Studies on Cobaloxime Compounds. V. Oxidation of Formaldehyde with Cobaloximes and Aquocobalamin

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Catalytic activities of cobaloximes in the oxidation of formaldehyde were compared with each other and with the activity of aquocobalamin (vitamin B_{12a}). All the cobaloximes showed much less activities than B_{12a}. Chlorocobaloximes had the highest activities. Reaction order was found to be -0.18 with respect to the concentration of hydrogen ion, 0.71 to that of formaldehyde, and 0.49 to that of chloroaquocobaloxime. In anaerobic conditions, the reduced-state B_{12r} considered to be an intermediate in the air-oxidation reaction of aldehyde was detected spectrochemically, but not the reduced-state of cobaloxime. A much slower reaction of aldehyde with cobaloximes as compared with cobalamin was attributed to the higher reduction potentials of the former.

Concerning the vitamin B_{12}-dependent enzymatic system which converts diols to the corresponding aldehydes, further oxidation reaction of the aldehydes might occur with the aid of the B_{12} component. The vitamin B_{12}-formaldehyde complex was reported to be a one-carbon unit precursor in the biosynthesis of methionine.[1] The catalytic activity of aquocobalamin in the air-oxidation of some aldehydes was reported by Komai *et al.*[2]

In the present work, oxidation reaction of aldehydes was examined with cobalamin and cobaloximes in order to compare their chemical reactivity; the resemblance of cobaloxime compounds to cobalamin compounds in chemical behavior has been described elsewhere.[3,4]

Experimental

Materials. All the cobaloximes were prepared as reported previously.[5] Aquocobalamin was kindly supplied by Eisai Co. Commercial G. R. grade formalin (containing about 37% formaldehyde) was diluted with distilled water to suitable concentration. Acetaldehyde, propionaldehyde and benzaldehyde were commercial E. P. grade reagents. All other reagents were G. R. grade obtained commercially.

Procedures. *pH Measurement*: A pH Meter, Toa Electronics Ltd., model HM-5A, was used for pH determination.

pH Change during the Course of the Oxidation Reaction of Aldehyde: The reactions were carried out as follows. To a mixture of 6.0 m*l* of 5×10^{-4} M aqueous solution of a complex and 5.0 m*l* of 1.0 M formaldehyde was added first 35 m*l* of distilled water, followed by adjustment of pH of the solution to 7.00 with 5 mM NaOH, and by final addition of distilled water, whereby total volume was brought up to 50.0 m*l*. Thus, the final concentrations of formaldehyde and the complex were 0.10 M and 6.0×10^{-5} M, respectively. The reactions were conducted at 40°C with shaking in aerobic conditions. At appropriate intervals, the pH of the solution was measured and 2.0 m*l*-aliquot was taken for analysis of the remaining aldehyde. Aldehyde content was determined by titration: 4 m of 1 N NaOH and 5.0 m*l* of 0.1 N iodine solution were added to the aliquot. After 20 min, 2.0 m*l* of 3 N H_2SO_4 and a small amount of starch solution were added. Excess iodine was titrated with 0.1 N $Na_2S_2O_3$, from which the amount of the remaining aldehyde in the aliquot was calculated.

Dependence of the Rate upon the Concentrations of Hydrogen Ion, Formaldehyde, and Cobaloxime: The reactions were conducted in 0.5 M potassium phosphate buffer and the residual aldehyde content was determined. The dependence was examined in the pH range 5.8—7.8; the concentration of formaldehyde (2.35×10^{-2} M—2.03×10^{-1} M), and that of chloroaquocobaloxime (1.04×10^{-3} M—4.01×10^{-3} M).

Paper Chromatography: Formic acid formed in the reaction mixture was extracted with ether, and the ether layer was shaken with 1% NH_4OH aqueous solution. Ammonium formate solution thus obtained was used for paper chromatographic analysis in a mixed solvent of 95% ethanol and concd. NH_4OH (100 : 1, by vol.). Formate was detected by a blue spot at R_f 0.30 ($\pm$0.04) by spraying a bromophenol blue solution, whereas the authentic sample gave a spot at R_f 0.28 ($\pm$0.05).

