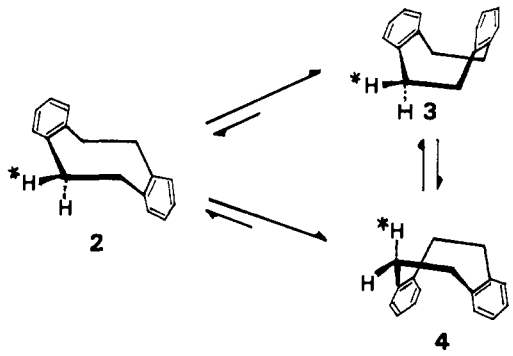
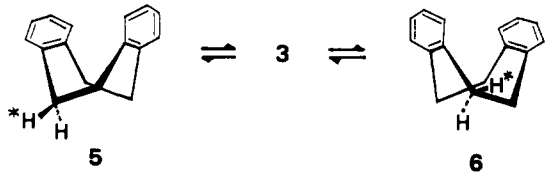


in an averaged symmetry equivalent to that of the boat **3**. Thus **3** and **4** represent rapidly equilibrating twist boat conformations and, being easier to visualize, are used to represent the effect of boat inversion and chair to boat interconversion.



The aromatic protons in conformations **5** and **6** are located in the shielding zone[14] of the aromatic ring across the boat. This effect, being more important for the boats than the chair, then suggests that the more intense high field component of the aromatic signal at $-100°$ is due to protons of the boat forms whereas the less intense low field component belongs to the protons of the chair. Thus, when the equilibrium **2** → **3** becomes rapid both types of aromatic protons become averaged together and one peak results.

The change from **3** to **4** causes an "outside" proton in **3** (starred proton) to become an "inside" proton in **4**. Thus, when boat inversion is slow on the nmr time scale, a complex pattern is expected for the methylene protons, and when it becomes rapid, all methylene protons of the boat family become equivalent, resulting in a singlet as observed. It should be noted that, because of the relatively small amount of chair form at $-100°$, the multiplet for the methylene protons of molecules in this conformation is barely visible compared to the larger signal for protons from molecules in the boat conformations. On the other hand, these protons appear responsible for part of the deformation (asymmetry) of the spectra at -100 and $-115°$.

Thus compound **1** has been found to exist as a mixture of conformations in solution. The interpretation of the spectral data suggests that a member of the boat family, most likely the twist boat, is present in a larger proportion than the chair form. This conclusion is particularly revealing since this compound has been reported to exist in a centrosymmetrical form (presumably the chair) in the crystalline state.[1] On the other hand, our nmr results agree partially with those from dipole moment studies[3] on the same molecule but differ in the nature of the predominant conformation. In view of the observed solvent dependence of the relative proportions of chair and boat conformations and the inherent difficulties of the dipole moment method, this difference does not appear too surprising.

(14) See L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, New York, N. Y., 1969, p 94.

Pmr studies[4] of 1,5-cyclooctadiene (**7**) were inconclusive since they failed to reveal a spectral change down to $-150°$. On the other hand, electron diffraction work[15] on this compound indicates that a mixture of chair and boat conformations exists with the latter predominating, whereas recent X-ray work on crystals of *syn*-3,7-dibromo-*cis*,*cis*-cycloocta-1,5-diene has shown that it possesses a twist-boat conformation.[10] Interestingly, theoretical predictions of the most stable conformation of **7** appear to disagree with each other; one calculation[7] favors a twist-boat conformation whereas the other[8] argues for a chair. It therefore appears that **1** and **7** might have similar conformational properties, the main differences between them being lower free energy values for the various equilibrations in **7**.

Future work on this subject will include a complete solvent study as well as the synthesis of deuterated derivatives to simplify the spectral changes thereby providing more precise information on the properties of the stable conformations and more accurate thermodynamic and kinetic parameters.

Acknowledgments. We are very thankful to Mr. Robert Mayer for technical assistance, Dr. T. Gough in whose laboratory R. W. prepared compound **1**, and the National Research Council of Canada for financial support.

(15) Personal communication from K. Hedberg to J. D. Dunitz, as quoted in footnote 26 of ref 9.

