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A Direct Method for Cobalt–Carbon Bond Formation in Cobalt(III)-Containing Cobalamins and Cobaloximes. Further Support for Cobalt(III) π Complexes in Coenzyme B_{12} Dependent Rearrangements

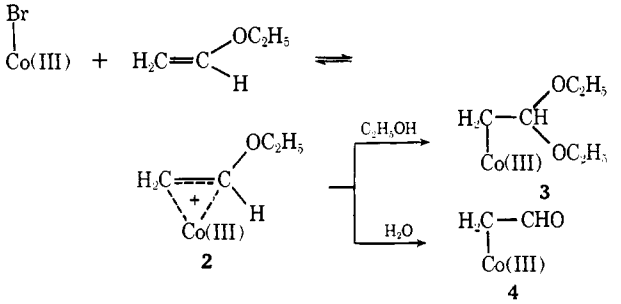
Sir:

The mechanism of action of coenzyme B_{12} dependent enzymes has been shown to involve a net substrate rearrangement in which a hydrogen exchanges places with an alkyl, acyl, or electronegative group on an adjacent carbon atom.[1-5] Recent observations suggest that an early step in the enzymatic reaction is the homolytic cleavage of the cobalt–carbon bond of the coenzyme,[2,6,7] and detailed information concerning the routes and stereochemistry of the hydrogen migration is known.[1,8-10] However, little is known about the mode of migration of the other group.[1-4,11-14] We recently reported evidence for $\sigma \rightleftharpoons \pi$ rearrangements in cobaloximes[15] and suggested a role for Co(III) π complexes in these enzymatically controlled rearrangements (Scheme I). The dioldehydrase reaction (ethylene glycol → acetaldehyde) has been chosen as a typical example. R–CH_2–Co(III) represents the enzyme–coenzyme complex where the methylene group is that of the 5′-deoxyadenosine moiety.

The principal intermediate in this reaction sequence is the π-complex **1**. Our observations on the solvolysis of 2-acetoxyethyl-*2*-^{13}C-(pyridine)cobaloxime[15] support the intermediacy of such a π complex, but in order to better characterize such species it was desirable to prepare Co(III) π complexes directly from Co(III) complexes and olefins.

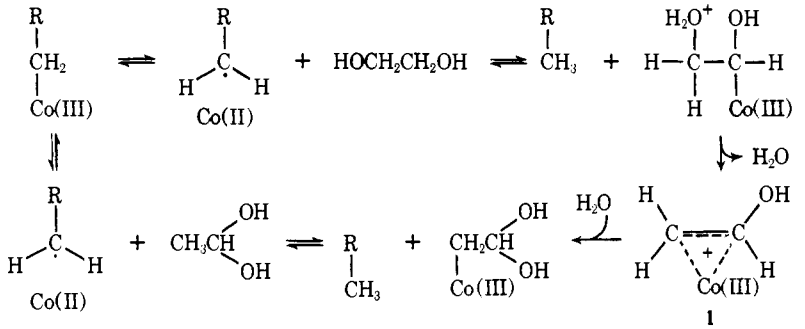
We have been unable, so far, to observe any interaction between ethylene and Co(III)-containing cobalamins or cobaloximes. This, however, is not too surprising since transition metal π complexes are, in general, most stable when the metal is in a low oxidation state and when the olefin is electron deficient.[16] The π complex sought would contain cobalt in its highest normal oxidation state. This suggested that electron-rich olefins should be used, and indeed the π complex proposed in the enzymatic system (Scheme I) contains such an electron-rich olefin (in this case enol acetaldehyde).

If reaction between a Co(III) cobaloxime and an electron-rich olefin, such as ethyl vinyl ether, were to give the π-complex **2**, then we anticipated that an ambient nucleophile would attack at the oxygen-bearing carbon atom of the complex to give a neutral species. When a methylene dichloride solution of bromo(pyridine)cobaloxime was treated with ethyl vinyl ether in the presence of triethylamine and ethanol, complete alkylation at the cobalt occurred to give a mixture of the acetal **3** and the aldehyde **4**.