Reduction of Cobaloxime by $NaBH_4$: Into 20 m*l* of 3.0×10^{-4} M solution of chloroaquocobaloxime was passed nitrogen gas and evacuated by a vacuum pump. After the N_2 passage-evacuation procedure was repeated five times, a small amount of solid $NaBH_4$ (*ca.* 1 mg) was added to the solution with stirring under a nitrogen atmosphere. Rapid color change in the solution from light yellowish brown to yellow took place, and the absorption maximum appeared at 463 mμ which is a characteristic band of cobaloxime(II), *i.e.*, $Co^{II}(DH)_2(H_2O)$ (DH: dimethylglyoximato monoanion). From the spectra for several concentrations of the cobaloxime, its molar extinction coefficient was determined to be 3.76 ($\pm$0.17)$\times10^3$.

Reduction of Cobaloxime and B_{12a} by Aldehydes: When formaldehyde was used instead of $NaBH_4$ at the final concentration 0.050 M, no reduction of chloroaquocobaloxime was observed even after 160 hr. However, this is ambiguous, since the hyperchromic shift of chloroaquocobaloxime occurs in aqueous solution. Therefore the reduction was examined for hydroxoaquocobaloxime (1.0×10^{-4} M) as well as aquocobalamin (3.0×10^{-5} M) with a large excess (hundreds times of the complex) of formaldehyde, acetaldehyde or benzaldehyde in 0.1 M potassium phosphate buffer (pH 7.1) at 40°C. Even after 120 hr no reduction was observed in the case of cobaloxime. On the contrary, B_{12a} was reduced by formaldehyde, acetaldehyde or benzaldehyde in 20 hr. This was demonstrated spectrochemically by the appearance of

1) B. W. Langer, Jr., and F. H. Kratzer, *Poultry Sci.*, **46**, 749 (1967); *Chem. Abstr.*, **67**, 9056*p* (1967).
2) T. Komai, S. Shimizu, R. Yamada, and S. Fukui, *Vitamins* (Japan), **35**, 395 (1967).
3) G. N. Schrauzer, *Accounts Chem. Res.*, **1**, 97 (1968); and the references cited therein.
4) Y. Hohokabe and N. Yamazaki, This Bulletin, **44**, 798 (1971).
5) N. Yamazaki and Y. Hohokabe, *ibid.*, **44**, 63 (1971).

the absorption maximum at 475 mμ in accord with the value reported in literature.[2]

Results and Discussion

It is expected that pH of the solution will decrease if oxidation of aldehyde takes place in the presence of a catalyst in aerobic conditions. The catalytic activities of several cobaloximes and aquocobalamin (B_{12a}) were examined in the oxidation of formaldehyde in the presence of air. Typical pH change during the course of the reaction is shown in Fig. 1. We see that

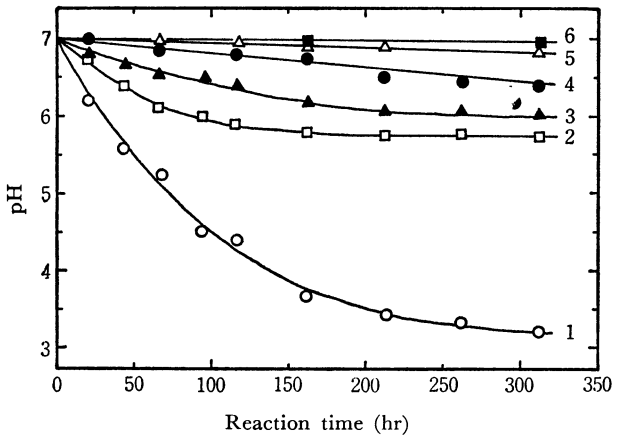


Fig. 1. pH change during the course of the oxidation reaction of formaldehyde.
[HCHO]=0.10 M, [Co]=6.0×10^{-5} M, at 40°C
1, B_{12a}; 2, $CoCl(DH)_2(H_2O)$; 3, $CoCl(DH)_2$(pyridine); 4, $CH_3Co(DH)_2(H_2O)$; 5, $Co(CN)(DH)_2(H_2O)$; 6, blank

