On Hydroxyalkylcobaloximes and the Mechanism of a Cobamide-Dependent Diol Dehydrase

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Abstract: Preparation and properties of a series of hydroxyalkylcobaloximes and of related substituted organocobaloximes are reported. The β-hydroxyalkylcobaloximes are more reactive than the simple alkylcobaloximes. The Co–C bonds are readily cleaved; in mildly acidic media olefins are formed, but in alkaline solution the products are aldehydes, ketones, or the respective aldols arising from the secondary condensation of the aldehydes. The γ-hydroxy-*n*-propylcobaloxime is about as stable as an alkylcobaloxime, but allyl- and crotylcobaloximes exhibit an acid sensitivity similar to that of β-hydroxyalkylcobaloximes. The mechanistic aspects of these degradation reactions are discussed and brought into relation with observations made in other laboratories on the chemical behavior of coenzyme B_{12} in the diol dehydrase of *Aerobacter aerogenes*. A mechanism for the action of the corrin coenzyme in this enzyme system is proposed.

Recent studies have demonstrated[2-6] that bis(dimethylglyoximato)cobalt complexes ("cobaloximes") are uniquely suited as models for the study of the properties of the cobalt ion in vitamin B_{12} and its biologically active derivatives. In the present paper we wish to report the results on various hydroxyalkylcobaloximes. Apart from the general synthetic interest, this study was also undertaken to obtain information on the possible mode of action of the cobinamide cofactor in a diol dehydrase of *Aerobacter aerogenes*.

Preparation of Hydroxyalkylcobaloximes. Hydroxyalkylcobaloximes are complexes of type I (see Figure 1) in which the hydroxyalkyl moiety is linked to the cobalt atom *via* a direct Co–C bond. These compounds may be obtained like other alkylcobaloximes by reacting hydroxyalkyl halides with cobaloximes (eq 1). A more convenient method for the preparation

$$\begin{matrix}(Co)^- \\ | \\ B\end{matrix} + X(CH_2)_xOH \longrightarrow \begin{matrix}(CH_2)_xOH \\ | \\ (Co) \\ | \\ B\end{matrix} + X^- \qquad (1)$$

X = *e.g.*, Br; B = *e.g.*, pyridine

of the β-hydroxyalkyl derivatives consists in the reaction of cobaloximes with epoxides. The Co^+ ion present in the cobaloxime reduced in alkaline solutions is strongly nucleophilic and capable of cleaving the epoxide ring (eq 2). Reaction 2 was originally observed with vitamin

$$\begin{matrix}(Co)^- \\ | \\ B\end{matrix} + \begin{matrix}O \\ / \quad \backslash \\ CH_2—CH_2\end{matrix} + H_2O \longrightarrow \begin{matrix}(CH_2)_2OH \\ | \\ (Co) \\ | \\ B\end{matrix} + OH^- \qquad (2)$$

B_{12s}.[7] An even better preparative procedure consists in using cobaloximes(II) as the starting cobalt complexes and molecular hydrogen as the reducing agent. Since this reaction is conducted in essentially neutral medium, the yields are particularly high (eq 3). A

$$2\begin{matrix}(Co^{II}) \\ | \\ B\end{matrix} + 2\begin{matrix}O \\ / \quad \backslash \\ CH_2—CH_2\end{matrix} + H_2 \longrightarrow 2\begin{matrix}(CH_2)_2OH \\ | \\ (Co) \\ | \\ B\end{matrix} \qquad (3)$$

similar ring opening reaction was observed with trimethylene oxide, although the 3-hydroxypropylcobaloxime is better obtained from 3-bromopropanol. The same product is also obtained in low yield by treating cobaloximes(II) with allyl alcohol and hydrogen. No reaction was observed with tetrahydrofuran. The 4-hydroxy-*n*-butylcobaloxime is conveniently obtained from 4-bromobutanol, however. Several β-hydroxyethylcobaloximes were also prepared with bases other than pyridine, *e.g.*, with H_2O or triphenylphosphine occupying the sixth coordination position of the cobalt atom.

