structure.[26] It is known that formation of clathrate hydrates results from a favorable enthalpy which is sufficient to counteract an unfavorable entropy.[27] A number of temperature studies on K_{diss} of apolar complexes have been made. For example, Wagner and coworkers[28] conducted temperature studies on the kinetically determined K_{diss} for the interactions of long-chain N-acylhistidine derivatives with *p*-nitrophenyl-N-dodecyl-N,N-dimethylammonioethyl carbonate ion. They reported temperature independence for K_{diss} from which they concluded that their data supported the hydrophobic model. The data in Table II can be used to support both models if one looks simply at the $\Delta H°$ and $\Delta S°$ values. They show that both entropic and enthalpic contributions to binding are important in cycloamylose complexes. To the extent that these cycloamylose complexes are able to simulate enzyme–substrate complexes, it appears worthwhile to be aware of the possibility that enzyme–substrate complex formation may also result from both entropic and enthalpic contributions to the free energy of binding. It should also be recognized that other factors such as van der Waals–London type forces may be involved in complex formation. Present theory does not allow a simple interpretation of the thermodynamic data in Table II.

(26) H. Frank and M. Evans, *J. Chem. Phys.*, **13**, 507 (1945); H. Frank and W.-Y. Wen, *Discussions Faraday Soc.*, **24**, 133 (1957).
(27) I. M. Klotz, "Horizons in Biochemistry," M. Kasha and B. Pullman, Ed., Academic Press, New York, N. Y., 1962, pp 523–550.
(28) R. G. Shorenstein, C. S. Pratt, C.-J. Hsu, and T. E. Wagner, *J. Amer. Chem. Soc.*, **90**, 6199 (1968).

Conclusion

Several observations have been made in this study of cycloheptaamylose complexes with mono-pCP esters of 3-substituted glutaric acids.

1. The rates in all cases are slower when cycloheptaamylose is present, due to preferential complexing of the unreactive or less reactive conformers which exist in solution.
2. The reactivity of the complexes depends upon the geometry of binding the esters to cycloheptaamylose. The specificity of this binding depends upon the 3-substituents.
3. This specificity of binding can be called a "negative specificity" because it results from lowering the ground-state energies of the esters.
4. There is no relationship between the relative reactivities of the free esters and the relative reactivities of the complexed esters. Consequently, the specificity of binding in the complexes can induce large changes in the overall relative reactivities of these esters.

This model study supports the idea that specificity in enzymic reactions may result from the geometry of binding the substrates to the enzymes, rather than resulting from the catalytic reactions themselves.

Acknowledgment. The authors thank Professor F. J. Kezdy for many helpful comments during the course of this work. Acknowledgment is also made to the National Institutes of Health and to the National Science Foundation for financial support.

Coenzyme B_{12} and Coenzyme B_{12} Model Compounds in the Catalysis of the Dehydration of Glycols[1]

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Abstract: Synthesis and reactions of 5′-deoxyadenosylcobaloximes are described. The model compounds of coenzyme B_{12} undergo Co–C bond cleavage with acids, cyanide ion, or on light irradiation just like coenzyme B_{12} itself. However, they are also alkali labile, yielding the cobaloxime(I) nucleophiles and β-elimination products of the adenosyl moiety. A similar Co–C bond cleavage reaction of coenzyme B_{12} was unknown previously but is shown to occur in strong alkali. The coenzyme B_{12} model compounds are only weak inhibitors in coenzyme B_{12} dependent enzymes and have no coenzyme activity. The mechanisms proposed for the action of coenzyme B_{12} in the diol dehydratase of Aerobacter aerogenes are discussed in the light of the new experimental evidence and with particular reference to the factors influencing the reactivity of 2-hydroxyethylcobalamin and -cobinamide derivatives previously assumed to be actual or functional intermediates in the enzymatic reaction. A new interpretation of the substrate–coenzyme hydrogen exchange in the glycol dehydratase reaction is given which is shown to be consistent with mechanistic postulates derived on the basis of model experiments. This leads to the formulation of a mechanism of coenzyme B_{12} action in the dehydration of glycols whose individual steps are in agreement with the chemical properties and reactions of all constituents and reactants of the system.

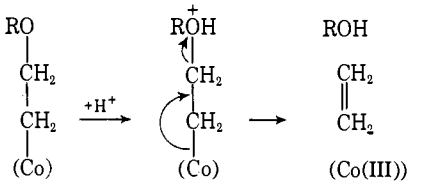
Coenzyme B_{12} dependent enzymes achieve intriguing molecular transformations presumably by virtue of the unique catalytic activity of the cobalt ion in the corrin system.[3] In the free coenzyme the cobalt ion is six-coordinated, however, and as such not accessible for any substrate. It is therefore plausible to suggest

(1) Supported by Grants GB 6174 and GP 12324 of the National Science Foundation.
(2) Petroleum Research Fund Predoctoral Fellow, 1968–1969, PRF-GF-62.

(3) See reviews by (a) H. A. Barker, *Biochem. J.*, **105**, 1 (1967); (b) F. Wagner, *Ann. Rev. Biochem.*, **35**, 405 (1966); (c) H. P. C. Hogenkamp, *ibid.*, 37, 225 (1968); (d) K. Bernhauer, *et al.*, *Angew. Chem.*, **75**, 1145 (1963), and references cited therein.

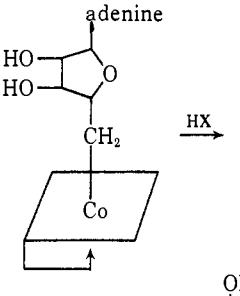
that the coenzyme is converted into an active form on interaction with the apoenzyme protein. This would require the cleavage of the Co–C bond and, perhaps, but not necessarily, the removal of the axial base (*e.g.*, 5,6-dimethylbenzimidazole). Whereas the latter reaction could occur simply by a protonation, the cleavage of the Co–C bond poses several mechanistic alternatives. The homolytic cleavage would yield a Co(II) corrin derivative, heterolytic cleavage could produce either a Co(III) or a Co(I) species. Whereas the Co(II) and Co(III) corrin derivatives are not particularly reactive except in simple displacement reactions involving axial ligands or in electron-transfer reactions, the Co(I) valence state is clearly much more reactive in view of its extraordinarily high nucleophilicity.[4,5] It is therefore an attractive hypothesis to regard the Co(I) derivatives of corrins as active forms of the coenzyme. If this is accepted the problem arises how the Co(I) nucleophiles can be generated from the coenzyme. In principle this could be possible indirectly by a reductive cleavage of the Co–C bond, but this is considered less likely in view of the requirement of a specific reducing system and the difficulty in formulating a reversible mechanism for such a process. A Co–C bond cleavage through a β elimination, on the other hand, could occur simply on interaction with a basic center on the apoenzyme, but no evidence is available that such a reaction can actually take place. We have studied this mechanistic possibility by first synthesizing 5′-deoxyadenosylcobaloximes as models of coenzyme B_{12} and will show in the present paper that Co–C bond cleavage through β elimination can indeed be achieved, both with the coenzyme model and the coenzyme itself. Thus a mechanism is available by which the most reactive reduced forms of corrin derivatives can be generated from the coenzyme without a specific requirement of a reducing agent. The Co(I) derivatives of enzyme-bound vitamin B_{12} compounds therefore could very well be active forms of the coenzyme. A reaction of particular interest in this context is the conversion of glycols into aldehydes by an enzyme system of Aerobacter aerogenes.[6] We have previously postulated[7] that the reaction proceeds enzymatically *via* 2-hydroxyalkylcobalt derivatives as actual or functional intermediates, utilizing the enzyme-bound coenzyme in form of a nucleophilic Co(I) derivative. This was supported by model experiments with cobaloximes, but so far not with cobalamin or cobinamide derivatives. We have since extended our work to include these and other cobalt chelates as catalysts for the conversion of glycol derivatives into aldehydes and will discuss the mechanism of this reaction in some detail.

