# Interaction of *cis*-Diamminediaquoplatinum(II) with Adenosylcobalamin and Alkylcobalamins

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The interaction of *cis*-diamminediaquoplatinum(II) nitrate with adenosylcobalamin and a series of alkylcobalamins was studied by carbon-13 nuclear magnetic resonance spectroscopy and by electronic spectroscopy. With these cobalamins *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ forms adducts in which N(3) of the 5,6-dimethylbenzimidazole moiety is co-ordinated to platinum(II) rather than to cobalt(III) of the corrin. The chemical shifts of the 5'-methylene carbon of adenosylcobalamin–platinum adduct and of the Co–methyl carbon of the methylcobalamin–platinum adduct are characteristic of these cobalamins in the ' base-off ' form. Furthermore, these cobalamin–platinum complexes have visible spectra identical to those of the cobalamins in acidic solution. The $^{13}C$ n.m.r. spectrum of the adenosylcobalamin–platinum complex suggests the presence of a second adduct in which platinum(II) complexes are co-ordinated to both the N(3) of the 5,6-dimethylbenzimidazole nucleotide and to N(7) of the 5'-deoxyadenosyl moiety. The rate of the alkylcobalamin–platinum(II) adduct formation is determined by the strength of the cobalt(III)–5,6-dimethylbenzimidazole co-ordinate bond. Thus, adenosylcobalamin reacts much faster than adenosylepicobalamin to form the cobalamin–platinum(II) adduct.

THE anti-tumor properties of *cis*-diamminedichloroplatinum(II) have been well documented and at present this platinum(II) complex alone or in combination with other drugs is used extensively in the treatment of gynecological tumors.[1,2] The treatment with *cis*-diamminedichloroplatinum(II) produces in some cases severe side effects such as hematological and renal toxicity and sometimes neurotoxicity. The peripheral neuropathy due to treatment with this platinum complex caught our attention because similar neurological symptoms are associated with vitamin $B_{12}$ deficiency.[3] Taylor and Hanna [4] and Agnes *et al.*[5,6] have demonstrated that methylcobalamin is dealkylated in the presence of platinum(II) and platinum(IV) salts, while more recently Yurkevich *et al.*[7] described complexes of methylcobalamin and adenosylcobalamin with tetrachloropalladate(II). These observations suggested that patients treated with platinum(II) complexes develop a vitamin $B_{12}$ deficiency because the interaction between the corrinoids and the chemotherapeutic agent causes inactivation of the corrinoid coenzymes. It has been shown that *cis*-diamminedichloroplatinum(II) and its hydrolysis product *cis*-diamminediaquoplatinum(II) react with acetamide [8] and purines and pyrimidines.[9] Yurkevich *et al.*[7] suggested that N(7) of the 5'-deoxyadenosyl moiety and N(3) of the 5,6-dimethylbenzimidazolyl ribonucleotide are the most probable co-ordination sites of palladium(II) in the adenosylcobalamin–palladium(II) complex. Thus, possible sites of interaction between the corrinoid coenzymes and the $Pt^{II}$ chemotherapeutic agents are the acetamide or propionamide side chains of the corrin ring, the 5,6-dimethylbenzimidazole nucleotide in the lower co-ordination position, and the 5'-deoxyadenosyl moiety in the upper co-ordination position.

In the present study we have investigated the interaction of *cis*-diamminediaquoplatinum(II) with adenosylcobalamin, methylcobalamin, and several alkylcobalamins.

## EXPERIMENTAL

*Materials.*—Hydroxycobalamin hydrochloride was purchased from Sigma Chemical Co. Other corrinoids were prepared from hydroxycobalamin by published procedures: alkylcobalamins,[10] adenosylcobalamin,[11] [$^{13}C$]methylcobalamin,[12] [5'-$^{13}C$]adenosylcobalamin,[13] and *cis*-diamminediaquoplatinum(II) nitrate.[14]

*Methods.*—Pulse Fourier-transform $^{13}C$ (25.2 MHz) n.m.r. spectra were obtained at 25 ± 0.5 °C using a Varian XL-100-15 spectrometer locked to the resonance (15.4 MHz) of the solvent $D_2O$ and interfaced to a Nova 1210 computer. The transients resulting from the application of 90° pulses in a spectral width of 2 000 Hz were accumulated at 4 096 points in the time domain and transformed into a 2 048 point spectrum in the frequency domain. The data acquisition time was 1 s and the spectra were obtained under conditions of simultaneous broad band (3 000 Hz) proton noise decoupling. Peak positions were determined by computer examination of the final Fourier-transformed spectrum and the chemical shifts were measured with respect to a tetramethylsilane external reference.