Properties. The hydroxyalkylcobaloximes are orange to yellow, crystalline, and quite air stable. They are somewhat soluble in water and in a variety of organic solvents. Their constitution follows unambiguously from the elemental analyses, nmr measurements, and degradation reactions to be discussed below. On heating to about 175° the β-hydroxyethylaquocobaloxime decomposed forming ethylene; propylene was obtained from the thermal decomposition of the β-hydroxy-*n*-propyl derivative. On irradiation of the hydroxyethylcobaloxime in water–methanol, acetaldehyde is formed. The β-hydroxy-*n*-propyl derivative yielded mainly acetone and small amounts of propylene under analogous conditions. Whereas Co-alkylcobaloximes are remarkably resistant even to concentrated acids, β-hydroxyalkylcobaloximes are readily decomposed in 0.1–0.3 *N* aqueous hydrochloric acid at room temperature (eq 4). This reaction formally resembles the acid degradation of hydroxyalkylmercurials[8] (eq 5).

$$\begin{matrix}CH_2CH(R)OH \\ | \\ (Co) \\ | \\ B\end{matrix} + HX \longrightarrow \begin{matrix}X \\ | \\ (Co) \\ | \\ B\end{matrix} + RCHCH_2 \qquad (4)$$

$$HOCH_2CH_2HgX + HX \longrightarrow H_2O + HgX_2 + CH_2{=}CH_2 \qquad (5)$$

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(2) G. N. Schrauzer and J. Kohnle, *Chem. Ber.*, **97**, 3056 (1964).
(3) G. N. Schrauzer, R. J. Windgassen, and J. Kohnle, *ibid.*, **98**, 3324 (1965).
(4) G. N. Schrauzer and R. J. Windgassen, *ibid.*, **99**, 602 (1966).
(5) G. N. Schrauzer, *Naturwissenschaften*, **53**, 459 (1966).
(6) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **88**, 3738 (1966).
(7) O. Müller and G. Müller, *Biochem. Z.*, **336**, 229 (1962).
(8) K. A. Hofmann and J. Sand, *Chem. Ber.*, **33**, 1344 (1900).

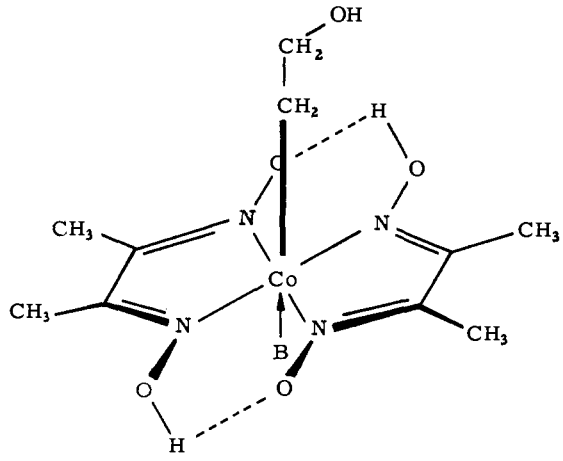
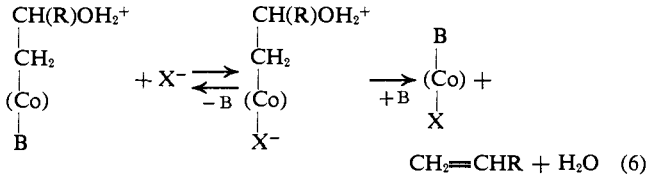


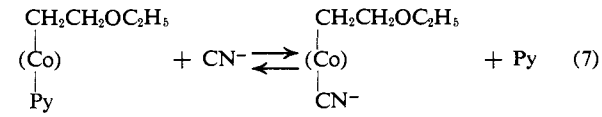
Figure 1. β-Hydroxyethylcobaloxime (B = *e.g.*, pyridine).

In contrast to the mercury compounds, the hydroxyalkylcobaloximes cannot be obtained from hydroxycobaloximes and olefins under a wide variety of conditions. In the acid-cleavage reaction of the hydroxyalkylcobaloximes the nucleophilicity of the acid anion and of the base component B are of special significance. Thus, solutions of β-hydroxyethylaquocobaloxime in 0.5 *N* H_2SO_4 or $HClO_4$ evolve ethylene at a hardly perceptible rate, whereas in dilute solutions of HCN the reaction is quite rapid. This implies that the formation of the olefin occurs by a process in which the initial oxonium ion formation is followed by the anionic displacement of the Co–C bond. The latter process probably involves *trans* attack of the anion, as indicated in eq 6. This is in accord with the marked dependence



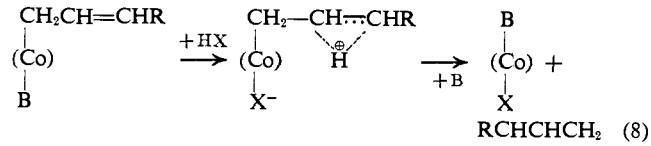
$$CH_2{=}CHR + H_2O \quad (6)$$

of the rate of ethylene evolution on the nature of the base component. In all hydroxyethylcobaloximes with readily exchanged axial ligands such as water or pyridine, the reaction with HCN or even mercaptans occurs quite readily, whereas in the case of phosphines as the base components, the olefin formation is very slow. We have also prepared β-ethoxyethylpyridinatocobaloxime. It is attacked by 0.1 *N* HCl, forming ethanol and ethylene. In aqueous 0.5 *N* KCN the ethoxyethyl group is not displaced at a rate greater than that observed for simple alkylcobaloximes and no ethylene forms on heating. The compound is, however, solubilized by base displacement (eq 7).



