The Vitamin B_{12} Coenzyme

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Vitamin B_{12} (cyanocobalamin; Figure 1, R = CN) was simultaneously isolated by Folkers[1] and Smith[2] in 1948, two decades after Minot and Murphy[3] reported the effectiveness of whole liver in the treatment of pernicious anaemia, a disease which is today effectively controlled by a 100-μg injection of B_{12}. The structure of vitamin B_{12}, the most complex nonpolymeric compound found in nature, was revealed by the crystallographic work of Hodgkin[4] aided by the chemical studies of Todd and Johnson.[5] It then came as a surprise when Barker[6] reported that a biochemically active form of the cobalamin (the vitamin B_{12} coenzyme) contained an adenine nucleoside, and that vitamin B_{12} was in fact an artifact produced, during its isolation, by reaction with cyanide ion.

The additional instability of the coenzyme toward light and acid[7] suggests why the coenzyme form of vitamin B_{12} remained undiscovered for as long as it did. As with vitamin B_{12}, the structure of the coenzyme was elucidated through the crystallographic studies of Hodgkin,[8] who showed that the general macrocyclic structure and peripheral substituents were the same for both cyanocobalamin and the vitamin B_{12} coenzyme and also demonstrated, a unique feature of the coenzyme, the covalent bond between cobalt and the 5′ carbon of an adenine moiety. This was the first example of a naturally occurring organometallic compound. Indeed to this day the vitamin B_{12} coenzyme and related alkylcobalamins represent the only known organometallic compounds of nature.

While the crystallographic studies elucidated the major structural features of the vitamin B_{12} coenzyme, they left open the possibility that the extent of the conjugated chromophore might be different in the coenzyme from that of B_{12} itself. This difference in the extent of oxidation of the chromophore was

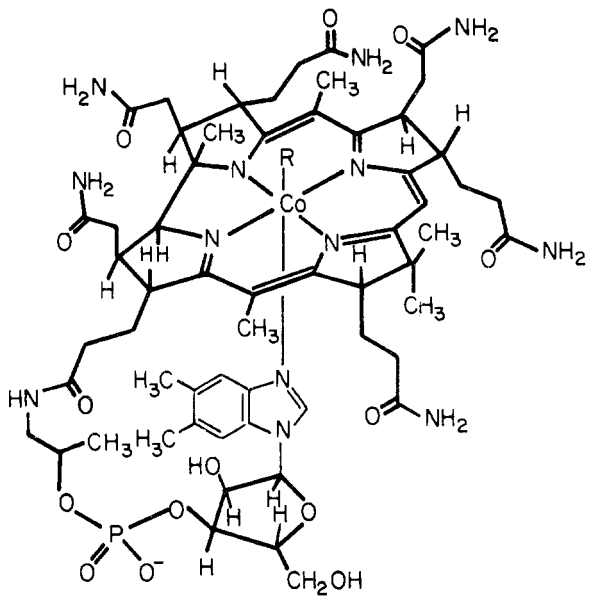

Figure 1.

suggested by studies on the formation of the coenzyme from vitamin B_{12}[9] and would have been consistent with the considerable differences in the optical spectra (Figure 2) of the coenzyme (orange-yellow) and B_{12} (red-purple).[10] The degree of unsaturation of the corrin chromophore was related to, and further complicated by, the oxidation state of the cobalt which is trivalent (diamagnetic) in vitamin B_{12}, but which had been reported to be paramagnetic by some and diamagnetic by others in the coenzyme. At this time the coenzyme had been prepared by an initial reduction of cyano- or hydroxocobalamin followed by alkylation with a suitable derivative of 5′-deoxyadenosine.[11] Thus the mode of formation did not define the oxidation state of the cobalt, and allowed for the possible reduction of the chromophore during formation of the coenzyme.

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David Dolphin was born in London in 1940 and for the next 5 years the city suffered the greatest damage in its long history. After high school education in London, he moved to the University of Nottingham where he received both his B.Sc. and Ph.D. degrees working with A. W. Johnson. Around this time Alan Johnson moved to Sussex; left with nowhere to go Dolphin moved to Harvard as a postdoctoral fellow with R. B. Woodward. After a further 8 years as a member of the Harvard chemistry department faculty, he left to take up his present position as associate professor at the University of British Columbia. The structure, synthesis, chemistry and biochemistry of porphyrins, vitamin B_{12}, and related macrocycles continue to be the main focus of his research.