Absorption spectra were recorded with a Cary Model 15 spectrophotometer; other visible and u.v. spectral measurements were made with a Zeiss PMQ II spectrophotometer. The kinetics of the reactions of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with the cobalamins was determined using the following procedure. At room temperature, an aliquot of a concentrated solution of alkylcobalamin and 0.033 $cm^3$ of a 0.4 mol $dm^{-3}$ solution of diamminediaquoplatinum were added to a volume of 0.1 mol $dm^{-3}$ sodium acetate, pH 5.5, sufficient to make a solution of 1.5 $cm^3$ volume and containing a large excess of the platinum(II) complex relative to the cobalamin (Table 1). The initial absorbance ($A_0$) at the maximum of the α band (see below) for each alkylcobalamin was recorded, and the samples were incubated at 50 ± 0.5 or 80 ± 0.5 °C. At selected time intervals the absorbance ($A_t$) was again recorded. The rates of reaction of platinum with

the cobalamins were calculated from the percent unreacted cobalamin $(A_t - A_\infty)/(A_0 - A_\infty)$ per min, using as a final point ($A_\infty$) the absorbance at the $\lambda_{max.}$ after the addition of 0.02 mol $dm^{-3}$ HCl (which converted all the cobalamin to the base-off form). Rate constants ($k_{obs.}$, Table 2) were calculated from plots of log $[(A_t - A_\infty)/(A_0 - A_\infty)]$ against time which were linear over four half-lives. The constants reported are the average obtained from five independent measurements and the range represents the maximum and minimum values obtained.

## RESULTS AND DISCUSSION

The interaction between the organocorrinoids and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ was investigated by $^{13}C$ n.m.r. spectroscopy using methylcobalamin and adenosylcobalamin selectively enriched with carbon-13 and by visible spectroscopy using adenosylcobalamin and a series of alkylcobalamins.

*Carbon-13 N.M.R. Spectroscopy.*—Figure 1 illustrates the $^{13}C$ n.m.r. spectra in the region of the $Co-^{13}CH_3$ resonance for $D_2O$ solutions of [$^{13}$C]methylcobalamin in the absence and in the presence of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$. In the presence of the platinum complex an additional resonance (0.04 p.p.m.) is evident upfield from the 7.86 p.p.m. resonance characteristic of methylcobalamin in the 'base-on' configuration.[12] The slow rate of increase in the intensity of this resonance is reminiscent of slow substitution reactions of square-planar platinum(II) complexes.[15] Because its chemical shift (0.04 p.p.m.) is similar to those of methylcorrinoids lacking a co-ordinated 5,6-dimethylbenzimidazole nucleotide (dbzm) (*e.g.* [$^{13}$C]methylcobinamide, 0.3 p.p.m.; [$^{13}$C]methyltrimethylbenzimidazolecobamide, 0.32 p.p.m.; and [$^{13}$C]methylcobalamin at pH <1, 0.2 p.p.m.[16]), we assign it to the $^{13}C$-methyl resonance of [$^{13}$C]methylcobalamin in which the benzimidazole is detached from the cobalt and is co-ordinated to platinum(II) through N(3), perhaps as $(H_3N)_2(H_2O)Pt$–(dbzm)(corrin)Co. Support for this assignment of the upfield resonance and the rejection of an alternative based on the co-ordination of platinum(II) to the peripheral acetamides or propionamides [17] of the corrin ring, derive from the following considerations.

Firstly, in earlier studies,[16] it has been shown that modification of the amide substituents by lactam or lactone formation or by hydrolysis shifts the $^{13}CH_3$ resonance of [$^{13}$C]methylcobalamin by less than 2 p.p.m. Second, the linewidths of the $^{13}C$ resonances of $^{13}CH_3$ groups bound to $Co^{III}$ in cobalamins containing a nitrogen base in the lower co-ordination position of cobalt are much broader than in cobinamides and related structures where a water molecule occupies the sixth co-ordination position.

