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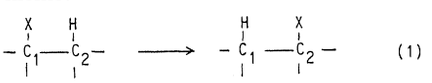
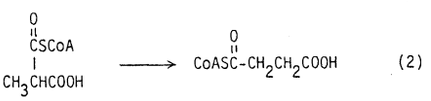
53. We are grateful to the National Science Foundation and the Petroleum Research Fund, administered by the American Chemical Society, for support of this work. We also thank A. T. Barfknecht and W. Tumas for their many suggestions and for their critical reading of the manuscript.

Mechanisms of Coenzyme B_{12}–Dependent Rearrangements

Jack Halpern

Coenzyme B_{12} (5′-deoxyadenosylcobalamin, abbreviated $AdCH_2$-B_{12}), whose structure is depicted in Fig. 1, serves as a cofactor for various enzymatic reactions (Table 1). [For comprehensive accounts of the chemistry and biochemistry of vitamin B_{12}, see (*1*, *2*).] A common feature of these reactions, depicted by Eq. 1, is the 1,2-interchange of a hydrogen atom and another substituent [X = OH, NH_2, C(=O)SCoA, $C(=CH_2)COOH$ or $CH(NH_2)COOH$] on adjacent carbon atoms of the substrate (*3*). Among these reactions, the methylmalonyl–coenzyme A (CoA) mutase rearrangement (Eq. 2) (*4*), whose mechanism is discussed in this article, is distinctive in that it plays a role in mammalian systems, whereas the other reactions occur only in microorganisms.


(1)


(2)

Enzymatic studies have provided convincing evidence for the essential features of the mechanistic scheme depicted by Fig. 2 (*5*–*7*). This mechanism encompasses the following sequence of steps: (i) enzyme-induced homolytic dissociation of the cobalt-carbon bond of coenzyme B_{12} to generate cob(II)alamin (also designated vitamin B_{12_r}) and a 5′-deoxyadenosyl radical (abbreviated $AdCH_2\cdot$), (ii) abstraction of a hydrogen atom (*8*) from the substrate to generate a substrate radical and 5′-deoxyadenosine ($AdCH_3$), (iii) rearrangement of the resulting substrate radical (either directly or through additional intermediate steps) to the corresponding product radical, and (iv) abstraction of a hydrogen atom from $AdCH_3$ by the product radical (*8*) to complete the rearrangement reaction.

The evidence (*5*–*7*) for this mechanistic scheme includes (i) the demonstration, with the use of deuterium tracers, that the migrating H atom is scrambled with the two methylene H atoms of $AdCH_2$-B_{12}, suggesting that the three H atoms become equivalent during the course of the reaction (consistent with the intermediate formation of $AdCH_3$), (ii) the direct spectroscopic (electronic and electron paramagnetic resonance) observation of the free radical intermediates (B_{12_r} and a carbon-centered free radical) in certain coenzyme B_{12}–dependent rearrangements (notably ethanolamine ammonia lyase and diol dehydrase); and (iii) the demonstration, in at least one case, of the reversible formation of $AdCH_3$ during the course of the enzymatic reaction.

Figure 2 represents the minimal mechanistic scheme that accommodates the above observations. One important feature not specifically depicted by this scheme is that both the coenzyme and the substrate are bound to the enzyme during the course of the reaction. This binding presumably dictates the chemical selectivity and regioselectivity of the H abstraction steps and the stereospecificity of the H-X interchange [for example, retention of the configuration at C-2 for methylmalonyl-CoA mutase (*4*) and inversion for diol dehydrase (*9*)]. The mechanism also may be incomplete in that there may be additional steps and intermediates, such as intermediate hydrogen carriers in the H transfer steps, as well as additional steps and intermediates (for example, carbonium ions, carbanions, or substrate-derived organocobalt complexes) in the rearrangement process itself (that is, the 1,2-migration of X).

Summary. Coenzyme B_{12} serves as a cofactor in various enzymatic reactions in which a hydrogen atom is interchanged with a substituent on an adjacent carbon atom. Measurement of the dissociation energy of the coenzyme's cobalt-carbon bond and studies of the rearrangement of model free radicals related to those derived from methylmalonyl–coenzyme A suggest that these enzymatic reactions occur through homolytic dissociation of the coenzyme's cobalt-carbon bond, abstraction of a hydrogen atom from the substrate by the coenzyme-derived 5′-deoxyadenosyl radical, and rearrangement of the resulting substrate radical. The only role thus far identified for coenzyme B_{12} in these reactions—namely, that of a free radical precursor—reflects the weakness, and facile dissocation, of the cobalt-carbon bond.

In this article, the following themes that relate to the mechanistic scheme of Fig. 2 for coenzyme B_{12}–dependent rearrangements are discussed: (i) the cobalt-carbon bond dissociation energy of coenzyme B_{12}, (ii) the factors that influence cobalt-carbon bond dissociation energies and that may contribute to the enzyme-induced bond weakening and dissocia-

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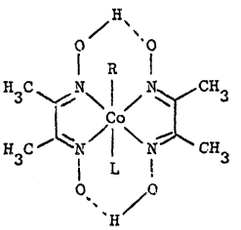
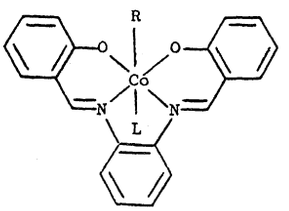
tion, (iii) the mechanism of the 1,2-migration of X, (iv) the role of the coenzyme and, in particular, of the cobalt atom, and (v) the role of the enzyme.

