Kinetics of Electron Transfer between Vitamin B_{12} Compounds[1]

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Abstract: The reaction of vitamin B_{12s} and hydroxocobalamin (B_{12b}) proceeds rapidly in basic, aqueous solution leading to the formation of vitamin B_{12r}. Kinetic studies were carried out at $\mu = 0.10$ M in the hydroxide range $0.00145\ M \leq [OH^-] \leq 0.025$ M. The results obeyed the rate expression $-d[B_{12s}]/dt = (a + b/[OH^-])[B_{12s}][B_{12b}]$ with $a = (5.80 \pm 0.77) \times 10^3\ M^{-1}\ s^{-1}$ and $b = 70.7 \pm 1.9\ s^{-1}$ at 25.0 °C. Activation parameters have been evaluated for both terms. The mechanism of this reaction is discussed, and the kinetic data are also used to show that methylation of B_{12r} *cannot* occur by the reverse process, base-promoted disproportionation of B_{12r}.

The Co(I) derivative of vitamin B_{12} (B_{12s}) is a strong reducing agent[2,3] as well as a powerful nucleophile.[4] This nucleophilicity is important in the synthesis of organocobalamins[5,6] and in enzymatic reactions,[7,8] and as a result has been extensively studied.[9] In contrast little is known about electron-transfer reactions of B_{12s} and related compounds.

One such reaction involving the transfer of an electron between B_{12s} and aquocobalamin, a Co(III) species, was first reported by Hill and coworkers in 1962:[10]

$$[Co(I)]^- + [Co(III)]^+ \rightarrow 2[Co(II)] \qquad (1)$$

As noted by Banks et al. in their study of the reduction of N_2O by B_{12s},[11] reaction 1 prevents product analysis from yielding information as to whether B_{12s} acts as a one- or two-electron reductant. Any Co(III) produced as the result of a two-electron transfer will react with B_{12s} to form Co(II) which is the one-electron oxidation product.

Reaction 1 is also the reverse of disproportionation which has been postulated as the source of B_{12s} in enzymatic reactions.[12] Although several groups have investigated this disproportionation,[3,13,14] little mechanistic information on the forward or reverse reaction has been reported.

This paper presents the results of a kinetic investigation of the rapid reaction between B_{12s} and hydroxocobalamin (B_{12b}) in basic, aqueous media and the possible mechanistic implications of these results. Since the completion of this work a report of a similar study in which B_{12s} was generated by pulse radiolysis has appeared.[15]

Experimental Section

Materials. Solutions of B_{12b} in dilute sodium hydroxide were prepared from commercially available hydroxocobalamin hydrochloride (Sigma Chemical Co.). These solutions were freshly prepared prior to each kinetic experiment to minimize complications from "self-reduction" of the B_{12b}.[16]

Solutions of B_{12s} in aqueous sodium hydroxide were prepared by the reduction of hydroxocobalamin with excess sodium borohydride in the presence of trace amounts of $PdCl_4^{2-}$ as catalyst. It was observed that the rate of formation of B_{12s} was dependent upon the hydroxide ion concentration. At hydroxide ion concentrations of 0.01 M, approximately 12 h was required for complete reduction, whereas lowering the hydroxide concentration to 0.001 M reduced the reduction time to approximately 30 min. Visual observations suggested the slowest step was reduction of B_{12r} to B_{12s}. To avoid transfer of dilute solutions ($\sim 10^{-4}$ M) of B_{12s}, which are exceptionally sensitive to oxygen, the reductions were effected directly in the reservoir syringes of the stopped-flow instrument.

Sodium hydroxide and sodium borohydride were used as purchased. An excess of borohydride, or any impurities introduced with it, had no effect upon the reaction of interest. Twice recrystallized sodium perchlorate was used to maintain ionic strength in kinetic experiments.

Kinetic Studies. Rate determinations were made spectrophotometrically with Durrum D-110 and Canterbury SF-3A stopped-flow spectrophotometers. Reaction progress was monitored at the 700-nm peak of B_{12s}. Hydroxocobalamin was maintained in sufficient concentration excess to ensure pseudo-first-order conditions. Data analysis was done graphically and with a PDP-15 computer interfaced to the Durrum instrument.