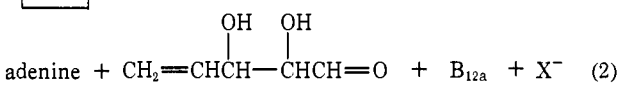
Synthesis of Deoxyadenosylcobaloximes. Coenzyme B_{12}[8] (5′-deoxyadenosylcobalamin) is a six-coordinated cobalt complex in which one of the axial positions is occupied by a nitrogen atom of 5,6-dimethylbenzimidazole, and the other position by an adenosyl group attached covalently through the 5′ carbon atom. The coordinated benzimidazole is not held very firmly;[9] its presence or absence changes the kinetic reactivity and the oxidation–reduction potential of the cobalt ion without causing fundamental changes in the chemical properties. Coenzyme B_{12} thus may be formally regarded as a substituted 2-alkoxyethylcobalt chelate. Most properties of 2-alkoxyethylcobalamins resemble those of normal alkylcobalamins. An important difference in behavior is noted in acidic solution leading to the fragmentation indicated



(1)

in eq 1.[7,10] Coenzyme B_{12} is similarly known to be acid





(2)

labile (eq 2).[11] Cleavage of the coenzyme by a reaction similar to eq 2 could in principle occur in enzymatic systems but we consider this an unlikely mechanism of coenzyme activation since the corrin species would be generated in the less reactive Co(III) form. Whereas 2-alkoxyethylcobaloximes and -cobalamins are remarkably resistant to cyanide ion,[7,11] coenzyme B_{12} reacts rapidly with Co–C bond cleavage, suggesting a special reactivity of this bond.[12] It appeared to be of interest, therefore, to synthesize 5′-deoxyadenosylcobaloximes, and to compare the properties of the Co–C bond in these model compounds with those of the coenzyme itself. While our work was in progress, Russian workers briefly reported the synthesis of 5′-deoxyadenosyl(pyridine)cobaloxime[13,14] **1**. We at first attempted our synthesis of **1** by treating cobaloximes with 2′,3′-isopropylidene-5′-tosyladenosine. The acid lability of the Co–C bond of the resulting product caused significant yield losses on saponification of the cyclic ketal, just as has been noted in the analogous synthesis of coenzyme B_{12}. The desired compound was obtained in good yield by using *p*-dimethylaminobenzaldehyde as the protecting agent[15] for the 2′,3′-adenosylhydroxyls in the synthesis

(4) G. N. Schrauzer, E. Deutsch, and R. J. Windgassen, *J. Am. Chem. Soc.*, **90**, 2441 (1968).
(5) G. N. Schrauzer and E. Deutsch, *ibid.*, **91** 3341 (1969).
(6) R. H. Abeles and H. A. Lee, *Ann. N. Y. Acad. Sci.*, **112**, 695 (1964).
(7) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **89**, 143 (1967).
(8) H. A. Barker, H. Weissbach, and R. D. Smyth, *Proc. Nat. Acad. Sci. U. S.*, 1093 (1958).
(9) (a) H. A. Barker in "Vitamin B_{12} and Intrinsic Factor," Enke-Verlag, Hamburg, 1961, p 82; (b) R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, R. G. Thorp, R. J. P. Williams, *J. Chem. Soc., A*, 2419 (1968).
(10) H. P. C. Hogenkamp, J. E. Rush, and C. A. Swenson, *J. Biol. Chem.*, **240**, 3641 (1965).
(11) H. Weissbach, J. N. Ladd, B. E. Volcani, R. D. Smyth, and H. A. Barker, *ibid.*, **235**, 1462 (1960).
(12) (a) O. Müller and G. Müller, *Biochem. Z.*, **335**, 340 (1962); (b) K. Bernhauer and O. Müller, *ibid.*, **334**, 199 (1961); (c) A. W. Johnson and N. Shaw, *J. Chem. Soc.* (London), 4608 (1962).
(13) I. N. Rudakova, B. N. Shevčenko, T. A. Posnelova, and A. M. Urkevitch, *Zhur. Obshch. Chim.*, **37** (99), 1748 (1967).
(14) A. M. Urkevitch, I. N. Rudakova, and H. A. Preodrashcenskij, *Chim. Prirod. Svedinenij*, **1**, 48 (1967).
(15) R. R. Schmidt and F. M. Huennekens, *Arch. Biochem. Biophys.*, **118**, 253 (1967).

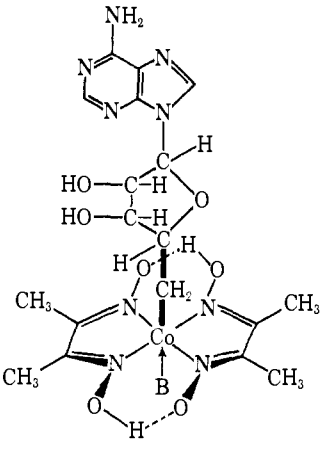
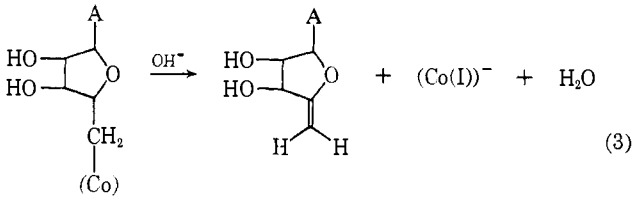


Figure 1. Structure of 5′-deoxyadenosylcobaloximes: B = pyridine (**1**); B = H_2O (**2**).

of the tosylate, as this can be conveniently removed from the adenosyl moiety prior to reaction with the cobaloxime(I).

Properties of 5′-Deoxyadenosylcobaloximes. 5′-Deoxyadenosyl(pyridine)cobaloxime (structure **1**, Figure 1) and the corresponding aquo derivative **2** are yellow, crystalline air-stable, light-sensitive compounds. They are somewhat more sensitive to acids than coenzyme B_{12}, decomposing measurably fast in 0.1 *M* aqueous HCl at room temperature, under conditions where coenzyme B_{12} remains essentially unchanged. The cleavage by cyanide ion takes place at a rate comparable to coenzyme B_{12}. The most remarkable property of deoxyadenosylcobaloximes is their alkali lability. Solutions of the compounds in 1 *M* aqueous NaOH decompose at significant rates to yield Co(I) nucleophiles which are readily recognized by their characteristic absorption spectra and elimination products of the 5′-deoxyadenosyl moiety. The principal reaction proceeds



(3)

according to eq 3 (A = adenine). Using 2′,3′-isopropylidene-5′-deoxyadenosyl(pyridine)cobaloxime and the corresponding derivative of coenzyme B_{12}, the unsaturated cleavage product of the adenosine moiety has been definitely identified. Some adenine is also formed presumably because of the instability of the 4′,5′-dehydro-5′-deoxyadenosine derivative under our reaction conditions.

Since β eliminations of 5′-deoxyadenosyl derivatives such as the tosylate take place under surprisingly mild conditions[16] reaction 3 thus is not unexpected and *per se* not even indicative of a special reactivity of the Co–C bond in the coenzyme. It has been stated occasionally that the Co–C bond is polarized and hence more reactive in the coenzyme than in other organocobalamins. The greater reactivity of the adenosylcobaloximes or -cobalamins is more likely caused by the presence of the electronegative oxygen in the sugar moiety, since β-alkoxyethylcobaloximes and -cobalamins are quite stable in alkaline medium.[17] Effects of ring strain upon the reactivity of the 4′-hydrogen must also be considered. Reaction 3 could in principle be reversible, but the direct demonstration of reversibility has so far not been possible.[17a]

The analogous cleavage of the Co–C bond of coenzyme B_{12} was demonstrated in subsequent experiments. Decomposition is measurably fast in 2 *M* NaOH at room temperature in the dark, yielding a dark brown solution containing vitamin B_{12r} and B_{12s}. The presence of vitamin B_{12s} was indicated by the appearance of the characteristic absorption at 285 mμ; upon the addition of methyl iodide, methylcobalamin is produced. The latter evidence is not entirely unambiguous, however, since Co(I) derivatives of dehydrocorrins are formed in alkaline medium by self-reduction.[18] Nevertheless, coenzyme B_{12} is alkali labile under conditions where simple alkylcobalamins remain unchanged. Our observations explain the earlier findings of Wagner and Bernhauer,[19] who noted a partial degradation of coenzyme B_{12} on air oxidation in boiling 0.1 *M* aqueous NaOH.

