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KINETICS AND MECHANISM OF THE REACTION BETWEEN METHYLCOBALAMIN AND MERCURIC CHLORIDE

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Summary

The reaction between the organometallic compound methylcobalamin and mercuric chloride has been studied at several temperatures by ultraviolet-visible spectrophotometric and electron capture gas chromatographic methods. The mechanism of the reaction is shown to involve a transfer of a methyl group as a carbanion from "base on" methylcobalamin to mercuric chloride and the activation parameters have been determined. The initial product is methylmercury rather than dimethylmercury. Where mercuric chloride is in excess no dimethylmercury can be detected as a product of the reaction. This mechanistic study is related to the environmentally important process of mercury methylation leading to the occurrence of toxic methylmercury species under natural conditions.

Introduction

Methylcobalamin, a naturally occurring coenzyme of vitamin B_{12}, is an organometallic compound having a methyl group to cobalt bond and has an empirical formula $C_{63}H_{91}O_{14}N_{13}PCo$. Although its structure is complex, the significant organometallic and environmental reactions mainly proceed on the basis that the compound is a five- or six-coordinate complex of cobalt(III), and therefore its structure can be represented as in Fig. 1. In the text methylcobalamin is referred to as $CH_3(B_{12})$.

The role of methylcobalamin in the environmental methylation of mercury has been indicated by Wood et al. [2] and Imura et al. [3]. The environment process is thought to involve a transfer of the axial methyl group from cobalt to mercury in a chemical reaction which is rendered catalytic by the enzymatic regeneration of methylcobalamin by microorganisms [2,4]. The key step in the process of mercury methylation can therefore be seen to be the breaking of the

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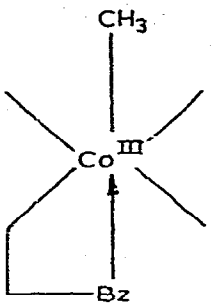
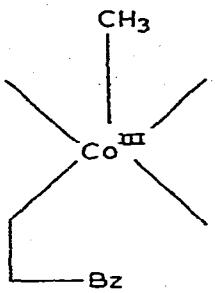


(Ia) Base on



(Ib) Base off

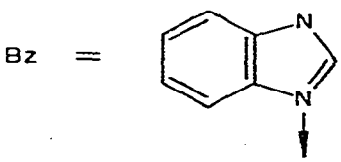


Fig. 1. Methyl cobalamin, $CH_3(B_{12})$. The benzimidazole nucleus $C_9H_9H_9N_2$, Bz) attached to the corrinoid the system may be coordinated ("base on") or not coordinated ("base off") to the axial coordination position of cobalt. (For detailed structural discussion see ref. 1.)

methyl group to cobalt bond and the transfer of this group to mercury.

There have been few quantitative attempts to derive the mechanism of this important reaction. The ability of methylcobalamin to methylate inorganic mercury species has been demonstrated by several groups [2,5,6] but in these cases no kinetic and mechanistic studies were initiated.

Only one detailed study of the reaction had been published prior to our work [7,8]. As a result it was concluded that in the reaction between methylcobalamin and mercuric acetate, an essentially electrophilic attack by a mercury species took place on methylcobalamin in its base coordinated configuration. This leads to the formation of monomethylmercury species and aquocobalamin (reaction 1).

$$CH_3(B_{12}) + Hg(OAc)_2 + H_2O \rightarrow H_2O(B_{12})^+ + CH_3Hg^+ + 2OAc^- \quad (1)$$

Kinetic work for this system was complex because of the rapid reaction-rate making stop flow methods necessary, by the number of species thought to be present in solution as a result of buffering and ionic strength maintainance, and by uncertainty about the mercury species involved. In fact it was not possible to specify the identity of the mercury species which attacked the methyl to cobalt bond.

This work also demonstrated the occurrence of a prior complexation between mercury and the benzimidazole nucleus of the cobalt system, leading to a reduction in the amount of methyl cobalamin present (reaction 2).

$$CH_3(B_{12}) + Hg(OAc)_2 \rightleftharpoons CH_3(B_{12})Hg^{2+} + 2OAc^- \quad (2)$$

In an attempt to clarify the mechanism of this environmentally important reaction we felt it would be desirable to investigate a less complex system where the reaction took place at a slower rate. In principle the system methylcobalamin/mercuric chloride in aqueous solution appeared likely to satisfy these criteria, and in addition is a suitable model system for the environmental reaction since mercury present, for example, under estuarine conditions may exist as chloride species [9].

