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THE EFFECT OF CHEMICAL MODIFICATIONS OF COBAMIDE CO-ENZYME ON THE RATE OF SUBSTITUTION OF *trans*-AXIAL LIGANDS

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SUMMARY

The effect of aglycone substitution in the 5'-desoxynucleoside part of cobamide co-enzyme**, modifications in the sugar part of desoxynucleoside ligand, the effect of desoxyglycosyl ligands and modifications in the "lower" axial position on the stability of Co–C and Co–N_{Bz} bonds in the cyanide cleavage reactions have been studied. Cyanide reactions involving octahedral derivatives of cobamide co-enzymes proceed via two steps: a fast step (k_1) corresponding to the substitution of the "lower" 5,6-dimethylbenzimidazole ligand, and a slow step (k_2) corresponding to the simultaneous cleavage of the Co–C and N–glycoside bonds. Other mechanisms are involved in cyanide reactions with analogues modified at the "lower" axial ligand which presumably possess a similar structure to penta-coordinated complexes.

INTRODUCTION

The properties of the organometallic σ Co–C bond in cobamide co-enzyme have recently been the subject of many studies in view of the discovery that a large number of biochemical processes occur in the presence of cobamide-dependent enzymes. Theories relating to the function of cobamide co-enzyme in enzymatic reactions have been based on the assumption that either a homolytic[1–3] or a heterolytic[4–6] cleavage of the Co–C bond occurs during the course of biological reactions. Homolytic cleavage of the cobalt–carbon bond should lead to cobalt(II) cobalamin, whereas heterolytic cleavage should lead to cobalt(III) or cobalt(I) cobalamin. As we have shown earlier[7], the cyanation reaction may provide a convenient model for heterolytic cleavage of the Co–C bond, thus enabling the quantitative estimation of *cis* and *trans* effects in cobalamins. Johnson and Shaw[8] have found that the cyanide reaction of DBC in the absence of light gives dicyanocobalamin, adenine and a sugar cyanhydrine which may be identified as the cyanhydrine of *D-erythro*-2,3-dihydroxypenten-4-al. Thus both *trans*-axial ligands were substituted. As far as the mechanism

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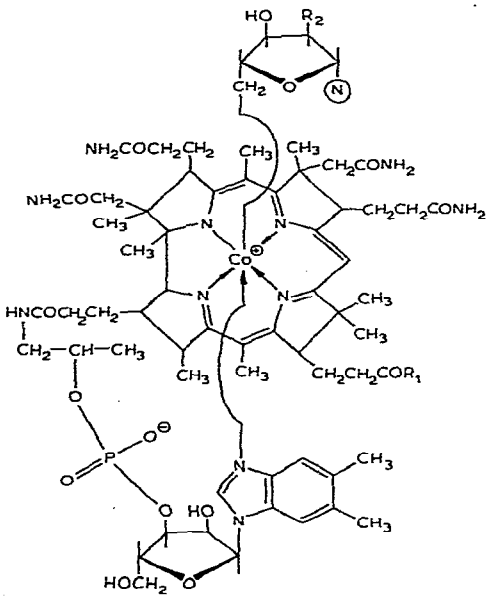
** Notation: cobamide co-enzyme [α-(5,6-dimethylbenzimidazolyl)-Co-5'-desoxyadenosylcobamide, DBC].

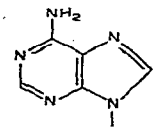
of this reaction is concerned, it has been assumed that it is similar to the alkaline hydrolysis of sulphonium nucleosides[9]. However, to date no detailed study of this reaction mechanism has been made.

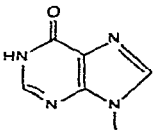
RESULTS

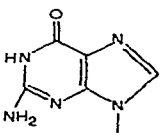
In the present work we have studied the effect of aglycone substitution in the

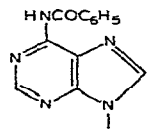
SCHEME 1

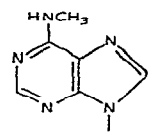


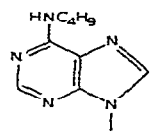
(I) ; (N) = 

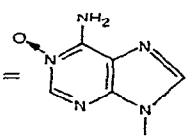
(II) ; (N) = 

(III) ; (N) = 

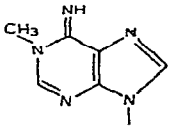
(IV) ; (N) = 

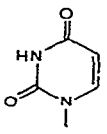
(V) ; (N) = 

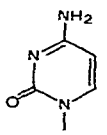
(VI) ; (N) = 

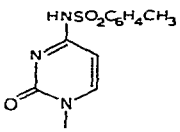
(VII) ; (N) = 

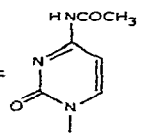
[In (I)–(XII) : R_2=OH ; R_1= NH_2 and in (XIII) and (XIV) : R_2=H ; R_1=NH_2]