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Concerning the Mechanism of Action of Coenzyme B_{12} in Dioldehydrase and Related Enzymes. Synthesis and Reactions of Postulated Organocorrin Intermediates

Sir:

Recently, new mechanisms of action of coenzyme B_{12} in dioldehydrase[1] and in related enzymes were postulated,[2-4] which differ substantially from the mechanism we proposed[5,6] on the basis of model experiments. A typical version of the potentially alternative mechanisms is shown in Scheme I, which since has been modified to apply to the mechanism of ethanolamine ammonia-lyase.[7] The new mechanisms suggest an initial homolysis of the Co–C bond of the coenzyme and several hypothetical reactions of postulated organocorrin intermediates or of free radicals derived from the substrate and

(1) Abbreviations used are: dioldehydrase, DL-1,2-propanediol hydrolase (EC 4.2.1.28); coenzyme B_{12}, α-(5,6-dimethylbenzimidazolyl)-*Co*-5′-deoxyadenosylcobamide; cobaloximes are derivatives of bis(dimethylglyoximato)cobalt.

(2) M. K. Essenberg, P. A. Frey, and R. H. Abeles, *J. Amer. Chem. Soc.*, **93**, 1242 (1971).

(3) R. H. Abeles, *Advan. Chem. Ser.*, No. 100, 346 (1971).

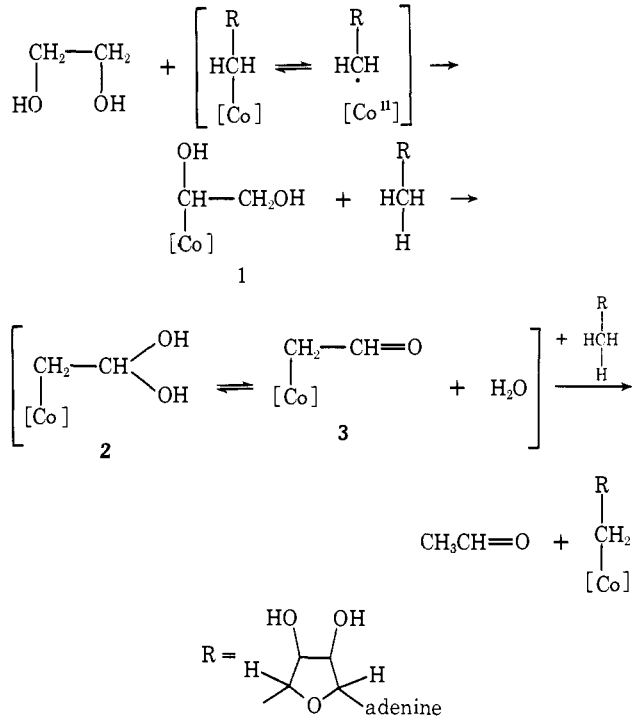
(4) S. A. Cockle, H. A. O. Hill, R. J. P. Williams, S. P. Davies, and M. A. Foster, *J. Amer. Chem. Soc.*, **94**, 275 (1972).

(5) G. N. Schrauzer and J. W. Sibert, *ibid.*, **92**, 1022 (1970).

(6) G. N. Schrauzer, *Advan. Chem. Ser.*, No. 100, 1 (1971).

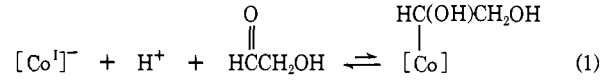
(7) T. J. Carty, B. M. Babior, and R. H. Abeles, *J. Biol. Chem.*, **246**, 6313 (1971).

Scheme I. Postulated Mechanism of Dioldehydrase



the coenzyme. We have investigated the synthesis of the assumed organocorrin intermediates and studied their reactions to provide evidence for or against these mechanisms from nonenzymatic model experiments. We have also studied reactions of free radicals derived from ethylene glycol in order to achieve its conversion to acetaldehyde by a free-radical process under mild conditions in the presence of cobalt complexes as catalysts.