These differential linewidths for the base-on and base-off complexes are also apparent in Figure 1 and presumably reflect chemical-exchange effects or scalar relaxation of the second kind at the $^{13}C$-methyl ligand by the directly bonded cobalt, which has a quadrupole moment and a resonance frequency close to that of carbon-13.[18] The changes in $^{13}C$ linewidths and in spin-lattice relaxation times that accompany the substitution of a nitrogen by an oxygen donor are not inconsistent with this interpretation.

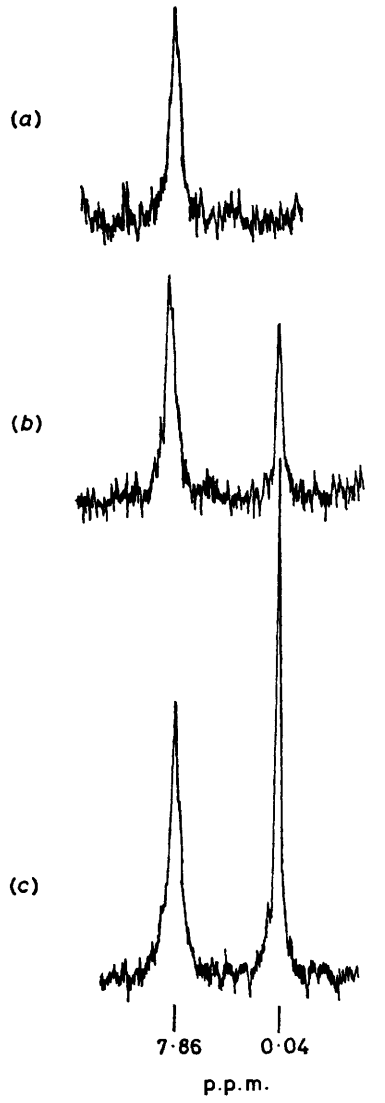
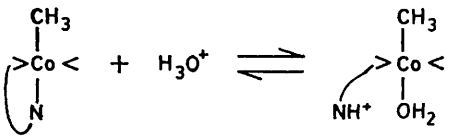



FIGURE 1 Proton noise decoupled $^{13}C$ n.m.r. spectra of [$^{13}$C]-methylcobalamin and *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$: (*a*) 16 mmol $dm^{-3}$ [$^{13}$C]methylcobalamin alone, 2 456 scans at 25 °C; (*b*) 16 mmol $dm^{-3}$ [$^{13}$C]methylcobalamin and 36 mmol $dm^{-3}$ *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, 2 425 scans at 25 °C, approximately 25 min subsequent to mixing the reagents; (*c*) solution (*b*), 5 975 scans at 25 °C after the solution had been incubated at 40 °C for 2.5 h

Finally, we note that base-on base-off equilibria in a wide variety of systems such as that shown below are




rapid on the $^{13}C$ n.m.r. time scale. It is apparent that the system described by Figure 1 is in the slow-exchange

limit. Thus, it is unlikely that co-ordination of the platinum(II) complex to one or two amide carbonyls would not only induce a displacement of the benzimidazole from the sixth co-ordination position of the cobalt atom but also reduce its rate of chemical exchange.

As shown in Figure 2, the interaction between [5′-$^{13}$C]adenosylcobalamin and the *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ ion is more complex. The reaction of the platinum(II) complex with the cobalamin results in the appearance of at least two additional resonances in the 19 p.p.m. region of the spectrum. This spectral region is characteristic of the carbon-13 chemical shift of the 5′-methylene carbon of adenosylcobalamin in the ' base-off ' form (19.8 p.p.m.).[13] In the context of the previous discussion, the narrow $^{13}$C resonance at 19.3 p.p.m. is assigned to the ' base-off ' complex: $[(H_3N)_2(H_2O)$-Pt(dbzm)(corrin)Co$]^{2+}$. The broad $^{13}$C-resonance centred at 18.0 p.p.m. may be a composite of two peaks and a reasonable assignment for one of these is a resonance for a complex in which both N(3) of the 5,6-dimethylbenzimidazole nucleotide and N(7) of the