After completion of our experimental work a further study of this reaction type became available [10]. This work clarified the identity of the mercury species involved in the prior complexation equilibrium (reaction 2) and proposed that two separate mechanistic routes may exist which do not occur simultaneously. When no free mercuric ion is present methylcobalamin is essentially uncomplexed by mercury and a direct methylation occurs as in reaction 1. However when sufficient Hg^{2+} is present, reaction 2 occurs with Hg^{2+} and a two step mechanism occurs (reaction 3).

$$CH_3(B_{12}) + Hg^{2+} \rightleftharpoons CH_3(B_{12})Hg^{2+} \quad (3a)$$

$$CH_3(B_{12})Hg^{2+} + Hg^{2+} + H_2O \rightarrow H_2O(B_{12})^+ + CH_3Hg^+ + Hg^{2+} \quad (3b)$$

The important points made in this study are the positive identification of Hg^{2+} as the only mercury species involved in reaction 2 [10] and the suggestion that where reaction 3 occurs all of the methylcobalamin is complexed and that in this situation the only methylating species is $CH_3(B_{12})Hg^{2+}$.

It appears therefore that although a reaction between methylcobalamin and mercuric chloride can be assumed to proceed essentially by a transfer of a methyl group with its set of electrons from cobalt to mercury, the reaction is likely to be more complex than the proposed reaction 4 might suggest.

$$CH_3(B_{12}) + HgCl_2 + H_2O \xrightarrow{k_1} H_2O(B_{12})^+ + CH_3Hg^+ + 2\,Cl^- \quad (4)$$

Several features are likely to be in doubt. First, it has been suggested that the initial product of the reaction is dimethylmercury and that the observed monomethylmercury found in the reaction mixture arises by reaction with excess inorganic mercury (reaction 5) or is an artefact of an extraction procedure [5].

$$(CH_3)_2Hg + HgCl_2 \rightarrow 2CH_3HgCl \quad (5)$$

This suggestion has been opposed [6] and in any case it appears that the rate constant for a second methylation step (reaction 6) is much lower than that of the first [8,11].

$$CH_3(B_{12}) + CH_3Hg^+ + H_2O \rightarrow H_2O(B_{12})^+ + (CH_3)_2Hg \quad (6)$$

We have therefore investigated the mercury products from the reaction with methylcobalamin and have studied them by electron capture gas chromatography.

Another feature that has to be considered is the configuration of methylcobalamin in the reaction. A pH-dependent equilibrium exists between the forms shown in Fig. 1, and both are in principle capable of donating a methyl group to mercury. However it is likely that the influence of the electron-donating benzimidazole group directly bound to cobalt in the six coordinate species (Fig. 1a) would make this a much more effective methylating agent for the electrophilic

mercury species than the five coordinate form (Fig. 1b). This has in fact been shown; however allowance for the presence of Ib must be made as its appearance would reduce the concentration of the actual methylating species Ia. However, under conditions of low pH methylcobalamin is present solely in configuration Ia and these conditions applied in our study [1]. In addition, however, in the presence of mercury species, the occurrence of reaction 3a has to be allowed for. This initial coordination of Hg^{2+} onto the benzimidazole base would lead to the generation of a base off species (Fig. 2, reaction 7).

In the presence of base on methylcobalamin (Ia) species II might again be expected to show a low rate of methylation by comparison. Transfer of the methyl group with its electrons might be expected to be severely hindered by the electrophilic mercury species coordinated to the benzimidazole group. However Chu suggests that where reaction 7 occurs and all of Ia is converted to II, the two stage mechanism (reaction 3) takes place. We were interested to see if the proposed two stage mechanism could apply to the reaction with mercuric chloride; particularly we wished to investigate the suggested total removal of Ia by Hg^{2+}. For mercuric chloride it seemed likely that not all of the efficient methylating species Ia would be removed by Hg^{2+} in view of the small ionisation of this molecule and the low concentration of Hg^{2+} [12]. If so the two stage mechanism would not be applicable to the reaction with mercuric chloride. In that case methylation would take place mainly through the remaining six coordinate methylcobalamin (Ia) and the function of the kinetic equations would then be to allow for and express its reduced concentration as a result of reaction 7. To obtain these these equations requires a statement of the concentration of Hg^{2+} in terms of the total concentration of added mercuric chloride (i.e. reaction 8)

$$HgCl_2 \overset{K_2}{\rightleftharpoons} Hg^{2+} + 2\,Cl^- \qquad (8)$$

(K_2 equilibrium constant)