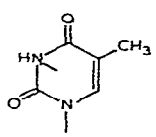
(VIII) ; (N) = 

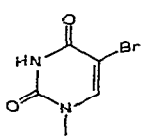
(IX) ; (N) = 

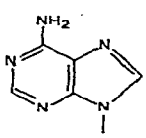
(X) ; (N) = 

(XI) ; (N) = 

(XII) ; (N) = 

(XIII) ; (N) = 

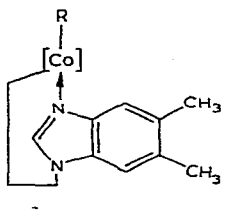
(XIV) ; (N) = 

(XV) ; (N) = 

(R_1= OH ; R_2 = OH)

5′-desoxynucleoside part of the DBC molecule, modifications in the sugar part of the desoxynucleoside ligand, the effect of desoxyglycosyl ligands and modifications in the "e" position of the corrine ring in the "lower" axial ligand on the stability of σ Co–C and Co–N_{Bz} bonds in the cyanide reaction. The structures of the compounds investigated are shown in Schemes 1 and 2. We have investigated in detail the kinetics of the cyanation reaction of cobamide co-enzyme and its various analogues by spectroscopy. The cyanide cleavage was carried out in the dark in a 0.1 *M* sodium bicarbonate buffer (pH 10.5) using a 0.1 *M* solution of potassium cyanide at 25°. In Fig. 1(a)

SCHEME 2



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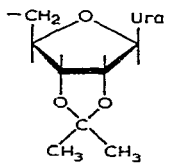

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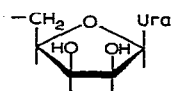

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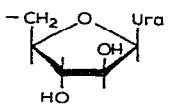

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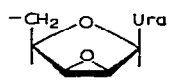

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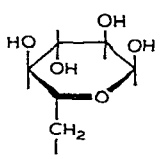

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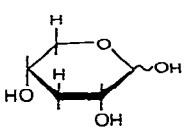

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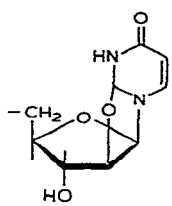

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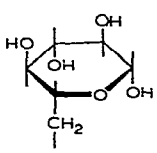
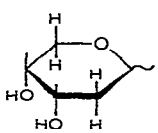
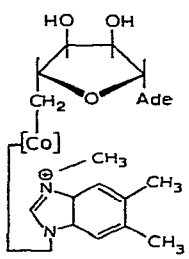


(XXIV) R =

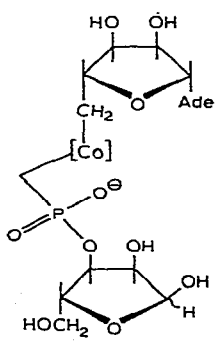


(XXV) R = CH_3

(XXVI)



(XXVII)



the spectra of DBC taken at various time intervals during the cyanation reaction are shown. It may be seen that both the spectra obtained after 3 min of the start of the reaction and subsequently exhibit three isosbestic points at 348, 391 and 535 nm. The presence of such isosbestic points demonstrates the formation of an intermediate product during the reaction which is converted relatively slowly to the final compound. The kinetics of the faster first step in the cyanation of cobamide co-enzyme may be readily followed from the rate of appearance of the isosbestic points. The kinetic curve for the reaction of DBC in terms of the isosbestic point at 348 nm is given in Fig. 1.(b) on a semilogarithmic scale. The linear plot obtained indicates that the first step is first-order with a rate constant (k_1) equal to 2.9×10^{-2} s^{-1}. A similar value for the rate constant k_1 has been obtained by following the appearance of the isosbestic point at 535 nm.