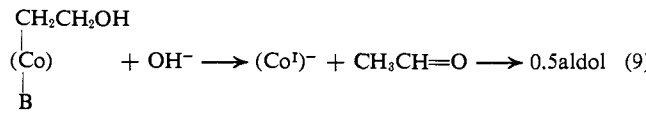
(7)

Upon careful neutralization crystals of the starting material precipitate, but the solution shortly becomes homogeneous again as ethylene evolution proceeds. Supporting evidence for the proposed protonation-substitution mechanism was gained by experiments with other functionally substituted cobaloximes. Thus, allyl- and crotylcobaloximes exhibit the same sensitivity toward acid as hydroxyalkyl species (eq 8).



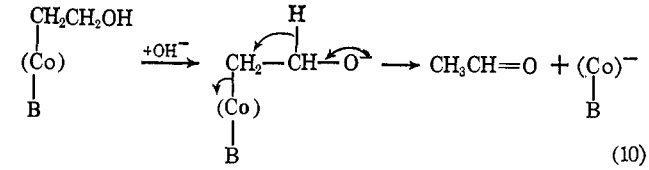
(8)

A similar formation of butene-1 was observed on acid treatment of crotylpentacyanocobaltate ion.[9] The 3-hydroxy-*n*-propylcobaloxime behaves more like a simple alkylcobaloxime. It is relatively insensitive to acids and forms allyl alcohol on thermal decomposition. It is of interest, however, that 3-bromo-*n*-propylcobaloxime[6] undergoes thermal decomposition to cyclopropane. In 0.5 *N* KCN solution cyclopropane is also rapidly evolved. The 4-hydroxy-*n*-butylcobaloxime, as well as the 4-bromobutyl derivative, did not exhibit any reaction substantially different from alkylcobaloximes. The β-hydroxyalkylcobaloximes are readily decomposed in mildly alkaline solutions under conditions where alkylcobaloximes remain unchanged. The Co–C bond is cleaved, for instance, in 0.05–0.1 *N* NaOH at room temperature. With the β-hydroxyethyl derivative, *acetaldehyde* and the blue-green cobaloxime$_s$ are formed (eq 9). The acetaldehyde undergoes



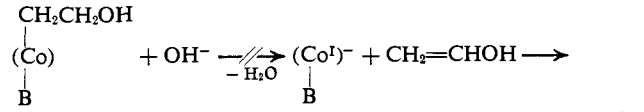
(9)

secondary condensation to aldol under these conditions. Under the same conditions β-hydroxy-*n*-propylcobaloxime gives high yields of *acetone*, whereas β-hydroxyisopropylcobaloxime and 2,3-dihydroxypropylcobaloxime yield propionaldehyde or 2-hydroxyacetone, respectively. We assume that the alkali-cleavage reaction is accompanied by a hydride shift, as indicated in eq 10.



(10)

The nature of the base in *trans* position appears to have little, if any, effect in this reaction; pyridinato- or tributylphosphinato-2-hydroxyethylcobaloxime both exhibit qualitatively about the same alkali sensitivity. On the other hand, the formation of the oxyanion in eq 10 is essential for the cleavage to occur and probably provides the main driving force for this reaction. This is strikingly demonstrated by the fact that β-ethoxyethylcobaloxime may be dissolved in 50% NaOH solution without decomposition. This seems to rule out a β-elimination mechanism accompanied by the formation of vinyl alcohols (or ethers, respectively) (eq 11).



(11)

(9) J. Kwiatek and J. K. Seyler, *J. Organometal. Chem.* (Amsterdam), **3**, 421 (1965).

