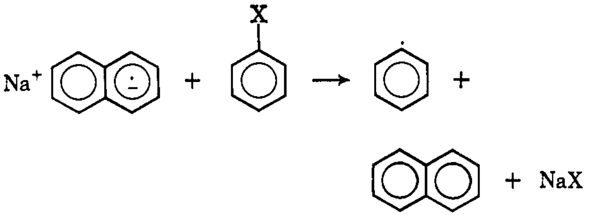
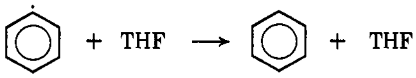


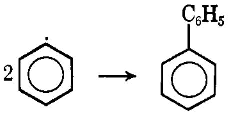
(4)



(5)

$$2\mathrm{THF}\cdot \xrightarrow{\text{combination}} \text{THF dimer} \qquad (6)$$

$$2\mathrm{THF}\cdot \xrightarrow{\text{disproportionation}} \text{several products} \qquad (7)$$



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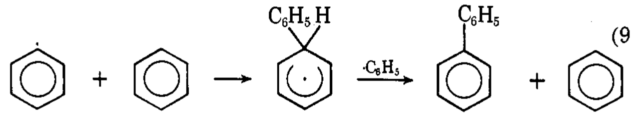

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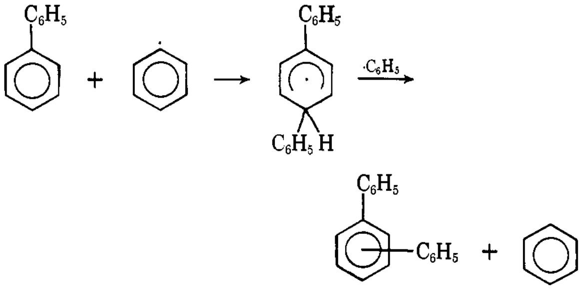

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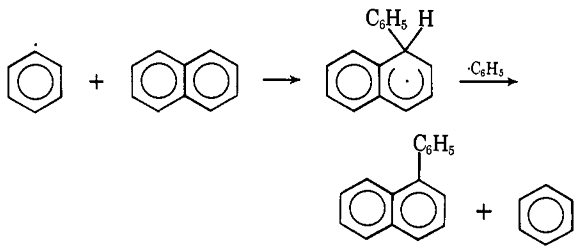
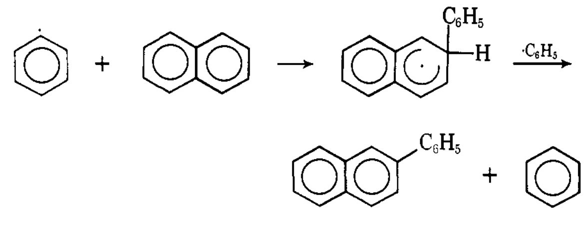


(11)



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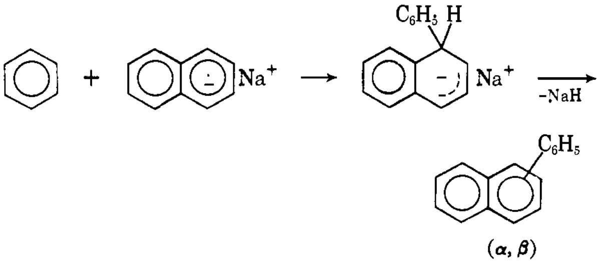

(13)

concentration of sodium naphthalene in the range of 1–2. (2) A small amount of THF dimer was observed by mass spectrometry (*m/e* 142) when sodium naphthalene (37.5 mmol) reacted with chlorobenzene (18.75 mmol) at 27° in THF. (3) The product distribution before and after hydrolysis is identical (Tables I and II).

It thus appears that the reaction is proceeding by a radical mechanism. From our analysis of products from D_2O treatment, it would appear that any combination mechanism would have to be predominately of radical nature. This interpretation agrees with the data of Müller and Roscheisen on reactions of disodium tetraphenylethylene with bromobenzene.[9] Furthermore, our product distributions for the reaction of sodium naphthalene with either bromobenzene or iodobenzene were consistent with Hey and coworkers[10] who report that the isomer distributions for the reaction of benzoyl peroxides with biphenyl yielded 49% *o*-, 23% *m*-, and 29% *p*-terphenyl. We thus believe that a radical mechanism best explains the observed products. Others have generated phenyl radical in the presence of aromatic solvents and have observed the same product distributions that we have isolated (biphenyl, isomeric terphenyl).[10–12]

A two-step mechanism of the type shown in eq 9, 10, 11, and 12 has also been reported by several investigators.[11,12]

Acknowledgment. The authors are pleased to acknowledge informative discussions with Professor H. J. Harwood. The authors also wish to thank The Firestone Tire & Rubber Co. for permission to publish this work.