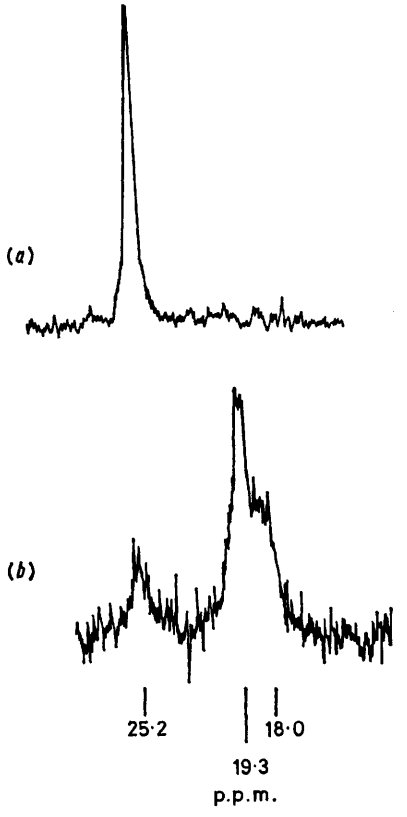



FIGURE 2 Proton noise decoupled $^{13}$C n.m.r. spectra of [5′-$^{13}$C]-adenosylcobalamin: (*a*) 15 mmol dm$^{-3}$ [5′-$^{13}$C]adenosylcobalamin, 2 456 scans; (*b*) 15 mmol dm$^{-3}$ [5′-$^{13}$C]adenosylcobalamin and 18 mmol dm$^{-3}$ *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$, pH 7, after incubation at 25 °C for 18 h, 4 313 scans

deoxyadenosyl ligand are co-ordinated to a $[Pt(NH_3)_2$-$(OH_2)]^{2+}$ moiety. The other resonance may involve a dimeric form of the latter complex as suggested by Yurkevich *et al.*[7] for the complex of adenosylcobalamin with lithium tetrachloropalladate(II). In this regard it is interesting to note that the $^{13}$C-methylene resonance of the parent ' base-on ' adenosylcobalamin at 25.2 p.p.m. is also severely broadened in the presence of the *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ ion. In like manner, this may be attributed to the formation of ' base-on ' complexes in which N(7) of the adenine moiety is co-ordinated to Pt$^{II}$.

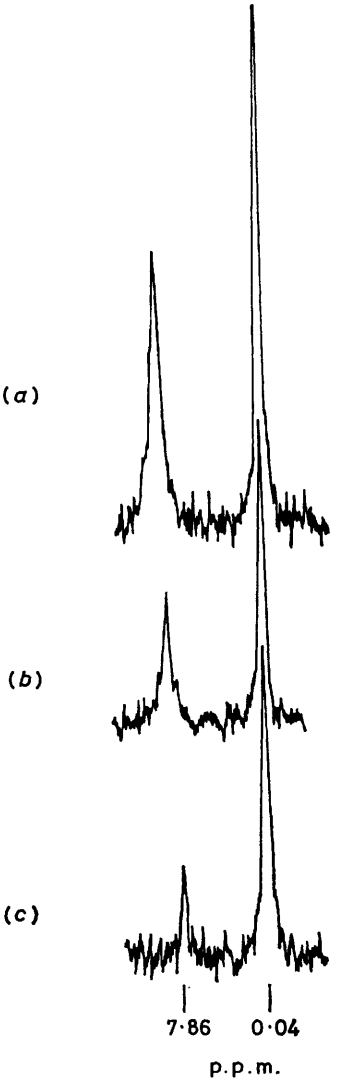



FIGURE 3 Proton noise decoupled $^{13}$C n.m.r. spectra in the $^{13}CH_3$–Co region for solutions of: (*a*) 16 mmol dm$^{-3}$ [$^{13}$C]-methylcobalamin and 36 mmol dm$^{-3}$ *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, 5 975 scans; (*b*) solution (*a*) +0.05 mmol dm$^{-3}$ $[PtCl_6]^{2-}$, 2 723 scans; (*c*) solution (*a*) +3 mmol dm$^{-3}$ $[PtCl_6]^{2-}$, 2 723 scans