In addition to the enzymatic studies cited above, various studies on model systems (involving modeling of both the coenzyme and substrates) have been important in elucidating aspects of the chemistry of coenzyme B_{12} and the mechanisms of its rearrangement reactions (*10*). Examples of organocobalt compounds that have been widely invoked as coenzyme B_{12} models in such studies include alkyl derivatives of bis(dimethylglyoximato)cobalt (**1**) (abbreviated $[RCo(DH)_2L]$, where DH_2 is dimethylglyoxime and L is an axial ligand such as water, pyridine, or a tertiary phosphine), and cobalt complexes of Schiff bases such as bis(salicyclaldehyde)phenylenediimine (**2**) (abbreviated [RCo(saloph)L]). With respect to properties that are recognizably relevant to the biochemical roles of coenzyme B_{12} (for example, redox properties and cobalt-carbon bond dissociation energies), the cobalt–Schiff base complexes appear to come closest to providing a model for coenzyme B_{12} (*10*).



$[RCo(DH)_2L]$

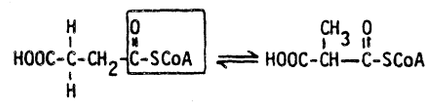
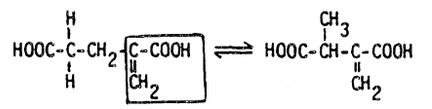
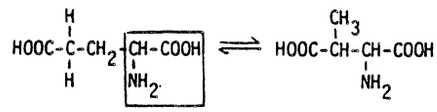
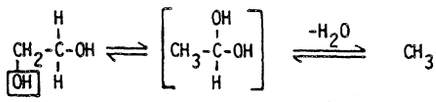
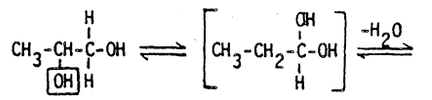
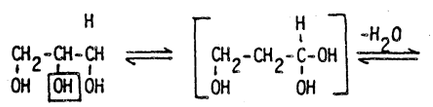
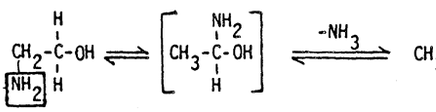
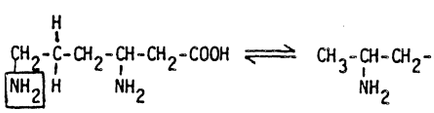
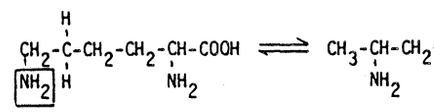
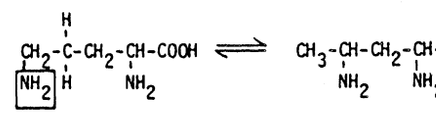
1



[RCo(saloph)L]

2

Table 1. Coenzyme B_{12}-Dependent Rearrangements*

Reaction	Enzyme
	Methylmalonyl-CoA mutase
	α-Methyleneglutarate mutase
	Glutamate mutase
	Diol dehydrase
	Diol dehydrase
	Glycerol dehydrase
	Ethanolamine ammonia lyase
	L-β-Lysine mutase
	D-α-Lysine mutase
	Ornithine mutase

*The migrating group that interchanges with an adjacent hydrogen atom is shown enclosed in a rectangle.

Cobalt-Carbon Bond Dissociation Energy of Coenzyme B_{12}

The role of coenzyme B_{12}, encompassed by the mechanism of Fig. 2, implies a very weak cobalt-carbon bond. A troublesome feature of this mechanistic scheme has been the absence until recently of precedents for such weak transition metal-alkyl bonds or, indeed, knowledge of transition metal-alkyl bond dissociation energies in general (*11*).

We have recently accomplished the measurement of the cobalt-carbon bond dissociation energy ($D_{Co\text{-}CH_2Ad}$) of coenzyme B_{12} by determining the kinetics of the bond dissociation process in aqueous solution. For this purpose, we used $[Co^{II}(DH)_2(H_2O)]$ (which forms a stronger Co-C bond than B_{12r}) to trap the $AdCH_2\cdot$ radical, in accord with Eqs. 3 to 5 (*12*).

$$AdCH_2\text{-}B_{12} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} AdCH_2\cdot + B_{12r} \quad (3)$$

$$AdCH_2\cdot + [Co^{II}(DH)_2(H_2O)] \xrightarrow{k_4} [AdCH_2\text{-}Co(DH)_2(H_2O)] \quad (4)$$

$$AdCH_2\text{-}B_{12} + [Co^{II}(DH)_2(H_2O)] \longrightarrow [AdCH_2\text{-}Co(DH)_2(H_2O)] + B_{12r} \quad (5)$$

Application of the steady-state approximation to this reaction sequence

yields the rate law corresponding to Eq. 6. Fitting our kinetic data (*12*) to Eq. 6 yielded $k_3 = 1.0 \times 10^{-4}$ sec^{-1} and $k_{-3}/k_4 = 1.1$ at 100°C. Measurement of the temperature dependence of k_3 yielded (after correction for the equilibrium involving dissociation of the pendant axial 5,6-dimethylbenzimidazole ligand to form the unreactive "base-off" form) $\Delta H_3^{\ddagger} = 28.6$ kcal/mol and $\Delta S_3^{\ddagger} = 2$ cal mol^{-1} K^{-1}. Earlier demonstrations that the recombinations of B_{12_r} with various free radicals including $AdCH_2\cdot$ are diffusion-controlled (*13*) permit the value of $\Delta H_{-3}^{\ddagger}$ to be estimated as about 2 kcal/mol. These data yield a value of about 26 kcal/mol for the Co-C bond dissociation energy of coenzyme B_{12} ($D_{Co\text{-}CH_2Ad} = \Delta H_3^{\ddagger} - \Delta H_{-3}^{\ddagger}$) in aqueous solution (*14*).