Intermediate **1**, believed[2-4] to result from ethylene glycol by hydrogen abstraction and subsequent combination with the cobalt(II) ion of enzyme-bound coenzyme, is formally the "corrinohydrin" of glycolaldehyde and thus should be at equilibrium with glycolaldehyde and the Co(I) nucleophile (vitamin B_{12s}, eq 1). How-



(1)

ever, no evidence for the existence of **1** was obtained. The characteristic blue-green color of vitamin B_{12s} persists over long periods of time in alkaline (pH 12–10) solutions containing excess glycolaldehyde or other acyloins such as 3-hydroxy-2-butanone, indicating that the position of equilibrium 1 is most likely far on the left. Glycolaldehyde was also not converted to acetaldehyde in solutions containing vitamin B_{12s} over a pH range of 12–4.7. We conclude from these observations that some of the essential reactions assumed in Scheme I do not occur. The only reaction of corrins with acyloins known at this time is the reduction of vitamin B_{12a} (hydroxocobalamin) to B_{12r} to B_{12s} in neutral or alkaline solution, respectively.[8]

To achieve the conversion of ethylene glycol to acetaldehyde by a cobalamin- or cobaloxime-catalyzed free-radical reaction, methylcobalamin or methyl(pyridine)-cobaloxime was photolyzed in anhydrous glycol under argon. This gave rise to the exclusive formation of methane rather than to a mixture of methane and ethane as observed in aqueous solution.[9] This indicates that the methyl radicals produced in glycol terminated to a substantial extent by abstraction of α hydrogen, thus generating the $\cdot CH(OH)-CH_2OH$ radical. However, no trace of acetaldehyde was observed; instead, the glycol radical terminated by dimerization to erythritol, traces of which were detected by tlc of the photolyzed reaction solution according to a published procedure.[10] Neither acetaldehyde nor its acetal with ethylene glycol was formed on thermolysis of various alkylcobalt complexes including coenzyme B_{12} in anhydrous ethylene glycol under anaerobic conditions. Photolysis in the presence of $FeCl_3$, as well as γ radiolysis of ethylene glycol, is known to produce acetaldehyde, erythritol, and other products.[10-12] However, the free-radical formation of acetaldehyde from ethylene glycol is evidently not promoted by cobalt chelates.

The assumed terminal organocorrin **3** was prepared by the reaction of vitamin B_{12s} with haloacetaldehydes.[13,14] Other authors[7] previously attempted to synthesize **3** but were unsuccessful and for this reason considered its Co–C bond as unstable. We have found **3** to be as stable as a typical organocorrin, and have established its structure unambiguously by 220-MHz ^1H nmr measurements, optical absorption spectroscopy, and degradation reactions. The characteristic triplet of the α proton of the cobalt-bound $CH_2CH{=}O$ group occurs at 9.01 ppm, J = 3.5 Hz. In the corresponding cobaloxime derivative (axial base, pyridine), the α hydrogen triplet is found at 9.10 ppm, J = 3.5 Hz. The optical absorption spectrum of **3** in aqueous solution (Figure 1) is that of a base-on alkylcobalamin; the pK_b of 5,6-dimethylbenzimidazole is 3.2 at 27° (determined spectrophotometrically by observing the characteristic red-yellow shift upon acidification[15]). On anaerobic photolysis under conditions described in ref 16, **3** decomposes quantitatively into CH_3CHO (identified by glpc, mass

(8) (a) G. N. Schrauzer, *Ann. N. Y. Acad. Sci.*, **158**, 526 (1969); (b) G. N. Schrauzer and L. P. Lee, *Arch. Biochem. Biophys.*, **138**, 16 (1970).

(9) (a) G. N. Schrauzer, J. W. Sibert, and R. J. Windgassen, *J. Amer. Chem. Soc.*, **90**, 6681 (1968); (b) G. N. Schrauzer, L. P. Lee, and J. W. Sibert, *ibid.*, **92**, 2997 (1970); (c) R. Yamada, S. Shimizu, and S. Fukui, *Biochem. Biophys. Acta*, **124**, 195 (1966).

(10) S. A. Barker, S. Brimacombe, and E. D. M. Eades, *Radiat. Res.*, **22**, 357 (1964).