Results

Initial experiments indicated that B_{12s} and B_{12b} react rapidly to produce a product with a visible spectrum identical with that of B_{12r},[6] consistent with observations of previous investigators.[10] The disappearance of B_{12s} was accurately described by the following rate expression:

$$\frac{-d[B_{12s}]}{dt} = k_1[B_{12s}][B_{12b}] \qquad (2)$$

for at least 80% of the reaction with $[B_{12s}]_0 = 2\text{–}5 \times 10^{-5}$ M and $[B_{12b}]_0 = 2.5 \times 10^{-5}\text{–}1.0 \times 10^{-3}$ M. Values of k_1 were typically reproducible to within 8%.

The kinetic effect of certain variables was explored and the results are summarized in Table I. Of particular concern were possible complications from the excess borohydride and $PdCl_4^{2-}$ used to produce the B_{12s}. Conditions were adjusted such that these reagents were not able to reduce significant amounts of the B_{12b} reactant or the B_{12r} product during the time required for complete reaction of B_{12b} and B_{12s}. Entries 1–5 show that these reagents have no effect on the value of k_1. Possible complications from the exposure of the reactants to

Table I.[a] Effect of Selected Variables on the Rate of Reaction of B_{12s} and B_{12b}

Entry	$[BH_4^-] \times 10^4$, M	$[PdCl_4^{2-}] \times 10^6$, M	μ, M	$10^{-4}k_1$, M^{-1} s^{-1}
1	0.94	1.4	0.10	1.15
2	1.8	1.4	0.10	1.23
3	2.7	1.4	0.10	1.31
4	2.7	0.72	0.10	1.37
5	2.7	2.9	0.10	1.39
6[b]	2.7	1.4	0.10	1.34
7[c]	0.87	1.4	0.10	1.39
8[c,d]	0.87	1.4	0.10	1.22
9[c]	0.87	1.4	0.019	1.06
10[c]	0.87	1.4	0.050	1.13
11[c]	0.87	1.4	0.20	1.46

[a] Solvent: H_2O; λ 700 nm; [NaOH] = 0.0094 M; T = 25.0 °C; $[B_{12s}]_0 = (2.0\text{–}4.0) \times 10^{-5}$ M; $[B_{12b}] = (4.7\text{–}6.0) \times 10^{-4}$ M; Durrum flow; equal [NaOH] in both reactant solutions; B_{12b} solutions used within 1 h of addition of NaOH. [b] B_{12b} solution aged for ~3.5 h prior to reaction. [c] [NaOH] in B_{12s} = 0.0010 M; [NaOH] in B_{12b} = 0.0178 M. [d] Canterbury flow.

Table II. Rate Constants:[a] Dependence upon Temperature and Hydroxide Ion Concentration

[OH⁻], M	$10^{-4}k_1$, dm³ mol⁻¹ s⁻¹, at temperature, °C				
	7.45	17.75	25.0	30.0	49.8
0.00145			5.54		
0.00200		2.81	3.98	5.78	
0.00400	0.635	1.74	2.24[b]	2.86	6.87
0.0094	0.377	0.870	1.27	1.70	3.97
0.0250	0.291	0.626	0.914	1.24	2.18

[a] In water evaluated at λ 700 nm with $[B_{12s}]_0 \simeq 3.0\text{–}4.5 \times 10^{-5}$ M and $[B_{12b}] \simeq 5 \times 10^{-4}$ M; $\mu = 0.10$ M (with $NaClO_4$). [b] $[OH^-] = 0.00440$ M.

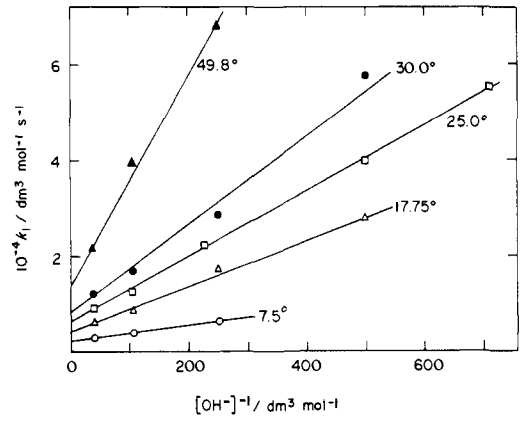


Figure 1. Plots showing the linear variation of the second-order rate constant k_1 with the inverse of $[OH^-]$ at various temperatures.