$$\frac{-d[AdCH_2\text{-}B_{12}]}{dt} = \frac{k_3k_4[AdCH_2\text{-}B_{12}][Co^{II}(DH_2)(H_2O)]}{k_{-3}[B_{12_r}] + k_4[Co^{II}(DH)_2(H_2O)]} \quad (6)$$

Factors Influencing Cobalt-Carbon Bond Dissociation Energies

Although the Co-C bond of coenzyme B_{12} is weak compared with typical covalent bonds in organic molecules, the value of k_3 at 30°C (that is, the rate constant for dissociation of the Co-C bond of the free coenzyme), calculated from the above values of $\Delta H_3^{\ddagger}$ and $\Delta S_3^{\ddagger}$, is only about 10^{-7} sec^{-1}. This is approximately 10^{-9} times ($\Delta\Delta G^{\ddagger} \sim 13$ kcal/mol) the values of the catalytic rate constants ($k_{cat} \sim 10^2$ sec^{-1}) that have been estimated for several coenzyme B_{12}–dependent enzymatic reactions, including

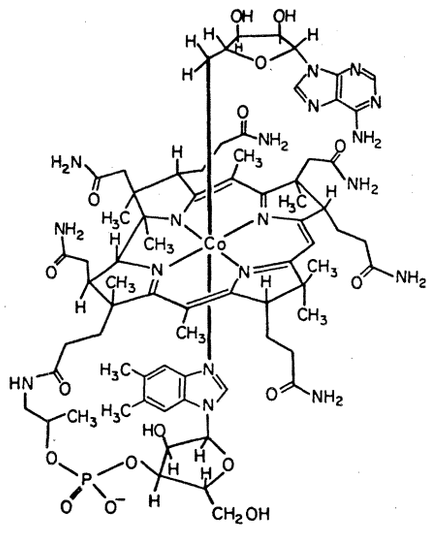


Fig. 1. Coenzyme B_{12}.

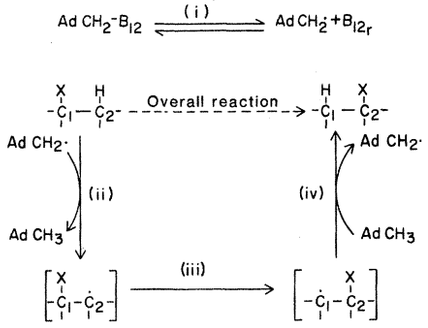


Fig. 2. Mechanistic scheme for coenzyme B_{12}–dependent rearrangements.

methylmalonyl-CoA mutase (*4*, *9*, *15*). Even though this discrepancy will be offset by the chain length of the catalytic cycle that is initiated by cleavage of the coenzyme Co-C bond, it is unlikely that the chain length is as high at 10^9. Thus, considerable further weakening of the Co-C bond, by interaction with the enzyme, appears to be required to achieve dissociation rates that are compatible with the enzymatic rates.

To identify the factors that might be responsible for this bond weakening and dissociation, we examined the influence of various electronic and steric parameters on the Co-C bond dissociation energies of some coenzyme B_{12} model compounds including [R-Co(DH)$_2$L] (**1**) and [R-Co(saloph)L] (**2**) (*16*). Values of $D_{Co\text{-}R}$ for [R-Co(saloph)L] were deduced from kinetic measurements analogous to those described above for coenzyme B_{12}, but with *n*-$C_8H_{17}SH$ used as the

$$[C_6H_5(CH_3)CH\text{-}Co(DH)_2L] \rightleftharpoons [Co^{II}(DH)_2L] + C_6H_5CH{=}CH_2 + \tfrac{1}{2}H_2 \quad (7)$$

$$C_6H_5CH{=}CH_2 + \tfrac{1}{2}H_2 \rightleftharpoons C_6H_5\dot{C}HCH_3 \quad (8)$$

$$[C_6H_5(CH_3)CH\text{-}Co(DH)_2L] \rightleftharpoons [Co^{II}(DH)_2L] + C_6H_5\dot{C}HCH_3 \quad (9)$$

radical trap (*17*). Values of the Co-C bond dissociation energies of a series of [$C_6H_5(CH_3)$CH-Co(DH)$_2$L] compounds were deduced either from kinetic measurements (*18*) or, in some cases, from measurements of equilibrium constants and of the enthalpy (ΔH_7°) of the reversible reaction depicted by Eq. 7, in combination with published data for ΔH_8° (−2.2 kcal/mol) (*19*). Thus, $D_{Co\text{-}R} \approx \Delta H_9^{\circ} \approx \Delta H_7^{\circ} + \Delta H_8^{\circ} \approx \Delta H_7^{\circ} - 2.2$ kcal/mol. The values of $D_{Co\text{-}R}$ resulting from these measurements are given in Table 2.