(1) E. L. Rickes, N. G. Brink, F. R. Konivszy, T. R. Wood, and K. Folkers, *Science*, **107**, 396 (1948).
(2) E. L. Smith and L. F. J. Paker, *Biochem. J.*, **43**, viii (1948).
(3) G. R. Minot and W. P. Murphy, *J. Am. Med. Assoc.*, **87**, 470 (1926).
(4) D. C. Hodgkin, J. Kamper, M. Mackay, J. W. Pickworth, K. N. Trueblood, and J. G. White, *Nature (London)*, **178**, 64 (1956).
(5) R. Bonnet, J. R. Cannon, V. M. Clark, A. W. Johnson, L. F. J. Parker, E. L. Smith, and A. R. Todd, *J. Chem. Soc.*, 1158 (1957).
(6) H. A. Barker, H. Weissbach, and R. D. Smyth, *Proc. Natl. Acad. Sci. U.S.A.*, **44**, 1093 (1958).
(7) D. Dolphin, A. W. Johnson, R. Rodrigo, and N. Shaw, *Pure Appl. Chem.*, **1**, 539 (1963).
(8) P. G. Lenhart and D. C. Hodgkin, *Nature (London)*, **192**, 937 (1961).
(9) F. Wagner and P. Renz, *Tetrahedron Lett.*, 259 (1963).
(10) D. Dolphin, *Methods Enzymol.* **18c**, 34 (1971).
(11) A. W. Johnson, L. Mervyn, N. Shaw, and E. L. Smith, *J. Chem. Soc.*, 4146 (1963).

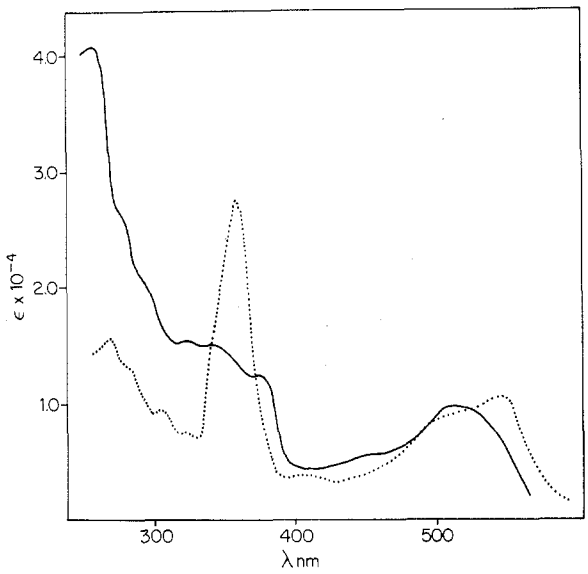


Figure 2. Optical absorption spectra (H_2O) of vitamin B_{12} (- - - -) and the vitamin B_{12} coenzyme (——).

These questions were resolved when it was shown[12] that both the extent of the conjugated chromophore and the oxidation state of the cobalt were the same for both B_{12} and the B_{12} coenzyme.

Thus the catalytic reduction of hydroxocobalamin (B_{12b}) with hydrogen and platinum oxide gave vitamin B_{12r}. B_{12r} had previously been assigned[13] as a cobalt(II) complex, and this was confirmed when 0.5 mol of hydrogen was consumed during the formation of B_{12r}.

$$\underset{B_{12b}}{\text{hydroxocobalamin Co(III)}} \xrightarrow{\frac{1}{2}H_2} \underset{B_{12r}}{\text{Co(II)}} + H^+$$

Similarly the reduction of methylcobalamin (identical in oxidation state with that of the coenzyme) also consumed 0.5 mol of hydrogen, when reduced,[12] to give 1 mol of methane and 1 mol of B_{12r}.

$$\text{methylcobalamin} \xrightarrow{\frac{1}{2}H_2} \text{methane} + B_{12r}$$

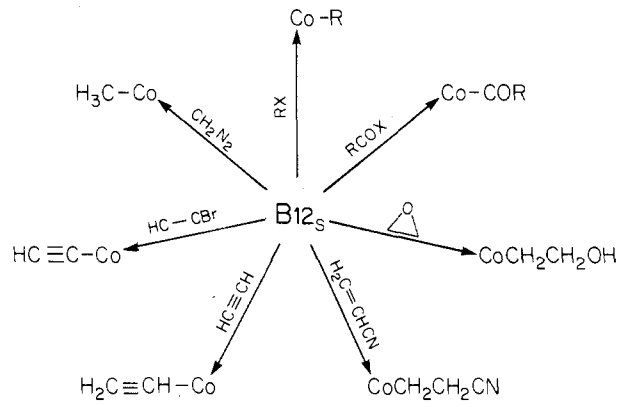
From the equations it is clear that the coenzyme and B_{12} share the same oxidation state for cobalt(III) and a chromophore of the same oxidation state, with the result that the coenzyme (Figure 1, R = 5′-deoxadenosyl) and the analogous alkylcobalamins can formally be considered as carbanions coordinated to trivalent cobalt.

Further reduction of B_{12r} gives a green complex, B_{12s}. Vitamin B_{12s} is rapidly oxidized by both oxygen and water at a pH below 8. One mole of B_{12b} and 1 mol of B_{12s} gives 2 mol of B_{12r}, establishing[14] B_{12s} as a monovalent cobalt complex. B_{12s} is a powerful nucleophile and reacts with a variety of alkylating agents to give the corresponding alkylcobalamin.[11] This oxidative addition to electrophiles provides a convenient route to both alkyl- and acylcobalamins, and provides a convenient, and commercial, route to the coenzyme.