Comparison with Coenzyme B_{12}

Coenzyme B_{12} (II; see Figure 2) is a β-alkoxyalkylcobalt derivative and is generally more sensitive to acids and bases than simple alkylcobalamins, but clearly more stable than β-hydroxyethylcobalamin, which recently was shown to rapidly decompose in dilute hydrochloric acid.[10] Coenzyme B_{12} is moderately stable in 0.1 *N* HCl at room temperature but readily decomposes at 100°.[11] Degradation also occurs in 0.1 *N* KCN.[12] Protonation of the basic adenyl group may be assumed to be the first step in the acid degradation of coenzyme B_{12}. This leads to a multicenter intermediate for decomposition, as has already been proposed by Johnson and Shaw.[11] The authors suggested *cis* attack of the cyanide at the Co–C bond, although displacement of the dimethylbenzimidazole ligand by cyanide may also be involved. Cyanide is known to displace the axial base in both methylcobalamin and methylcobaloximes.[6]

On the Mechanism of the Diol Dehydrase of *Aerobacter aerogenes*

The observed properties of β-hydroxyalkylcobaloximes in alkaline solution strongly suggest that similar organocobalamin derivatives may be intermediates in the diol dehydrase action of an enzyme system of *Aerobacter aerogenes*. This diol dehydrase converts 1,2-glycols to aldehydes at the optimal pH of about 8[13,14] and was shown to probably proceed in two steps[15] (eq 12). Dimethylbenzimidazolyl cobinamide

$$2\ \underset{\displaystyle CH_2OH}{\overset{\displaystyle RCHOH}{|}} \longrightarrow RCH_2CH(OH)CH(R)CH{=}O \xrightarrow[K^+,Mn^{2+}]{} 2RCH_2CH{=}O \qquad (12)$$

5′-deoxyadenosine apparently is the only active cofactor. The synthetic Co-alkylcorrins proved to be effective antagonists. It is considered likely that the first reaction follows through the aldehyde step which, under the alkaline reaction conditions, undergoes secondary condensation to aldol. Abeles and Lee[14] suggested that rupture of the Co–C bond of the coenzyme could result in the formation of a "nucleophilic cobalt." The model experiments described in this paper show that this is indeed possible under physiologically plausible conditions.[16]

The dehydration of 1,2-glycols to aldehydes is well-known but *in vitro* requires acidic conditions. The dehydration of, *e.g.*, 1,2-propanediol yields propionaldehyde and not acetone since alkyl groups facilitate the removal of the 2-hydroxyl group (eq 13). It is

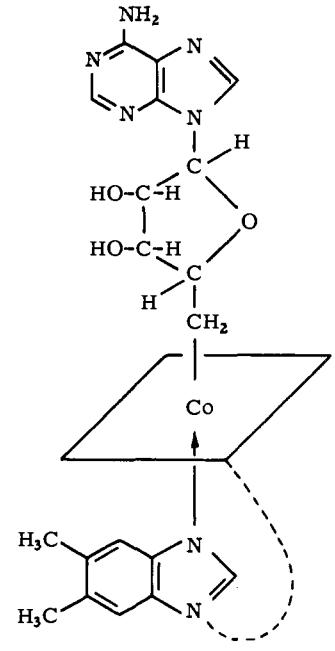
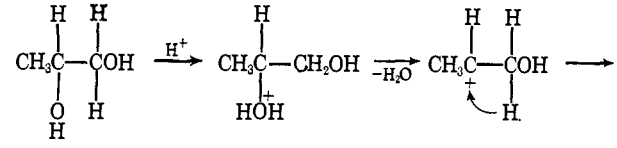


Figure 2. Coenzyme B_{12}.

thus necessary to propose a reasonable mechanism for the dehydration of a glycol under enzymatic conditions in an essentially alkaline medium. The fact that we have succeeded in converting cobaloxime derivatives of glycols into aldehydes under mildly alkaline conditions



$$CH_3CH_2CH{=}O + H^+ \qquad (13)$$

leads us to propose the following mechanism for the coenzyme B_{12} dependent part of the enzymatic dehydration: step 1, cleavage of the Co–C bond in the dimethylbenzimidazolylcobamide 5′-deoxyadenosine to the nucleophilic cobalt(I) derivative of the corrin;[17] step 2, alkylation of the cobalt with the intermediate formation of a β-hydroxyethylcobinamide derivative; step 3, cleavage of the Co–C bond and concerted conversion of the organic residue into the aldehyde and regeneration of the nucleophilic cobalamin(I) compound; step 4, aldehyde condensation to aldol.

Since 1,2-glycols do not react with cobaloximes or vitamin B_{12s} *in vitro*, it must be assumed that the diol is converted into an active form prior to the reaction with the cobalt. It is conceivable that this occurs by the interaction of the glycol with the enzyme, yielding a reactive species $X\cdots CH_2CH_2OH$. The nature of "X" is unknown. This "active" glycol is expected to react with the reduced cobinamide. The collapse of the intermediate Co-β-hydroxyethyl species to the aldehyde could well occur by a hydride shift, which would be basically consistent with the observed lack of hydrogen exchange with the solvent.[18] Quite recently, Abeles and Zagalak[19] rejected a hydride shift mechanism