The second, slower step in the reaction also obeys first-order kinetics as is evident from the linearity of plot of log ($D_\infty - D$) vs. time at times greater than 3 min [Fig. 1c)]. The rate constant for this first-order reaction as estimated from the slope of this plot is equal to 8.4×10^{-4} s^{-1} and coincides with the rate constants computed

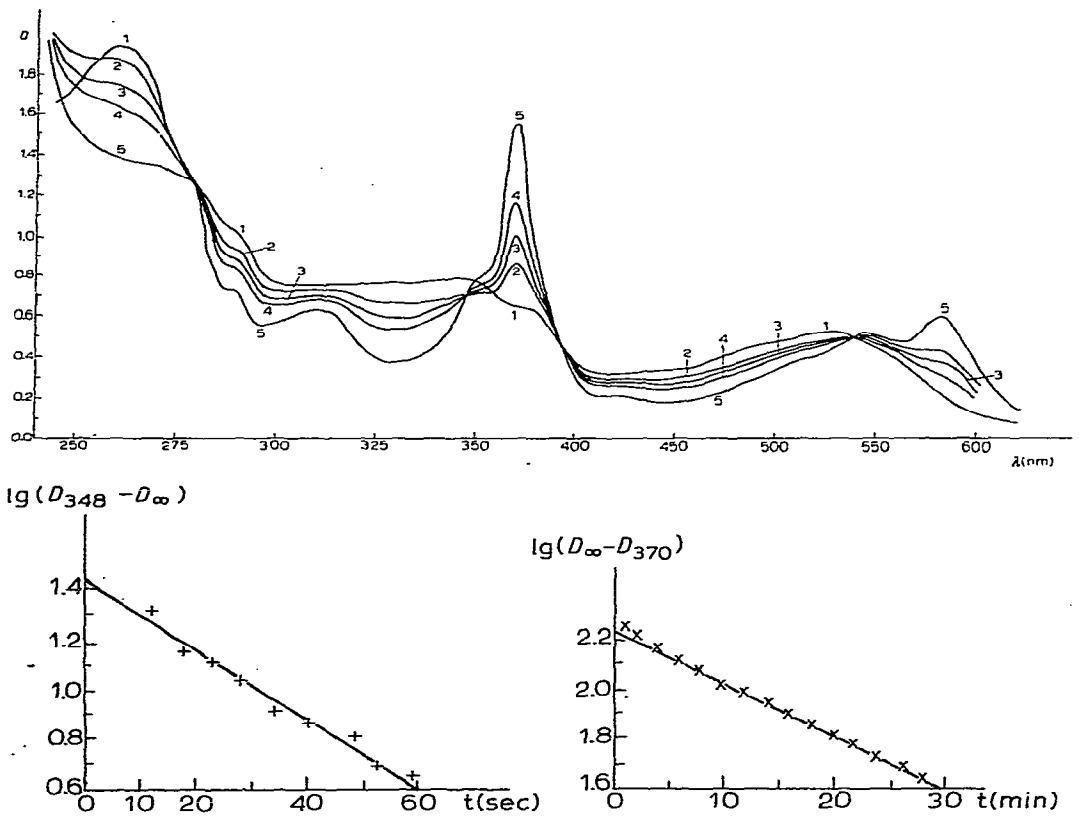


Fig. 1. The kinetics of the cyanide reaction of DBC co-enzyme; 0.1 *M* KCN; 0.1 M sodium bicarbonate buffer pH 10.5; 5×10^{-5} *M* DBC co-enzyme. (a) Spectra of the reaction mixture taken at the following time intervals (min): 0–1, 2–3, 3–9, 4–16, 5–∞. (b) Kinetic plot of log ($D_{348} - D_\infty$) *vs. t* at λ 348 nm. (c) Kinetic plot of log ($D_\infty - D_{370}$) vs. t at λ 370 nm. (D_∞ in these two cases means the respective optical densities at $t = \infty$).

from the kinetic curves obtained at 260, 330 and 583 nm.

It should be noted that the above values of k_1 and k_2 were obtained using a potassium cyanide concentration (0.1 *M* KCN) much greater than that of the cobamide co-enzyme (30–50 μM). For this reason, both these rate constants should be regarded as the pseudo-first-order constants corresponding to second-order constants divided by the cyanide ion concentration.