(11) H. Inoue, K. Tamaki, N. Komakine, and E. Imoto, *Bull. Soc. Chem. Jap.*, **39**, 1577 (1966).

(12) P. J. Venter, H. J. Van der Linde, and R. A. Basson, *J. Chem. Soc., Chem. Commun.*, 187 (1972).

(13) Vitamin B_{12s} was generated from 100 mg of crystalline hydroxocobalamin (Merck) by reduction with 3 g of 80% aqueous hydroxybutanone solution in 10 ml of 0.3 *N* aqueous NaOH under argon. Conversion to vitamin B_{12s} is complete in 5 min. To this solution 1 ml of freshly distilled chloro- or bromoacetaldehyde was added and the reaction mixture was allowed to stand for 12 hr at 27°. Excess haloacetaldehyde and hydroxybutanone were removed by successive repeated extraction first with ether and later with chloroform. The solution was neutralized with acetic acid and the corrin was isolated by phenol extraction as described in ref 14. The cobinamide of **3** was synthesized analogously. The same organocorrins were obtained from haloacetaldehydes and vitamin B_{12s} prepared with $NaBH_4$ as the reducing agent in 0.1 *N* NaOH. It is essential to destroy excess $NaBH_4$ with acetone prior to the addition of the haloacetaldehyde.

(14) H. A. Barker, R. D. Smyth, and H. P. C. Hogenkamp, *Biochem. Prep.*, 10, 27 (1963).

(15) H. P. C. Hogenkamp, J. E. Rush, and C. A. Swenson, *J. Biol. Chem.*, **240**, 3641 (1965).

(16) Reaction tubes containing the organocorrins in aqueous solution under argon were irradiated with a 150-W GE projector spot lamp 20 cm away. During the illumination a stream of cold air was blown over the tubes to prevent the temperature of the solutions from rising above 35°.

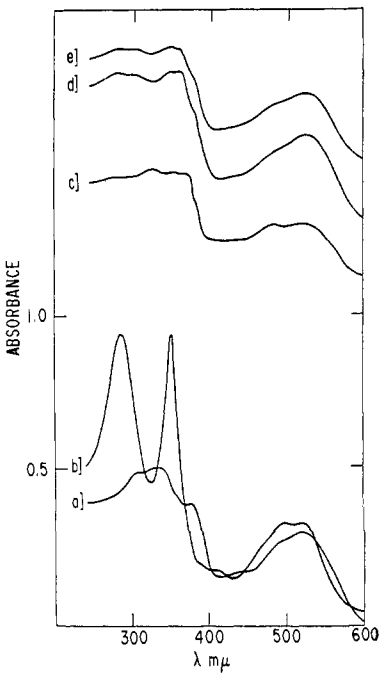
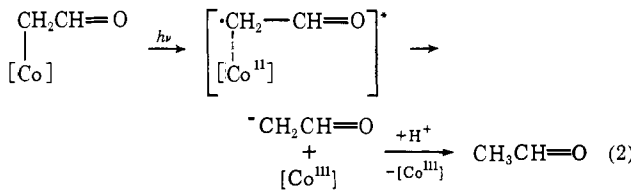
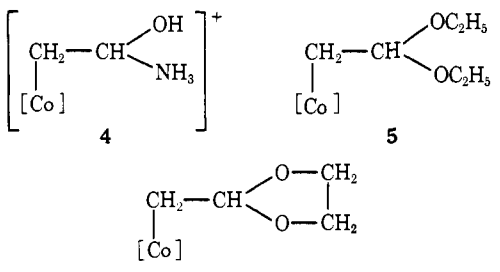


Figure 1. Optical absorption spectra of organocobalamins and -cobinamides in 0.15 *M* aqueous phosphate buffers of pH as indicated: (a) **3** at pH 5.8; (b) **3** after photolysis under argon; (c) cobinamide of **5** at pH 11.5; (d) **6** at pH 10.5; (e) **5** at pH 11. Spectra of photolyzed solutions of **5** and **6** are virtually identical with b.