conditions of high pH for significant periods prior to reaction were shown unimportant; entry 6 derives from an experiment in which the B_{12b} solution was exposed to 0.01 M NaOH for an extended period prior to the reaction with B_{12s}. Similar possible effects on the B_{12s} solutions were examined by lowering the concentration of hydroxide in the B_{12s} solution as in entry 7, which greatly reduces the time required for reduction to occur and thus the time in which the B_{12} species are exposed to hydroxide ion. No effect on k_1 was observed when the preparation of B_{12s} was varied in this manner. Only a small increase in k_1 was observed with ionic strength variation from 0.019 to 0.20 M.

The kinetic effects of hydroxide ion concentration and of temperature variation are summarized in Table II. The data at each temperature are consistent with the expression

$$k_1 = a + b/[OH^-] \quad (3)$$

Linear plots of k_1 vs. $[OH^-]^{-1}$ for $0.0015 < [OH^-] < 0.025$ M are shown in Figure 1. Points at $[OH^-] > 0.05$ M lie above the lines shown, and studies at $[OH^-] < 10^{-3}$ M are limited by the hydrolysis of excess borohydride which alters $[OH^-]$. Least-squares analysis of the kinetic data at each temperature yields the values of a and b given in Table III.

Activation enthalpies and entropies for each rate constant were evaluated from data at 17–50 °C according to the Eyring relation from plots of $\ln k/T$ vs. $1/T$. The results are $\Delta H_a^{\ddagger} = 27 \pm 1$ kJ mol⁻¹, $\Delta S_a^{\ddagger} = -83 \pm 4$ J mol⁻¹ K⁻¹; $\Delta H_b^{\ddagger} = 34 \pm 2$ kJ mol⁻¹, and $\Delta S_b^{\ddagger} = -94 \pm 7$ J mol⁻¹ K⁻¹. The values of k_1 are represented by these parameters to 4.9% mean deviation between experimental and calculated values.[4]

After this work was completed there appeared in the literature[15] an independent evaluation of k_1. These authors generated B_{12s} using pulse radiolysis and measured the rate of its decay at various pHs (5.8–11.0), at apparently a single concentration of aquocobalamin(III). They find a rate constant

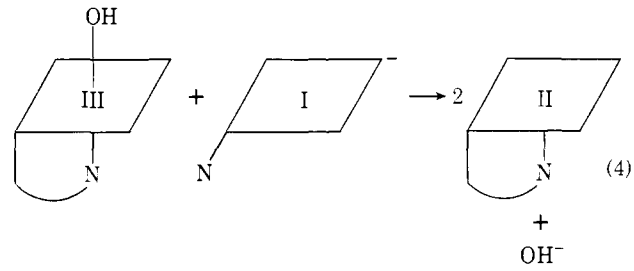
Table III. Least-Squares Values of Rate Constants a and b at Various Temperatures: $k_1 = a + b/[OH^-]$

T, °C	$10^{-3}a$, dm³ mol⁻¹ s⁻¹	$10^{-2}b$, s⁻¹
7.45	2.15 ± 0.18	0.166 ± 0.012
17.75	4.28 ± 0.74	0.484 ± 0.026
25.0	5.80 ± 0.77	0.707 ± 0.019
30.0	6.65 ± 2.11	0.996 ± 0.074
49.8	14.4 ± 2.6	2.20 ± 0.17

$k_1 = 3 \times 10^7$ dm³ mol⁻¹ s⁻¹, roughly 10^3 times higher than our values. Also they find that the rate is independent of pH over the range cited, despite this range lying on both sides of the pK_a value for B_{12a}. We are at a loss to identify the problem, but do note that were an impurity present which was highly reactive toward B_{12s}, then such a pattern might be seen.

Discussion

B_{12} Compounds. Changes in coordination number[17-19] accompany the net reaction, eq 4, characterized[3] by an equilibrium constant $K_4 = 10^{7.65}$ M based on reduction potentials and the acidity constant[10,21] of aquocobalamin(III) ($pK_a = 7.8$).