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[9] E. Müller and R. Roscheisen, *Chem. Ber.*, **91**, 1106 (1958).

[10] J. I. G. Dadogan, D. H. Hey, and G. H. Williams, *J. Chem. Soc.*, 794 (1964). For a more extensive compilation, see: (a) C. Walling, "Free Radicals in Solution," Wiley, New York, N. Y., 1956, p 484; (b) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Wiley, New York, N. Y., 1959, p 722.

[11] W. A. Pryor, "Free Radicals," McGraw-Hill, New York, N. Y., 1966, pp 256–257.

[12] D. H. Hey, M. J. Perkins, and G. H. Williams, *J. Chem. Soc.*, 3412 (1964).

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The Mechanism of Coenzyme B_{12} Action in Dioldehydrase

Sir:

Recently,[1] a mechanism of action of coenzyme B_{12} in dioldehydrase[2] was postulated on the basis of extensive model studies, in which the corrin Co(I) nucleophile derived from coenzyme B_{12}[3] functions as the actual catalytic intermediate. In this communication we demonstrate the validity of this mechanistic proposition on the basis of additional model reactions and parallel experiments with the reacting dioldehydrase holoenzyme of *Aerobacter aerogenes.*[4]

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[1] G. N. Schrauzer and J. W. Sibert, *J. Amer. Chem. Soc.*, **92**, 1022 (1970).

[2] Dioldehydrase in DL-1,2-propanediol hydrolyase, E.C. 4.2.1.28.

[3] Coenzyme B_{12} is α-(5,6-dimethylbenzimidazolyl)-*Co*-5′-deoxyadenosylcobamide.

[4] Purified dioldehydrase was obtained from Dr. R. H. Abeles, Brandeis University, Waltham, Mass., or purchased from Calbiochem, Los Angeles, Calif. The activities of the enzyme from both sources differed, although their qualitative behavior was identical.

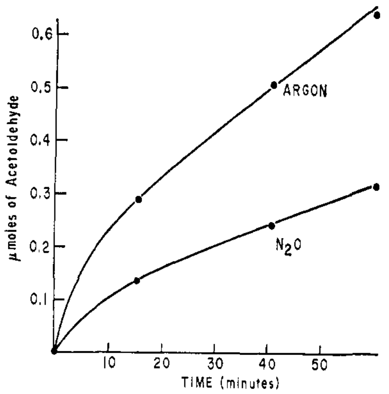


Figure 1. Inhibition of acetaldehyde formation from 2-bromoethanol by N_2O with β-cyanoethyl(pyridine)cobaloxime as the catalyst. The reaction solution contained the following in a final volume of 2.0 ml: β-cyanoethyl(pyridine)cobaloxime, 0.04 mmol; 2-bromoethanol, 0.25 mmol; potassium phosphate buffer (pH 12.0), 0.1 *M*. After the time indicated under argon or N_2O at 37°, the reaction was quenched with 0.5 ml of 2 *N* HCl. The acetaldehyde formed was assayed colorimetrically as its 2,4-dinitrophenylhydrazone at 540 mμ.