Additional support for these assignments is provided by a study of the demethylation of methylcobalamin. It has been shown [4-6,19] that methylcobalamin is readily demethylated by Pt$^{IV}$ in the presence of catalytic

$$CH_3(B_{12}) + PtCl_6^{2-} \xrightarrow{PtCl_4^{2-}} H_2O(B_{12}) + CH_3PtCl_5^{2-} + Cl \quad (1)$$

amounts of Pt$^{II}$, *viz.* equation (1). As illustrated in Figure 3, demethylation of methylcobalamin *in the base-*

*on configuration* by $PtCl_6^{2-}$ also occurs readily in the presence of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$. The reduced reactivity of the ' base-off ' methylcobalamin platinum(II) complex parallels the observation of Taylor and Hanna [4] that methylcobinamide is demethylated very slowly by the $PtCl_4^{2-}$–$PtCl_6^{2-}$ redox switch couple. In the redox switch reaction of $PtCl_4^{2-}$–$PtCl_6^{2-}$ with methylcobalamin, it has been proposed that $PtCl_4^{2-}$ forms a weak outer-sphere complex with the acetamide and/or propionamide side chains of the corrin ring, thus either labilizing the methyl group to direct electrophilic attack by $PtCl_6^{2-}$ or steering the $PtCl_4^{2-}$ close to the organometallic bond where its propinquity would facilitate a redox switch reaction [equation (2)]. Although a related mechanism

$$CH_3\text{-}(B_{12})\text{-}Pt^{II}Cl_4^{2-} + Pt^*Cl_6^{2-} \longrightarrow H_2O(B_{12}) + CH_3PtCl_5^{2-} + Pt^*Cl_4^{2-} + Cl^- \quad (2)$$

may be operative in the systems we have studied, we have not been able to detect a weak complex between

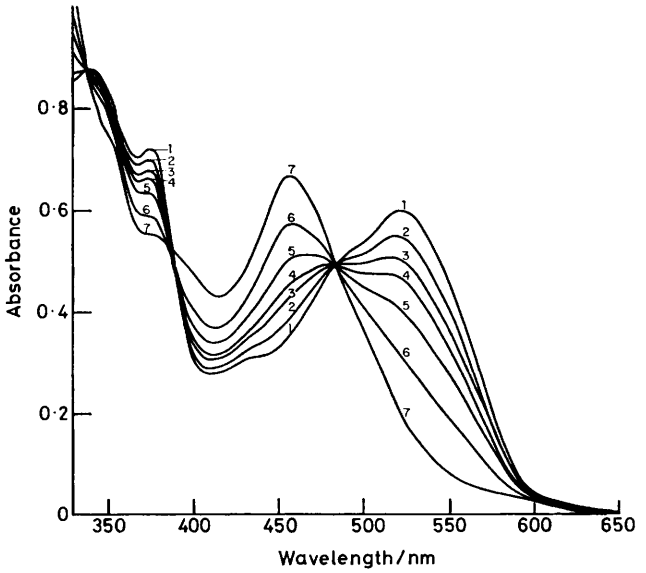



FIGURE 4 Absorption spectra of 0.075 mmol $dm^{-3}$ adenosylcobalamin in 0.1 mol $dm^{-3}$ sodium acetate buffer pH 5.5 in the presence of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ at 50 °C. At time zero (1); 75 (2); 255 (3); 430 (4); 830 (5), and 1 490 min (6) subsequent to mixing the reagents. Adenosylcobalamin 0.075 mmol $dm^{-3}$ in 0.1 mol $dm^{-3}$ HCl (7)

*cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and methyl- or adenosyl-cobalamin by either $^{13}C$ n.m.r. or electronic absorption spectroscopy. Even so, it should be possible to form one between *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and the corrin ring that would resemble that for $PtCl_4^{2-}$. In the case of the latter, the acetamide amide protons could hydrogen-bond to the chloride ligands; in the former, the acetamide carbonyls could hydrogen-bond to the acidic protons of the co-ordinated water molecules.