The final point we wished to clarify concerns the identity of the mercury species which is methylated. In their semi-quantitive study Bertillson and Neujahr assumed that for the mercuric chloride reactions Hg^{2+} was the sole reacting species despite its low concentration in the reaction system [6]. In their studies Chu and De

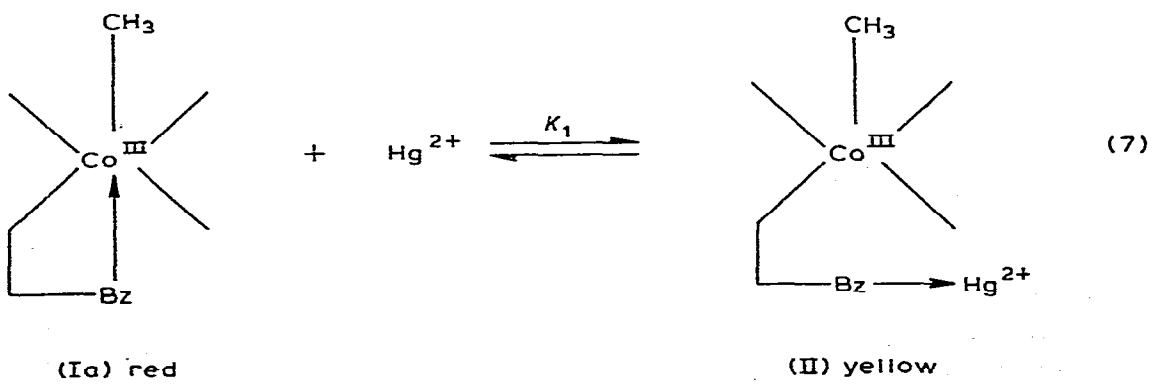


Fig. 2. Coordination of methylcobalamin with mercury species. (Equilibrium constant K_1.)

Simone et al. used the total added mercuric acetate concentrations in their calculations i.e. a tacit assumption that all the mercury species present were methylated at the same rate. We have tried therefore to distinguish between the unionised mercuric chloride molecule and Hg^{2+} as the dominant reacting mercury species in the methylation reaction.

Finally we believe that the mercuric chloride system is not only a realistic model for the environmental process but that this system eliminates the possibility present with mercuric acetate that methylmercury could be generated from the acetate methyl group on exposure to light, a reaction that has been shown to occur under certain conditions [13].

Results and discussion

Initially we decided to determine if simple second order kinetic equations would be an adequate description. In such circumstances the overall reaction is given by reaction 4 and the rate by eq. a. However our results were derived from pseudo first order conditions with excess mercury and for each run at 298 K at a particular concentration of mercuric chloride a pseudo first order rate constant k_{obs} was obtained (eq. b).

$$\text{Rate} = k_1[CH_3(B_{12})][HgCl_2] \quad (a)$$

$$\text{Rate} = k_{obs}[CH_3(B_{12}]_T \quad (b)$$

$[CH_3(B_{12})]_T$ = concentration of methyl cobalamin added initially.

$$k_{obs} = k_1[HgCl_2] \text{ if } [CH_3(B_{12})] = [CH_3(B_{12})]_T \quad (c)$$

and

$$\log_{10} k_{obs} = \log_{10} k_1 + \log_{10} [HgCl_2] \quad (d)$$

A plot of eq. d produces a linear graph giving an intercept value of k_1 equal to 3.2 $dm^3\ mol^{-1}\ sec^{-1}$ and a slope of 0.85. The value of 0.85 is near enough to the theoretical value of 1.0 for a simple second order process to be considered as reasonable experimental error. Alternatively it could imply mechanistic complexity for the reaction leading to a real divergence from simple second order rate equations. We therefore undertook the derivation of a second set of kinetic equations allowing for the various equilibria that may be undergone in solution as these equilibria may make the concentrations and possibly identities of the reacting species very different from these existing on addition of the reagents. These kinetic derivations were based on the occurrence of reactions 4, 7 and 8.