These observations are consistent with the quenching of a π complex either by ethanol, to give the acetal, which is stable under the reaction conditions, or by water, to give a hemiacetal which then collapses to the

Scheme I. Proposed Mechanism for the Rearrangements Controlled by B_{12} Dependent Enzymes



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(1) M. K. Essenberg, P. A. Frey, and R. H. Abeles, *J. Amer. Chem. Soc.*, **93**, 1242 (1971).
(2) S. A. Cockle, H. A. O. Hill, R. J. P. Williams, S. P. Davies, and M. A. Foster, *ibid.*, **94**, 275 (1972).
(3) T. J. Carty, B. M. Babior, and R. H. Abeles, *J. Biol. Chem.*, **246**, 6313 (1971).
(4) W. W. Miller and J. H. Richards, *J. Amer. Chem. Soc.*, **91**, 1498 (1969).
(5) R. L. Switzer, B. G. Baltimore, and H. A. Barker, *J. Biol. Chem.*, **244**, 5263 (1969).
(6) B. M. Babior, T. H. Moss, and D. C. Gould, *ibid.*, **247**, 4389 (1972).
(7) T. H. Finlay, J. Valinsky, K. Sato, and R. H. Abeles, *ibid.*, **247**, 4197 (1972).
(8) P. A. Frey, M. K. Essenberg, and R. H. Abeles, *ibid.*, **242**, 5369 (1967).
(9) R. G. Eager, B. G. Baltimore, M. M. Herbst, H. A. Barker, and J. H. Richards, *Biochemistry*, **11**, 253 (1972).
(10) M. Sprecher, M. J. Clark, and D. B. Sprinson, *J. Biol. Chem.*, **241**, 872 (1966).
(11) B. Babior, *ibid.*, **245**, 6125 (1970).
(12) J. Rétey, A. Umani-Ronchi, J. Seibl, and D. Arigoni, *Experientia*, **22**, 502 (1966).
(13) J. N. Lowe and L. L. Ingraham, *J. Amer. Chem. Soc.*, **93**, 3801 (1971).
(14) P. Dowd and C. S. Nakagawa, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1173 (1972).
(15) R. B. Silverman, D. Dolphin, and B. M. Babior, *J. Amer. Chem. Soc.*, **94**, 4028 (1972).
(16) M. Herberhold, "Metal π-Complexes," Vol. II, Part 1, Elsevier, New York, N. Y., 1972.

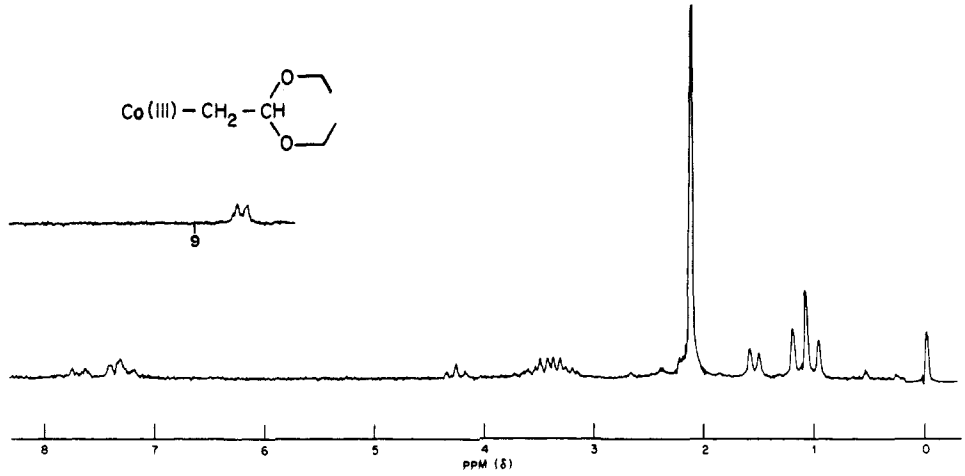


Figure 1. Nmr spectrum (60 MHz) of 2,2-diethoxyethyl(pyridine)cobaloxime (**3**) in $CDCl_3$.

