# Studies on Cobaloxime Compounds. VI. Decomposition of Formic Acid with Cobaloximes and Cobalamins

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Catalytic activities of cobaloximes in the decomposition reaction of formic acid were compared with each other and with those of cobalamins. In the cobalamin series, the activity decreased in the order aquocobalamin (vitamin $B_{12a}$) > methylcobalamin > cyanocobalamin (vitamin $B_{12}$). In the cobaloxime series, the activity decreased in the order $Co(OH)(DH)_2(H_2O) > CoCl(DH)_2(H_2O) > Co(CN)(DH)_2(H_2O) > CH_3Co(DH)_2(H_2O)$. Cobalamins had much greater activities than the corresponding cobaloximes. The reaction proceeded with uptake of oxygen. No hydrogen gas was evolved in the reaction. The reaction rate slightly decreased with the increase of pH between 6.0 and 7.0. The reaction rate changes with 0.39 power of formic acid concentration less than $1\times10^{-3}$ M (comparable to that of cobaloxime), and becomes independent of the concentration greater than $1\times10^{-3}$ M. It changed with 0.89 power of the concentration of hydroxoaquocobaloxime. Aquocobalamin was reduced to $B_{12r}$ in the presence of formic acid but not hydroxoaquocobaloxime in anaerobic conditions. The catalytic activities of cobaloximes lower than the corresponding cobalamins were attributed to the higher reduction potentials of the former.

So far, comparison between cobalamin and cobaloxime has been made in the reaction with CO,[1,2] $O_2$,[3] aldehyde,[4] electron transfer reaction,[5] photodecomposition reaction,[6,7] and other chemical and biochemical reactions.[8,9] Catalytic decomposition of formic acid by cobalamins was briefly described by Utsumi *et al.*[10]

In the present work, catalytic decomposition of formic acid by several cobaloximes and cobalamins was examined in order to compare their catalytic activities, and the results are given herewith.

## Experimental

*Materials.* Preparation of the cobaloximes was described previously.[11] Cobalamins were kindly supplied by Eisai Co. All other reagents were commercial products of G. R. grade.

*Procedures.* *Decomposition Reaction*: The reactions were followed by means of the conventional Warburg manometric method.[12] A typical reaction mixture with a total volume of 4.0 m*l* contained $6\times10^{-3}$ M of formic acid and $6\times10^{-4}$ M of cobalamin or cobaloxime in 0.5 M phosphate buffer (pH 6.00). Two manometers were used for following a run, one with a chamber containing 0.2 m*l* of 20% KOH with a small filter paper to absorb $CO_2$ gas evolved in the reaction, and another without the alkali chamber. The pressure change resulting from the uptake of oxygen was followed with the former, and the total pressure change due to the difference between the uptake of oxygen and the evolution of $CO_2$ with the latter. Each reading was corrected by that of a thermobarometer. The reaction was carried out at 40°C with shaking at a rate of 100 rpm.

*Reduction of Cobalamin or Cobaloxime with Formic Acid in Anaerobic Conditions*: Reduction was examined for hydroxoaquocobaloxime ($1.0\times10^{-4}$ M) as well as aquocobalamin ($3.0\times10^{-5}$ M) in the presence of $5\times10^{-3}$ M formic acid in 0.1 M phosphate buffer (pH 7.1) at 40°C under a nitrogen atmosphere, in the same way as reported previously.[4] $B_{12a}$ was reduced in 20 hr which was demonstrated spectrochemically by the appearance of an absorption maximum at 475 m$\mu$. No reduction was observed in the case of hydroxoaquocobaloxime even after 200 hr.

*Gas Chromatography*: The gases in the reaction mixture were examined by means of gas chromatography for hydrogen gas, which might be formed by non-oxidative decomposition of formic acid. For detection a Shimadzu Gas Chromatograph, model GC-4APT was used with nitrogen gas as carrier at the flow rate of 40 m*l*/min. Moelcular Sieves-13X (60—80 mesh) preheated at 500°C for 2 hr and cooled was packed in a steel column (3 mm × 3 m). The detector temperature was 30°C, and the column temperature 20°C. Authentic hydrogen gas gave a retention time 89.4 sec. The gas phase of the reaction mixture with $B_{12a}$ or chloroaquocobaloxime was thus examined and no hydrogen gas was detected.