Detailed derivations of the kinetic equations are made in the Experimental section, but the main conclusions are now outlined. Under pseudo first order conditions if all of the base on methylcobalamin (Ia) is not removed by Hg^{2+} then in effect a combination of a direct methylation and the two stage process could occur simultaneously with a contribution by $CH_3(B_{12})\ Hg^{2+}$ (II) depending on its concentration. However as we have already suggested, in this system $[CH_3(B_{12})Hg^{2+}]$ is low and experimental observations verify this [14]. Therefore our initial equations were derived to test the system on the basis that base on methylcobalamin is the main methylating species and mercuric chloride the substrate, but that the concentration of methylcobalamin is lower than that added to the

reaction system, i.e.

$[CH_3(B_{12})]_T = [CH_3(B_{12})]$ reacting + $[CH_3(B_{12})Hg^{2+}]$

Similarly an expression for the various mercury species present in terms of the concentration of mercuric chloride added initially ($[HgCl_2]_T$) was also derived. Substitution in eq. a and equating a and b then gives an expression (e) from which the rate constant k_1 (reaction 4) can be derived.

$$\frac{1}{k_{obs}} = \frac{K_1K_2}{k_1[Cl^-]^2} + \frac{1}{k_1[HgCl_2]_T}\left(\frac{K_2}{[Cl^-]^2} + 1\right) \quad \text{(e)}$$

If the factor $K_2/[Cl^-]^2$ is much less than unity then e can be further simplified (eq. f).

$$\frac{1}{k_{obs}} = \frac{K_1K_2}{k_1[Cl^-]^2} + \frac{1}{k_1[HgCl_2]_T} \quad \text{(f)}$$

This appears to be reasonable as from reaction 8 the factor is equal to $[Hg^{2+}]/[HgCl_2]$ and $HgCl_2$ is little ionised under these conditions [12]. If eq. f holds and the non-constant factor $K_1K_2/k_1[Cl^-]^2$ is small in comparison with $1/[HgCl_2]$, a plot of $1/k_{obs}$ against $1/[HgCl_2]_T$ should be linear with a slope of $1/k_1$. In fact an excellent straight line is obtained with slope 0.30 giving a value for k_1 of 3.3 $dm^3\ mol^{-1}\ sec^{-1}$ (Fig. 3). This derivation has postulated mercuric chloride as the species that is methylated. If instead we assume Hg^{2+} to be the dominant species to be methylated then eq. a now becomes:

$$\text{Rate} = k_1[CH_3B_{12}][Hg^{2+}] \quad \text{(g)}$$

Similar substitution produces another equation for k_1 (eq. h).

$$\frac{1}{k_{obs}} = \frac{K_1}{k_1} + \frac{1}{k_1[HgCl_2]_T}\left(\frac{[Cl^-]^2}{K_2} + 1\right) \quad \text{(h)}$$

To simplify this equation in order to obtain a plot of $1/k_{obs}$ versus $1/[HgCl_2]_T$ would we believe be unjustified as $[Cl^-]^2/K_2$ cannot be $<<1$. A small value for $[Cl^-]^2/K_2$ would require $HgCl_2$ to be very considerably ionised under the reaction conditions. In view of this and the linearity of the plot of eq. f over a range of mercuric chloride concentrations we suggest that the dominant methylated species is mercuric chloride as indicated in reaction 4.

Further evidence for this is provided by the value of our rate constant compared with those calculated for other mercury systems in the reaction with methylcobalamin. The mechanism of De Simone et al. is similar to ours except for the extent of the initial equilibrium; the basic methylation is a transfer from base on methylcobalamin to (an assumed) unionised mercuric acetate molecule. This gives a value for the equivalent rate constant of $3.7 \times 10^2\ dm^3\ mol^{-1}\ sec^{-1}$ and indeed the analogous one step mechanism of Chu also gives a similar value ($3.5 \times 10^2\ dm^3\ mol^{-1}\ sec^{-1}$. If in these cases the dominant species methylated is in fact not Hg^{2+} but the unionised molecule, then the slower rate we have observed for $HgCl_2$ seems reasonable as stronger Hg—Cl bonds have to be broken in the reaction [14].