Rate data of reactions of coenzyme B_{12} and of 5′-deoxyadenosyl(pyridine)cobaloxime are summarized in Table I. The model compound is about 10^2 times more reactive with acid and alkali, but reacts with cyanide at about the same rate as coenzyme B_{12} itself. The Co–C bond in coenzyme B_{12} thus is not kinetically labilized through steric or electronic effects of the corrin moiety. The coenzyme and its model both are photosensitive to about the same extent, indicating a great similarity of the ground state and excited state properties of the Co–C bonds in both compounds.

Table I. Pseudo-First-Order Rate Constants of Decomposition of 5′-Deoxyadenosylcobalamin (Coenzyme B_{12}) and of 5′-Deoxyadenosyl(pyridine)cobaloxime (Coenzyme B_{12} Model), with Acid, Cyanide and NaOH at 27°. Estimated Error in k = ±5%

Reaction	Pseudo-first-order rate constants, sec^{-1} — Coenzyme B_{12}	Coenzyme B_{12} model
Reaction with 1 *M* HCl	7.4×10^{-5}	2.2×10^{-3}
Reaction with 0.01 *M* KCN	8.3×10^{-3}	2.3×10^{-4}
Decomposition by 1 *M* NaOH	5.0×10^{-6}	1.6×10^{-3}

The deoxyadenosylcobaloximes were routinely tested for inhibitory activity in coenzyme B_{12} dependent enzyme reactions such as the diol dehydratase of Aerobacter aerogenes, the ribonucleotide reductase of L. leichmanii[20] and the methane producing enzyme of M. omelianskii.[21] The observed inhibitory effects were

(16) (a) J. R. McCarthy Jr., M. J. Robins, and R. K. Robins, *Chem. Commun.*, 536 (1967); (b) M. J. Robins, L. B. Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **88**, 1549 (1966).

(17) G. N. Schrauzer and R. J. Windgassen, *ibid.*, **89**, 1999 (1967).

(17a) Note Added in Proof. The reversibility of the reaction of tetrahydrofurfurylcobalamin with base has now been demonstrated. Vitamin B_{12s} was found to react with 2-methylenetetrahydrofuran to give the tetrahydrofurfuryl derivative.

(18) L. P. Lee and G. N. Schrauzer, *ibid.*, **90**, 5274 (1968), and references cited therein.

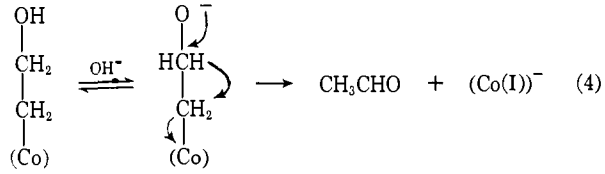
(19) F. Wagner and K. Bernhauer, *Ann. N. Y. Acad. Sci.*, **112**, 580 (1964).

(20) Inhibition experiment by Dr. D. Jacobson, Scripps Clinic and Research Institute, La Jolla, Calif.

(21) Experiments performed by Dr. J. M. Wood, Department of Biochemistry, The University of Illinois, Urbana, Ill.

small, however, only about equivalent to adenosine. The coenzyme models were furthermore incapable of replacing the coenzyme as cofactor in all cases studied.

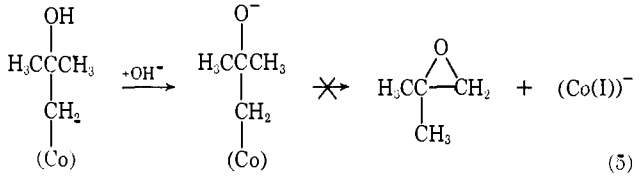
The Alkali Cleavage of Hydroxyethylcobalt Chelates. The alkali cleavage of 2-hydroxyethylcobaloximes could proceed either *via* a 1,2-hydride shift (eq 4) or a β elimination. All available evidence favors the 1,2-hydride



(4)

shift mechanism, which, in addition, is similar to the proposed intermediate steps in the conversion of ethylene oxide to acetaldehyde by hydridocobalt tetracarbonyl,[22] and the $PdCl_2$-catalyzed oxidation of ethylene to acetaldehyde.[23] Co–C bond cleavage through β elimination in this case is unlikely in view of the complete alkali resistance of 2-alkoxyethylcobaloximes (heating in 50% KOH causes no decomposition). β elimination, if at all favored, should proceed more readily with the 2-alkoxyethyl derivatives, however, since formation of the 2-oxyethylcobalt anion according to eq 4 must reduce the acidity of the 2-protons.

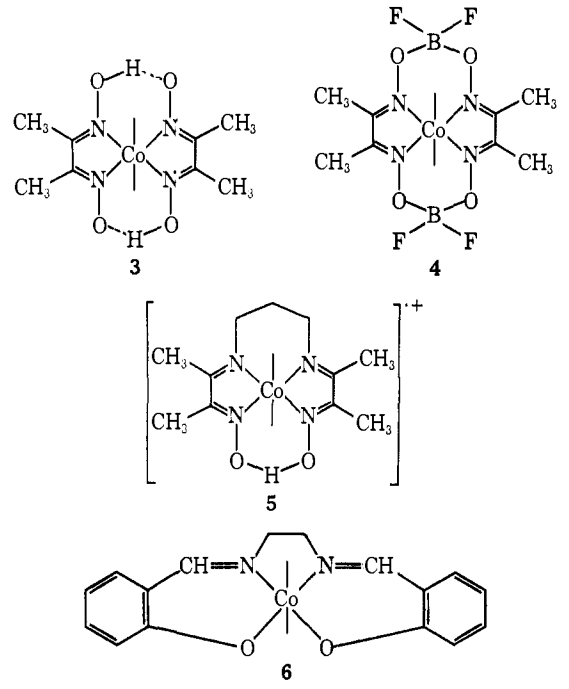
Johnson, *et al.*,[24] suggested that the initial product of alkali cleavage of 2-hydroxyethylcobalt chelates should be ethylene oxide, which would subsequently be converted into ethylene glycol. To demonstrate if epoxide formation can take place under these conditions we have synthesized 2,2-dimethyl-2-hydroxyethylcobaloxime. Substitution of the two 2-hydrogens by methyl groups should, if anything, favor epoxide forma-



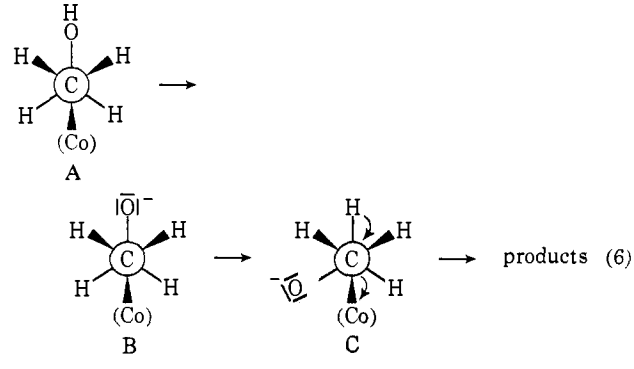
(5)

tion according to eq 5. However, the compound shows no tendency to decompose in concentrated alkaline solutions on heating, indicating that reaction **5** is not favored. Ethylene glycol, furthermore, is not formed in the alkali degradation of hydroxyethylcobaloximes or cobalamins. In an attempt to prove the intramolecular migration of the hydride ion during the alkali cleavage 2-hydroxyethylcobaloximes were decomposed in D_2O. Unfortunately, product exchange was too rapid under even the mildest conditions of decomposition.