The Co-R bond dissociation energies listed in Table 2 range from 17 to 25 kcal/mol, approaching the value deduced above for coenzyme B_{12} itself. The influence of electronic factors is most clearly revealed by the values of $D_{Co\text{-}R}$ for [$C_6H_5(CH_3)$CH-Co(DH)$_2$L] where L is pyridine (py) or a para-substituted pyridine (so that steric influences are constant) (*13*). For this series, $D_{Co\text{-}R}$ increases systematically with the basicity of the axial pyridine ligand, as depicted also by the plot of $D_{Co\text{-}R}$ versus pK_a in Fig. 3. The probable explanation for this trend is that dissociation of the Co-C bond, in accordance with the generalized representation of Eq. 10, involves reduction of the cobalt—that is, a decrease in the formal oxidation state of cobalt from +3 to +2. Thus, more basic ligands are expected to stabilize the parent cobalt(III)-alkyl relative to the cobalt(II) dissociation product and, hence, to increase the bond dissociation energy.

$$[L_5Co^{III}\text{-}R^-] \rightleftharpoons [L_5Co^{II}] + R\cdot \quad (10)$$

Comparisons of some of the data in Table 2 reveal the importance of steric influences on Co-R bond dissociation energies. Thus the large (~7 kcal/mol) difference between the Co-C bond dissociation energies of [$CH_3CH_2CH_2$-Co(saloph)py] and [$(CH_3)_3CCH_2$-Co(saloph)py] presumably is due largely to steric factors. The important influence of steric factors also is revealed by the trend of values of $D_{Co\text{-}R}$ for the series of [$C_6H_5(CH_3)$CH-Co(DH)$_2$(PR_3)] compounds that exhibit a marked inverse dependence (Fig. 4) on the size [as measured by the "cone angle" (*20*)] of the phosphine ligand. Thus, $D_{Co\text{-}R}$ ranges

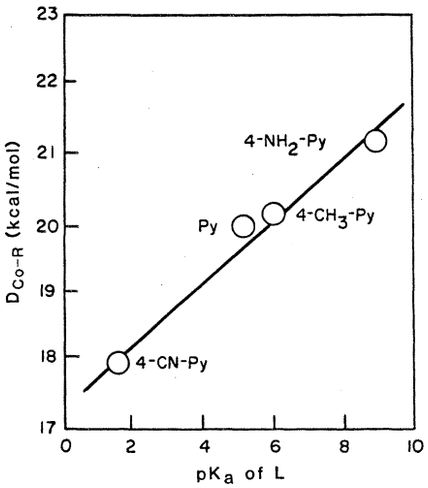


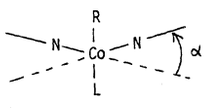
Fig. 3. Dependence of the Co-C bond dissociation energy of [$C_6H_5(CH_3)$CH-Co(DH)$_2$L] on the pK_a of L (*19*).

Table 2. Cobalt-carbon bond dissociation energies.

Cobalt alkyl	D_{Co-R} (kcal/mol)	Reference
$[CH_3CH_2CH_2$-Co(saloph)(py)]	25	(*17*)
$[(CH_3)_2CH$-Co(saloph)(py)]	20	(*19*)
$[(CH_3)_3CCH_2$-Co(saloph)(py)]	18	(*19*)
$[C_6H_5CH_2$-Co(saloph)(py)]	22	(*19*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(4$-NH_2-py)]	21	(*19*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(4$-CH_3-py)]	20	(*19*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2$(py)]	20	(*19*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(4$-CN-py)]	18	(*19*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2$(imidazole)]	21	(*19*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(PMe_2Ph)]$	24	(*18*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(P(CH_2CH_2CN)_3)]$	20	(*18*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(PBu^n_3)]$	21	(*18*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(PEtPh_2)]$	19	(*18*)
$[C_6H_5(CH_3)CH$-Co$(DH)_2(PPh_3)]$	17	(*18*)
Ado-CH_2-B_{12}	26	(*12*)

from about 24 kcal/mol for L = PMe_2Ph (cone angle 122°) to 17 kcal/mol for L = PPh_3 (cone angle 145°).

Evidence for the importance of steric influences on Co-C bond stability also is provided by the results of x-ray structural determinations on a series of [R-Co$(DH)_2$L] compounds (Table 3) (*21*). These measurements reveal significant lengthening of the Co-C bond with increasing steric bulk of R and L, as well as significant sterically induced conformational distortions from planarity of the bis(dimethylglyoximate) structure, reflected in variations of the dihedral angle α between the planes of the two dimethylglyoximate ligands (**3**).

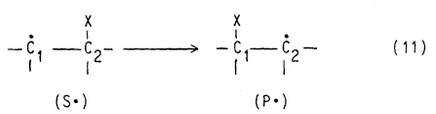


3

The structure of coenzyme B_{12}, as revealed by x-ray diffraction studies, shows evidence of similar steric crowding. The Co-C bond is quite long (2.05 angstroms) and the Co-C-C bond angle of 125° is much larger than the tetrahedral value of 109.5°, apparently reflecting repulsions between the 5′-deoxyadenosyl group and substituents on the corrin ring (*16*). Consistent with this is the identification of several close contacts (~3 Å) between atoms of the 5′-deoxyadenosyl group and atoms of the corrin ring and its substituents (*22*). In light of these considerations it seems highly likely that the enzyme-induced coenzyme Co-C bond weakening is due to steric influences—namely, an upward conformational distortion of the corrin ring that increases the steric repulsion of the 5′-deoxyadenosyl substituent and induces dissociation of the Co-C bond (*22, 23*). The results of the cited structural and bond dissociation studies on coenzyme B_{12} model compounds support the plausibility of this view and suggest that only a modest distortion of the already crowded coenzyme molecule is sufficient to effect the necessary Co-C bond weakening.