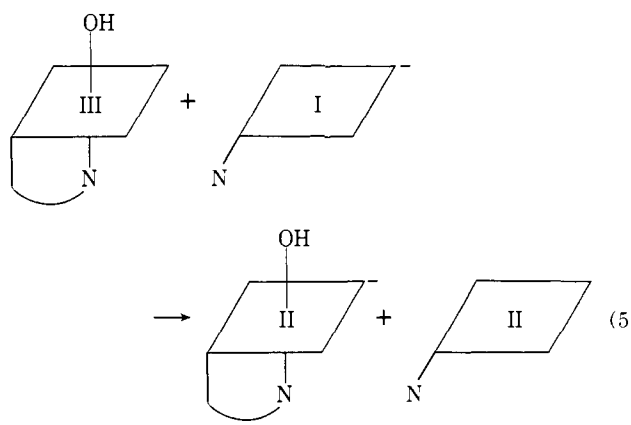
(4)

The predominant species of the B_{12} compounds are these. At pH 11–13, the predominant form of Co^{III}(corrin) is the six-coordinate complex, hydroxocobalamin, or vitamin B_{12b}.[20,21] The d^8 cobalt(I) complex, B_{12s}, exists as a four-coordinate complex in which the dimethylbenzimidazole is not coordinated.[17,18] The Co^{II}(corrin) exists as a rapidly equilibrating pair of four- and five-coordinate complexes, with the latter predominating by a factor of 62:1.[18,19]

Clearly a key question in the mechanism is: at what stage do the changes in coordination geometry accompanying reaction 4 occur? The mechanism consists of two parallel paths along which the transition states differ by a single proton or hydroxide.

Pathway a. Consider first parameter a, which characterizes the pathway with a rate independent of $[OH^-]$. Two mechanisms seem feasible, as follows (eq 5-12b).

Mechanism a-1



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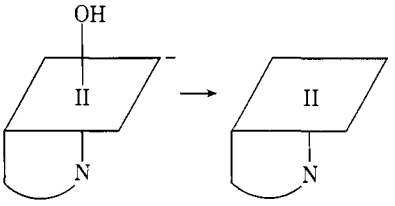

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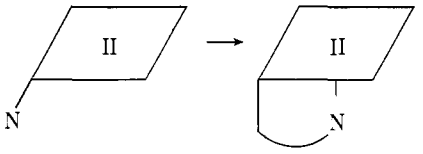
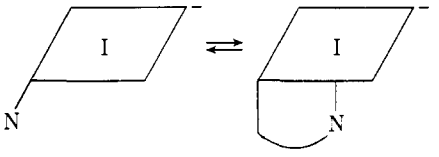


(7)

$$-d[B_{12S}]/dt = k_5[B_{12b}][B_{12S}] \quad (8)$$

Mechanism a-2



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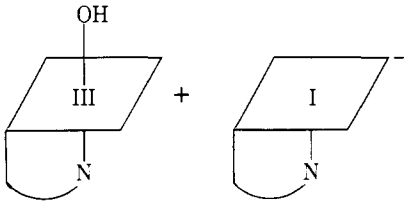

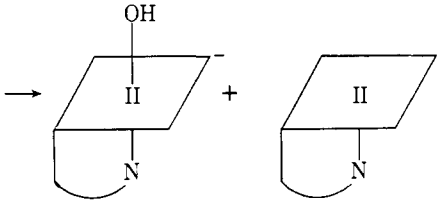

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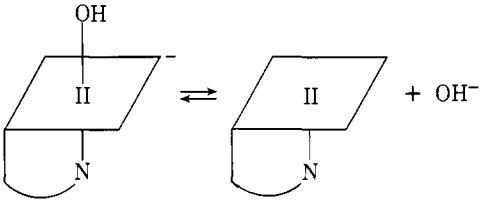


(11)

$$\frac{-d[B_{12S}]}{dt} = \left(\frac{k_9k_{10}}{k_{-9} + k_{10}[B_{12b}]}\right)[B_{12b}][B_{12S}] \quad (12a)$$

$$\frac{-d[B_{12S}]}{dt} \simeq \left(\frac{k_9k_{10}}{k_{-9}}\right)[B_{12b}][B_{12S}] \quad (12b)$$