(10) H. P. C. Hogenkamp, J. E. Rush, and C. A. Swenson, *J. Biol. Chem.*, 240, 3641 (1965).
(11) A. W. Johnson and N. Shaw, *J. Chem. Soc.*, 4608 (1962).
(12) D. Dolphin, A. W. Johnson, and R. Rodrigo, *Ann. N. Y. Acad. Sci.*, 12, 596 (1964). The same authors also reported that the reaction of coenzyme B_{12} with HCN is markedly faster than with KCN. This has been questioned by one referee, who has pointed out that this does not agree with his own observations. The disagreement may be caused by the fact that Dolphin, *et al.*, used more concentrated solutions of HCN than the other worker. Similarly, methylcobalamin is usually described as stable toward cyanide. Using concentrated cyanide solutions it is readily converted into dicyanocobalamin, however.[6]
(13) R. H. Abeles and H. A. Lee, Jr., *J. Biol. Chem.* 236, 2347 (1961).
(14) R. H. Abeles and H. A. Lee, Jr., *Ann. N. Y. Acad. Sci.*, 112, 695 (1964).
(15) J. Pawelkiewicz and B. Zagalak, *ibid.*, 112, 703 (1964).
(16) Another example for the heterolytic cleavage of the Co–C bond in a cobalamin derivative was recently reported by R. Barnett, H. P. C. Hogenkamp, and R. H. Abeles, *J. Biol. Chem.*, 241, 1483 (1966).
(17) This could be possible, *e.g.*, by interaction of the coenzyme with an SH or S^- group of the enzyme protein.
(18) A. M. Brownstein and R. H. Abeles, *J. Biol. Chem.*, 236, 1199 (1961).
(19) R. H. Abeles and B. Zagalak, *ibid.*, 241, 1245 (1966).

on the basis of additional experimental data. When a mixture of 1,2-propanediol (containing tritium on C-1) and unlabeled ethylene glycol was added to the diol dehydrase, the resulting acetaldehyde contained tritium in the methyl group. It was later shown[20] that hydrogen is also transferred from the substrate glycol to the 5′-deoxyadenosine in the course of the enzymatic reaction.

We believe that these results do not eliminate the hydride shift mechanism. It is equally possible that the hydrogen exchange takes place between the diols during their attachment to the enzyme, a reaction which may well be independent of, or not directly connected with, the actual hydride transfer. *Glycol aldehyde* was found to inhibit the dehydrase action.[14] It is conceivable that the initial activation of this compound and its attaachment to the corrin cobalt proceed just as that of glycol. The resulting organocobalamin derivative containing the $Co—CH_2CH=O$ unit may be too stable for further reaction and thus act as the inhibitor. This is supported by the fact that the corresponding cobaloxime, $O=CHCH_2Co(D_2H_2)Py$, which we have also prepared, is stable at pH 11.[21]

In a recent note Yamada, *et al.*,[22] reported experiments which seem to exclude β,γ-dihydroxy-*n*-propylcobamide derivatives as the intermediates in the glycol dehydrase system. According to our experience with the corresponding cobaloxime derivative, this is indeed unlikely since only allyl alcohol and hydroxyactone are formed on decomposition of this complex in acidic or alkaline solution, respectively. The likely organocobamide derivative would have to be the β-hydroxyisopropylcobalt compound, $HOCH_2CH(CH_3)Co$. The corresponding cobaloxime has been prepared (see above) and produces propionaldehyde on decomposition in alkaline medium. It remains to be determined if the β-hydroxyisopropylcobamide is sufficiently stable to be isolated and how it behaves on addition to the enzyme.

Experimental Section

The preparation of hydroxyalkylcobaloximes *via* cobaloximes and halohydrins does not offer new details over analogous reactions with alkyl halides as the alkylating agents, for which general procedures have already been outlined.[6] We therefore include only the most convenient methods of synthesis for several representative compounds. Yields in cobaloxime chemistry are almost generally high and in many cases near to quantitative. They are therefore not reported, especially in view of the general availability of the starting materials. The cobaloximes are reasonably air stable and can be handled without special precautions. Although dilute solutions are sensitive to light, storage of the solid compounds in tinted bottles did not indicate appreciable decomposition over a period of several weeks. For preparative work, light sensitivity of the compounds may be neglected.

Infrared spectra and decomposition points of the hydroxyalkylcobaloximes are not given as they are of little diagnostic value. The nuclear magnetic resonance spectra of several representative hydroxyalkylcobaloximes were recorded and were found to be in agreement with the proposed structures. They did not show any unusual features and, therefore, are likewise not reported in detail.