Mechanism of Substrate Rearrangement

The least well understood and most controversial aspect of the mechanism of coenzyme B_{12}–dependent rearrangements continues to be the mechanism of the rearrangement step itself—that is, of the 1,2-migration of X (Eq. 11).



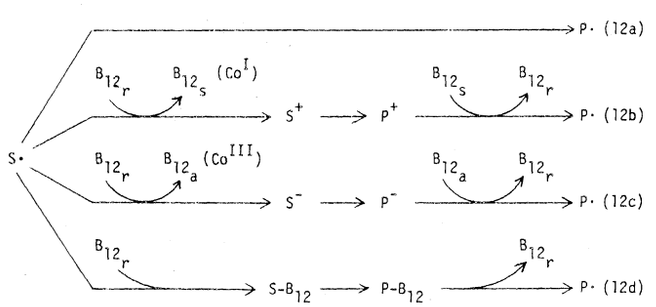
(11)

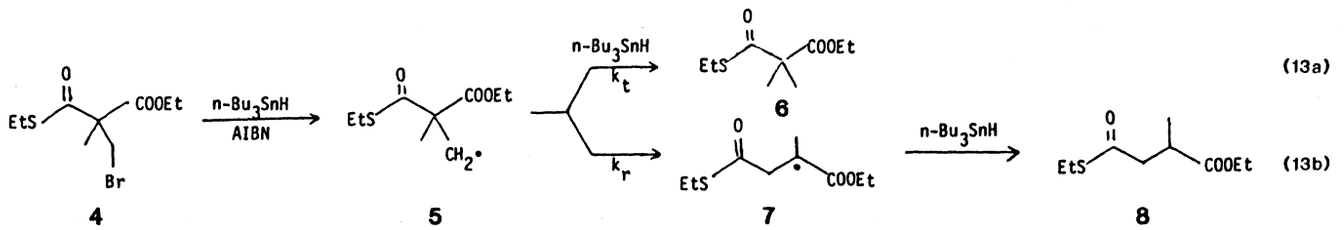
The mechanism depicted by Fig. 2 implies that the rearrangement is triggered by H atom abstraction from the substrate to generate the substrate radical, S· but does not require that the rearrangement involving the 1,2-migration of X (to yield, ultimately, the product radical P·) actually occur at the free radical stage. Possible alternatives to such a direct rearrangement (that is, to Eq. 12a) include rearrangement via intermediate carbonium ions or carbanions (generated by oxidation or reduction of S· by B_{12_r}) or via an organocobalt intermediate formed by combination of S· with B_{12_r} (Eqs. 12b, 12c, and 12d), respectively).

Free radical rearrangement pathways. One problem with the proposal of rearrangement at the initially formed free radical stage (Eq. 12a) is that such a 1,2-migration in a free radical is precedented for only one of the coenzyme B_{12} substrates, α-methyleneglutarate, which involves migration of a substituted vinyl group [–C(=CH_2)COOH] (*24*). For the other B_{12} substrates, migration of X [–OH, $-NH_2$, –C(=O)SCoA or $-CH(NH_2)$COOH] has not previously been observed in model free radicals. However, it should be noted that those coenzyme B_{12}–dependent reactions whose rates have thus far been reported are fairly slow ($k_{cat} \approx 10^2$ sec^{-1} for methylmalonyl-CoA mutase and diol dehydrase) (*4, 9, 15*). Radical rearrangement processes compatible with this time scale may well have escaped detection in earlier studies of free radical rearrragements, most of which were restricted to much shorter time scales.

Testing whether a given substrate radical, S· (or appropriate model thereof) would rearrange spontaneously on the time scale of coenzyme B_{12}–dependent reactions required generating the free radical unambiguously, under conditions in which its lifetime was fairly long ($\geq 10^{-2}$ second) and, preferably, susceptible to measurement and systematic variation. Furthermore, it seemed preferable to accomplish this in the absence of any cobalt complexes to eliminate the issue of possible cobalt participation in such rearrangements.

We have recently accomplished this for a radical EtSC(=O)C(CH_3)(CH_2·)COOEt (**5**) that models the substrate radical of the methylmalonyl-CoA mutase reaction [that is, CoASC(=O)CH(CH_2·)COOH] (*25*). The procedure used was an adaptation of the one used earlier by Walling and Cioffari (*26*) to study the rearrangement of the 5-hexenyl radical. The model radical **5** was generated from the corresponding bromide **4** by reaction with *n*-Bu_3Sn· [generated by reaction of *n*-Bu_3SnH with 2,2′-azobisisobutyronitrile (AIBN)] and the competition between direct trapping with *n*-Bu_3SnH (k_t) to yield **6** and





rearrangement (k_r), followed by trapping of the rearranged radical **7** to yield **8** as shown in Eq. 13, was monitored as a function of the initial n-Bu_3SnH concentration. Only the direct trapping product (**6**) and that resulting from 1,2-migration of the thioester group (**8**) were formed, and they were obtained together in essentially quantitative yield. No other product, notably that resulting from migration of the ester group, were detected.