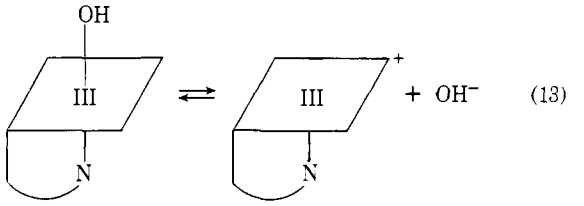
In both schemes 1 mol of Co(II) product emerges as the hydroxo complex, eq 5 and 10, which must subsequently dissociate by loss of OH^-, eq 6 and 11. The distinction between the two lies in whether the other cobalt coordinates axial dimethylbenzimidazole prior to reduction, as in eq 9, or after, as in eq 7. Since both mechanisms lead to the same rate law, no rigorous kinetic distinction is possible. Arguments based on rates and energetics lead us to favor a-1 over a-2, as follows.

Mechanism a-1 is characterized by $k_5 = 5.8 \times 10^3\ M^{-1}\ s^{-1}$ and also entails reaction 7 which poses neither an appreciable thermodynamic nor kinetic barrier:[18] $K_7 = 62$, $k_7 = 10^5\ s^{-1}$, $k_{-7} = 1.6 \times 10^3\ s^{-1}$.

The alternative mechanism a-2 requires in step 9 ligand coordination to the d^8 Co(I) complex prior to electron transfer. Taking $K_9 < 10^{-3}$ gives[22] $k_{10} > 5.8 \times 10^6\ M^{-1}\ s^{-1}$ and requires k_{-9} to be $>10\ s^{-1}$. There seems little reason to suggest this more drastic conformational change coupled with the requirement of faster electron transfer. For this reason, mechanism a-1 seems preferable to a-2, but the case is hardly a compelling one.

Pathway b. The inverse $[OH^-]$ dependence of b suggests either prior loss of OH^- (mechanism b-1) or conversion of hydroxocobalamin to aquocobalamin prior to electron transfer (mechanism b-2).

Mechanism b-1



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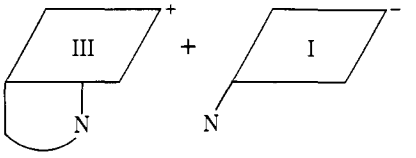

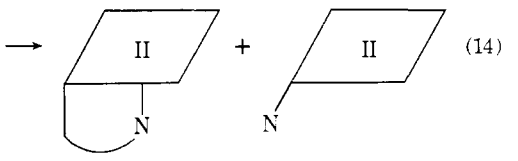

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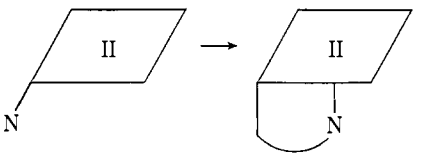
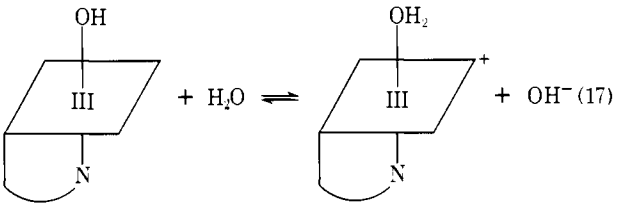


(15)

$$\frac{-d[B_{12S}]}{dt} = \left(\frac{k_{13}k_{14}}{k_{-13}[OH^-] + k_{14}[B_{12S}]}\right)[B_{12b}][B_{12S}] \quad (16a)$$

$$\frac{-d[B_{12S}]}{dt} \simeq \left(\frac{k_{13}k_{14}}{k_{-13}}\right)\frac{[B_{12b}][B_{12S}]}{[OH^-]} \quad (16b)$$

Mechanism b-2



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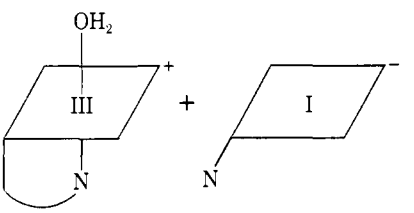