*Electronic Absorption Spectroscopy.*—Addition of an excess of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ to adenosylcobalamin in buffered aqueous solution at pH 5.5 causes a slow colour change from red to yellow (Figure 4). As discussed above, the spectrum due to the yellow corrinoid has been assigned to the ' base-off ' adenosylcobalamin–diammineaquoplatinum(II) adduct. Isosbestic points at 483 and 388 nm are consistent with the occurrence of a single chemical reaction. Photolysis of the adenosylcobalamindiammineaquoplatinum(II) complex does not produce aquocobalamin but a new corrinoid with absorption maxima at 518, 488, and 408 nm. These spectral properties are characteristic of diaquocobinamide and of aquocobalamin in strong acid, [20] suggesting that the photolysis product is aquocobalamin in the ' base-off ' form with N(3) of the 5,6-dimethylbenzimidazole nucleotide co-ordinated to the platinum(II) complex.

TABLE 1

Conditions used in the determination of the kinetics of the reaction of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ (8.8 mmol $dm^{-3}$) with cobalamins

| Compound | Concentration, mmol $dm^{-3}$ | $\theta_c$/°C | $\lambda_{max.}$/nm [a] | $\varepsilon$ [b] |
|---|---|---|---|---|
| Methylcobalamin | 0.069 | 50 | 519 | 8 700 |
| Ethylcobalamin | 0.081 | 50 | 509 | 8 800 |
| Propylcobalamin | 0.077 | 50 | 508 | 8 700 |
| Methylepicobalamin | 0.089 | 80 | 528 | 7 500 |
| Adenosylepicobalamin | 0.089 | 80 | 527 | 7 800 |
| Methylcobalamin | 0.080 | 80 | 519 | 8 700 |
| Adenosylcobalamin | 0.087 | 80 | 522 | 8 000 |
| Carboxymethylcobalamin | 0.090 | 80 | 523 | 7 300 |
| Vinylcobalamin | 0.075 | 80 | 520 | 9 300 |
| Ethoxycarbonylmethylcobalamin | 0.108 | 80 | 524 | 6 200 |

[a] Maximum of the α band at 20 °C. [b] Molar absorptivity at the maximum of the α band.

Similar red to yellow colour changes characteristic for a number of other alkylcobalamins in the presence of *cis*-$[Pt(NH_3)_2(OH_2)_2][NO_3]_2$ and the kinetics of these reactions have been determined by following the rate of decrease of the absorbance at the position of the maximum of the α band (*e.g.* 522 nm for adenosylcobalamin, Figure 4). As shown in Table 1, the reactions were studied under pseudo-first-order conditions, the platinum(II) complex being present in a large excess relative to the cobalamin. Because the reactions are very slow, most of the kinetic measurements were made at 80 °C. In the case of the thermally unstable ethylcobalamin [21] and propylcobalamin the kinetics were studied at 50 °C, well below the thermal decomposition temperatures. A summary of the derived rate constants is presented in Table 2 (see Experimental section).

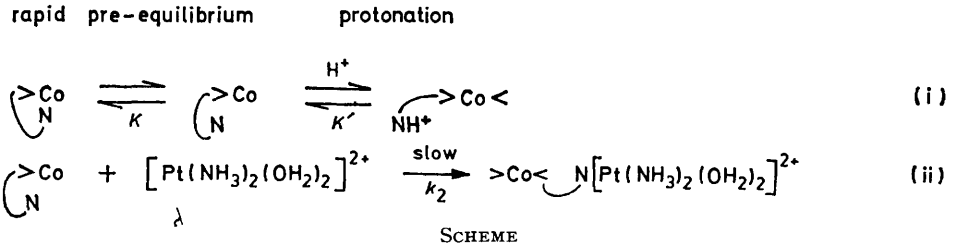
TABLE 2

Kinetic data for the reactions of alkylcobalamins with *cis*-diamminediaquoplatinum(II) nitrate at 80 °C

| Nature of the alkyl group | $pK_a$ | $10^3 k_{obs.}$ /$min^{-1}$ |
|---|---|---|
| Methyl epi | 2.2 | 3.15 ± 0.34 |
| Methyl | 2.72 | 8.03 ± 0.12 |
| Adenosyl epi | 2.8 | 8.05 ± 0.47 |
| Adenosyl | 3.45 | 19.1 ± 0.4 |
| Carboxymethyl | 2.20 | 7.09 ± 0.57 |
| Ethoxycarbonylmethyl | 2.25 | 2.84 ± 0.27 |
| Vinyl | 2.4 | 2.81 ± 0.44 |
| Methyl * | 2.72 | 0.474 ± 0.05 |
| Ethyl * | 3.87 | 1.29 ± 0.17 |
| Propyl * | 3.84 | 1.31 ± 0.05 |

* Determined at 50 °C.