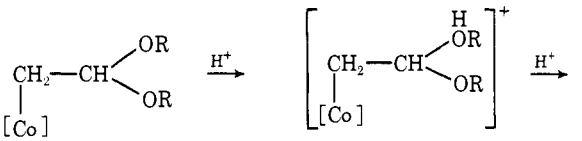
spectrometry, and a spot test[17]) and hydroxocobalamin (identified by its characteristic absorption spectrum; vitamin B_{12r} was not detectable at any point during the photolysis), indicating the preferential termination of the photochemically generated $\cdot CH_2CH{=}O$ radicals according to eq 2. This is the expected behavior of



inductively stabilized free radicals generated on photolysis of organocobalt complexes.[16] The cobinamide and cobaloxime[18] corresponding to **3** also produce acetaldehyde and Co(III) derivatives of the parent chelates on photolysis, the cobaloxime also on thermolysis.[18] The cobaloxime does not form a stable hydrate



or adduct with ammonium ion corresponding to **4** (the C=O band at 1672 cm^{-1} in the ir spectrum of the cobaloxime remains unchanged even in a pellet of NH_4Br). Several acetals of **3** were obtained by the reaction of vitamin B_{12s} with acetals of haloacetaldehyde. Absorption spectra of **5** and **6** in alkaline aqueous solution are shown in Figure 1. In contrast to **3**, the acetals **5** and **6** are stable in solution only above pH 8 and decompose during phenol extraction.[19] At lower pH, decomposition with Co–C bond heterolysis occurs, affording hydroxocobalamin and mixtures of acetal, acetaldehyde, and the corresponding alcohol (all identified by glpc). The rate law of decomposition is $-d[\text{complex}]/dt = k[\text{complex}][H^+]$; values for k at 27° are 2050 sec^{-1} for **5**, 6.0 sec^{-1} for **6**, and 26,000 sec^{-1} for the cobinamide of **5**, respectively. The anomalous lability of the Co–C bond in these corrins on protonation is attributed to a steric effect of the corrin ligand; this Co–C bond heterolysis is formulated in eq 3. In con-



$$[Co^{III}] + CH_3CH{=}O + 2ROH \quad (3)$$

trast to the behavior of the corrin derivatives, the cobaloxime acetaldehyde acetal with R = C_2H_5 retains its Co–C bond and undergoes simple acetal hydrolysis instead.[18] Anaerobic photolysis of **5** and **6** in alkaline solution yields hydroxocobalamin and acetaldehyde acetal; acidification of the reaction solutions after photolysis affords acetaldehyde and the corresponding alcohols in the amounts expected.

The mechanism in Scheme I and related versions thereof[20] must be considered as improbable if only in view of the tendency of **3** and of related compounds to undergo Co–C bond *heterolysis* rather than *homolysis*, but also because of the completely different affinity relationships between the reactants in the enzyme and for other reasons.[21-23]

Acknowledgment. This work was supported by Grant No. GP 284485 X from the National Science Foundation.

(17) F. Feigl, "Spot-Tests in Organic Analysis," 6th ed, Elsevier, New York, N. Y., 1966, p 414.

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

(19) The cobalamin acetals **5** and **6**, as well as the cobinamide corresponding to **5**, were prepared as outlined in ref 13, using the corresponding acetals of chloroacetaldehyde for the reaction with vitamin B_{12s}. After removal of excess organic compounds by extraction with ether and chloroform the reaction solutions were not neutralized but instead evaporated at room temperature in the dark to one-third of the original volume. Subsequent addition of dry acetone (100 ml) caused the precipitation of the solid corrins together with some inorganic salt, which was not removed in order to maintain the compounds in the alkaline environment at all times.

(20) T. H. Finlay, J. Valinsky, K. Sato, and R. H. Abeles, *J. Biol. Chem.*, **247**, 4197 (1972).

(21) The mechanistic significance of the esr signals observed in glyceroldehydrase[4] is open to question in view of the recent observations of Hamilton, *et al.*, on ribonucleotide reductase.[22]

(22) J. A. Hamilton, Y. Tamao, R. L. Blakley, and R. E. Coffman, *Biochemistry*, 11, 4696 (1972).

(23) G. N. Schrauzer, J. A. Seck, and R. J. Holland, *Z. Naturforsch. B*, in press.

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