B_{12s} + 5′-deoxadenosyl tosylate →
B_{12} coenzyme + *p*-toluenesulfonate

Most of the reactions of B_{12s} (Scheme I) are consistent with those of a powerful nucleophile. However, B_{12s} reacts with diazomethane to give methylcobalamin,[15] a reaction reminiscent of a transition-metal hybride. This dichotomous reactivity prompted us[12] to suggest that B_{12s} could best be formulated with the equilibrium:

Scheme I



$$\overset{\cdot\cdot}{H}\ \text{Co(III)} \rightleftharpoons \text{Co(I)} + H^+$$

Recently Schrauzer[16] has presented evidence which supports this formulation.

There are three routes for the cleavage of the cobalt–carbon bond of alkylcobalamins:

$$\text{Co–R} \rightarrow \text{Co(II)} + \text{R}\cdot \quad (1)$$

$$\text{Co–R} \rightarrow \text{Co(I)} + \text{R}^+ \quad (2)$$

$$\text{Co–R} \rightarrow \text{Co(III)} + \text{R}^- \quad (3)$$

Specific examples of these reactions are shown in Scheme II.

The displacement of cobalt–alkyl ligands by CN^- was discovered shortly after alkylcobalamins were first isolated, and it was initially proposed[17] that this reaction was a direct displacement of the ligand by CN^-. This, however, appears not to be the case. The reaction of CN^- with coenzyme (see Scheme II) and with methylcarboxymethyl-B_{12} have now been investigated in more detail. (The latter reaction yields dicyano-B_{12} and methyl acetate.) These reactions involve an initial displacement of a benzimidazole by CN^- in an equilibrium step,[18] followed by a unimolecular cleavage of the C–Co bond. A secondary deuterium isotope effect (V_H/V_D = 1.07) is observed, at high pH, with monodeuterated methylcarboxymethyl-B_{12}. Studies with stereospecifically labeled monodeuteriomethylcarboxymethyl-B_{12} show that the solvent proton is added to methyl acetate 75% of the time from the solvent side, i.e., the reaction pro-

(12) D. Dolphin, A. W. Johnson, and R. Rodrigo, *Ann. N.Y. Acad. Sci.*, **112**, 590 (1964).
(13) B. Jaselskis and H. Diehl, *J. Am. Chem. Soc.*, **76**, 4345 (1954).
(14) J. A. Hill, J. M. Pratt, and R. J. P. Williams, *J. Theor. Biol.*, **3**, 423 (1962).
(15) O. Müller and G. Müller, *Biochem. Z.*, **336**, 229 (1962).
(16) G. N. Schrauzer and R. J. Holland, *J. Am. Chem. Soc.*, **93**, 4060 (1971).
(17) D. Dolphin, A. W. Johnson, and R. Rodrigo, *Ann. N.Y. Acad. Sci.*, **112**, 590 (1964).
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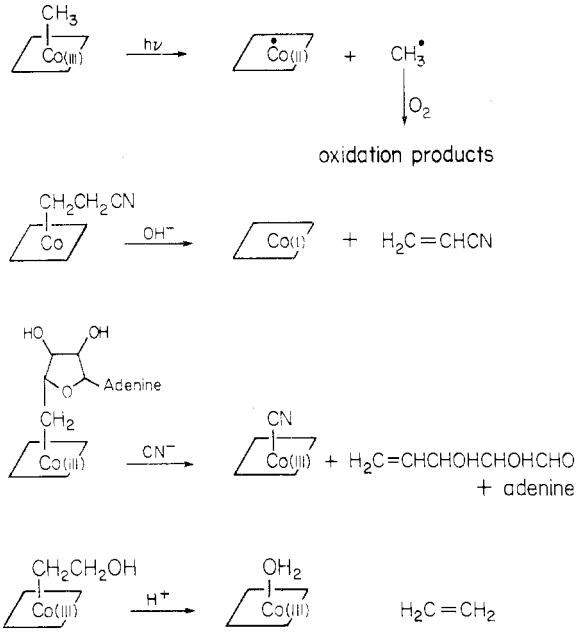
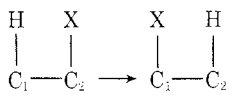
Scheme II



ceeds predominantly with the inversion at the C–Co bond.[19] These results suggest an intermediate formation of an enol-anion–B_{12} ion pair.

The coenzyme form of the vitamin was first observed[6] as a cofactor in the enzymatic conversion of glutamate to β-methylaspartate by glutamate mutase (reaction 1, Figure 3). At the present time ten distinct enzymatic reactions, requiring the B_{12} coenzyme as a cofactor, have been reported[20] (Figure 3). Along with glutamate mutase, two other reactions involving a carbon skeletal rearrangement have been described. Methylmalonyl-COA mutase (reaction 2, Figure 3) brings about the interconversion of methylmalonic and succinic acids and represents the only known reaction to occur in mammals which requires the B_{12} complex. The third skeletal rearrangement is catalyzed by α-methyleneglutarate mutase (reaction 3, Figure 3).

All of the reactions shown in Figure 3 with the exception of ribonucleotide reductase (reaction 10) can be generalized as the migration of a hydrogen from one carbon atom to an adjacent one with the concomitant migration of a group X from the adjacent carbon atom to the one to which the hydrogen was originally bound.