The question of the initial equilibrium (reaction 7) remains. If in our system the prior equilibrium involved mercuric chloride rather than Hg^{2+} then the

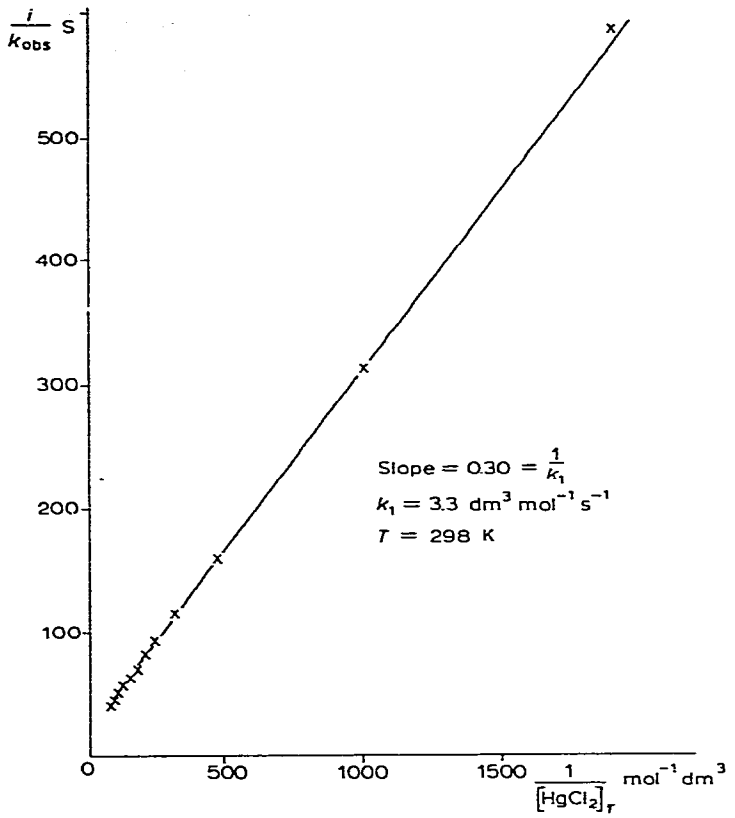


Fig. 3. Determination of rate constant k_1.

kinetic derivations would produce a slightly different version of eq. f viz. eq. i.

$$\frac{1}{k_{obs}} = \frac{K_1^*}{k_1} + \frac{1}{k_1[HgCl_2]_T} \qquad \text{(i)}$$

K_1^* is an analogous equilibrium constant to K_1 (reaction 7). The plot to test this is the same as that to test eq. f. However in the case of eq. i the intercept can be evaluated and K_1^* calculated. When this is done for our system the value of K_1^* is found to be 74.3. Such a value implies the extensive presence of $CH_3(B_{12})Hg^{2+}$ in the equilibrium and this is incompatible with the observed non appearance of a yellow colour that would exist if $CH_3(B_{12})Hg^{2+}$ predominated in solution on addition of mercuric chloride to methylcobalamin. Our results therefore suggest that any prior equilibrium involves Hg^{2+} rather than mercuric chloride, but the near coincidence in our values of k_1 when derived either from assumptions of simple second order kinetics assuming no prior equilibrium, or from the assumption of an equilibrium demonstrates that the extent of the prior equilibrium of reaction 7 is small.

We have also made measurements at three other temperatures in order to obtain the activation parameters of the reaction using the Arrhenius equation.

Values for the energy of activation E (52.4 kJ), the frequency factor A (5.3×10^9 sec^{-1}), the enthalpy of activation $\Delta H^{\theta\neq}$ (49.9 kJ) and the entropy of

activation $\Delta S^{\theta\neq}$ (—58.7 kJ) have been obtained by the normal methods.

The significance of the value for the entropy of activation is that it suggests the incorporation of another molecule in the transition state i.e. a mechanism of the type suggested in reaction 4 rather than for example an intramolecular transfer to an already bound mercury species [15]. The frequency factor A is relatively low as might be expected for a reaction involving a complex molecule such as methylcobalamin where the effective collisions are likely to be a small proportion of the total.