Factor Influencing the Alkali Lability of Hydroxyethylcobalt Chelates. To study the effects of the in-plane ligands and of the axial bases on the alkali lability of hydroxyethylcobalt compounds the corresponding derivatives of chelates **3–6** were synthesized according to published methods.[25] All hydroxyethyl complexes decompose in alkaline medium yielding acetaldehyde as the initial product, following a second-order rate law $-d(R\text{-}Co)/dt = k_2(R\text{-}Co)(OH^-)$ (where R-Co symbolizes the hydroxyethylcobalt derivative). The second-order rate constants determined spectrophotometrically are listed in Table II. Alkali lability evidently increases with decreasing nucleophilicity of the Co(I) species, or, in equivalent terms, with decreasing Co(II)/Co(I) reduction potential of the parent chelates.[25] The variation of the axial bases effects the rates of alkali cleavage only to a minor extent. This is not unexpected in view of the relatively small effects of axial bases on the Co(I) nucleophilicity of cobaloximes.[4,5] Hydroxyethylcobalamin and -cobinamide are more resistant to alkali than expected from the nucleophilicity of the Co(I) species. In the cobalamin derivative a pronounced stabilizing effect of the axial base 5,6-dimethylbenzimidazole is observed, which is in contrast with the minor effects of axial bases on the rate of cleavage in the cobaloxime series (Table II).



Although the greater alkali resistance of hydroxyethylcorrin derivatives *per se* suggests a somewhat higher electron density around the cobalt ion, the relatively small differences of the nucleophilicities of the Co(I) derivatives of cobaloximes and cobalamins cannot be solely responsible for the differences in reactivity. The steric requirements for Co–C bond cleavage could well be another factor. The conversion of hydroxyethylcobalt chelates to acetaldehyde and the Co(I) nucleophiles may be written configurationally as shown in eq 6.



(6)

(22) L. Eisenmann, *J. Org. Chem.*, 27, 2706 (1962).
(23) See, *e.g.*, P. M. Henry, Advances in Chemistry Series, No. 70, American Chemical Society, Washington, D. C., 1968, p 126.
(24) D. A. Clarke, D. Dolphin, R. Grigg, A. W. Johnson, and H. A. Pinnock, *J. Chem. Soc.* (London), *C*, 881 (1968).
(25) G. N. Schrauzer, J. W. Sibert, and R. J. Windgassen, *J. Am. Chem. Soc.*, 90, 6681 (1968).

Table II. Second-Order Rate Constants of Decomposition of Hydroxyethylcobalt and Related Chelates by Aqueous NaOH at 27°, Products of Decomposition and Average Co(I) Nucleophilicities

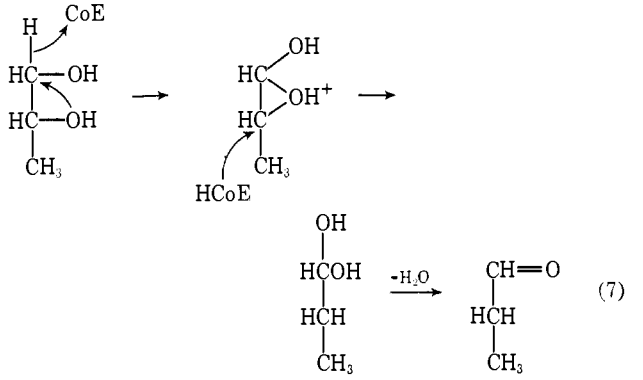
R	Chelate[a]	Axial base	Reaction products[b]	k_2, l./mole sec[c]	Nucleophilicity[d]
$HOCH_2CH_2$	**3**	H_2O (OH^-)	CH_3CHO	9.6×10^{-3}	14.3
	3	Benzimidazole	CH_3CHO	1.6×10^{-3}	14.1
	3	Pyridine	CH_3CHO	4.3×10^{-2}	13.8
	4	Pyridine	CH_3CHO	2.9×10^{-1}	12.2
	5	H_2O (OH^-)	CH_3CHO	1.2×10^{-2}	13.2
	6	H_2O (OH^-)	CH_3CHO	5.2×10^{-4}	14.6[g]
	Cobalamin	5,6-Dimethylbenzimidazole	CH_3CHO	1.5×10^{-4}	14.4
	Cobinamide	H_2O (OH^-)	CH_3CHO	9.9×10^{-4}	14.4
$HOCH(CH_3)CH_2$	**3**	Pyridine	CH_3COCH_3	1.4×10^{-2}	13.8
$HOCH_2CH(OH)CH_2$	**3**	Pyridine	CH_3COCH_2OH[e]	8.8×10^{-3}	13.8
2-$HO(C_6H_{10})$	**3**	Pyridine	*f*	4.9×10^{-4}	13.8

[a] Numbers refer to structures shown in this paper. [b] Glpc analysis in gas and liquid phase, not including secondary products due to aldol condensation. [c] Error limits ±5%. [d] Pearson nucleophilicities, see ref 4, 5. [e] Initial product. [f] Product not determined. [g] Approximate value.

The configuration of lowest energy is **6A** whose conversion to the anion **6B** occurs without the rotation of the 2-carbon atom. The concerted 1,2-hydride shift Co–C bond cleavage, however, is expected to proceed with low energy of activation only *via* **6C**. Hence, in this case rotation of the 2-carbon atom is required which may be more difficult in the cobalamin than the cobaloxime. Removal of the 5,6-dimethylbenzimidazole acting as the axial base may increase the structural nonrigidity of the corrin ligand or induce a conformation change facilitating the conversion of **6B** to **6C**. In *trans*-2-hydroxycyclohexylcobaloxime the equivalent conversion of **6B** into **6C** is not possible without considerable distortion of the complex molecule. Accordingly, this hydroxyalkylcobaloxime is exceptionally resistant to alkali and its eventual degradation in strong alkali may proceed by a different mechanism (*i.e.*, β elimination). The considerable alkali stability of hydroxyethylcobalt porphyrin,[24] on the other hand, is a consequence of the high Co(II)/Co(I) reduction potential of the parent chelate.[25,26]

The diminution of electron density on the cobalt ion in hydroxyethylcobalt chelates effectively increases the alkali lability, as follows from the comparison of the rates of alkali decomposition of chelates **3** and **4**. In **4** the electron density on cobalt is substantially reduced through the electron-withdrawing effect of the two BF_2 groups. This causes a drop in the Co(I) nucleophilicity from 13.8 to 12.2 (with pyridine as the axial base).[5] A similar reactivity change could be achieved in the case of corrins on interaction with polar groups of the apoenzyme protein. In addition, conformational changes of the corrin ligand may be induced through the binding of the coenzyme to the enzyme protein. The reactivity of corrins in enzymatic reactions thus may be much greater than in nonenzymatic systems.