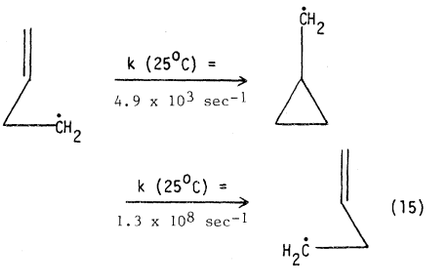
According to the scheme of Eq. 2

$$\frac{d[\mathbf{6}]}{d[\mathbf{8}]} = \frac{k_t[n\text{-}Bu_3SnH]}{k_r} \quad (14)$$

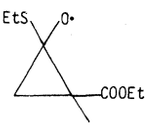
Fitting the results of our measurements to Eq. 14 in combination with published data for k_t (*27*) yielded the values k_r (60.5°C) = 24 sec^{-1}, $\Delta H_r^{\ddagger}$ = 13.8 kcal/mol, and $\Delta S_r^{\ddagger} = -11$ cal mol^{-1} K^{-1}. The value of k_r at 30°C, calculated from these activation parameters, is 2.5 sec^{-1}. This is about 1/40 of the estimated value of k_{cat} for the methylmalonyl-CoA mutase reaction. However, this relatively modest rate difference could well be accommodated by the chemical and structural differences between the model radical **5** and the methylmalonyl-CoA mutase radical, as well as by effects of interaction of the (enzyme-bound) substrate with the enzyme—for example, hydrogen bonding to the sulfur atom or conformational influences.

Related experiments in which the carbanion corresponding to **5**—namely, $EtSC(=O)C(CH_3)(CH_2^-)COOEt$, was generated by reduction of **4** with sodium naphthenide, revealed that rearrangement, while rapid, was less selective than for the free radical, yielding products resulting from migration of both the thioester and ester groups, together with other unidentified products (*25*). The formation of such a substrate carbanion [presumably by electron transfer between the initially formed B_{12_r} and substrate radical (Eq. 12c)] is expected to be highly unfavorable and thus to constitute a much less likely pathway than the alternative free radical rearrangement process that now has been shown to be a chemically viable pathway.

As already noted, rearrangement at the free radical stage [involving 1,2-migration of the $-C(=CH_2)COOH$ group] also seems likely for the α-methyleneglutarate mutase reaction, since analogous 1,2-migrations of vinyl groups in related radicals, via intermediate cyclopropylmethyl radicals, are well documented (for example, Eq. 15) (*28*).



It is likely that 1,2-migration of the thioester group proceeds through the analogous cyclopropyloxy radical **9**.



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Carbonium ion rearrangements. The facile 1,2-migration of saturated groups, such as OH, NH_2, and $CH(NH_2)COOH$, in free radicals is neither precedented nor is it expected on theoretical grounds.

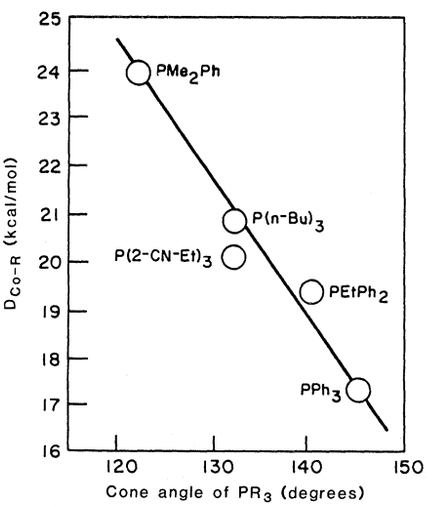
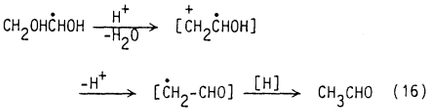


Fig. 4. Dependence of the Co-C bond dissociation energy of $[C_6H_5(CH_3)CH\text{-}Co(DH)_2(PR_3)]$ on the cone angle of PR_3 (*18*).

Acid-promoted dehydration of 1,2-diol radicals—for example, of 1,2-dihydroxyethyl to form acetaldehyde (Eq. 16)—has been observed (*29*) and has been invoked as a model of the dioldehydrase reaction (*4, 30, 31*). However, it is unlikely that such dehydration proceeds by 1,2-OH migration as does the enzymatic reaction [although it has been suggested that such migration may be accomplished at the radical stage through a sequence involving dehydration of the bound $CH_2(OH)\dot{C}HOH$ radical and rehydration of the resulting bound $\dot{C}H_2CHO$ radical (*30*)].



Since 1,2-migration of OH or NH_2 in carbonium ions is expected to be a facile process, the alternative possibility of mechanisms corresponding to Eq. 12b warrants consideration. We have previously proposed such a mechanism for enthanolamine ammonia lyase (*32*), which is adapted in Fig. 5 for the corresponding diol dehydrase reaction. Steps i, ii, and vii of this mechanism correspond to steps i, ii, and iv of the mechanistic scheme of Fig. 2, and the other steps depict the carbonium ion rearrangement. Although the initial heterolytic cleavage of the coenzyme B_{12} Co-C bond to form the unstabilized primary $AdCH_2^+$ carbonium ion is highly unfavorable, the considerably enhanced stability of the oxocarbonium ion, $CH_2(OH)\overset{+}{C}HOH$, could provide the necessary driving force for the proposed electron transfer (step iii) after the H-atom transfer (step ii). Conversely, the ensuing 1,2-OH shift and dehydration to form the unstable $\overset{+}{C}H_2CHO$ ion, would generate a driving force for reversal of the electron transfer step (that is, for step vi). Direct evidence for such a mechanistic scheme and, in particular, for the intermediacy of vitamin B_{12_s} (Co^I) in any coenzyme B_{12}–dependent reaction is at present lacking.