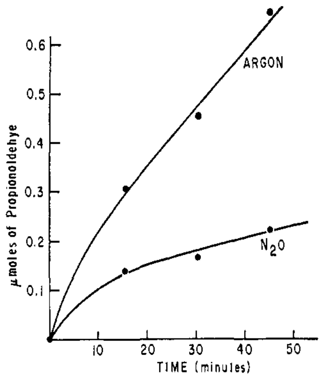
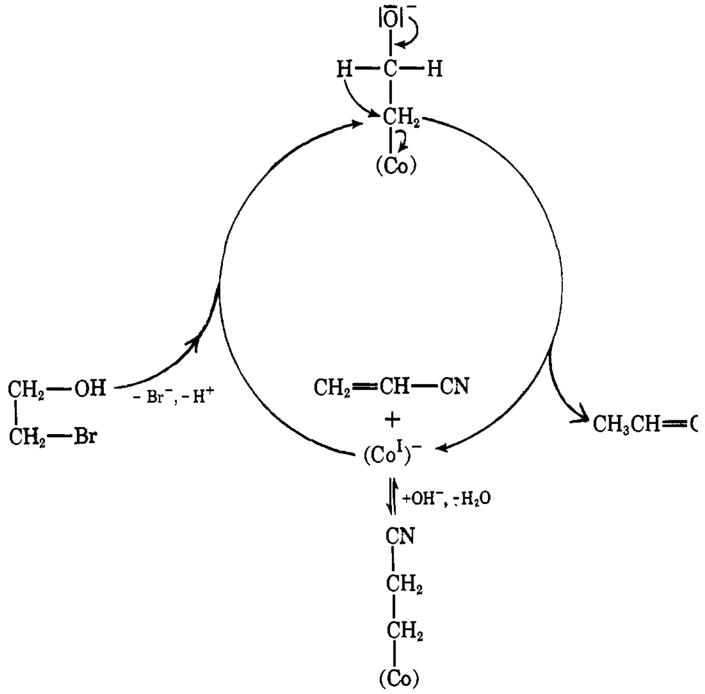


Figure 2. Inhibition of propionaldehyde formation by dioldehydrase with N_2O. The reaction solutions contained the following in a final volume of 1 ml: apoenzyme, 0.3 unit; DL-1,2-propanediol, 0.025 *M;* coenzyme B_{12}, 0.018 μg; potassium phosphate buffer (pH 8.0), 0.02 *M*. The incubations were carried out for the time indicated under argon or N_2O at 37°. The propionaldehyde was assayed colorimetrically as its 2,4-dinitrophenylhydrazone at 540 mμ.

Nitrous oxide, N_2O, is remarkably inert at room temperature and normal pressure. In homogeneous solution it reacts only with a small number of transition metal salts, complexes, and organometallic compounds. The reduction of N_2O to N_2 and H_2O by the Co(I) derivatives of vitamin B_{12}, cobaloximes,[5] and other cobalt chelates, first observed by Banks, *et al.*,[6] is particularly noteworthy as it proceeds readily in neutral or mildly alkaline medium. Since N_2O does not react with Co(II) or Co(III) chelates, a *selective* scavenger for nucleophilic Co(I) intermediates is available which was utilized as a probe in reacting dioldehydrase.

The Co(I) scavenging ability of N_2O was first demonstrated in a nonenzymatic model system consisting of β-cyanoethyl(pyridine)cobaloxime as the "coenzyme," and 2-bromoethanol as the substrate. In alkaline solution, β-cyanoethylcobaloxime undergoes Co–C bond cleavage to yield the cobaloxime(I) nucleophile,[7] in formal analogy to the behavior of coenzyme B_{12}.[1] The cobaloxime(I) nucleophile in turn catalyzes the conversion of 2-bromoethanol into acetaldehyde under anaerobic conditions. The reaction proceeds as summarized in Scheme I. Essential steps in this catalytic conversion of a glycol derivative into acetaldehyde are believed[1] to parallel the enzymatic process of coenzyme B_{12} dependent 1,2-diol dehydration. If this model reaction is carried out under 1 atm of pure N_2O instead of argon, the production of acetaldehyde is substantially inhibited (Figure 1) due to the oxidation of the catalytically active cobaloxime(I) by N_2O. Applying N_2O in similar fashion to reacting dioldehydrase holoenzyme in the presence of 1,2-propanediol, a significant inhibition of propionaldehyde production relative to identical runs under argon is reproducibly observed (Figure 2). The inhibiting effect of N_2O is particularly pronounced at low levels of added coenzyme.

Scheme I. Cobaloxime(I)-Catalyzed Conversion of 2-Bromoethanol into Acetaldehyde[a]



[a] (Co) denotes the cobaloxime moiety; the axial base component (pyridine) is not shown.