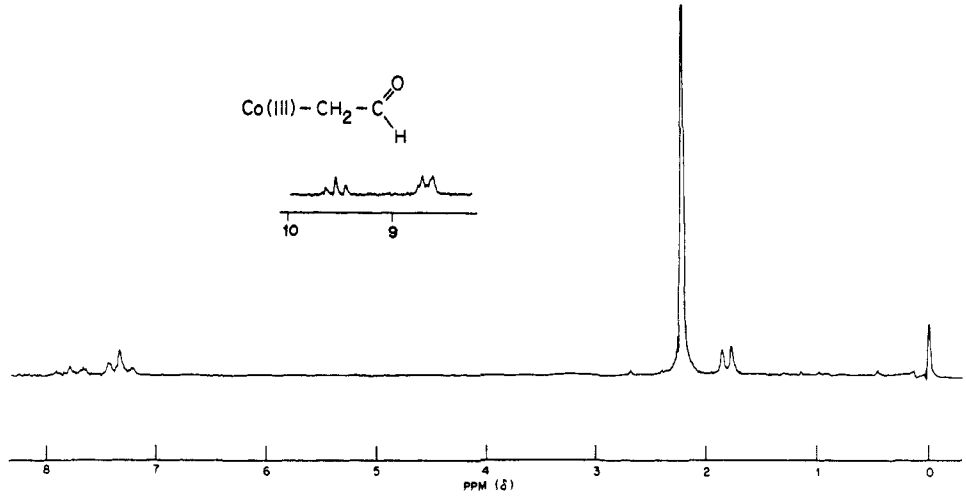


Figure 2. Nmr spectrum (60 MHz) of formylmethyl(pyridine)cobaloxime (**4**) in $CDCl_3$.

observed aldehyde. Furthermore, as increasing amounts of water were added to the reaction mixture, larger amounts of the aldehyde were formed at the expense of the acetal.

In order to characterize these alkylcobaloximes, reduced cobaloxime (Co(I)) was treated with the diethyl acetal of 2-bromoacetaldehyde. This gave a product which was identical with the acetal **3** prepared *via* the ethyl vinyl ether route.[17] Thus, the acetal **3** has, *inter alia*, bands in the nmr spectrum (Figure 1) at δ 1.09 (6 H, t), 1.55 (2 H, d), 3.15–3.6 (4 H, m), 4.27 (1 H, t). Hydrolysis of this acetal with aqueous acetic acid gave an aldehyde which was again identical with the aldehyde **4**.[17] The nmr spectrum (Figure 2) has bands at δ 1.83 (2 H, d) and 9.55 (1 H, t).