The Mechanism of Substrate–Coenzyme Hydrogen Exchange in the Diol Dehydratase Reaction. A Reinterpretation. Extensive studies of the enzymatic conversion of ethylene glycol and propane-1,2-diol to acetaldehyde and propionaldehyde, respectively, with the glycol dehydratase enzyme of Aerobacter aerogenes[6] led to the following mechanistic postualtes.[3c] (a) The reaction involves a displacement of a hydroxyl group by hydrogen, *e.g.*, from C-1 to C-2 of propanediol. (b) The essential initial step in the reaction is the abstraction of a hydride ion from C-1 of propanediol. (c) This hydride ion is transferred to the coenzyme where it becomes equivalent with the two 5′-hydrogen atoms of the adenosyl moiety. (d) The resulting carbonium ion derived from the glycol substrate rearranges by the transfer of one hydroxyl group from C-2 to C-1. (e) The rearrangement process is completed by the addition of a hydride ion from the coenzyme–hydride complex to C-2 of propanediol. The propionaldehyde–hydrate is subsequently stereospecifically dehydrated to propionaldehyde. The suggested scheme is summarized in eq



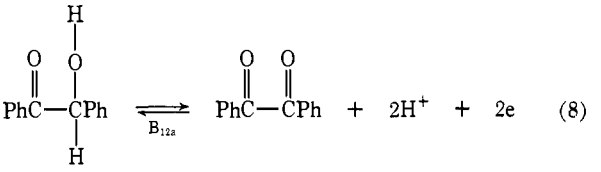
(7)

7 (CoE = coenzyme B_{12}–enzyme complex). Equation 7 has only formal significance and as such cannot be readily correlated with known chemistry. Thus, the conversion of the glycol to the aldehyde is formulated as an oxidation–reduction reaction in which the enzyme-bound coenzyme is assumed to mediate the energetically unfavorable abstraction of a hydride ion from the diol. This reaction, if at all possible, would require a much greater oxidation potential than is available in this system. Even the abstraction of a hydrogen atom would seem to be unlikely for similar reasons. The product of the presumed hydride abstraction is a protonated form of glycolaldehyde. Its conversion to the hydrate of the aldehyde *via* the epoxide-type intermediate in eq 7 thus far has no parallel in organic chemistry. Apparent support for a glycolaldehyde intermediate could be constructed from Abeles' observation[6] of the inhibitory effect of glycolaldehyde in the diol dehydratase

(26) The Co(I) nucleophile of cobalt porphyrins cannot be generated in aqueous alkaline medium due to the high Co(II)/Co(I) reduction potential.[25] The 2-hydroxyethyl derivatives of cobalt porphyrins accordingly are essentially alkali stable, in contrast to the corresponding cobaloxime and cobinamide derivatives. Similarly, the 2-cyanoethylcobalt porphyrins are very resistant to alkali, whereas the cobaloximes and cobalamins are alkali labile.

reaction. During these experiments it was noted that glyoxal is formed, and this was considered consistent with the hypothesis that the enzyme on incubation with glycolaldehyde is capable of reductively cleaving the Co–C bond.[27]

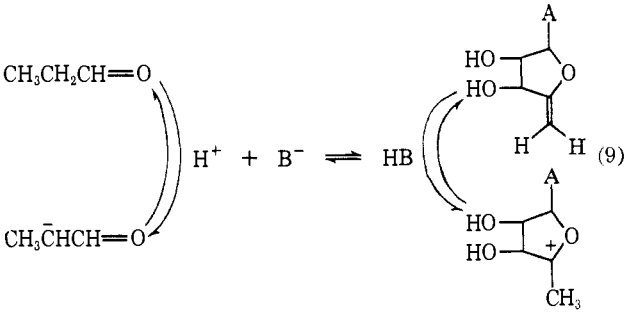
A reductive Co–C bond cleavage of coenzyme B_{12}, organocobalamins, or -cobaloximes cannot be achieved with acyloins under nonenzymatic conditions. Glyoxal, furthermore, *also* inactivates the coenzyme–enzyme complex and produces similar changes of the optical spectrum as does glycolaldehyde,[27] even though it would act as an oxidizing, and not a reducing agent in this system. Recent work has established that vitamin B_{12a} is rapidly reduced by acyloins in general,[28] and that vitamin B_{12a} and cobaloximes are powerful catalysts of oxidation–reduction reactions of acyloins and 1,2-diketones. The mechanism of these reactions will be discussed elsewhere. Their existence must be considered in interpreting the enzyme inactivation by glycolaldehyde or glyoxal. Although a direct reductive or oxidative Co–C-bond cleavage by glycolaldehyde or glyoxal is unlikely, the available evidence nevertheless suggests that the Co–C bond in the coenzyme–enzyme complex is broken. The cobalt ion in the corrin would consequently be accessible for oxidation–reduction reactions involving glycolaldehyde or glyoxal, respectively. Reactions of this type can be performed nonenzymatically. For example, the oxidation–reduction of benzoin and benzil is catalyzed by vitamin B_{12a} (eq 8). The coen-



(8)

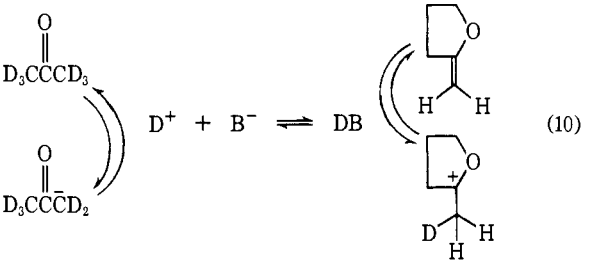
zyme–enzyme complex, finally, is sensitive to oxygen, whereas coenzyme B_{12} is not. This again suggests that the Co–C bond is broken and that the corrin present is in a reduced, oxygen sensitive form.[28a] In summary, eq 7 thus may be regarded as a formalism accounting for observed hydrogen exchange phenomena in which the actual chemical processes are left unspecified. We will show in the next sections that an alternative description is possible which appears to be in agreement with the chemical properties of the constituents of this system.

A Reinterpretation of the Substrate–Coenzyme Hydrogen Exchange Reaction. The hydrogen-exchange reactions between coenzyme B_{12} and the glycol substrates in the diol dehydratase are relevant to the mechanism of the conversion of glycols to aldehydes if, and only if, it can be rigorously proved that the hydrogen exchange takes place during, and not before or immediately after, the actual formation of product. This is very difficult to ascertain, even in the absence of any evidence to the contrary. It has been shown, however, that if propionaldehyde is incubated with diol dehydratase containing 5′-tritiated coenzyme considerable transfer of tritium into the propionaldehyde takes place.[29a] This has been interpreted to suggest that an early step in the reaction is practically irreversible.[29] However, the experiment *de facto* demonstrates product–coenzyme hydrogen exchange, which thus could be responsible for all the hydrogen exchange phenomena in this system. The exchange of protons between the deoxyadenosyl fragment and the product aldehyde is readily envisaged as it can occur as a general acid–base-catalyzed process. Assuming that the coenzyme underwent Co–C bond cleavage to yield enzyme-bound 4′,5′-didehydro-5′-deoxyadenosine or a similar 4′,5′ unsaturated adenosine derivative, the transfer of protons can be formulated as



(9)

shown in eq 9. To demonstrate the validity of eq 9 we have carried out several mechanistically equivalent exchange reactions. Using 2-methylenetetrahydrofuran as a model for 4′,5′-didehydro-5′-deoxyadenosine and acetone-d_6 as the source of labile deuterium reaction 10 was verified using small amounts of CH_3COOD or of Al_2O_3 as catalysts. No exchange occurred without added catalysts. The deuterium–hydrogen exchange



(10)

between acetone-d_6 and 2-dihydropyran was demonstrated similarly (see Experimental Section). We therefore consider eq 9 as a possible mechanism of the substrate–coenzyme hydrogen transfer observed in the diol dehydratase reaction. Catalysis of eq 9 would require the presence of a general Brønsted acid or base in the vicinity of the coenzyme. The reaction could in principle also be catalyzed by the Co(I) nucleophile itself, although this is difficult to demonstrate and presently considered to be less likely. When propanediol-1-^{3}H is incubated together with unlabeled ethylene glycol, the products acetaldehyde and propionaldehyde both contain tritium, consistent with our mechanism. If the enzymatic dehydration of propanediol-1-^{3}H is carried out in the presence of acetaldehyde, tritium is only found in the propionaldehyde and not in the acetaldehyde.[30] This is not in conflict with the postulated mechanism,

(27) O. W. Wagner, H. A. Lee, Jr., P. A. Frey, and R. H. Abeles, *J. Biol. Chem.*, **241**, 1751 (1966).