These experiments permit the unambiguous conclusion that the Co–C bond of the coenzyme in the reacting dioldehydrase holoenzyme is cleaved and that the corrin is present in the nucleophilic Co(I) state. The specificity of N_2O for Co(I) derivatives clearly indicates that the Co(I) nucleophile is the actual catalyst in the conversion of the substrate to the aldehyde, thus providing firm grounds for the rejection of mechanistic alternatives involving Co(II) or Co(III) corrin derivatives as catalysts and free-radical type derivatives of the substrates as reactive intermediates in dioldehydrase and related enzymes.[8,9] A common feature of these hypothetical mechanisms is that they involve unusual C–H cleavage reactions and rearrangement processes which are not observed in cobalamin and cobaloxime chemistry. On

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[5] Cobaloximes are vitamin B_{12} model compounds derived from bisdimethylglyoximatocobalt.

[6] R. G. S. Banks, R. J. Henderson, and J. M. Pratt, *Chem. Commun.*, 387 (1967).

[7] G. N. Schrauzer, J. H. Weber, and T. M. Beckham, *J. Amer. Chem. Soc.*, **92**, 7078 (1970).

[8] M. A. Foster, H. A. O. Hill, and R. J. P. Williams, *Biochem. Soc. Symp.*, **31**, 187 (1970).

[9] B. M. Babior, *J. Biol. Chem.*, **245**, 6125 (1970).

the other hand, the results of coenzyme tritium labeling studies[10] can be readily interpreted[11] in terms of our mechanism,[1] which is uniquely supported by stoichiometric and catalytic model reactions, as well as by the N_2O inhibition experiments described in this communication.

In applying N_2O as a potential inhibitor to other coenzyme B_{12} dependent enzyme reactions we have to date been unable to observe inhibiting effects with ribonucleotide reductase from *Lactobacillus leichmannii* and with methylmalonyl-CoA mutase from *Propionibacterium shermanii.*[12] In contrast to dioldehydrase, which is inactivated by oxygen, these enzymes operate aerobically just as well as anaerobically. The absence of an inhibiting effect of N_2O thus does not rule out the possibility that the Co(I) nucleophile is present in the active form of the enzyme; it may be that the active site is merely more protected against oxidation by either O_2 or N_2O.

Acknowledgments. This work was supported by Grant No. GP 12324 from the National Science Foundation.

(10) P. A. Frey, M. K. Essenberg, R. H. Abeles, and S. S. Kerwar, *J. Amer. Chem. Soc.*, 92, 4488 (1970).

(11) (a) The principal objection raised[10] against our mechanism[1] is derived from the results of tritium labeling experiments. The tritium incorporation from labeled substrate into the enzyme-bound coenzyme increases the radioactivity of the coenzyme 200–700 times compared to the activity of product propionaldehyde in the reaction solution. This rules out equilibration between product propionaldehyde in the solution with enzyme-bound coenzyme under the reaction conditions. We assume, however, that the H–T exchange takes place between the enzyme-bound coenzyme and newly formed propionaldehyde at the active site, *prior* to equilibration with propionaldehyde outside the enzyme.

(12) Inhibition experiments were performed by Dr. D. Jacobsen (Scripps Clinic and Research Foundation, La Jolla, Calif.) and Dr. J. D. Brodie, State University of New York at Buffalo.

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Hydridocobaloximes

Sir:

Hydridocobaloximes, $H\text{–}Co(dmg)_2B$ (dmg = dimethylglyoximato monoanion, B = axial base), are the Brønsted acids corresponding to the cobaloxime(I) nucleophiles, and are postulated intermediates in certain cobaloxime reactions under neutral reducing conditions.[1–4] In previous attempts at synthesis, only the corresponding cobaloxime(II) derivatives were obtained, suggesting that hydridocobaloximes are inherently unstable species. We have now found that the stability of hydridocobaloximes is sensitively influenced by the nature of the axial base component B. Whereas hydridocobaloximes in which the axial ligands are predominantly σ-bonding nitrogen bases are indeed short-lived and difficult to isolate, relatively stable hydrides are formed if the axial ligands are π-bonding trialkylphosphines. The hydride $H\text{–}Co(dmg)_2P(n\text{-}C_4H_9)_3$, **1**, for example, is obtained by reducing $Cl\text{–}Co(dmg)_2P(n\text{-}C_4H_9)_3$ with excess $NaBH_4$ in pH 7 phosphate-buffered 50% (volume) aqueous methanol.[5] The black, oxygen-sensitive crystals of **1** precipitate out of the reaction solution with methanol of crystallization. *Anal.* Calcd for $C_{20}H_{42}N_4O_4PCo\cdot 2CH_3OH$: C, 47.37; H, 9.05; N, 10.07; P, 5.56; Co, 10.74. Found: C, 47.95; H, 9.65; N, 9.78; P, 5.68; Co, 10.95. The compound loses the methanol on heating *in vacuo* to 75° and starts to decompose with hydrogen evolution at 150°. *Anal.* Calcd for the methanol-free **1**, $C_{20}H_{42}N_4O_4PCo$: C, 48.77; H, 8.59; N, 11.37; Co, 11.96. Found: C, 48.32; H, 8.21; N, 11.20; Co, 11.32. The hydride **1** is also formed on careful acidification of alkaline solutions of the corresponding cobaloxime(I) nucleophile. Prepared in this fashion, **1** was apparently in our hands in 1965, but was at this time considered to be a cobaloxime(II) derivative.[1] It is now apparent that cobaloximes(II) with $P(n\text{-}C_4H_9)_3$ as the axial base disproportionate readily even in neutral solution. Unlike the Co(I) nucleophile, **1** is freely soluble in nonpolar hydrocarbon solvents and may be *quantitatively extracted into n-hexane or benzene.* The pK_a of **1** was estimated to be 10.5 by phase-distribution measurements between 50% aqueous methanol and *n*-hexane and is in agreement with the result of a previous indirect determination.[1] The constitution of **1** is supported by infrared and nmr measurements. In the infrared spectrum (Nujol mull) a band at 2240 cm^{-1} is assigned to the Co–H stretch; the Co–D stretch in the deuteride occurs at 1680 cm^{-1}. The hydride and deuteride bands disappear upon exposure of the infrared disks to air. Similar bands were found to be absent in the infrared spectra of a variety of cobaloxime(II) and alkylcobaloxime derivatives. In the ^1H nmr spectrum in *n*-hexane a broad signal of relative intensity 1 at δ 6.0 ppm is assigned to the resonance of the cobalt-bound hydrogen, consistent with the polarization $H^{\delta+}\text{–}Co^{\delta-}$ of the cobalt–hydrogen bond. The Co–H signal is absent in the spectrum of the deuteride and disappears on passing air through the nmr sample solution containing the hydride. The signal of the dmg methyl protons occurs at 1 ppm and coincides with the signals of the tributylphosphine ligand. The anomalously high chemical shift of the dmg protons indicates a partial negative charge of the $Co(dmg)_2$ moiety.

In the reduction of halocobaloximes(III) with $NaBH_4$ in neutral buffered solution transient blue species are frequently observed, indicating the formation of unstable hydridocobaloximes under these special conditions of reduction. Utilizing the solubility of

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(1) G. N. Schrauzer, R. J. Windgassen, and J. Kohnle, *Chem. Ber.*, **98**, 3324 (1965).

(2) G. N. Schrauzer, and R. J. Windgassen, *J. Amer. Chem. Soc.*, **89**, 1999 (1967).

(3) E. N. Sal'nikova and M. L. Khidekel', *Izv. Akad. Nauk, Ser. Khim.*, **1**, 223 (1967).

(4) M. Naumburg, K. N.-V. Duong, and A. Gaudemer, *J. Organometal. Chem.*, **25**, 231 (1970).

(5) In a typical experiment, 5 g of $Cl\text{–}Co(dmg)_2P(n\text{-}C_4H_9)_3$ [see G. N. Schrauzer, *Inorg. Syn.*, 11, 62 (1968)] was suspended in 250 ml of 50% (v/v) aqueous methanol. A total of approximately 15 g of solid primary and tertiary sodium phosphate was added to adjust the pH close to 7. After most of the alkali phosphate dissolved a freshly prepared solution of 1.5 g of $NaBH_4$ in 25 ml of water was added gradually over a period of 30 min. Occasionally, small amounts of methanol had to be added to reduce excessive foaming. During the last 15 min of $NaBH_4$ addition the reaction solution was kept anaerobic by a blanket of nitrogen. A nearly black solid precipitates which was collected by filtration under nitrogen gas. The hydride was washed with water to remove inorganic salts and dried *in vacuo* at 75° for 12 hr. The isolated yield was 4.3 g of dry hydride, or 92%, based on cobaloxime starting material. The product slowly oxidizes on contact with air, but may be stored for at least several days in argon-filled ampoules. Decomposition was noted on prolonged storage.