B_{12a} acted as a much better catalyst than cobaloximes. After 300 hr the initial concentration of formaldehyde 0.10 M decreased to 0.082 M in the presence of B_{12a} with a concentration 6.0×10^{-5} M, which shows that the reaction proceeded catalytically. Of the cobaloximes, chlorocobaloximes exhibited the highest activity. The catalytic activity decreased in the order $B_{12a} \gg CoCl(DH)_2(H_2O) > CoCl(DH)_2$(pyridine) $> CoCl(DH)_2$(Copoly-AM-VPy) $> CH_3Co(DH)_2(H_2O) > Co(OH)(DH)_2(H_2O) \simeq Co(OH)(DH)_2$(Copoly-AM-VPy) $\simeq CH_3Co(DH)_2$(pyridine) $> Co(CN)(DH)_2(H_2O)$ as determined from the decrease of the pH of the reaction mixture, where DH denotes dimethylglyoximato monoanion and Copoly—AM-VPy a low molecular weight copolymer of acrylamide and 4-vinylpyridine.[6] The reason for the higher activity of chlorocobaloximes than hydroxo derivatives cannot be presented. Their higher activity than methyl or cyano derivatives might be due to weaker Co–Cl bond than Co–C bonds. Cyanocobalamin was reported to have no catalytic activity in the oxidation of aldehyde.[2] The oxidation of propionaldehyde was observed to take place much more slowly than that of formaldehyde in the presence of these catalysts.

Dependence of the reaction rates upon the concentrations of formaldehyde (2.35×10^{-2}—2.03×10^{-1} M), chloroaquocobaloxime (1.04×10^{-3}—4.01×10^{-3} M) and the acidity (pH 5.82—7.78) was examined.

6) N. Yamazaki and Y. Hohokabe, *Chem. Commun.*, **1968**, 829.

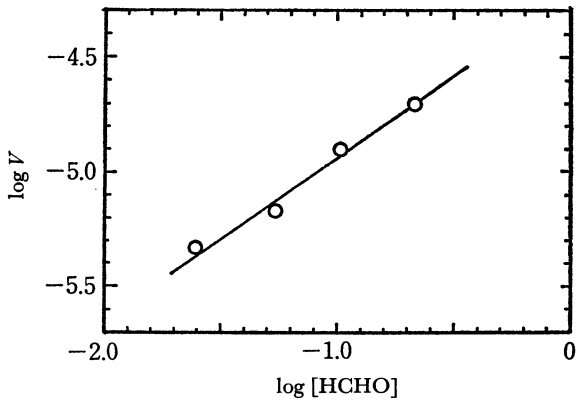


Fig. 2. Dependence of rate upon the concentration of formaldehyde.
[$CoCl(DH)_2(H_2O)$]=3.0×10^{-3} M, pH 7.0, at 40°C

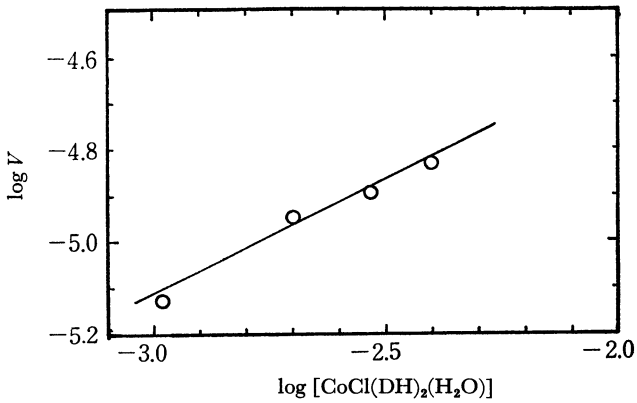


Fig. 3. Dependence of rate upon the concentration of chloroaquocobaloxime.
[HCHO]=0.10 M, pH 7.0, at 40°C