The conversion of propylene glycol to propionaldehyde by diol dehydrase (reaction 4, Figure 3), which also converts ethylene glycol to acetaldehyde, of glycerol to β-hydroxypropionaldehyde by glyceroldehydrase (reaction 5, Figure 3), and of ethanolamine to acetaldehyde by ethanolamine ammonia-lyase (reaction 6, Figure 3) all involve the elimination of either water or ammonia from the substrate. Nonetheless, they still can be generalized as a mutual 1,2 shift of hydrogen and hydroxyl (or amide) followed by loss

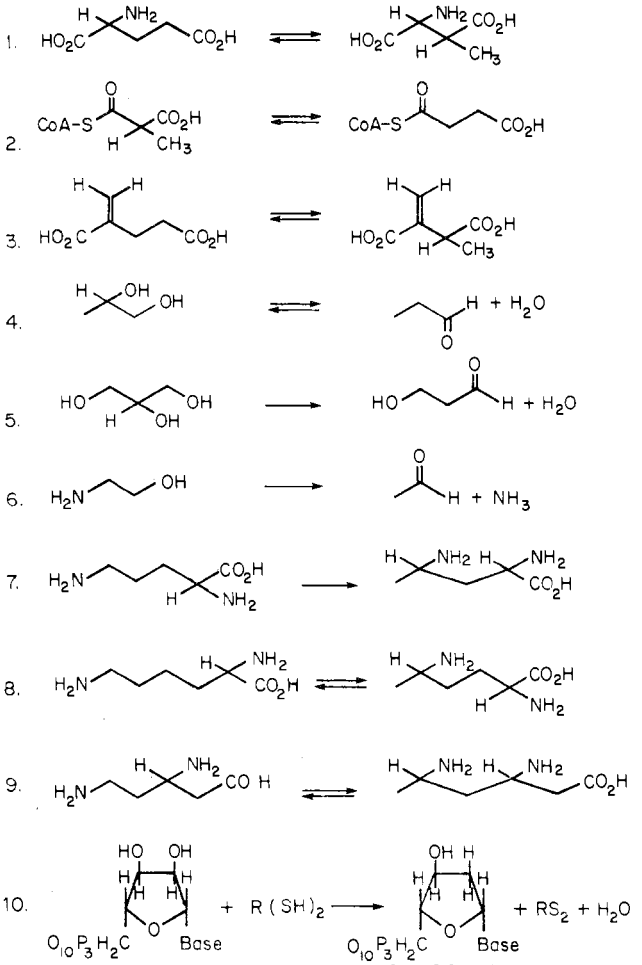
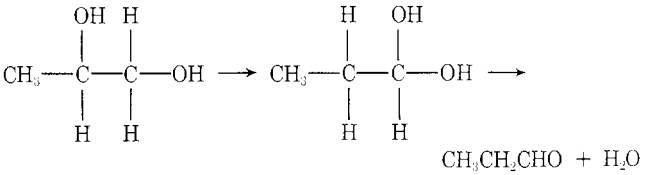


Figure 3. Enzymic rearrangements catalyzed by the vitamin B_{12} coenzyme.

of water (or ammonia) to give aldehyde, e.g.



The intermediacy of the geminol diol is confirmed by the work of Arigoni and Retey,[21] who showed that not only are the initial migrations stereospecific but also the degradation of the geminol diol is a stereospecific enzymatic reaction with only one of the two prochiral hydroxyl groups being eliminated.

While the amino acid rearrangements (reactions 7–9, Figure 3) clearly involve 1,2 rearrangements, it is not immediately apparent that the conversions of ribose to deoxyribose by ribonucleotide reductase can also be so characterized. If it *were* strictly analogous to the reaction catalyzed by diol dehydrase, the reduction of the C-2′ OH group of the nucleotide would involve the following steps: (1) hydrogen transfer from C-3′ to C-2′ and concomitant transfer of OH from C-2′ to C-3′—this leads to the formation of a 3′,3′-diol; (2) reduction of the 3′,3′-diol to a hydroxyl group—this reduction must proceed with stereospecific removal of one of the OH^- groups, since experiments with ^{18}O-labeled substrates show[22,23] that the

(19) W. Reenstra, W. P. Jencks, and R. H. Abeles, unpublished data.
(20) H. A. Barker, *Annu. Rev. Biochem.*, **41**, 55 (1972).
(21) J. Retey, A. Umani-Ronchi, J. Seible, and D. Arigoni, *Experientia*, **22**, 502 (1966).

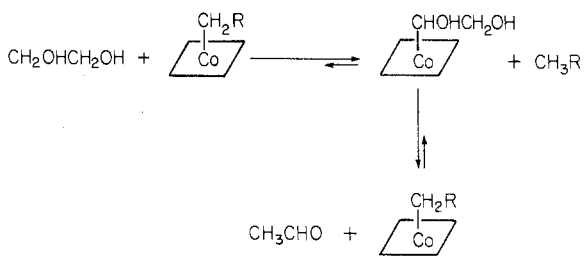
C-3′ OH is not lost while C-2′ OH is. However, solvent hydrogen is incorporated into C-2′ position of the reaction product and *not* in the C-3′ position.[24,25] These results make it appear unlikely that a mechanism analogous to that of diol dehydrase is involved. The mechanism of this reaction probably does not involve participation of the C-3′ position.