The kinetics of the cyanide reactions of cobamide co-enzyme analogues also indicate that these reactions occur by a two-step mechanism. For 5′-desoxy[6-*exo*-*N*-benzoyladenosyl]cobalamin, methylcobalamin, 5′-desoxyuridylcobalamin and 5′-desoxycytidylcobalamin, the constants k_1 and k_2 were determined as described above for cobamide co-enzyme. When changes in the optical density at the isosbestic points corresponding to the first step were small, the "difference" method (see experimental section) was used to calculate the results. Thus, the results shown in Fig. 2

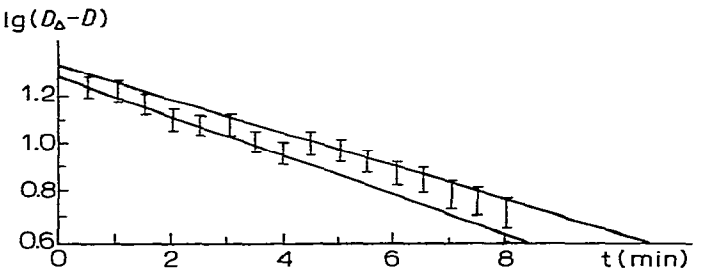


Fig. 2. The kinetics of the cyanide reaction of glucopyranosylcobalamin (α-anomer) using the same conditions as in Fig. 1. Concentration of the DBC co-enzyme analogue 1.5×10^{-5} M. Kinetic plot of log ($D_\Delta - D$) *vs.* t where D and D_Δ are the respective optical densities at t and $(t+\Delta)$.

illustrate the calculation of the rate constant using the "difference" method for the α-anomer of glucopyranosylcobalamine (XXI). The rate constant computed from the slope of the linear plot of log (D_Δ–D) against t (where D and D_Δ are the optical densities at the time t and $(t+\Delta)$ respectively where $\Delta = 4$ min) is equal to 2.6×10^{-3} s^{-1}. The cyanation rate constants for purine and pyrimidine 5′-desoxynucleosylcobalamins (Scheme 1) are listed in Table 1.

It is interesting to note that the chemical modification of the amide group of the corrine macrocycle does not affect the rate of scission of the Co–C bond in the DBC analogue. The cyanation kinetics of "e"-carboxy-DBC (XV) are similar to those of the cyanide cleavage of the co-enzyme. The value obtained in this study for the rate constant (k_2) for the analogue (XV) is equal to 8.2×10^{-4} s^{-1}, which is close to the value of k_2 for the co-enzyme.

Table 2 gives the cyanation rate constants of 5′-desoxyuridylcobalamins modified in the sugar part of the 5′-desoxynucleoside ligand (Scheme 2). Analogues with the same aglycone structure were chosen. A study of these analogues enabled the effect of modifying the sugar part of the structure on the stability of the Co–C bond to be examined. From the data obtained it follows that substitution of ribose for 2,3-isopropylideneribose and lyxose in the 5′-desoxynucleoside ligand does not affect

TABLE 1

RATE CONSTANTS FOR THE PSEUDO-FIRST-ORDER CYANIDE REACTION OF PURINE AND PYRIMIDINE 5'-DESOXYNUCLEOSYLCOBALAMIN[a]

No.	*5'-Desoxynucleosylcobalamin*	*Rate constants for cyanide reaction*	
		$10^2 k_1$ (s^{-1})	$10^4 k_2$ (s^{-1})
(I)	5'-Desoxyadenosyl-	2.9 ±0.1	8.4 ±0.4
(II)	5'-Desoxyinosyl-	0.28±0.05	3.9 ±0.1
(III)	5'-Desoxyguanosyl-	0.7 ±0.1	2.0 ±0.1
(IV)	5'-Desoxy[6-*exo*-*N*-benzoyladenosyl]-	2.6 ±0.1	0.44±0.02
(V)	5'-Desoxy[6-*exo*-*N*-methyladenosyl]-	1.0 ±0.1	7.5 ±0.3
(VI)	5'-Desoxy[6-*exo*-*N*-butyladenosyl]-	0.88±0.05	6.7 ±0.2
(VII)	1-*N*-Oxide 5'-desoxyadenosyl-	1.8 ±0.2	0.12±0.01
(VIII)	5'-Desoxy[1-*N*-methyladenosyl]-	0.37±0.02	8.0 ±0.1
(IX)	5'-Desoxyuridyl-	2.4 ±0.1	2.9 ±0.1
(X)	5'-Desoxycytidyl-	1.7 ±0.2	3.5 ±0.2
(XI)	5'-Desoxy[4-*exo*-*N*-tosylcytidyl]-	0.6 ±0.1	0.97±0.05
(XII)	5'-Desoxy[4-*exo*-*N*-acetylcytidyl]-	2.3±0.2	0.66±0.02
(XIII)	5'-Desoxytymidyl-	0.84±0.06	0.27±0.01
(XIV)	5'-Desoxy[5-bromouridyl]-	0.12±0.02	0.42±0.02