Our general observations complement those made by Robinson et al. [14] on various methylcobalamin/mercury(II) systems. As in our case Robinson et al. do not observe any appearance of the (base off) yellow colour in the chloride case when sodium chloride is added in excess. Similarly acetate complexes react faster than chloride complexes. However there are considerable differences in reaction conditions in the two studies (particularly the use of excess chloride or acetate in acid solution in Robinson et al.'s work) and it is not clear what mechanistic parallels can be drawn between the two cases.

With regard to the possible occurrence of dimethylmercury in the reaction, using the method described in the experimental section we were unable to detect any trace of this material when 60 μmol of methylcobalamin was treated with 1000 μmol of mercuric chloride. Had dimethylmercury been generated in the first instance it is unlikely that it would not have been detected by this system. When 60 μmol of methylcobalamin was treated with only 100 μmol of mercuric chloride, a trace of dimethylmercury was detected, but the former conditions only are those of the mechanistic study. For this reason we discounted the occurrence of reaction 6 in our kinetic work.

Experimental

Methylcobalamin was obtained from Glaxo Ltd. or Sigma Corporation. Mercuric chloride was analytical reagent grade.

Solutions of methylcobalamin for reaction were prepared in distilled water that had been deaerated by boiling, with subsequent cooling under oxygen-free nitrogen gas. The solutions were kept in a refrigerator in opaque bottles and precautions were taken to exclude light. It was found that a 1.3×10^{-3} mol dm^{-3} solution was stable for at least one week under these conditions.

Reactions were normally carried out under pseudo first order conditions with excess of mercuric chloride, and were followed by conventional ultraviolet/visible spectroscopic methods on a Unican SP800 instrument. Methylcobalamin was measured by recording the strong absorbance at 350 nm of the aquocobalamin product. An attempt was made to measure the reaction rate under pseudo first order conditions with methylcobalamin in excess, but at the concentrations used ([$HgCl_2$] = 6×10^{-6} mol dm^{-3} initially) mercury was lost by adsorption onto the vessel walls at a comparable rate to the reaction. As we did not wish to change the pH conditions from that of the main study, we did not proceed further with this approach to the reaction.

In the experiments to investigate dimethylmercury generation this product was estimated by gas chromatography after conversion to methylmercury by reaction 5. The method used is a simplified version of that described previously [4].

Kinetic derivations

The total concentration of methylcobalamin species in the reaction mixture is the sum of the concentrations of the "base on" and "base off" species

$$[CH_3(B_{12})]_T = [CH_3(B_{12})] + [CH_3(B_{12})Hg^{2+}]$$

where $[CH_3(B_{12})]_T$ is the total methylcobalamin added initially. The concentrations of the two individual species are related by the equilibrium constant K_1 of reaction 7.

$$K_1 = \frac{[CH_3(B_{12})Hg^{2+}]}{[CH_3(B_{12})]\,[Hg^{2+}]}$$

The concentrations of the individual species can therefore be expressed as functions of K_1 and $[CH_3(B_{12})Hg]$ as follows:

$$[CH_3(B_{12})]_T = \frac{[CH_3(B_{12})Hg^{2+}]}{K_1[Hg^{2+}]} + [CH_3(B_{12})Hg^{2+}]$$

$$[CH_3(B_{12})Hg^{2+}] = \frac{[CH_3(B_{12})]_T + K_1[Hg^{2+}]}{1 + K_1[Hg^{2+}]} \text{ and similarly}$$

$$[CH_3(B_{12})] = \frac{[CH_3(B_{12})]_T}{1 + K_1[Hg^{2+}]}$$

Likewise the concentration of Hg^{2+} and $HgCl_2$ in terms of the amount of mercuric chloride originally added, $[HgCl_2]_T$, can be expressed in terms of the relevant equilibrium constant K_2, viz.:

$$[Hg^{2+}] = \frac{[HgCl_2]_T \cdot K_2}{K_2 + [Cl^-]^2} \text{ and}$$

$$[HgCl_2] = \frac{[HgCl_2]_T[Cl^-]^2}{K_2 + [Cl^-]^2}$$

If the overall methylation reaction is given by reaction 4 (eq. a), its rate is as follows:

$$\text{rate} = k_1[CH_3(B_{12})][HgCl_2]$$

The concentrations of the individual species can now be substituted to give an expression involving only the concentration of the species initially added to the reaction mixture:

$$\text{Rate} = \frac{k_1[CH_3(B_{12})]_T}{1 + K_1[Hg^{2+}]} \cdot \frac{[HgCl_2]_T\,[Cl^-]^2}{K_2 + [Cl^-]^2}$$