## Results and Discussion

Decomposition of formic acid with cobalamins or cabaloximes proceeded quantitatively with consumption of oxygen. The $CO_2/O_2$ ratios were 1.6—1.0. Initial rates for the decomposition of formic acid with some cobalamins and cobaloximes are summarized in Table 1. Aquocobalamin was found to be the most powerful catalyst among the cobalamins or cobaloximes examined. Methylcobalamin was the second. Cyanocobalamin showed the lowest activity among cobalamins, in good agreement with the results by Utsumi *et al.*[10] In the cobaloxime series, the catalytic activity decreased in the order hydroxo- > chloro- > cyano- > methyl-aquocobaloxime.

Dependence of rate on the concentration of formic acid, hydroxoaquocobaloxime and pH was investigated. At a concentration comparable to that

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Table 1. Initial rates for the decomposision of formic acid with cobalamins and cobaloximes[a)]

| Material[b)] | Initial rate, M/min |
|---|---|
| Aquocobalamin | $5.70\times10^{-5}$ |
| Methylcobalamin | $9.48\times10^{-6}$ |
| Cyanocobalamin | $1.29\times10^{-6}$ |
| $Co(OH)(DH)_2(H_2O)$ | $1.92\times10^{-6}$ |
| $CoCl(DH)_2(H_2O)$ | $3.31\times10^{-7}$ |
| $Co(CN)(DH)_2(H_2O)$ | $2.64\times10^{-7}$ |
| $CH_3Co(DH)_2(H_2O)$ | $1.92\times10^{-7}$ |

a) $[HCOOH]=6.0\times10^{-3}$ M, $[Co]=6.0\times10^{-4}$ M, in 0.5 M phosphate buffer (pH 6.00), at 40°C. (total volume: 4.0 m*l*). The estimated relative errors are not more than 5%.

b) DH denotes dimethylglyoximato monoanion.

of cobaloxime, the rate increased with the substrate concentration, the reaction order being about 0.4. However, at a higher concentration, the rate curves become flat as shown in Fig.1. The rate increased with the concentration of cobaloxime, the reaction order being 0.89 as shown in Fig 2. A slight decrease in rate with the increasing of pH was unexpected (Fig. 3), since the coordination of formate anion to the complex was considered to be easier than that of the protonated form of formic acid. No reason can be given at present.

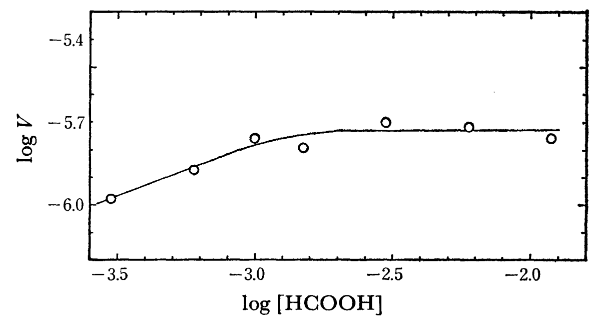

Fig. 1. Dependence of rate upon the concentration of formic acid.
$[Co(OH)(DH)_2(H_2O)]=6.0\times10^{-4}$ M, in 0.5 M phosphate buffer (pH 6.00), at 40°C