β-Hydroxyalkylcobaloximes. A suspension of 23.8 g (0.1 mole) of $CoCl_2 \cdot 6H_2O$ and 23.2 g (0.2 mole) of dimethylglyoxime was stirred under nitrogen in 300 ml of methanol until all cobalt chloride had dissolved, and then 8.0 g (0.2 mole) of NaOH in 30 ml of water was added. After 15 min of stirring the nitrogen was replaced by hydrogen and 0.15 mole of an epoxide (ethylene oxide, propylene oxide, glycidol, styrene oxide, cyclohexene oxide, etc.) was added. On further rapid stirring approximately 1.2 l. of hydrogen was consumed in about 15 min, and on cessation of gas uptake an orange solution of the cobaloxime with suspended salt resulted. The solution was filtered, concentrated to about 100 ml, and diluted with water to precipitate the product. When the aquocobaloximes were too soluble to permit isolation, the less soluble pyridinatocobaloximes were formed by adding pyridine to the solutions.

β-Hydroxyethylaquocobaloxime ($HOCH_2CH_2Co(D_2H_2)OH_2$): *Anal.* Calcd for $C_{10}H_{21}N_4O_6Co$: C, 34.10; H, 6.01; N, 15.91. Found: C, 34.36; H, 6.22; N, 16.04.

β-Hydroxyethylpyridinatocobaloxime ($HOCH_2CH_2Co(D_2H_2)C_5H_5N$): *Anal.* Calcd for $C_{15}H_{24}N_5O_5Co$: C, 43.59; H, 5.86; N, 16.95. Found: C, 43.32; H, 5.98; N, 16.63.

β-Hydroxy-*n*-propylpyridinatocobaloxime ($CH_3CH(OH)CH_2Co(D_2H_2)C_5H_5N$): *Anal.* Calcd for $C_{16}H_{26}N_5O_5Co$: C, 44.97; H, 6.13; N, 16.39. Found: C, 44.91; H, 6.21; N, 16.44.

2,3-Dihydroxypropylpyridinatocobaloxime ($HOCH_2CHOHCH_2Co(D_2H_2)C_5H_5N$): *Anal.* Calcd for $C_{16}H_{26}N_5O_6Co$: C, 43.35; H, 5.91; N, 15.80. Found: C, 43.17; H, 6.08; N, 15.63.

β-Ethoxyethylpyridinatocobaloxime ($CH_3CH_2OCH_2CH_2Co(D_2H_2)C_5H_5N$). This complex was prepared from 2-bromoethyl ether by the previously published procedure;[2,5] orange crystals from methanol-water. *Anal.* Calcd for $C_{17}H_{28}N_5O_5Co$: C, 46.26; H, 6.40; N, 15.87. Found: C, 46.38; H, 6.65; N, 15.77.

2-Phenyl-2-hydroxyethylpyridinatocobaloxime ($HOC(C_6H_5)HCH_2Co(D_2H_2)C_5H_5N$): *Anal.* Calcd for $C_{21}H_{28}N_5O_5Co$: C, 51.53; H, 5.77; N, 14.31. Found: C, 51.30; H, 5.63; N, 14.56.

2-Hydroxycyclohexylpyridinatocobaloxime ($CH_2(CH_2)_3CH(OH)CHCo(D_2H_2)C_5H_5N$): *Anal.* Calcd for $C_{19}H_{30}N_5O_5Co$: C, 48.82; H, 6.47; N, 14.99. Found: C, 48.99; H, 6.68; N, 15.24.

2-Hydroxyoctylpyridinatocobaloxime (*n*-$C_6H_{13}CH(OH)CH_2Co(D_2H_2)C_5H_5N$): *Anal.* Calcd for $C_{21}H_{36}N_5O_5Co$: C, 50.70; H, 7.30; N, 14.08. Found: C, 50.50; H, 7.56; N, 13.72.

3-Hydroxy-*n*-propylpyridinatocobaloxime ($HO(CH_2)_3Co(D_2H_2)C_5H_5N$): *Anal.* Calcd for $C_{16}H_{26}N_5O_5Co$: C, 44.97; H, 6.13; N, 16.39. Found: C, 44.59; H, 5.79; N, 16.41. (This complex was prepared from trimethylene oxide. The identical material was obtained from 3-bromopropanol.)

4-Hydroxy-*n*-butylpyridinatocobaloxime ($HO(CH_2)_4Co(D_2H_2)C_5H_5N$). *Anal.* Calcd for $C_{17}H_{28}N_5O_5Co$: C, 46.26; H, 6.40; N, 15.87. Found: C, 45.97; H, 6.08; N, 16.03 (prepared from 4-bromo-*n*-butanol).

