 460 nm) without the introduction of any obvious anomalies, despite the fact that the αβ absorption bands could in principle exhibit a very complex pattern of variation in wavelengths and intensities corresponding to several independently variable components of the interaction of the different organo-ligands with cobalt and the corrin ring. The higher-energy transitions of the γ band do in fact show a slightly different order and seem to be more sensitive to substitution on $C_\beta$ (see series III). Our results therefore show that changes in the structure of the ligands are seen mainly as changes in a single variable by both the *cis* (corrin) and *trans* (dbzm) ligands, and hence presumably also by the Co atom. The data of Table 3(*b*) show that this variable, whatever it is, clearly distinguishes Et from Me, $R_{dbc}$, $Bu^i$, and np, yet cannot readily distinguish between Me, $C_3$, and $R_{dbc}$, between $Bu^i$ and $C_4$, or between $Pr^i$, np, $C_5$, and $C_6$. The results of *X*-ray diffraction provide a clue as to the nature of this variable.

The Co–$C_\alpha$–$C_\beta$ bond angle of 125°, which is observed in dbc,[13] provides direct evidence for severe strain around $C_\alpha$. Since $R_{dbc}$ and Me occupy very similar positions in the ligand order (Table 3), this strongly suggests that there must be considerable strain around $C_\alpha$ even in the case of Me. It is, therefore, not surprising that the Co atom can so clearly ' see ' the difference between Me and Et and that the effects of the ligands can be critically dependent on relatively small changes in the electron density around $C_\alpha$; the difference between Et and $R_{dbc}$, for example, can probably be ascribed to the electron-withdrawing effect of the O atom at $C_\beta$, which in turn reduces the repulsions around $C_\alpha$. Equally interesting is the fact that, even though the ' tetrahedral ' Co–$C_\alpha$–$C_\beta$ bond angle in dbc is 125°, the $C_\alpha$–Co–$N_{eq}$ bond angles remain relatively undistorted (*e.g.* 89 and 93°) and that, while the co-ordinated dbzm is tilted with Co–$N_{dbzm}$–C bond angles of 123 and 132° [due to contact between the H atom on C'(4) of dbzm and the C(5)–C(6) segment of the corrin ring], the $N_{eq}$–Co–$N_{dbzm}$ bond angles remain relatively undistorted (*e.g.* 88 and 92°); [13] a similar tilt without significant change in $N_{eq}$–Co–$N_{dbzm}$ is found in all other cobalamins. This provides direct evidence that steric compression around the donor atom (X) of the co-ordinated axial ligand cannot be relieved to any significant extent by distortion of the X–Co–$N_{eq}$ bond angles; this is not surprising in view of the high electron density around the Co–X axis provided by the lone pairs on the Co and the equatorial N atoms. It is therefore reasonable to assume that any partial relief of steric compression will most probably be achieved by slight changes in the Co–X bond length; these may well be too small to be detected by *X*-ray analysis. We therefore suggest that the main ' variable ', which determines the position of the organo-ligand in the experimentally observed orders, is the Co–C bond length, which increases from Me to $Pr^i$.* Although $B_{12s}$ apparently reacts with t-butyl halides, neither we nor others [14] have been able to detect any t-butylcobalamin; $Bu^t$ would presumably occupy a position in the ligand order even beyond that of $Pr^i$. A change in the Co–C bond length will, of course, be accompanied by a change in the degree of hybridisation of the 3*d* with the 4*s* and 4*p* orbitals and in the electron density distribution in the partially covalent Co–C bond. This means that, although changes in the Co–C bond length may be caused primarily by steric effects (within the ligand or between the ligand and the corrin ring with its substituents), they will be transmitted to the Co atom and hence to the other ligands primarily as an electronic effect.

Our experimental results therefore provide direct evidence that there is a good parallel between the effects of the organo-ligands (R) on the spectra and on $pK_{obs.}$ and that there is no fundamental difference between primary and secondary alkyl ligands or between substitution on

* *Note added at proof:*—It has recently been shown by *X*-ray analysis that replacing Me by $Pr^i$ in the ' cobaloximes ' [CoR-$(dmg)_2(py)$] increases the Co–C bond length from 1.998 to 2.085 Å; see L. G. Marzilli, P. J. Toscano, L. Randaccio, N. Bresciani-Pahor, and M. Calligaris, *J. Amer. Chem. Soc.*, 1979, **101**, 6754.

$C_\alpha$ and $C_\beta$ (*i.e.* between the effects of decreasing and increasing Co–$C_\alpha$–$C_\beta$ respectively). Evidence for the predominance of steric over electronic effects in the series of ligands studied is provided by a comparison of the effects of R on the p$K$ values of cobalamins and of substituted organic acids, and also by the fact that the assumption of steric compression around $C_\alpha$ leads naturally to an explanation of the effects of R on both spectra and p$K_{obs.}$. Our experimental results can be combined with $X$-ray evidence to build up a simple and self-consistent picture of the steric effects of organo-ligands, in which the main features appear to be the following. (i) The main focus of steric compression is $C_\alpha$ due to repulsions between the bonding electron pairs on $C_\alpha$ on the one hand and the lone pairs on Co and the equatorial N atoms on the other. (ii) There is compression around $C_\alpha$ even in the case of Me. (iii) The compression is greater in the six-co-ordinate ' base-on ' complexes (A), but still detectable in the five-co-ordinate ' base-off ' complexes (E) where the Co atom is probably displaced out of the equatorial plane. (iv) The main variable, as ' seen ' by Co and the other ligands, is the Co–C bond length and the associated degree of hybridisation of the 3*d* with the 4*s* and 4*p* orbitals (*i.e.* an ' electronic ' effect), whatever the ultimate steric origin of the change in this bond length.

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