[a] Conditions: 0.1 M sodium bicarbonate buffer, pH 10.5, 0.1 M KCN, 25°

TABLE 2

RATE CONSTANTS FOR THE PSEUDO-FIRST-ORDER CYANIDE REACTION OF 5'-DESOXYURIDYLCOBALAMIN ANALOGUES MODIFIED IN THE SUGAR PART OF THE DESOXYNUCLEOSIDE LIGAND[a]

No.	*Desoxynucleoside ligand*	*Rate constants for cyanide reaction*	
		$10^2 k_1$ (s^{-1})	$10^4 k_2$ (s^{-1})
(IX)	5'-Desoxyuridyl-	2.4 ±0.1	2.9 ±0.1
(XVI)	5'-Desoxy-2',3'isopropylideneuridyl	1.1 ±0.1	3.0 ±0.2
(XVIII)	5'-Desoxy-1 (β-D-lyxofuranosyl)uracylyl-	0.41±0.02	3.2 ±0.1
(XIX)	5'-Desoxy-1(2',3'-epoxy-β-D-lyxosyl)uracylyl-	0.24±0.01	1.3 ±0.2
(XX)	5'-Desoxy[2,2'-anhydro-1-(β-D-arabinosyl)uracylyl]-	1.3 ±0.1	1.4 ±0.1
(XXVII)	5'-Desoxy-1(β-D-arabinofuranosyl)uracylyl-	0.25±0.01	0.69±0.02

[a] Conditions: as for Table 1.

the stability of the Co–C bond and partially stabilizes the Co–N_{Bz} bond in the corresponding analogues. On the other hand, replacement of arabinose by ribose causes stabilization of the Co–C and Co–N_{Bz} bonds (by 4- and 10-fold, respectively). The stability of the Co–C bond in the epoxy-(XIX) and anhydro-(XX) analogues is two times greater than that in 5'-desoxyuridylcobalamin.

It seemed of interest to investigate the behaviour of the various co-enzyme analogues minus their respective purine and pyrimidine fragments. In methylcobalamin (XXV), the Co–C bond is stable to attack by cyanide in the absence of light whilst under the same conditions cleavage of the Co–N_{Bz} bond occurs. The value of the rate constant k_1 measured for this cobalamin at 348 nm was equal to 2.8×10^{-2} s^{-1}

which is close to that for DBC (I) (Table 1). A study of cyanation kinetics of apurine analogues of DBC [(XVI)-(XIX)] showed that the Co–C bond in these compounds is stable to attack by cyanide and that in this respect they are similar to the simplest alkylcobalamins. The stability of the Co–N_{Bz} bond in apurine analogues of cobamide co-enzyme (Table 3) is an order of magnitude greater than that of DBC and methylcobalamin. The kinetic data obtained demonstrate the important role played by aglycone in determining the high lability of the Co–C bond in the co-enzyme.

TABLE 3

RATE CONSTANTS FOR THE PSEUDO-FIRST-ORDER CYANIDE REACTION OF METHYLCOBALAMIN AND Co–C-GLYCOSYL ANALOGUES OF COBAMIDE CO-ENZYME[a]

No.	*Cobalamin*	*Rate constants for cyanide reaction*	
		$10^2 k_1$ (s^{-1})	$10^4 k_2$ (s^{-1})
(XXI)	6-Desoxy-α-glucopyranosylcobalamin	0.26 ± 0.02	0.065 ± 0.005
(XXII)	6-Desoxy-β-glucopyranosylcobalamin	0.18 ± 0.03	0.11 ± 0.1
(XXIII)	3-Desoxyxylopyranosylcobalamin	0.42 ± 0.03	0.034 ± 0.002
(XXIV)	1-Desoxy(2-desoxyribopyranosyl)cobalamin	0.31 ± 0.02	0.031 ± 0.003
(XXV)	Methylcobalamin	2.8 ± 0.3	

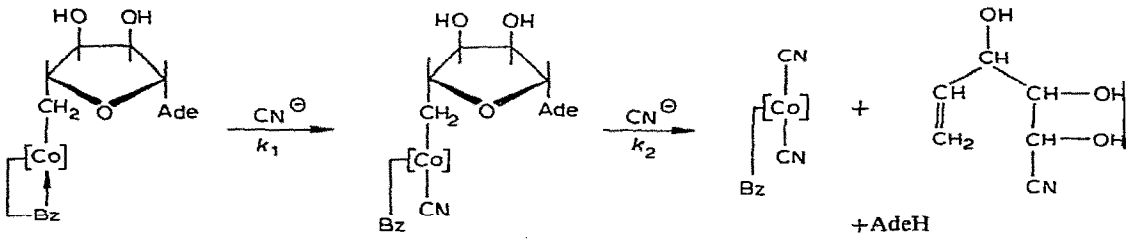
[a] Conditions: as for Tables 1 and 2.