Evidence available so far suggests that all of the reactions may have some important features in common. In the following section, we shall summarize the currently known facts about these reactions, with special emphasis on mechanistic studies with diol dehydrase.

Hydrogen-Transfer Step

In the rearrangements controlled by diol dehydrase the hydrogen which migrates to the adjacent carbon does so without incorporation of protons from water.[26] The stereospecificity of this reaction is such that the choice as to which hydrogen atom migrates depends upon the chirality of the carbon to which it migrates. Thus, using (*R,R*)- and (*R,S*)-1,2-propanediol-*1-d*, we showed[27] that deuterium was transferred from C-1 of the (*R,R*) substrate while protium migrated from the (*R,S*) isomer. Furthermore, in the case of diol dehydrase the migrating atom replaces the migrating group X with inversion of configuration at C-2.[28,29] A similar inversion of configuration is observed in glutamate mutase,[30] while both methylmalonyl-CoA mutase[31] and ribonucleotide reductase[32,33] show retention at "C-2". Despite the stereospecificity of these hydrogen-transfer steps they do not necessarily proceed by a "direct intramolecular" process, for when tritiated propanediol and unlabeled ethylene glycol were incubated together with diol dehydrase tritium was found in the acetaldehyde.[34] In addition to this and in apparent contradiction to the specificity of these reactions, it was found that both of the C-5′ hydrogen atoms of the coenzyme were replaced by tritium when 1,2-propanediol-*t* was used as substrate, and that the coenzyme could transfer tritium from either of the C-5′ positions to product.[35] Thus one of the roles of the coenzyme is to act as a hydrogen carrier.

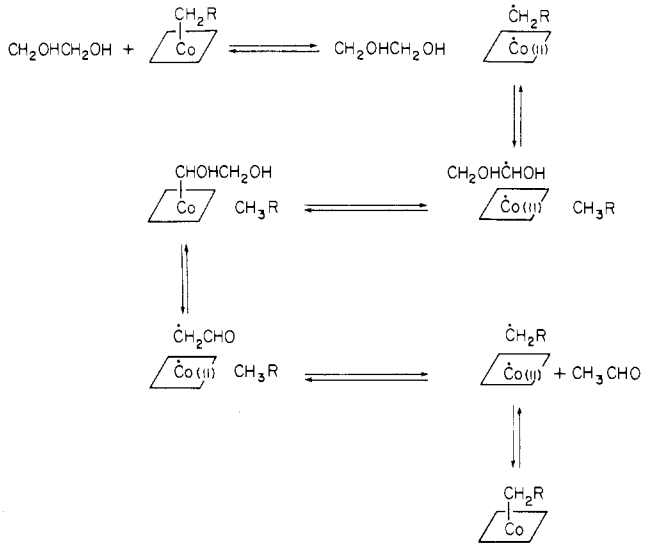
From a number of different kinetic approaches it was concluded[35] that an enzyme-bound intermediate exists in the conversion of 1,2-propanediol to propionaldehyde in which the hydrogen abstracted from the substrate becomes equivalent with the two hydrogens at the C-5′ position of the coenzyme. These observations, together with the previously mentioned fact that the two stereochemical nonequivalent C-5′ hydrogens of the coenzyme participate in the reaction, led to the suggestion[35] that 5′-deoxyadenosine, derived from the adenosyl moiety of the coenzyme, is an intermediate in the reaction. Experiments with methylmalonyl-CoA mutase have also led to the conclusion that an intermediate occurs in which a substrate-derived hydrogen and the two C-5′ hydrogens of the coenzyme become equivalent. The intermediate involvement of 5′-deoxyadenosine was also proposed for this reaction.[36] Subsequently confirmation for the intermediate participation of 5′-deoxyadenosine was obtained[37] by direct isolation of this compound from enzymic reactions. When the enzyme–coenzyme complex was dissociated after reaction with several substrate analogues (glycol aldehyde, chloroacetaldehyde with diol dehydrase; ethylene glycol with ethanolamine deaminase), extensive conversion of the adenosyl portion of the coenzyme to 5′-deoxyadenosine was observed. Although these compounds have many properties of substrates, they reacted stoichiometrically with enzymes, and were not subject to catalysis. Small amounts of 5′-deoxyadenosine were detected when the catalytic action of ethanolamine deaminase on aminoethanol was interrupted.[38] Recently it was demonstrated[39] that 5′-deoxyadenosine is reversibly formed when ethanolamine deaminase catalyzes the conversion of 2-amino-1-propanol to propionaldehyde. When the enzyme is denatured during the catalytic process, 80% of the enzyme-bound coenzyme is converted to 5′-deoxyadenosine. The 5′-deoxyadenosine contains hydrogen (tritium) derived from the substrate. When 2-amino-1-propanol is removed from the enzyme–coenzyme complex prior to dissociation, all of the original coenzyme can be recovered. Thus the reversible formation of 5′-deoxyadenosine during the catalytic process is established. The following is a minimal reaction sequence showing the role of 5′-deoxyadenosine in the catalytic conversion of ethylene glycol to acetaldehyde:



So far it has not been possible to achieve incorporation of added 5′-deoxyadenosine into the coenzyme when it is added during the catalytic process. This is probably due to the fact that the nucleotide is tightly

(22) M. Follman and H. P. C. Hogencamp, *Biochemistry*, **8**, 4372 (1969).
(23) H. Follman and H. P. C. Hogencamp, *J. Am. Chem. Soc.*, **92**, 671 (1970).
(24) T. J. Batterham, R. K. Ghambeer, R. L. Blakley, and C. Brownson, *Biochemistry*, **6**, 1203 (1967).
(25) C. E. Griffin, F. D. Hamilton, S. P. Hopper, and R. Abrams, *Arch. Bioch. Biophys.*, **126**, 905 (1968).
(26) R. H. Abeles and H. A. Lee, Jr., *Bookhaven Symp. Biol.*, **15**, 310 (1962).
(27) P. A. Frey, G. L. Karabatsos, and R. H. Abeles, *Biochem. Biophys. Res. Commun.*, **18**, 551 (1965).
(28) B. Zagalak, P. A. Frey, G. L. Karabatsos, and R. H. Abeles, *J. Biol. Chem.*, **241**, 3028 (1966).
(29) J. Retey, A. Umani-Ronchi, and D. Arigoni, *Experientia*, **22**, 502 (1966).
(30) M. Sprecher, R. L. Switzer, and D. B. Sprinson, *J. Biol. Chem.*, **241**, 864 (1966).
(31) M. Sprecher, M. S. Clark, and D. B. Sprinson, *J. Biol. Chem.*, **241**, 872 (1966).
(32) T. J. Batterham, R. K. Ghambeer, R. L. Blakley, and C. Brownson, *Biochemistry*, **6**, 1203 (1967).
(33) C. E. Griffin, F. D. Hamilton, S. P. Hopper, and R. Abrams, *Arch. Biochem. Biophys.*, **126**, 905 (1968).
(34) P. A. Frey and R. H. Abeles, *J. Biol. Chem.*, **241**, 2732 (1966).
(35) P. A. Frey, M. K. Eisenberg, and R. H. Abeles, *J. Biol. Chem.*, **242**, 5369 (1967).
(36) J. H. Richards and W. W. Miller, *J. Am. Chem. Soc.*, **91**, 1498 (1969).
(37) O. W. Wagner, H. A. Lee, Jr., P. A. Frey, and R. H. Abeles, *J. Biol. Chem.*, **241**, 1751 (1966).
(38) B. M. Babior, *J. Biol. Chem.*, **245**, 1695 (1970).
(39) B. M. Babior, T. J. Carty, and R. H. Abeles, *J. Biol. Chem.*, **249**, 1689 (1974).

Scheme III



bound to the enzyme during turnover and does not exchange with exogenous nucleoside.

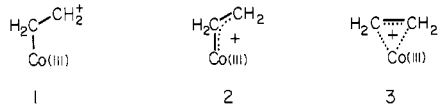
Mechanism of Action

The isolation of 5′-deoxyadenosine and hydroxocobalamin from the functioning enzyme suggests that breaking of the cobalt–carbon bond of the coenzyme is a necessary step in the catalytic cycle. Evidence is now accumulating that this bond-breaking is homolytic and gives B_{12r} (Co(II)) and the C-5′ methylene radical.[40] Such nonenzymatic homolytic cleavage induced both thermally and photochemically is well documented for cobalamins[7] and the chemically related cobaloximes.[41]

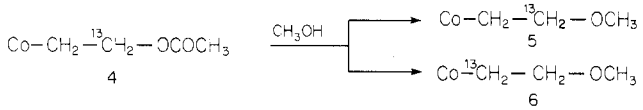
With this additional evidence the minimal mechanism shown above can be expanded as shown in Scheme III. The principal features are the homolytic cleavage of the cobalt–carbon bond of the coenzyme and abstraction of a hydrogen atom from substrate by the C-5′ methylene radical to give a methyl group at C-5′ and a substrate radical. Combination of the divalent cobalt and the substrate radical generates a new alkylcobalamin with the substrate as ligand, the result being a transalkylation of the cobalt. Rearrangement of the substrate ligand while bonded to, and under the influence of, the cobalt, followed by a reverse transalkylation (via a radical pathway), produces product and regenerates the coenzyme.

A major problem which remains to be answered for any mechanistic proposal, including the one above, is how does the rearrangement occur. For while the above mechanism is consistent with all of the observations made on the enzymatic reactions, including the pathway of the hydrogen migration, it says nothing about the rearrangement itself, especially the route of the migrating group X. This is an area to which we have recently turned our attention following a report by Golding[42] that ethanolysis of 2-acetoxyethyl(pyridine)cobaloxime gave 2-ethoxyethyl(pyridine)cobaloxime. The reaction followed first-order kinetics with a rate similar to that observed with trityl acetate.

On the assumption that this reaction proceeds via an initial breaking of the carbon–oxygen bond, then three extreme electronic forms of the intermediate can be envisioned: the primary carbonium ion **1**, the delocalized carbonium ion **2**, or the π complex **3**.