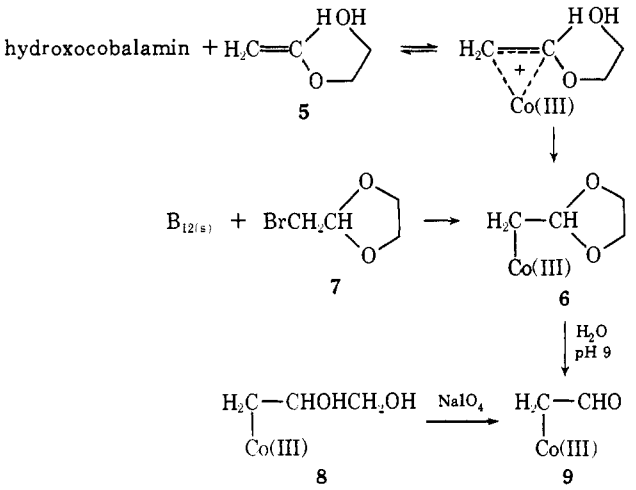
It is generally assumed that cobaloximes provide good models for cobalamins. Nonetheless, in order for π complexes to be considered as intermediates in the B_{12} controlled rearrangements, it was necessary to show that the same chemistry was operative with cobalamins. To demonstrate this, 2-hydroxyethyl vinyl ether (**5**) was chosen as the olefin, since it is soluble in water, the solvent of choice for cobalamins. Moreover, an internal cyclization between the π complex, if formed, and the β-hydroxyl group might lead exclusively to acetal, with no aldehyde formation, even though the reaction was carried out in water. When hydroxocobalamin, in water, was treated with a 100-fold excess of the vinyl ether **5** in the presence of triethylamine, the acetal **6** was formed quantitatively. The half-life ($t_{1/2}$) for this reaction at room temperature was *ca.* 4 hr. Furthermore, this acetal **6** was identical with that prepared from B_{12s} (Co(I)) and the bromoacetal **7**.[18] In water at pH 9 this acetal is hydrolyzed to formylmethylcobalamin (**9**). This conversion of the acetal to the aldehyde gives additional proof of the structural assignments made since the aldehyde **9** derived from the acetal **6** was found to be identical with formylmethylcobalamin[18] prepared by the method of Abeles,[19] which involves the periodate cleavage of 2,3-dihydroxypropylcobalamin (**8**).

The reaction between bromo(pyridine)cobaloxime and vinyl ether **5** once again produced a quantitative

(17) The alkylcobaloximes prepared by either route had identical spectroscopic (ir, nmr, uv) properties, which were consistent with the assigned structures.

(18) The alkylcobalamins prepared by two different routes are shown to be identical by a comparison of their electronic absorption spectra and by showing that they have identical R_f values in three solvent systems on cellulose tlc. The system *n*-butyl alcohol–ethanol–water (10:3:7) containing 0.5% concentrated NH_4OH by volume is capable of cleanly separating 1,3-dioxa-2-cyclopentylmethyl-, methyl-, 2,2-diethoxyethyl-, formylmethyl-, cyano-, and hydroxocobalamin.

(19) R. H. Abeles, T. Carty, and E. Krodel, unpublished results.



yield of cyclic acetal,[17] but this reaction was now slower ($t_{1/2}$ *ca.* 7 hr) than that with the cobalamin. Finally, the reaction between hydroxocobalamin and ethyl vinyl ether, in ethanol, gave a quantitative yield of the acetal, 2,2-diethoxyethylcobalamin, which was also prepared from B_{12s} and the corresponding bromoacetal.[18] Once again, as in the cobaloxime case, increasing amounts of water in this reaction gave increasing amounts of the aldehyde **9**.[18]

We have shown that the reactions of trivalent cobalt-containing cobalamins and cobaloximes with enol ethers can be formulated as proceeding *via* the formation of a Co(III) π complex, which can be quenched by ambient nucleophiles to give stable alkylcobalamins and cobaloximes. The observation of such reactions strengthens our hypothesis that the intermediacy of π complexes can explain the rearrangements controlled by coenzyme B_{12} dependent enzymes. These π complexes could be generated by the enzymatic removal of a group from a carbon atom β to the cobalt. This is a process which would clearly be assisted by the cobalt, for we have already seen that labilization of the "β groups" is greater with the cobalamins than with the cobaloximes. Thus, while the 2,2-diethoxyethylcobalamin is solvolyzed in water at pH 9, at room temperature, to the formylmethylcobalamin, solvolysis of the corresponding cobaloxime acetal requires 0.1 *M* acetic acid.