(28) Unpublished work, with Dr. R. J. Windgassen and L. P. Lee. Also see G. N. Schrauzer, *Ann. N. Y. Acad. Sci.*, **158**, 526 (1969).

(28a) NOTE ADDED IN PROOF. Esr spectra of the coenzyme–enzyme complex have been run and are similar to those obtained with vitamin B_{12r} [see G. N. Schrauzer and L. P. Lee, *J. Am. Chem. Soc.*, **90**, 6541 (1968)], indicating cleavage of the cobalt–carbon bond. However, speculations based on the esr spectra of coenzyme B_{12}–enzyme complexes must be interpreted with care since the B_{12r} may be an artifact due to the extreme photolability and the acid and base sensitivity of the coenzyme and the instability of the Co(I) nucleophile.

(29) P. A. Frey, M. K. Essenberg, and R. H. Abeles, *J. Biol. Chem.*, **242**, 5369 (1967).

(30) R. H. Abeles and B. Zagalak, *ibid.*, **241**, 1246 (1966).

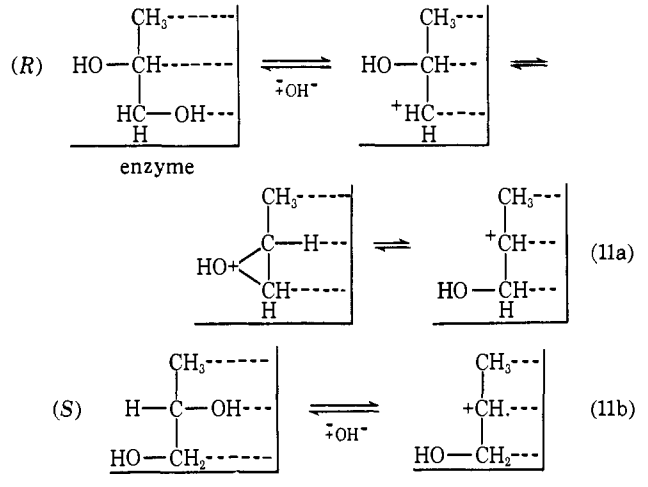
since the hydrogen-exchange reaction is assumed to take place at the active site. In the presence of substrate propanediol the binding of extraneous acetaldehyde to the active site is likely to be difficult due to the difference between substrate and product binding constants, which is expected to be large.

It is tempting to postulate related mechanisms for the observed hydrogen exchange reactions between coenzyme and the substrates or derivatives thereof, in other coenzyme B_{12} dependent processes. These matters will be discussed in a forthcoming paper, however.

The Possible Function of Coenzyme B_{12} in the Diol Dehydratase Reaction. The accumulated evidence led us to propose an alternative mechanism of the enzymatic diol dehydration which is supported by model experiments.

(a) An early step in the reaction causes the reversible cleavage of the Co–C bond in the enzyme–coenzyme complex by a reaction similar to eq 3. For this the specific interaction of the coenzyme with a basic center of the apoenzyme is required. Co–C bond cleavage or enzyme activation does not occur with organocobalamins in place of the coenzyme. 2-Hydroxyethylcobalamin or methylcobalamin are inhibitory.[31]

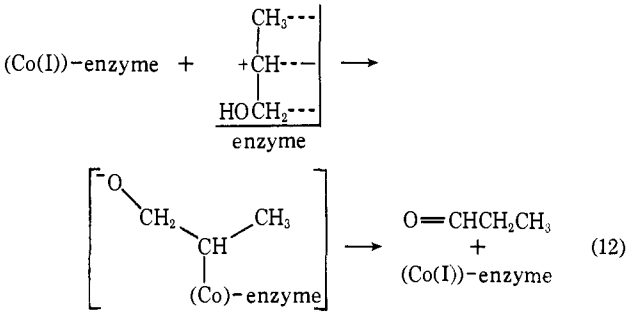
(b) The relative chemical inertness of glycols and the pronounced substrate specificity and stereoselectivity of the enzymatic reaction necessitates the assumption of discrete substrate binding equilibria causing the activation on C-2 of propanediol through the formation of an insipient carbonium ion. Depending whether the substrate is (*R*)- or (*S*)-propanediol, formation of the inductively stabilized carbonium ion proceeds with or without migration of the hydroxyl group from C-2 to C-1 and without changing the conformation on C-2, thus providing an alternative interpretation of the ^{18}O isotope distribution experiments of Rétey and Arigoni[32] in accordance with known reactions of glycol derivatives under



solvolysis conditions (eq 11a,b)[33] (the dotted lines symbolize enzyme protein–substrate binding interactions).

Equations 11a and 11b are also consistent with the established stereochemical course of the enzymatic reaction on C_1 of 1,2-propanediol.[34]

(c) Reaction of the activated glycol with the enzyme-bound Co(I) nucleophile presumably occurs as a SN1-type process with retention of configuration on C-2.[35] The resulting 2-hydroxyalkylcobalt intermediate or transition state on interaction with a basic center localized on the apoenzyme produces the aldehyde by a 1,2-hydride shift with the simultaneous regeneration of the Co(I) nucleophile (eq 12), and inversion of configuration on C-2.



(d) The transfer of hydrogen between the coenzyme adenosyl fragment and the product aldehyde is considered to occur as a product exchange, taking place by eq 8 or an equivalent process independent of the glycol-aldehyde conversion.

The kinetic isotope effect of the enzymatic reaction according to our mechanism would be associated with the 1,2-hydride shift in eq 12. Experiments with 2-hydroxypropylcobalamin-d_1 and 2-hydroxypropyl(pyridine)cobaloxime-2-d_1 indicate a H/D kinetic isotope effect of 5.5 on decomposition in 2 *N* NaOH. This is less than the H/D effect observed[34] for the enzymatic reaction (H/D observed approximately 10). Enzymatic H/D effects are usually larger than nonenzymatic effects in comparable reactions. The postulated mechanism has the unquestionable advantage over eq 7 in that the intermediate reaction steps have all essentially been demonstrated by plausible model reactions, utilizing the coenzyme or its models in their most reactive forms as part of an experimentally established catalytic cycle. In contrast and in spite of considerable efforts, we have been unable to duplicate any of the reaction steps postulated in eq 7 in nonenzymatic model systems. The new interpretation of the substrate–coenzyme transfer or exchange phenomena will facilitate the interpretation of other coenzyme B_{12} dependent enzymatic processes. Whereas it now appears that the hydrogen-exchange reactions are not directly connected with the actual catalytic processes they seem to demonstrate that derivatives of the substrates, of reactive intermediates, or of the products, must pass through the close vicinity of the coenzyme during the enzymatic reaction. That the hydrogen

(31) R. H. Yamada, T. Kato, S. Shimizu, and S. Fukui, *Biochim. Biophys. Acta*, 97, 353 (1965).

(32) J. Rétey, A. Umani-Ronchi, J. Seibl, and D. Arigoni, *Experientia*, 22, 502 (1966).

(33) (a) See, *e.g.*, R. Livingston in "Technique of Organic Chemistry," Vol. 8, Part I, S. L. Friess, E. S. Lewis, and A. Weissberger Ed., Interscience Publishers, New York, N. Y., 1961, p 158. (b) Analogous enzymes such as the coenzyme B_{12} dependent glycerol dehydratase and the ethanolamine deaminase are expected to differ from the diol dehydratase mainly with respect to the specificity of substrate binding and activation.