Studies of the effect of the ligand 5,6-dimethylbenzimidazole on the rate of cleavage of the *trans*-Co–C bond have been undertaken for the two co-enzyme analogues (XXVI) and (XXVII) in which Co–N coordination does not occur. The rate of cleavage of the Co–C bond in these corrinoids is considerably different from that of DBC. The spectra of compounds (XXVI) and (XXVII) obtained during the course of the cyanide reaction exhibit three isobestic points at 348, 382 and 492 nm, respectively, the two latter points differing from those obtained for DBC. A plot of log $(D_\infty - D)$ against t indicates the occurrence of two different processes in these reactions. It is interesting that in these cases even after an interval of 3 min the spectra of the resulting mixtures are similar to that of dicyanocobalamin. The reaction rate constants (k_1) estimated from the slope of the plot of log $(D_\Delta - D)$ against t are also the same as those for the analogues (XXVI) and (XXVII) and are equal to 1.3×10^{-2} s^{-1}. The values of k_2 found in such cases are 500 times greater than the k_2 value for cobamide co-enzyme whose neutral solution contains 90% of the 6-coordinated octahedral form. The calculated rate constants for the slower reaction process in the corrinoids (XXVI) and (XXVII) are close to the k_2 value for cobamide co-enzyme suggesting the presence of small amounts of the equilibrium octahedral forms of cobalamin in the analogues (XXVI) and (XXVII) which cleave on reaction with cyanide ions via the standard two-step mechanism.

DISCUSSION

The results obtained indicate that the cyanide reaction of DBC involves two steps, the fast step (k_1) probably corresponding to a substitution of the "lower"

5,6-dimethylbenzimidazole ligand while the slow step (k_2) corresponds to a simultaneous cleavage of the Co–C and N–glycoside bonds.



Schrauzer *et al.*[10] showed in 1970 that in the presence of excess potassium cyanide the reaction of cobamide co-enzyme is first order with a rate constant of 8.3×10^{-3} s^{-1} when the concentration of potassium cyanide was 0.01 *M* (27°, 525 nm). It should be noted that the k_1 values obtained in the present study are close to the values of the pseudo-first-order rate constants of Schrauzer, and from this it follows that Schrauzer's constant *k* corresponds to the more rapid substitution of the 5,6-dimethylbenzimidazole ligand rather than to cleavage of the Co–C bond.

From the data obtained it follows that substitution of adenine in the desoxynucleoside ligand of DBC in place of hypoxanthine and guanine leads to a negligible increase in the stability of the Co–C bond relative to the stability of this bond in cobamide co-enzyme. A simultaneous increase in the stability of the Co–N_{Bz} bond is also observed in these analogues. Introduction of electron-releasing substituents into the 6-*exo*-amino group in the 1-*N* position of the purine nucleus does not change the stability of the Co–C bond but does tend to increase the stability of the Co–N_{Bz} bonds in these analogues. On the other hand, introduction of the electron-attracting groups to the 6-*exo*-*N* and 1-*N* positions of adenine increases the stability of the Co–C bond but has little effect on the Co–N_{Bz} bond. A similar increase in stability of the Co–C bond has been observed with the introduction of electron-attracting groups (*p*-toluene sulfonyl and acetyl) into the *exo*-cyclic amino group of cytosine in 5′-desoxycytidylcoabalamin. In these analogues (as in the purines), the stability of the Co–N_{Bz} bond is hardly affected. Introduction of an electron-donating group (CH_3) or electron-attracting substituent (Br) into position 5 of uracyl considerably increases the stability of the Co–C bonds in 5′-desoxytymidylcobalamin (XIII) and 2′,5′-dideoxy-5-bromouridylcobalamin (XIV) and simultaneously increases the stability of the Co–N bond in these analogues.