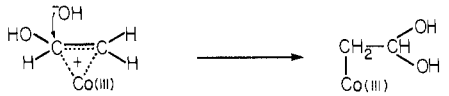
In order to establish whether the reaction proceeded via an unsymmetric carbonium ion or the symmetric π complex, we carried out the solvolysis of 2-acetoxyethyl(pyridine)cobaloxime (**4**) in methanol and found[43] that the product contained equal amounts of **5** and **6**. This showed that at some stage during the



solvolysis the cobalt becomes bonded equally to both carbon atoms of the ligand. While there are various ways of describing the electronic structure of such an intermediate, the simplest is that of the olefin π complex.

What role can such a complex play in the B_{12}-dependent enzymatic rearrangements? Transalkylation of the coenzyme by substrate would generate a new alkylcobalamin, and for each of the ten enzymatic systems described above the new alkylcobalamin has a leaving group on the carbon β to the cobalt. In the case of diol dehydrase and ethanolamine ammonia-lyase the leaving groups are water and ammonia. For methylmalonyl-CoA one is required to have an acyl group migrate with its bonding electrons, a reaction for which there is ample precedent.[44] Metal-promoted loss of substituents from the β atom of a metal alkyl are well known and account for much of the catalytic activity of organometallic systems.[45] Such metal participation undoubtedly accounts for the fast rate of solvolysis of the β-acetoxyethyl(pyridine)cobaloxime discussed above. In an enzymatic reaction, such as that catalyzed by diol dehydrase, loss of the β-hydroxy group could generate the olefin π complex.

Clearly the hydroxyl-bearing carbon atom is better able to stabilize the positive charge than the other carbon atom of the olefin. Thus, if the cobalt bound olefin is attacked by a nucleophile, addition will occur at the hydroxyl-bearing carbon to regenerate a new σ complex.



The sequence of steps involving the loss of a β substituent from the cobalt σ complex to generate a co-

(40) T. H. Finlay, J. Valinsky, A. S. Mildvan, and R. H. Abeles, *J. Biol. Chem.*, **248**, 1285 (1973); B. M. Babior and D. C. Gould, *Biochem. Biophys. Res. Commun.*, **34**, 441 (1969); B. M. Babior, T. H. Moss, and D. C. Gould, *J. Biol. Chem.*, **247**, 4389 (1972).

(41) G. N. Schrauzer, *Acc Chem. Res.*, **1**, 97 (1968).

(42) B. T. Golding, H. L. Holland, U. Honn, and S. Sakrikor, *Angew. Chem., Int. Ed. Engl.*, **9**, 955 (1970).

(43) R. B. Silverman and D. Dolphin, *J. Am. Chem. Soc.*, **94**, 4028 (1972).

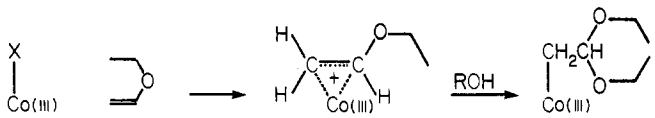
(44) R. M. Acheson, *Acc. Chem. Res.*, **4**, 177 (1971).

(45) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry", 3rd ed, Interscience, New York, N.Y., 1972, Chapter 24.

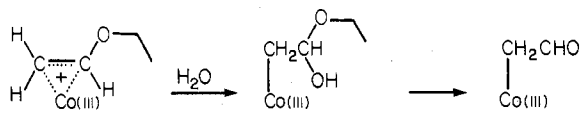
balt π complex, followed by readdition of the leaving group to the π complex and regeneration of a new σ complex, brings about the overall rearrangement catalyzed by the B_{12} coenzyme. A second transalkylation between 5′-deoxyadenosine and the rearranged substrate alkyl would regenerate coenzyme and product.

The critical intermediate in this rearrangement is the Co(III) olefin π complex, and one might ask how reasonable an intermediate is it? Olefin π complexes of transition metals are well known;[46] however, the majority of such systems derive from the combination of the transition metal in a low oxidation state (electron rich) with an olefin bearing electron-withdrawing groups (electron poor). Under these conditions the synergic bonding, involving interaction between the filled π orbitals of the olefin and the empty d orbitals of the metal, and the filled d orbitals of the metal and the π^* olefin orbital, is optimized. Nonetheless one can imagine that the reverse of this situation, an electron-deficient metal and an electron-rich olefin, could give rise to strong synergic bonding and stable olefin complexes. This situation would obtain for the olefin π complex we propose in the diol dehydrase catalyzed rearrangement where the olefin (enol acetaldehyde) is electron rich and the metal (trivalent cobalt) electron deficient.

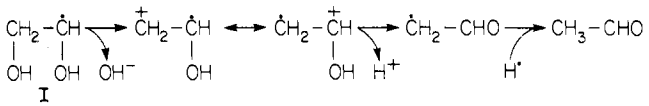
In order to place cobalt π complexes on a more substantial footing, we have sought to prepare and characterize such systems. However, we have so far been unable to spectroscopically detect any interaction between Co(III) complexes and suitable electron-rich olefins such as enol ethers. If an olefin π complex were indeed formed between a trivalent cobalt complex and an enol ether, then, in the presence of a nucleophile, such as an alcohol, one would anticipate addition of the nucleophile to the oxygen-bearing carbon, to give the σ-bonded acetal.