4-Bromo-*n*-butylpyridinatocobaloxime ($Br(CH_2)_4Co_1(D_2H_2)C_5H_5N$): A suspension of 47.6 g (0.2 mole) of $CoCl_2 \cdot 6H_2O$ and 46.4 g (0.4 mole) of dimethylglyoxime in 750 ml of methanol was stirred until all the cobalt chloride had dissolved. After adding 16.0 g (0.4 mole) of NaOH in 50 ml of water, the suspension was stirred under nitrogen for 10 min. Next, 30 g of 1,4-dibromobutane was added followed by 8.0 g (0.2 mole) of NaOH. After 10 min the reaction mixture was filtered and diluted with 2 l. of water. Excess of the dibromobutane was removed in an air stream. On adding 16.0 g (0.2 mole) of pyridine, a yellow precipitate formed which was collected, washed, dried, and then dissolved in the minimum amount of boiling methanol. On cooling and standing, crystals of 1,4-tetramethylenebis(pyridinatocobaloxime) formed. From the filtrate, on dilution with water, 19.6 g (39%) of 4-bromobutylpyridinatocobaloxime was obtained.

Anal. Calcd for $C_{17}H_{27}N_5O_4CoBr$: C, 40.49; H, 5.39; N, 13.89; Br, 15.85. Found: C, 40.68; H, 5.22; N, 13.61; Br, 15.33.

The following two complexes were obtained from 2-hydroxyethylaquocobaloxime on reaction with the respective bases.

2-Hydroxyethylbenzimidazolocobaloxime ($HOCH_2CH_2Co(D_2H_2)C_7H_6N_2$): *Anal.* Calcd for $C_{17}H_{25}N_6O_5Co$: C, 45.14; H, 5.57; N, 18.59. Found: C, 44.89; H, 5.73; N, 18.35.

2-Hydroxyethyltriphenylphosphinatocobaloxime ($HOCH_2CH_2Co(D_2H_2)P(C_6H_5)_3$): *Anal.* Calcd for $C_{28}H_{34}N_4O_5CoP$: C, 56.38; H, 5.75; N, 9.39. Found: C, 56.09; H, 5.98; N, 9.14.

Degradation Experiments. The degradation reactions mentioned in this paper require no detailed experimental description. The identification of the products was achieved by gas–liquid partition chromatography and mass spectroscopy. The aldehydes and aldols were also characterized as the *p*-nitrophenylhydrazones and compared (melting point, mixture melting point, and infrared spectrum) with authentic samples. Only one specific example of a degradation experiment will be reported.

(20) P. A. Frey and R. H. Abeles, *J. Biol. Chem.*, **241**, 2732 (1966).
(21) G. N. Schrauzer and R. J. Windgassen, to be published.
(22) R. H. Yamada, T. Kato, S. Shimizu, and S. Fukui, *Biochim. Biophys. Acta*, **97**, 353 (1965).

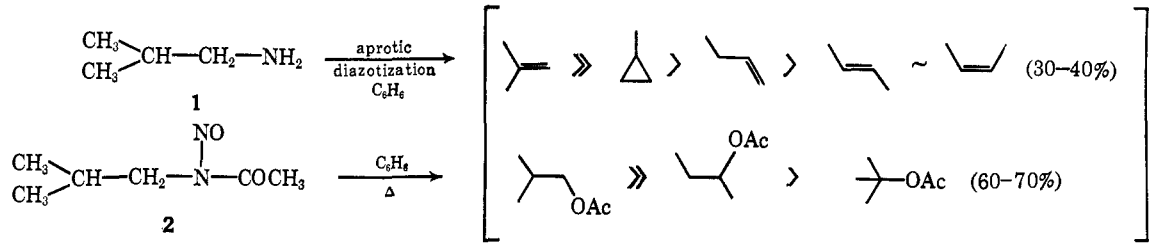
Degradation of β-Hydroxyisopropylpyridinatocobaloxime. The cobaloxime (5.0 g) was suspended in 25 ml of 1 *N* NaOH and the mixture was gently warmed. Samples of the volatile organic decomposition products were continuously withdrawn out of the gas phase and analyzed by programmed mass spectroscopy. Acetone was the only product detected. After careful neutralization with dilute H_2SO_4 the reaction mixture was fractionally distilled. Acetone, isolated in the over-all yield of 69% of the theoretical amount, was identified by gas chromatography and mass spectroscopy.

Communications to the Editor

Concerning the Mechanisms of Alkylamine Diazotization and N-Alkyl-N-nitrosoacetamide Decomposition[1]

Sir:

In attempts to determine the nature of the intermediates involved in formation of bicyclobutane by aprotic diazotization of cyclopropylcarbinylamine,[2] reactions were conducted using DOAc and amine-d_2,[3] wherein the bicyclobutane formed contained significant amounts of deuterium.[4] In order to elucidate the mechanism(s) by which this may have occurred, a study of the diazotization process was initially undertaken using isobutylamine (**1**).