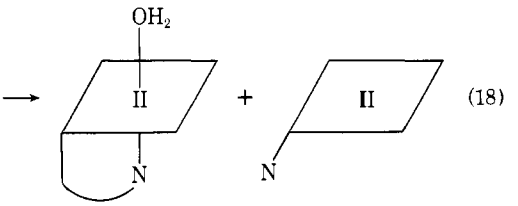

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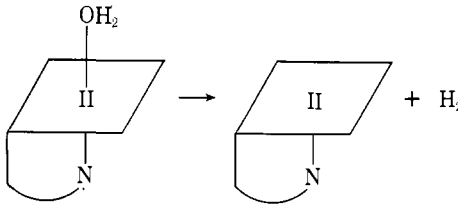

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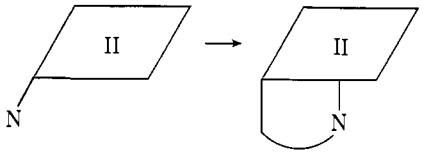

(20)

$$\frac{-d[B_{12s}]}{dt} = \left(\frac{k_{18}K_{17}}{[OH^-] + K_{17}}\right)[B_{12b}][B_{12s}] \quad (21a)$$

$$\frac{-d[B_{12s}]}{dt} \simeq k_{18}K_{17}\frac{[B_{12b}][B_{12s}]}{[OH^-]} \quad (21b)$$

The apparent first-order rate constant for hydroxide dissociation from hydroxocobalamin(III) has not been measured directly, but k_{13} can readily be inferred from data of Thusius[23,24] as 5×10^{-4} s^{-1}. Thus, from eq 16b and parameter b, the ratio $k_{14}/k_{-13} = 1.4 \times 10^5$. Reduction of the general rate law of eq 16a to form 16b, which is compatible with the experimental form, requires that k_{14}/k_{-13} be $\ll [OH^-]/[B_{12s}]$. In our kinetic experiments the latter ratio ranged broadly (during the first 2 half-lives) from 10 to 10^4. These requirements are mutually incompatible; mechanism b-1 is invalid.

Mechanism b-2 gives $b = k_{18}K_{17}$. The value of b, 71 s^{-1}, together with $K_{17} = 6.3 \times 10^{-7}$ M (from pK_a for aquocobalamin[18,21] and pK_w) afford $k_{18} = 1.1 \times 10^8$ M^{-1} s^{-1}.

Rates of electron transfer from B_{12s} to B_{12b} (k_5) and to B_{12a} (k_{18}) can be directly compared, and prove to be quite different: $k_{18}/k_5 = 1.9 \times 10^4$. If axial water is bound by cobalt(III) much less tightly than hydroxide ion is, then a lower transition-state energy might be expected considering that one feature of the activation is addition of an electron to an antibonding orbital which destabilizes axial coordination (and leads eventually to 5-coordination for Co(II)).

Disproportionation of B_{12r}. The reverse of eq 4 is of intrinsic interest, for it controls the rate of generation of reactive B_{12s}. Also, Yamada[14] has suggested that disproportionation of B_{12r} controls the formation of methylcobalamin (CH_3-B_{12}) from CH_3I and B_{12r}.

Application of the principle of microscopic reversibility affords the rate law in eq 22, valid at least under the range of concentrations employed here.

$$d[B_{12s}]/dt = (c[OH^-] + d)[B_{12r}]^2 \quad (22)$$

$$c = 1.3 \times 10^{-4}\ M^{-2}\ s^{-1}$$

$$d = 1.6 \times 10^{-6}\ M^{-1}\ s^{-1}$$

The reverse reaction occurs quite slowly. Even at 1 M OH^-, conversion of B_{12r} (say initially at 1×10^{-4} M) to CH_3-B_{12} would require 7.2 years (!) for 75% completion; the observed reaction reaches completion within 24 h.[14] Similar arguments against B_{12r} disproportionation as the route to CH_3-B_{12} were advanced by Birke and coworkers,[3] although without benefit of kinetic data.

Direct reaction of B_{12r} with CH_3I is required to account for the slow but appreciable formation of CH_3-B_{12}. Bläser and Halpern[25] find a direct reaction between B_{12r} and alkyl iodides, including CH_3I. The rate law is $k[CH_3I][B_{12r}]^2$. Clearly a mechanism other than disproportionation is responsible. Studies[26] of the disproportionation of Co(II)$(dmgH)_2$ and of its direct reaction with CH_3I support a similar formulation, although in this case disproportionation occurs more rapidly and the processes appear competitive.

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References and Notes

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