A reasonable path for the reaction which is consistent with the chemistry of platinum(II) complexes [15] and cobalamins is outlined below and involves the rapid pre-equilibrium between the ' base-on ' and ' base-off ' form followed by a slow reaction of the ' base-off ' form with $Pt^{II}$. The observed rate constant then should be proportional to $k_2K$. The parameter $K$ has never been measured directly but recent studies [21] suggest that it is very small. An indirect measure of $K$ is provided by $K_A(\equiv K'K)$, the protonation constant for the complete set of reactions in (i) of the Scheme below. Since the long α-D-ribofuranose-3-phosphate side chain is identical




SCHEME

for all cobalamins studied, the protonation constant $K'$ and the rate constant $k_2$ should exhibit little variation with the nature of the alkyl group bound to cobalt. If the above scheme is correct, we might expect that the observed rate constant ($k_{obs.}$) would be proportional to $K_A$. As shown in Figure 5, this expectation is met.

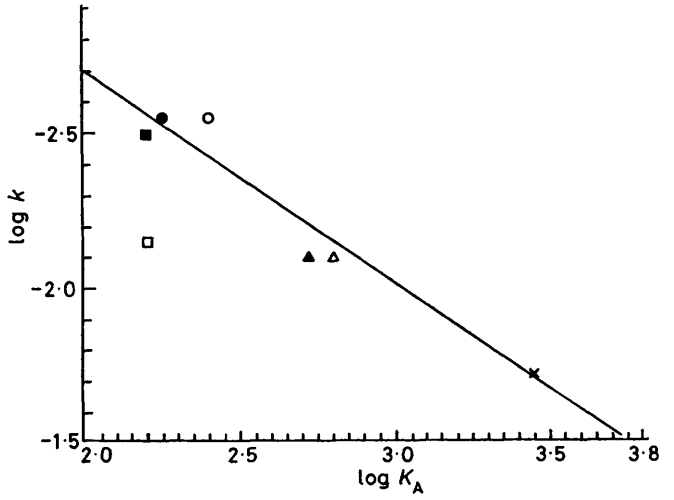



FIGURE 5 Log–log plot of the rate constant for the reaction of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with cobalamins against the constant for the protonation of the benzimidazole moiety of the cobalamin. Ethoxycarbonylmethyl (●), vinyl (○), methyl epi (■), carboxymethyl (□), methyl (▲), adenosyl epi (△), and adenosyl (×)

The deviation of carboxymethylcobalamin from the plot is not understood at this time, but it may involve complexation of $Pt^{II}$ with the charged carboxymethyl group ($Co-CH_2-CO_2^-$) as well. In summary, the kinetic data are consistent with a reaction which involves competition between the electrophilic platinum(II) complex and the $Co^{III}$ of the corrin ring for the lone pair of electrons on N(3) of the 5,6-dimethylbenzimidazole nucleotide.

*Conclusion.*—The data presented in this paper clearly demonstrate that *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ interacts with adenosylcobalamin and a series of alkylcobalamins to form adducts in which N(3) of the 5,6-dimethylbenzimidazole moiety is co-ordinated to the platinum(II) complex. In addition, adenosylcobalamin forms a complex in which a $Pt^{II}$ is co-ordinated to both N(3) of the benzimidazole nucleotide and N(7) of the 5'-deoxyadenosine nucleoside.

Since adenosylcobamides, such as Coα-aquo-Coβ-adenosyl(3,5,6-trimethylbenzimidazolyl)cobamide in which the lower ligand is prevented from co-ordinating to the cobalt atom, are inhibitory in all the adenosylcobalamin-dependent enzymatic systems,[22] it would be expected that the adenosylcobalamin–diammineaquoplatinum(II) adduct is also unable to function as a coenzyme. Enzymatic studies with the cobalamin–platinum(II) adduct are presently under investigation.

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