Aprotic diazotization[5] of **1**-d_2 in benzene, chloroform, or mesitylene gave C-4 hydrocarbon mixtures that incorporated deuterium to the extent of ~36% d_1 and ~12% d_2.[6] Products obtained by diazotization in ethylene glycol-d_2 (EG-d_2) incorporated deuterium to a similar but somewhat smaller degree (Table I). In more protic systems such as DOAc and D_2O–DOAc, deuterium uptake was diminished[7] and limited to monodeuteration. Inasmuch as it was reported previously[5] that aprotic diazotization of amines and thermal decomposition of N-alkyl-N-nitrosoacetamides under similar conditions afford the same products, presumably *via* common intermediates, the decomposition of N-isobutyl-N-nitrosoacetamide (**2**) was also investigated in deuterium-labile media (Table I).

The decomposition of **2** in the presence of *only 1 equiv* each of D_2O and hexanol-*d*, in order to simulate as closely as possible the conditions of aprotic diazotization, *gave products which incorporated deuterium to the same extent* (51% d_0, 40% d_1, 9% d_2). Furthermore, decomposition of **2** in more highly protic media (Table I) results in less deuterium uptake.

The data must be rationalized by mechanisms which accommodate the following experimental observations for primary aliphatic systems: (a) the incorporation of one and two deuterium atoms/molecule in products from both aprotic diazotization of amines and thermal decomposition of N-alkyl-N-nitrosoacetamides in deuterium-labile environments; and (b) an increase of solvating power of the media and/or a decrease in apparent pH markedly diminishes the extent of deuteration.

The results in aprotic media are best explained by the following reaction scheme[8] which involves the formation of covalent diazonium acetate[8b,9] (**3**) as the common primary intermediate. Structure **3** should prevail over **3a** and would give **4**[10] *via* cyclic elimination of acetic acid.[7b] Addition of DOAc would lead to **5** and **5a**, incorporating one deuterium atom at this stage. By similar processes **7** and **7a** could be formed, thus incorporating two atoms of deuterium. The formation of non-, mono- and dideuterated products can occur as depicted arising from **3a**, **5a**, and **7a**, respectively.[11]

(1) Financial support (Grant No. GP-3976) from the National Science Foundation is gratefully acknowledged.

(2) J. Bayless, L. Friedman, J. A. Smith, F. B. Cook, and H. Shechter, *J. Am. Chem. Soc.*, **87**, 661 (1965).

(3) *I.e.*, $RND_2 + D^+ + R'ONO \longrightarrow$ products derived from $RN_2^+ + D_2O + R'OD$.

(4) Unpublished results.

(5) J. H. Bayless, F. D. Mendicino, and L. Friedman, *J. Am. Chem. Soc.*, **87**, 5790 (1965).

(6) By low-voltage mass spectrometry on total hydrocarbon fraction. Individually trapped isomers (methylcyclopropane, isobutylene, 1-butene) showed essentially the same degree of deuterium incorporation. Nmr analysis of the isobutyl acetate indicated the same amount of deuterium and that it was present only at C-1.

(7) (a) *Cf.* A. Streitwieser, Jr., and W. D. Schaeffer, *J. Am. Chem. Soc.*, **79**, 2888 (1957); (b) *ibid.*, **79**, 2893 (1957).

(8) (a) The possibility of diazo–diazonium ion equilibration for diazoalkanes was previously recognized but was not definitively resolved: R. Huisgen and H. Reimlinger, *Ann.*, **599**, 183 (1956); (b) *ibid.*, **599**, 161 (1956).

(9) E. H. White, *J. Am. Chem. Soc.*, 77, 6014 (1955).

(10) Diazoalkanes are formed in the thermal decomposition of the N-nitrosoamides of primary carbinamines: E. H. White and C. A. Aufdermarsh, Jr., *J. Am. Chem. Soc.*, **83**, 1174 (1961), and references contained therein. (b) On the other hand, N-nitrosoamides of secondary carbinamines presumably decompose without the involvement of diazoalkane intermediates: E. H. White and C. A. Aufdermarsh, *ibid.*, **83**, 1179 (1961). Aprotic diazotization of *sec*-butylamine-d_2 in benzene with 1 equiv of DOAc is in agreement with this; *i.e.*, only ~3% monodeuteration is observed.

(11) Diazoester—diazonium acylate ion-pair equilibria have been postulated for the decomposition of N-nitrosoamides of secondary carbinamines.[10b]