Thus the observed cyanation rate constants of 6-coordinate purine and pyrimidine analogues of cobamide co-enzyme suggest that the rate of cleavage of Co–C and Co–N bonds by cyanide depends on the aglycone structure present in 5′-desoxynucleoside of the DBC co-enzyme analogues. It is important to note that the strength of the bonding between the central cobalt atom and the "lower" and "upper" axial ligands, which are spatially remote and not conjugated to the adenine nucleus, is sensitive to chemical modification of the heterocyclic base. Two possible mechanisms may be proposed to explain this apparent transmission effect; "π-interaction" between the corrine macrocycle and the adenine nucleus or the simultaneous cleavage of the Co–C and N–glycoside bonds in nucleosylcobalamins during the cyanation reaction. The first mechanism is apparently in accord with the known X-ray data[11], re-

garding the proximity of the adenine and corrine cycles in the cobamide co-enzyme. According to this mechanism, changes in the rate of substitution of *trans*-axial ligands may be interpreted in terms of changing conformational and electronic effects. The conformation change ("bending") of the corrine macrocycle may be detected by means of circular dichroism (CD) spectra. These spectra are quite sensitive to both *trans*-axial ligand substitution[12] and inversion at only one asymmetric atom of the corrine macrocycle, *e.g.* to C-13 inversion[13]. A study of the CD spectra of cobamide co-enzyme analogues has shown that such spectra are more sensitive to modification in the macrocycle than are the corresponding absorption spectra.

It is interesting that in all the co-enzyme analogues studied the short-wave region (220–300 nm) of the CD spectrum is the most sensitive to chemical modifications. This is most apparent in the CD spectra of apurine DBC analogues [(XXI)-(XXIV)] which contain no adenine chromophore active in this part of the spectrum. It was found that the CD spectra of these analogues are very similar to the corresponding spectra for DBC in the 300–600 nm region of the spectrum. The short-wave region of the spectrum, however, had a narrow negative band ($[\Delta\varepsilon]_{260\ nm}$ −8.0) and a positive maximum ($[\Delta\varepsilon]_{235\ nm}$ +10), differences which could be attributed to conformational changes in the molecule on removal of aglycone from the "upper" axial ligand.

The CD spectra of the purine and pyrimidine analogues in which the aglycone part of the desoxynucleoside ligand was modified were not very different from the spectrum of the co-enzyme over the range 300–600 nm indicating that in these cases no noticeable conformation changes occurred upon substitution in the molecule.

Considerable differences were observed in the CD spectra of analogues of 5'-desoxyuridylcobalamin modified in the sugar part of the molecule. Thus, the CD spectrum of the epoxy analogue (XIX) exhibits a large negative maximum ($[\Delta\varepsilon]_{252\ nm}$ − 10.1) while the anhydro analogue (XX) has in addition an intense negative band in the UV part of CD spectrum for which $[\Delta\varepsilon]_{265nm}$ is −17.0. The observed differences between the CD spectra of (XIX) and (XX) and that of 5'-desoxyuridylcobalamin (IX) are probably due to conformation changes in the corrine chromophore when the carbohydrate residue of the axial ligand is modified.

On the basis of this viewpoint, it is interesting that when a change in a given conformation obviously distorts an assumed "π-interaction" between the heterocyclic aglycone and corrine macrocycle, *e.g.* in cobalamin (XX), no essential difference is observed in the rate constants for the cyanide reaction which is attributable to a change in the Co–C bond stability. This observation suggests that simultaneous cleavage of the N–glycoside bond and elimination of the "upper" axial ligand is a preferable mechanism.

A decisive factor in slowing down the cleavage of the Co–C bond is the electronegativity at *N*-9 and *N*-3 in glucoside purine and pyrimidine bases respectively. We have shown earlier[7] that a good linear relationship exists between the magnitude of the positive charge on the glucoside nitrogen of ribonucleotide and the rate constant k_2 for cleavage of the organometallic bond in the presence of cyanide. In such cases the rate observed on substituting both axial ligands in the co-enzyme is the maximum value and this does not change on introducing electron-donating alkyl groups into the adenine nucleus. However, the rate changes considerably if electron-attractive groups are introduced into the purine nucleus. The reported kinetic param-

eters for the acidic hydrolysis of nucleosides confirm the fact that alkyl substituents have little effect on the rate of N–glucoside bond cleavage. Hence the stability of the glucoside bond in 1-*N*-methyladenosine and 6-*exo*-methyladenosine is virtually the same as that in adenosine[14–17].