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Conformational Determination in Paramagnetic Metal Complexes

Sir:

When a labile complex is formed in solution between a diamagnetic ligand and a paramagnetic species, the nuclear magnetic resonance signals from those ligand nuclei closest to the site(s) of interaction are preferentially broadened. This effect has been widely employed in establishing paramagnetic metal binding sites.[1-4] However, the implied quantitative relationship between resonance line broadening and the electron–nucleus distances, r_i, has not been exploited.[5]

The conditions for which meaningful values of r_i can be obtained from paramagnetically broadened nuclear resonance lines are established herein. Experimental values for a variety of labile paramagnetic complexes are in excellent agreement with known structures.

The paramagnetic contribution to the spin–spin relaxation time, T_2, of a diamagnetic nucleus in a labile complex is given by[6] eq 1 when relaxation is modulated

$$\frac{1}{T_2} = \left(\frac{1}{15}\right)S(S+1)g^2\beta^2\gamma_I\left(\frac{N_s n}{N_I}\right)\left(\frac{1}{r_i^6}\right)\left[7\tau_c + \frac{13\tau_c}{1+\omega_s^2\tau_c^2}\right] + \left(\frac{1}{3}\right)S(S+1)\frac{A^2}{\hbar^2}\left(\frac{N_s n}{N_I}\right)\left[\tau_e + \frac{\tau_e}{1+\omega_s^2\tau_e^2}\right] \quad (1)$$

by isotropic rotation of the complex. S is the effective electron spin quantum number, n is the number of identical nuclei coupled to the electron, and N_s and N_I are the total number of paramagnetic species and diamagnetic ligand molecules per cubic centimeter. A is the isotropic hyperfine coupling constant which is a complicated function of r_i. g, β, and γ_I have their usual meanings.

The two terms in eq 1 represent dipolar and scalar relaxation components with motional correlation times,[6] τ_c and τ_e, respectively. In cases where dynamic scalar coupling is relatively inefficient[7,8] (*e.g.*, between protons and labile paramagnetic metal ions other than lanthanide and actinide ions), r_i can be estimated (assuming $A = 0$) from a plot of T_2^{-1} *vs.* N_s, provided that τ_c and n are known for each proton.

The very sensitive distance dependence (r_i^{-6}) of dipolar relaxation actually enables meaningful values of r_i to be obtained even when some scalar relaxation occurs. Furthermore, only small errors result if it is assumed[9] that τ_c is the same for all protons in the complex. Thus, relative r_i values can be obtained directly from the relative slopes of $[nT_2]^{-1}$ *vs.* N_s plots.

Measurements of T_2 for individual peaks in a high resolution nmr spectrum are usually accomplished by measuring resonance line widths, $\Delta\nu_{1/2}$ ($=[\pi T_2]^{-1}$).[10] However, many useful resonance lines exhibit multiplet

(1) N. C. Li, R. Scruggs, and E. D. Becker, *J. Amer. Chem. Soc.*, **84**, 4650 (1962).
(2) G. L. Eichhorn, P. Clark, and E. D. Becker, *Biochemistry*, **5**, 245 (1966).
(3) H. Sigel and D. B. McCormick, *J. Amer. Chem. Soc.*, **93**, 2041 (1971).
(4) A. W. Missen, D. F. S. Natusch, and L. J. Porter, *Aust. J. Chem.*, **25**, 129 (1972).
(5) K. G. Morallee, E. Nieboer, F. J. C. Rossotti, R. J. P. Williams, A. V. Xavier, and R. A. Dwek, *Chem. Commun.*, 1132 (1970).
(6) D. R. Eaton and W. D. Phillips, *Advan. Magn. Resonance*, **1**, 103 (1965).
(7) A. Abragam, "Principles of Nuclear Magnetism," Clarendon Press, Oxford, England, 1961.
(8) R. A. Dwek, O. W. Howarth, D. F. S. Natusch, and R. E. Richards, *Mol. Phys.*, **13**, 457 (1967).
(9) D. Doddrell and A. Allerhand, *J. Amer. Chem. Soc.*, **93**, 1558 (1971).
(10) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 1, Pergamon Press, Oxford, 1965.