This is what we observed.[47] Thus treatment of hydroxocobalamin with ethyl vinyl ether and triethylamine in absolute ethanol gave the corresponding 2,2-diethoxyethylcobalamin which crystallized directly from the reaction mixture. The intermediate olefin complex is intercepted by small amounts of water in the reaction mixture, and formylmethylcobalamin,[48] a proposed intermediate in the diol dehydrase catalyzed rearrangement[49] of ethylene glycol, is produced.



An alternative mechanism for the cobalt-catalyzed rearrangement has been proposed by Golding.[50] The mechanism differs from one shown above in that the rearrangement does not proceed through an ionic mechanism but through a radical rearrangement via the epoxy radical. Which mechanism obtains must await further studies with the enzymes and with model systems. The feasibility of a radical mechanism for the conversion of ethylene glycol to acetaldehyde was recently demonstrated.[51] The following reaction sequence was proposed:



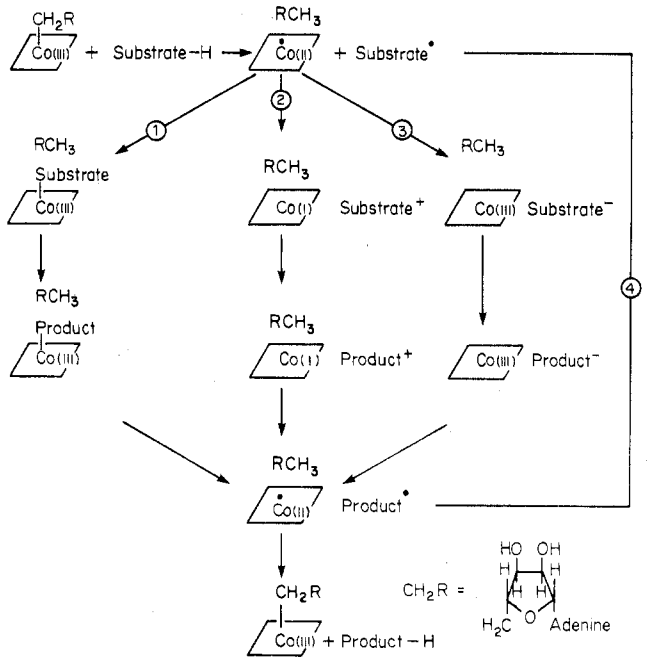
I

Radical I is produced by the action of Fenton's reagent on ethylene glycol. This reaction might well be a model reaction for the enzymic conversion of ethylene glycol to acetaldehyde. It might be interesting to establish whether this reaction proceeds with OH^- migration, analogous to the enzymic reaction.

The possibility should also be considered that not *all* reactions involving B_{12} coenzyme proceed through the same mechanism. Some reactions could involve radical rearrangements, others carbonium or carbanion rearrangements. An initial step in all reactions could be the homolytic cleavage of the carbon–cobalt bond and abstraction of a substrate hydrogen atom. This leads to the formation of Co(II) and a substrate radical, which react in either of four ways shown in Scheme IV. Pathway 1 represents the rearrangement discussed above; pathway 4, a radical rearrangement as proposed by Golding; 2 and 3, carbonium ion and carbanion rearrangements. A carbanion mechanism has been proposed for the reaction catalyzed by methylmalonyl-CoA isomerase.[52] So far no mechanisms involving carbonium ion rearrangements (2) have been suggested.

Although the mechanism of the rearrangement is uncertain at this time, experimental data obtained

Scheme IV



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with diol dehydrase[40,53] and ethanolamine deaminase[54] provide strong support for the homolytic cleavage of the carbon–cobalt bond of the enzyme-bound coenzyme at an early stage in the reaction.

How does the carbon–cobalt bond become activated toward homolytic cleavage? We have considered the possibility that this activation may be the result of the distortion of the corrin ring brought about through interaction of the amide groups on the periphery of the corrin with amide groups or other groups of the enzyme protein which can participate in hydrogen bonding. It is interesting to note that hydrolysis of one of the amide groups, probably the E group, leads to complete loss of coenzymic activity. This observation has been made for two enzymes, ribonucleotide reductase[55] and diol dehydrase.[56] Esterification of the carboxyl group restores partial coenzymic activity. The role of the peripheral amide groups of the coenzyme in the catalytic process is now under investigation.

In addition to the mechanisms discussed here, an entirely different mechanism has been proposed by Schrauzer.[57] According to this mechanism the conversion of the diol to the aldehyde proceeds via a 1,2-hydride shift and the coenzyme does not function as a hydrogen-transfer agent. We believe, and have pointed out so on several occasions, that such a mechanism is not consistent with the experimental data available from enzyme studies.

We thank all of our colleagues who, for the past decade, have been involved in the work described here. The work has been supported by the National Science Foundation and the National Institutes of Health of the United States, and the National Research Council of Canada.

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