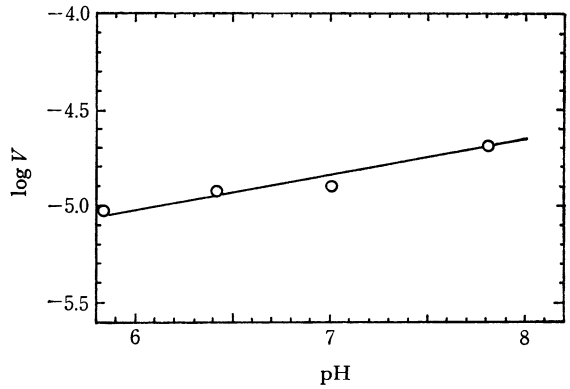


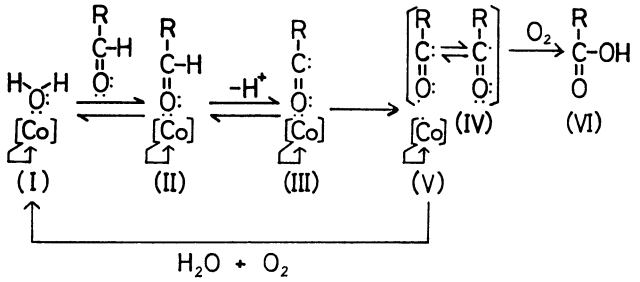
Fig. 4. Dependence of rate upon the pH.
[HCHO]=0.10 M, [$CoCl(DH)_2(H_2O)$]=3.0×10^{-3} M, at 40°C

The results are shown in Figs. 2, 3, and 4. The reaction rate increased with concentrations of aldehyde or cobaloxime, and slightly with pH. The reaction order was found to be −0.18 with respect to [H^+]; 0.71 to the concentration of aldehyde and 0.49 to that of cobaloxime.

The oxidation reaction of formaldehyde in the presence of chloroaquocobaloxime proceeded too slowly; only twice the number of moles of the aldehyde, as compared to the cobaloxime added, was oxidized after about 400 hr. Thus cobaloximes

had a quite low catalytic activity, though B_{12a} acted as a better catalyst as mentioned above. The formation of formic acid was confirmed by paper chromatagraphy. Authentic formic acid gave a spot at R_f 0.28 (±0.05) in 95% ethanol - *concd.* NH_4OH (100 : 1, by vol.), whereas the sample gave a spot at R_f 0.30 (±0.04).

A mechanism for the oxidation of aldehyde was proposed as follows.[2]



1) Aldehyde coordinates to the Co atom in exchange for H_2O of B_{12a}. 2) Proton leaves from the aldehyde-cobalamin complex. 3) The unstable intermediate thus formed splits to give an acyl radical and vitamin B_{12r}. 4) Under aerobic conditions, the acyl radical is attacked by oxygen molecule and converted to carboxylic acid. 5) B_{12r} is reoxidized to aquocobalamin (B_{12a}).

A similar mechanism could be applied to the reaction with cobaloximes. Much lower catalytic activity of cobaloximes as compared with aquocobalamin might be due to higher reduction potentials of the former. Thus, the difficulty of reduction of cobaloxime by aldehydes was demonstrated spectrophotometrically: Since chloroaquocobaloxime shows a hyperchromic shift in the visible region due to the aquation reaction, it is difficult to detect the formation of the reduced-state of cobaloxime spectro-chemically. Hence, the reaction of aldehydes with hydroxoaquocobaloxime was examined in an aerobic conditions, as well as with aquocobalamin. In the case of B_{12a}, with formaldehyde, acetaldehyde or benzaldehyde, the absorption maximum attributable to B_{12r} was observed at 475 mμ in 20 hr, which disappeared in contact with oxygen giving rise to the restoration of the spectrum of the original solution. On the other hand, in the case of cobaloxime, the absorption maximum at 463 mμ attributable to cobaloxime (II) could not be observed even after 120 hr under the same conditions.

Thus, it is concluded that the reduction of cobaloximes with aldehydes, if any, takes place much more slowly, than that of B_{12a}, and the low catalytic activity of the former can be attributed to their higher reduction potentials. This is supported by the observation that cobalamins are reduced at less negative potentials than the corresponding cobaloximes in the polarographic reduction,[7] and the reduction of cobaloximes by thiols is much slower than that of cobalamins.[8]

7) Y. Hohokabe and N. Yamazaki, This Bulletin, **44**, 1563 (1971).

8) G. N. Schrauzer and J. W. Sibert, *Arch. Biochem. Biophys.*, **130**, 257 (1969).