Rearrangement via organocobalt intermediates. Other mechanistic proposals have invoked the intermediacy of organocobalt adducts, arising from Co-C

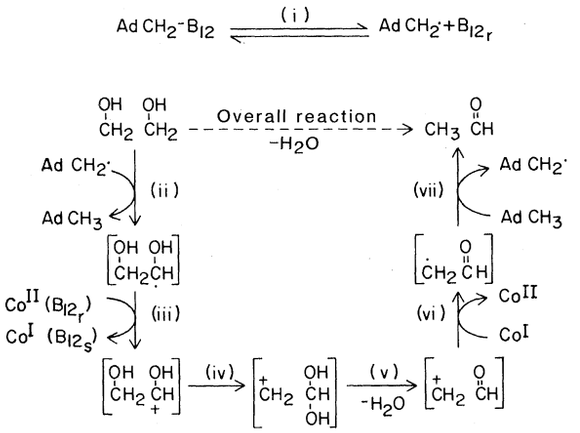
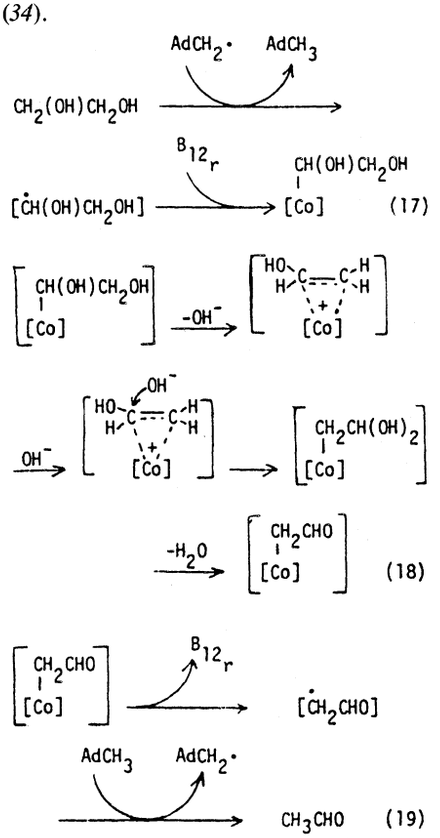


Fig. 5. Possible mechanism of the diol dehydrase rearrangement.

Table 3. Structural data for [R-Co(DH)$_2$L] compounds (*21*).

R	L	Co-C (Å)	Co-L (Å)	α (degrees)
CH_3	H_2O	1.990	2.058	−4
CH_3	Pyridine	1.998	2.068	+3.2
CH_3	PMe_3	2.015	2.294	+4.0
CH_3	PPh_3	2.026	2.418	+14.0
$CH_2C(CH_3)_3$	H_2O	2.044	2.056	−7
$CH_2C(CH_3)_3$	Pyridine	2.060	2.081	−5.2
$CH_2C(CH_3)_3$	PMe_3	2.084	2.316	−5
$CH_2C(CH_3)_3$	PPh_3	2.118	2.460	+2
$CH(CH_3)_2$	Pyridine	2.085	2.099	+4

bond formation between B_{12_r} and the substrate radical (*33–36*). One such proposal for the diol dehydrase reaction, which derives some support from model experiments, is depicted by Eqs. 17 to 19 (*34*).



Assessment of the validity of this mechanistic proposal, as well as that of Fig. 5, must await further evidence. In this connection, recent model studies suggest that Co-CH_2CHO is not an intermediate in the formation of CH_3CHO (*30*).

Several model experiments, such as those depicted by Eqs. 20 to 22, have been reported, that attempt to probe the possible role of organocobalt intermediates in B_{12}-dependent rearrangements by generating organocobalt adducts containing cobalt-bonded groups intended to model those derived from coenzyme B_{12} substrates (*37–39*).

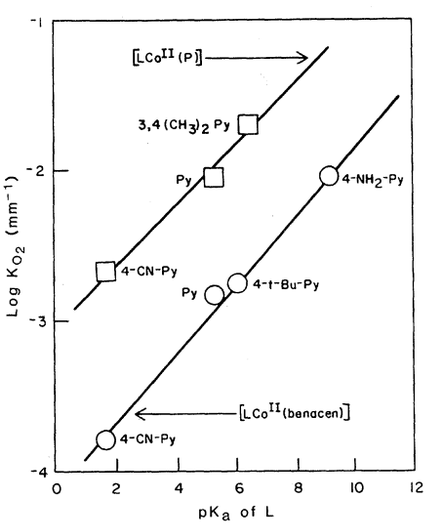
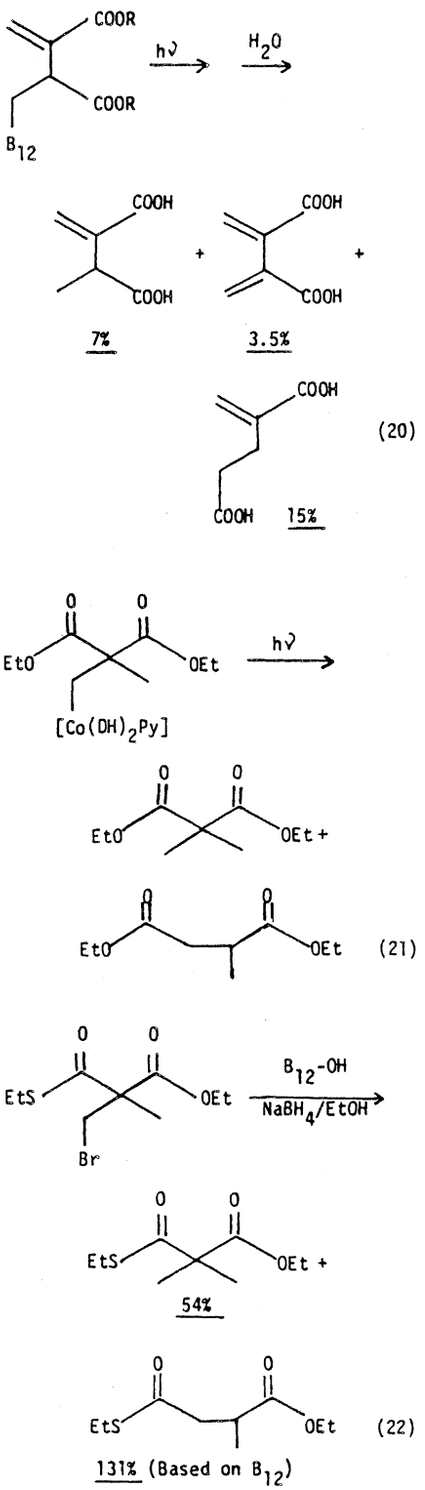