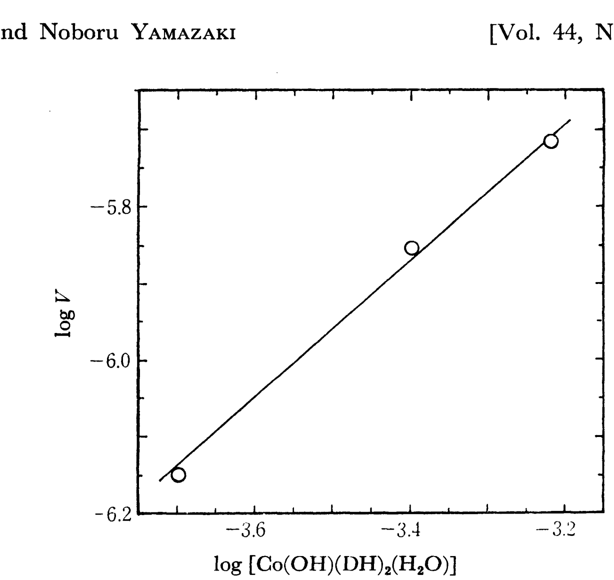

Fig. 2. Dependence of rate upon the concentration of hydroxoaquocobaloxime.
$[HCOOH]=6.0\times10^{-3}$ M, in 0.5 M phosphate buffer (pH 6.00), at 40°C

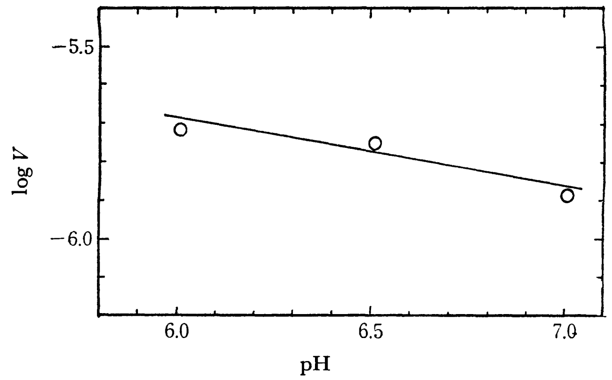

Fig. 3. pH Dependence of rate.
$[HCOOH]=6.0\times10^{-3}$ M, $[Co(OH)(DH)_2(H_2O)]=6.0\times10^{-4}$ M, in 0.5 M phosphate buffer, at 40°C

Change of activity with the variation of the axial ligand in cobalamins and cobaloximes suggests that the formate coordinate to the cobalt atom in the sixth position as in acetate anion,[13] though the formation constant of acetate anion is very small. Thus two electrons are considered to be transferred from the formate anion coordinated to $Co^{3+}$ in the complex to Co atom, producing $CO_2$ and $H–Co^{1+}$. The $Co^{1+}$ species formed is quite unstable under these conditions and reoxidized to $Co^{3+}$. Otherwise, $HCO_2\cdot$ and $Co^{2+}$ would be formed *via* one-electron transfer process, and oxidized by oxygen to $CO_2+HO\cdot$ and $Co^{3+}$, respectively. However, no detailed mechanism can be presented at present. The over-all decomposition reaction of formic acid in the presence of the cobalt catalyst in aerobic conditions may be written as

$$HCOOH + \tfrac{1}{2}\cdot O_2 + Co^{3+} \rightarrow CO_2 + H_2O + Co^{3+}.$$

Thus the $CO_2/O_2$ ratio should be 2.0 theoretically; the observed values were higher than unity, supporting the equation. Oxygen might be consumed by side reaction such as ligand oxidation of the complexes.[10] Much lower catalytic activities of cobaloximes as compared with cobalamins might be attributed to their higher reduction potentials, as in the oxidation of aldehyde.[4] An extremely slow reduction of cobaloximes by thiols in contrast to cobalamin has recently been demonstrated.[5] Reduction of $B_{12a}$ with formic acid to $B_{12a}$ was demonstrated spectrochemically by the appearance of the absorption maximum at 475 mμ in anaerobic conditions (pH 7.1). In contrast, reduction of hydroxoaquocobaloxime was not observed under similar conditions. Although $B_{12s}$ might be expected to be formed in the reaction of $B_{12a}$ and formic acid on the basis of the above mechanism, it was considered unstable under the conditions (pH 7.1) and converted to $H\cdot+B_{12r}$ under a nitrogen atomosphere,[14] its presence being demonstrated in the visible spectrum. The hydrogen radical would attack the oxygen molecule in aerobic conditions and hence no hydrogen gas would be evolved, in agreement with the results we obtained.

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