The kinetic data obtained for the cyanide reaction of corrinoids (XXVI) and (XXVII) which contain no Co–N_{Bz} bonds indicate that a change occurs in the two-step mechanism observed for octahedral derivatives of DBC. These data may be interpreted on the basis of the assumption mentioned above[18] that a change in the geometry of the cobalt atom occurs in organocobalamins which do not contain the strong 5,6-dimethylbenzimidazole ligand. A 5-coordinate cobalt atom in such complexes may be forced out of the plane of the corrine macrocycle, producing a square-pyramid arrangement and facilitating nucleophilic attack by the cyanide ion. Such an assumption may be confirmed by an analysis of CD spectral curves. The CD spectrum of the analogue (XXVI) is very different from that of the co-enzyme in that it has a very strong maximum at $[\Delta\varepsilon]_{332\ nm}+21.4$ [DBC has two small positive maxima in the UV region at $[\Delta\varepsilon]_{298\ nm}+4.8$ and $[\Delta\varepsilon]_{332\ nm}+0.8$].

Similar changes of the positive maximum at 332 nm have been observed in the CD spectrum of the co-enzyme at a pH value of 3.0 and at higher temperature (75°). They are probably due to a decrease in the coordination of the "lower" ligand.

Chemical investigations of analogues of DBC in which the coordinated "lower" ligand is absent have led to a clearer understanding of the mechanism of the activation of the Co–C bond in the co-enzyme during its combination with apo-enzyme in biocatalysis. Many of the purine and pyrimidine analogues of DBC which have been synthesized act as concurrent inhibitors of glyceroldehydratase[19] and this may also be an indication of the influence of chemical modifications on the reversible cleavage of Co–C bonds. These data confirm the specific role of the adenine fragment which determines both the chemical and biocatalytical properties of cobamide co-enzyme.

EXPERIMENTAL

Studies of the cleavage of cobamide co-enzyme and its analogues in the presence of the cyanide ion were performed under aerobic conditions in the absence of light. A sample (2.8 ml) of DBC solution or its analogue in 0.1 *M* sodium bicarbonate buffer (pH 10.5) was added to a 1 ml quartz cuvette and the reaction started by the addition of 0.2 ml of a 1.5 *M* solution of potassium cyanide at 25°. The concentration of DBC or its analogue was 30–50 μM. A spectrophotometric method was employed for studying the kinetic behaviour during the reaction. Optical densities were measured on a Pye Unicam SP 800 spectrophotometer using a SP 22 recorder. At low percentage conversions, the 0.1 optical density unit/20 cm scale was used, while at high percentage conversion the 1.0 optical density unit/20 cm scale was employed.

The CD spectra were measured on a Jasco ORD/UV/CD-5 instrument using 0.2 *M* potassium phosphate buffer solution (pH 8.0). The concentration of DBC and its analogues used for measuring was 25–60 μM, the cuvette path length being equal to 0.5–2 cm. The error in the measurements never exceeded 5% over the 350–600 nm region of the spectrum and 10% over 220–350 nm. The purine and pyrimidine analogues of 5′-desoxynucleoside DBC were prepared by alkylation of Vitamin B_{12s}

with the appropriate 2',3'-phenylboric esters of 5'-O-tosylnucleosides[20,21].

5'-Desoxyuridylcobalamin derivatives modified in the sugar part of an axial nucleoside ligand were synthesized by a general procedure[21] employing the respective 5'-O-mesyl- and tosyl-uridines as the alkylating agents.

Apurine 6-desoxyglucosyl DBC analogues were obtained from Vitamin B_{12s} and 6-bromo-6-desoxyglucose and tosyl ribose compounds[22].

DBC analogues modified at the "e" position of the corrine ring and at the "lower" axial ligand were prepared from the respective cobalt(I) derivatives and the 2',3'-phenylboric ester of 5'-O-tosyladenosine.

Computation of the rate constant by the "difference" method

The kinetic curve corresponding to the first step in the mechanism may be described by the following equation:

$$D = D_\infty - (D_\infty - D_0) \cdot e^{-k_1 \cdot t} \quad (1)$$

where D_0, D and D_∞ are the optical densities at $t=0$, $t=t$ and $t=\infty$ respectively. The presence of the second slower step complicates the determination of D_∞. When D_∞ is not known, the constant k_1 may be obtained by the "difference" method which is a modification of Guggenheim's procedure[23]. If two points with coordinates (D, t) and $(D_\Delta, t+\Delta)$ are chosen on the D–t curve, equation (1) is valid for the first point and the relationship $D_\Delta = D_\infty - (D_\infty - D_0) \cdot e^{-k_1(t+\Delta)}$ is valid for the second point. Eliminating D_∞, we obtain the following linear relationship for the determination of the rate constant:

$$\log (D_\Delta - D) = \log \{D_0 - D_\infty) \cdot (1 - e^{k_1 \cdot t})\} - k_1 t / 2.3$$

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