(34) B. Zagalak, P. A. Frey, G. L. Karabatsos, and R. H. Abeles, *J. Biol. Chem.*, 241, 3028 (1966).

(35) A SN2 displacement of the 2-OH group of propanediol by the Co(I) nucleophile, though possible in principle, is considered less likely in view of the observed net inversion of configuration at C-2 and the ^{18}O isotope distribution results. Retention of configuration, although a planar carbonium ion is formed, has been explained in other cases by the enzyme holding the carbonium ion tight enough to prevent rotation. See, for example, L. L. Ingraham, "Biochemical Mechanisms," John Wiley & Sons, Inc., New York, N. Y., 1962, p 51.

transfer or exchange reactions could be side reactions has recently been suggested as a possibility in the methylmalonyl–succinyl–CoA mutase reaction;[36] the hydrogen transfer effects in this reaction could indeed be interpreted on a basis similar to that suggested for the reactions discussed in the present paper. Nevertheless, the integrated process of the enzymatic dehydration of glycols is likely to be still more complicated than indicated. It appears, however, that the suggested mechanism represents essential reaction steps involving the coenzyme.

Experimental Section

Starting Materials. Vitamin B_{12a} (Hydroxocobalamin), factor B (cyanoaquocobinamide) and 5′-deoxyadenosylcobalamin (coenzyme B_{12}) were obtained from Merck Sharp and Dohme Laboratories, Rahway, N. J. All other starting materials for the synthesis of the cobalt complexes described were commercially available.

Synthesis of 5′-Deoxyadenosylcobaloximes. The coenzyme models were synthesized from adenosine-5′-tosylate prepared according to Schmidt and Huennekens.[15] To a solution of 1.19 g (0.005 mole) of $CoCl_2 \cdot 6H_2O$ in 20 ml of methanol was added 1.16 g (0.010 mole) of dimethylglyoxime under argon. This was followed by 0.8 g (0.01 mole) of 50% wt NaOH solution, 0.5 g (0.005 mole) of pyridine, and 0.2 g of $NaBH_4$. After stirring for 5 min the solution had turned blue-green. At this point 2.1 g (0.005 mole) of adenosine-5′-tosylate was added and the solution was stirred for 10 min.

After the color of the reaction solution turned brown, air was passed through it for 20 min, 30 ml of H_2O was added, and the solution cooled in ice. Yellow crystals of 5′-deoxyadenosyl(pyridine)-cobaloxime separated and were collected by filtration: yield, 2.3 g (74% based on adenosine tosylate). The complex has no sharp melting point and starts to decompose above 175°. *Anal.* Calcd for $C_{23}H_{31}N_{10}O_7Co$: C, 44.65; H, 5.05; N, 22.66. Found: C, 44.91; G, 5.38; N, 22.48.

5′-Deoxyadenosyl(aquo)cobaloxime was prepared similarly except that the addition of pyridine was omitted. The compound crystallizes with difficulty and was in most cases employed in solution.

Alkali Decomposition. 2′,3′-Isopropylidene-5′-deoxyadenosyl-(pyridine)cobaloxime (0.050 g) was decomposed in alkaline medium under the conditions given in Table III. The isopropylidene derivative was used for the identification of the alkali cleavage products in view of the instability of the free adenosine derivatives formed in the alkali treatment.[16] The products were identified by thin layer chromatography on cellulose. The identification of cobaloxime(I) in the alkali decomposition was carried out separately, both with the 5′-deoxyadenosyl(pyridine)cobaloxime and the 2,3-isopropylidene ketal, respectively. The Co(I) species were identified unambiguously by the characteristic low energy bands in the absorption spectra at 15,600, 18,182 and 21,830 cm^{-1}, respectively. 2′,3′-Isopropylidene-5′-deoxyadenosylcobalamin was decomposed in 5% potassium *t*-butoxide in *t*-butyl alcohol, and the cleavage products were identified as in the case of the cobaloxime derivative.

Alkali Degradation of 5′-Deoxyadenosylcobalamin. The coenzyme was dissolved in water to a concentration of 200 μg/ml. The solution was transferred into a uv cell fitted with a serum cap and first flushed with argon (99.998%). Subsequently deoxygenated NaOH solution was added until the solution was 2 *M* in NaOH and 100 μg/ml in coenzyme B_{12}. The course of the degradation was followed by monitoring the decrease of absorbance at 525 mμ, yielding a straight line $\log[OD_t - OD_{t+\Delta t}]$ plot consistent with a pseudo-first-order reaction. Similar experiments at 1 and 5 *M* NaOH gave the same second-order rate constant of 5.3×10^{-7} l./mole sec. The formation of vitamin B_{12s} was indicated by the appearance of a band at 385 mμ which is characteristic of the Co(I) derivative. The concurrent formation of vitamin B_{12r} was indicated by the brown color of the reaction solution. Addition of methyl iodide to a solution of coenzyme B_{12} in 2 *M* NaOH after decomposition indicated the formation of some methylcobalamin (identified by tlc using butyl alcohol-2-propanol–water–acetic acid = 100:100:100:3 as the solvent and cellulose as the adsorbant).

(36) W. W. Miller and J. H. Richards, *J. Am. Chem. Soc.*, **91**, 1498 (1969).

Table III. Alkali Decomposition of 2′,3′-Isopropylidene-5′-deoxyadenosylcobaloxime and -cobalamin Derivatives. Identification of Cleavage Products by Chromatography on Cellulose

Compound	R_f values in indicated solvent[a]			
	I		II	
2′,3′-Isopropylidene-5′-deoxyadenosyl-(pyridine)cobaloxime in 3 *M* aqueous NaOH	0.86	0.59	0.81	0.34
Same, in 5% K-*t*-butoxide in *t*-butyl alcohol	0.86	0.59	0.81	0.34
2′,3′-Isopropylidene-5′-deoxyadenosyl-cobalamin in 5% K-*t*-butoxide in *t*-butyl alcohol	0.86	0.59	0.81	0.34
Adenine[b]		0.59		0.35
2′,3′-Isopropylidene-4′,5′-didehydro-5′-deoxyadenosine[c]	0.85		0.81	

[a] Solvent I: *n*-butyl alcohol–acetic acid–water = 4:1:5; solvent II, *n*-butyl alcohol–water = 86:14 (vol). [b] The relative amounts of adenine formed were about equal to the product with R_f = 0.86. [c] Prepared by the method of J. R. McCarthy.[16]

Acid and Cyanide Decomposition of Coenzyme B_{12} and the Model Compounds. The rates of acid and cyanide degradation of coenzyme B_{12} and the pyridinecobaloxime model compound were determined at 25° in 1 *M* HCl and 0.01 *M* KCN aqueous solutions, respectively. The reactions were followed by observing the decrease of the absorbance at 525 mμ in the case of coenzyme B_{12} and at 445 mμ for the cobaloxime model compound. The reactions were cleanly pseudo first order under these conditions.

Synthesis of the 2-Hydroxyalkylcobalt Complexes. All 2-hydroxyalkylcobaloxime derivatives were prepared as described previously.[7] The hydroxyalkyl derivatives of chelates **4**, **5**, and **6** were prepared by treating the corresponding Co(I) derivatives with ethylene oxide. The method for the generation of the Co(I) nucleophile of chelate **6** was described elsewhere.[25] Hydroxyethylcobalamin was prepared by the method of Müller and Müller and purified through phenol extraction.[37] Hydroxyethylcobinamide was prepared and purified analogously. The derivative of chelate **5** was isolated as the perchlorate, the procedure of work-up was identical with that reported for the preparation of corresponding alkylcobalt derivatives: 2-hydroxyethylbis(salicylaldehyde) ethylenediiminecobalt (*Anal.* Calcd for $C_{18}H_{19}N_2O_3Co$: C, 59.00; H, 5.23. Found: C, 59.03; H, 5.25); 2-hydroxyethylbis(butanedione monoxime)-(propylenediimine)cobalt perchlorate (2-hydroxyethylcobalt derivative of the perchlorate of chelate *5*) (*Anal.* Calcd for $C_{13}H_{28}N_4O_7$-CoCl (the monohydrate): C, 35.73; H, 6.00. Found: C, 36.00; H, 5.97); 2,2-dimethyl-2-hydroxyethyl(pyridine)cobaloxime (from cobaloxime(I) and isobutylene oxide) (*Anal.* Calcd for $C_{17}H_{28}$-N_5O_5Co: C, 46.25; H, 6.39. Found: C, 46.30; H, 6.45).