Fig. 6. Effect of the axial ligand (L) on the equilibrium constant for the reversible binding of O_2 to protoporphyrin IX ester cobalt(II) [LCo^{II}(P)] and *N,N'*-ethylenebis(benzoylacetiminato)-cobalt(II) [LCo^{II}(benacen)] in toluene (*42, 43*).



Decomposition of such adducts under various conditions (photochemical, thermal, reductive) did, in some cases, lead to products that result from 1,2-migration, for example, of –C(=CH_2)COOR, –COOEt, and –COSEt in the examples

of Eqs. 20, 21, and 22, respectively (*31–39*). Although such observations have been interpreted as supporting the role of a substrate-derived organocobalt intermediate in related coenzyme B_{12}–dependent rearrangements (*37, 38*) this conclusion does not seem warranted. Thus, it is possible that the deliberately synthesized organocobalt compounds in these experiments serve simply as precursors of organic free radicals that are generated by homolysis of the cobalt-carbon bonds (for example, photochemically in Eqs. 20 and 21) or of carbanions generated under reducing conditions (Eq. 22).

Several other mechanistic proposals that have been advanced for coenzyme B_{12}–dependent rearrangements invoke substrate-derived organocobalt intermediates but do not include the cobalt-carbon homolysis step and the free radical intermediates that characterize the mechanistic scheme of Fig. 2 and its variants (*40, 41*). These proposals are not readily reconciled with the available evidence. Indeed, at this stage there is no convincing evidence, from either enzymatic or model system studies, for the formation of substrate-derived organocobalt adducts in coenzyme B_{12}–dependent rearrangements or for any role of such adducts in the rearrangement mechanisms.

Concluding Remarks

A combination of enzymatic and chemical studies, including studies on model systems, has resulted in considerable progress toward establishing the validity of the mechanistic scheme depicted by Fig. 2. At least for methylmalonyl-CoA mutase and α-methyleneglutarate mutase it seems likely that the substrate rearrangement step itself (the migration of X) occurs at the free radical stage. For other substrates the actual rearrangement mechanism remains to be elucidated.

The principal, if not the only, role of coenzyme B_{12} in these enzymatic processes appears to be that of a free radical precursor, a role that utilizes the weakness of the cobalt-carbon bond. The use of an organometallic molecule for this purpose seems appropriate since it is difficult to conceive of a stable organic molecule that would undergo thermal dissociation under such mild conditions to generate a highly reactive primary radical. At this stage there is no convincing evidence that the coenzyme or the cobalt atom plays any other role—for example, that of mediating the rearrangement step itself.

The reversible cobalt-carbon bond dissociation of coenzyme B_{12} and related organocobalt compounds, depicted by Eq. 10, corresponds formally to an inner sphere redox process. There is at least a formal analogy between this process and the reversible binding of dioxygen by cobalt(II) and iron(II) complexes (such as myoglobin) as shown in Eqs. 23 and 24. This parallel is quite far-reaching and is reflected in trends in the dependence of the Co-O_2 (and presumably Fe-O_2) bond dissociation free energies and enthalpies that parallel those of Co-R bond dissociation energy trends, as revealed by a comparison of Figs. 3 and 6 (*42, 43*). Indeed, typical Co-O_2 bond dissociation energies in such reversible dioxygen carriers lie in the range 10 to 20 kcal/mol, which is not far from the range of typical cobalt-alkyl bond dissociation energies.

$$[L_5Co^{III}\text{-}O_2^{\cdot-}] \rightleftharpoons [L_5Co^{II}] + O_2 \quad (23)$$

$$[L_5Fe^{III}\text{-}O_2^{\cdot-}] \rightleftharpoons [L_5Fe^{II}] + O_2 \quad (24)$$

In the light of these considerations, the role of coenzyme B_{12} in biological systems may be described as that of a "reversible free radical carrier," analogous to the role of myoglobin or hemoglobin as a "reversible dioxygen carrier." Thus, coenzyme B_{12} fulfills its biochemical role by serving as a "free radical reservoir" from which 5′-deoxyadenosyl radicals are reversibly released under mild conditions, just as oxyhemoglobin serves as a reservoir for the storage and reversible release of dioxygen. Significant questions that warrant further attention relate to the alternative choices of cobalt and iron, as well as of the corrin and porphyrin ligand systems, for these parallel functions.

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