$[Hg^{2+}]$ must be removed from this expression i.e.:

$$\text{Rate} = \frac{k_1[CH_3B_{12}]_T}{\dfrac{1 + K_1[HgCl_2]_T\,K_2}{K_2 + [Cl^-]^2}} \cdot \frac{[HgCl_2]_T[Cl^-]^2}{K_2 + [Cl^-]^2}$$

Expansion of this expression produces (eq. j):

$$\text{Rate} = \frac{k_1[CH_3B_{12}]_T\,[HgCl_2]_T\,[Cl^-]^2}{K_1[HgCl_2]_T\,K_2 + K_2 + [Cl^-]^2} \qquad \text{(j)}$$

Under pseudo first order conditions with an excess of mercuric chloride k_1 in eq. a can be replaced by a pseudo first order rate constant k_{obs} (eq. b).

$$\text{Rate} = k_{obs}[CH_3(B_{12})]_T$$

Comparing equations a and j:

$$k_{obs} = \frac{k_1[HgCl_2]_T\,[Cl^-]^2}{K_1[HgCl_2]_T\,K_2 + K_2 + [Cl^-]^2}$$

Inverting this we obtain (eq. e):

$$\frac{1}{k_{obs}} = \frac{K_1K_2}{k_1[Cl^-]^2} + \frac{1}{k_1[HgCl_2]_T}\left(\frac{K_2}{[Cl^-]^2} + 1\right) \qquad \text{(e)}$$

If the factor $K_2/k_1[Cl^-]^2$ is much less than 1 then eq. e can be simplified (eq. f):

$$\frac{1}{k_{obs}} = \frac{K_1K_2}{k_1[Cl^-]^2} + \frac{1}{k_1[HgCl_2]_T} \qquad \text{(f)}$$

Although the factor $K_1K_2/k_1[Cl^-]^2$ is not a constant, if it is small in relation to $1/k_1[HgCl_2]_T$ then a plot of $1/k_{obs}$ versus $1/[HgCl_2]_T$ will be linear with a slope equal to $1/k_1$. This plot is made in Fig. 3.

Using similar reasoning we have examined the implications if the species receiving the methyl group was Hg^{2+} rather than mercuric chloride. In this case the rate of the reaction is given as follows: Rate = $[CH_3(B_{12})]\,[Hg^{2+}]$ and as before we express this in terms of the concentrations of the species added initially viz.:

$$\text{Rate} = \frac{k_1[CH_3(B_{12})]_T}{1 + K_1[Hg^{2+}]} \cdot \frac{[HgCl_2]_T\,K_2}{K_2 + [Cl^-]^2} \text{ and further:}$$

$$\text{Rate} = \frac{k_1[CH_3(B_{12})]_T}{1 + \dfrac{K_1[HgCl_2]_T\,K_2}{K_2 + [Cl^-]^2}} \cdot \frac{[HgCl_2]_T\,K_2}{K_2 + [Cl^-]^2}$$

On expansion we find:

$$\text{Rate} = \frac{k_1[CH_3(B_{12})]_T[HgCl_2]_T\,K_2}{K_2 + [Cl^-]^2 + K_1[HgCl_2]_T\,K_2}$$

Again the rate is equal to $k_{obs}\,[CH_3(B_{12})]_T$ and therefore:

$$k_{obs} = \frac{k_1[HgCl_2]_TK_2}{K_2 + [Cl^-]^2 + k_1[HgCl_2]_TK_2}$$

On inversion we have:

$$\frac{1}{k_{obs}} = \frac{K_1}{k_1} + \frac{1}{k_1[HgCl_2]_T}\left(\frac{[Cl^-]^2}{K_2} + 1\right)$$

In our system $[Cl^-]^2/K_2$ is large and therefore may not be ignored compared to unity.

Acknowledgements

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