Product Analysis. The products of the decomposition of the 2-hydroxyalkylcobalt chelates were analyzed by glpc, utilizing a 1/8 in. × 5 ft Poropak Q column and a flame ionization detector. The reaction solutions were swept out with argon and volatile decomposition products condensed in a −78° trap. Acetaldehyde and acetone were the only products detected and identified by glpc and mass spectroscopy, from the decomposition of the 2-hydroxyethyl- and 2-hydroxypropylcobalt chelates studied. Analysis of the solution revealed the presence of acetaldehyde and aldol condensation products in the case of the reaction solutions of the alkali degradation of the 2-hydroxyethylcobalt complexes. No other products could be detected. In the case of the 2-hydroxypropylcobalt complex decomposition the liquid phase contained acetone, some diacetone alcohol, and traces of mesityl oxide.

Kinetic Measurements. The kinetic runs were made on a Beckman DK-2A recording spectrophotometer at a wavelength in the visible region characteristic of the alkylcobalt complex present at the beginning of the reaction (*e.g.*, 440–460 mμ for the cobaloximes, and chelates **4**–**6**, and 525 mμ for cobalamin and cobinamide derivatives). The base-catalyzed decompositions were generally run aerobically under pseudo-first-order conditions at a rate such that the initially produced Co(I) species was oxidized much more rapidly than it was formed. In several cases the rate determinations were made anaerobically, the observed rates were identical within experi-

(37) O. Müller and G. Müller, *Biochem. Z.*, **336**, 229 (1962).

mental error with those obtained aerobically. (The anaerobic experiments require the strict exclusion of oxygen and thus were less convenient to perform.) The runs were not thermostated and were performed at 27 ± 2°.

Rate Law. The reactions of the 2-hydroxyalkylcobalt chelates with alkali under pseudo-first-order conditions all gave straight $\log(OD_t - OD_\infty)$ *vs.* time plots; most rate constants listed in Table II were calculated using the Guggenheim treatment.[38] All the reactions were run in water or 10% methanol in water. No observable rate differences were observed between the two solvent systems. The base-catalyzed decomposition of the 2-hydroxyalkylcobalt chelates obeyed a second-order rate law, $-d[CoR]/dt = k_2[CoR][OH]$. The concentration of OH^- was varied by a factor of 10 with no change in the observed second-order rate constants.

Hydrogen–Deuterium Exchange Experiments. The deuterium-exchange reaction between acetone-d_6 and 2-methylenetetrahydrofuran[39] and 2-dihydropyran was demonstrated as follows. Nmr tubes were charged with 0.7 ml of 2-methylenetetrahydrofuran or 2-hydropyran, respectively, and with 0.2 ml of acetone-d_6 and of 0.1 ml of acetic acid-d_1. The tubes were sealed and the exchange was followed by nmr. The disappearance of the complex spin-coupling pattern of the nmr spectrum of 2-methylenetetrahydrofuran was accompanied by the appearance of the multiplet due to partially protonated acetone at 2.1 ppm. In the case of 2-dihydropyran the disappearance of the H_2 multiplet at 4.6 ppm and collapse of the H_1 doublet at 6.3 ppm in the dihydropyran spectrum proved the substitution of hydrogen by deuterium in the 3 position of 2-dihydropyran. Again, a multiplet due to partially protonated acetone was observed at 2.1 ppm. Similar exchange experiments were carried out with neutral aluminum oxide (chromatographic quality) as the catalyst, except that the reaction was carried out at 65° in a sealed vial. For the nmr measurements the solutions were freed of Al_2O_3 by filtration.

Determination of H/D Isotop eEffect. 2-Hydroxypropylcobalamin-2-*d* was synthesized from vitamin B_{12s} and 1-chloro-2-propanol-2-d_1 and purified by phenol extraction. The rate of alkali decomposition was determined in 2 *M* aqueous NaOH at 27°.

Acknowledgment. We thank Professor R. H. Abeles (Brandeis University) for a gift of purified diol dehydratase.

(38) E. A. Guggenheim, *Phil. Mag.*, 7, Vol. 2, 538 (1926).
(39) M. F. Ansell and D. A. Thomas, *J. Chem. Soc.*, 1163 (1958).

The Synthesis and Pharmacological Properties of [1-(δ-Mercaptovaleric acid)]-oxytocin, a Homolog of Deamino-oxytocin Containing a Twenty-Two-Membered Ring[1,2]

Wolfgang Fraefel[3] and Vincent du Vigneaud[4]

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Abstract: [1-(δ-Mercaptovaleric acid)]-oxytocin, a homolog of deamino-oxytocin in which the size of the disulfide ring is increased from 20 to 22 members by the formal insertion of two methylene groups at position 1, has been synthesized by the Merrifield solid phase method. This homolog does not exhibit avian vasodepressor or oxytocic activity but possesses a small inhibitory activity against the oxytocic activity of oxytocin itself. The mixed disulfide of cysteine and δ-mercaptovaleric acid has been synthesized, and its chromatographic behavior in the Beckman-Spinco amino acid analyzer has been established.

As has been shown in earlier studies,[5,6] the pharmacological activities of deamino-oxytocin, a highly active analog of oxytocin (Figure 1), are strongly dependent on the size of the disulfide ring. Thus [1-(γ-mercaptobutyric acid)]-oxytocin, a homolog of deamino-oxytocin in which the β-mercaptopropionic acid residue at position 1 is replaced by γ-mercaptobutyric acid to form a 21-membered ring, possesses approximately 3 units/mg of oxytocic activity and no detectable avian vasodepressor activity.[5] [1-(Mercaptoacetic acid)]-oxytocin, a deamino-oxytocin homolog in which a methylene group has been formally removed from the residue at position 1 to form a 19-membered ring, possesses 25 units/mg of oxytocic activity and 4 units/mg of avian vasodepressor activity.[6] Deamino-oxytocin possesses approximately 800 units/mg of oxytocic and 975 units/mg of avian vasodepressor activity.[7]

This paper deals with the synthesis of [1-(δ-mercaptovaleric acid)]-oxytocin, in which the β-mercaptopropionic acid residue of deamino-oxytocin is replaced with a δ-mercaptovaleric acid residue. Thus two methylene groups are introduced at position 1 adjacent to the disulfide bridge to form a 22-membered ring. For the synthesis of the desired deamino-oxytocin homolog, the Merrifield solid phase method was used.[8–10] The synthesis was carried out in eight cycles as described for the solid phase synthesis of deamino-oxytocin,[11] except that the glycine-resin used was in the unnitrated form and in the last cycle of the synthesis, S-benzyl-δ-mercaptovaleric acid was used instead of S-benzyl-β-mercaptopropionic acid. The cleavage of the final protected polypeptide from the resin support was achieved with

(1) This work was supported by Grant No. HE-11680 from the National Heart Institute, U. S. Public Health Service, and by a grant from Geigy Chemical Corporation.
(2) All optically active amino acid residues are of the L variety.
(3) Geigy Chemical Corporation Fellow.
(4) To whom correspondence and reprint requests should be addressed.
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