**CHEMICAL AND BIOCHEMICAL STUDIES ON
VITAMIN B₁₂ AND ITS RELATED COMPOUNDS**
**XV. PROPERTIES AND PHOTOCHEMICAL DECOMPOSITIONS OF SOME HYDROXYALKYLCOBALAMINS
AND HYDROXYALKYLCOBINAMIDES¹**

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(Received March 23, 1965)

It has been known that cobamide coenzymes discovered by Barker *et al.* (1) are very unstable in light and easily decompose by illumination. DBCC yields as photolysis products OH-B₁₂ and adenosine-5'-aldehyde (2) or adenosine-5'-carboxylic acid (3) under aerobic conditions, and OH-B₁₂ and 8,5'-cycloadenosine (4,5) under anaerobic conditions, respectively. Studies on the photochemical decomposition of DBCC made an important contribution on the elucidation of its structure.

The participation of Co-methylcobalamin as a methyl donor in methionine synthesis from homocysteine (6) has been ascertained in photochemical reaction. The involvement of this compound in enzymatic synthesis of both methionine (7) and methane (8) provided the hypothesis that an exchange of the ligand by cleavage of the cobalt-carbon bond would play an important role in the functions of DBCC. Nevertheless, there are only a few examples studying photolysis of synthetic light-sensitive vitamin B₁₂ derivatives which contain a carbon-cobalt linkage.

This paper deals with the syntheses and properties of several light-labile hydroxyalkyl derivatives of cobalamin and cobinamide as the model compounds of DBCC in photochemical reactions. Possibility of these compounds to be the intermediates of 1,2-glycol-deoxyaldehyde conversion catalyzed by DBCC (9) or cobinamide coenzyme (10) was also considered. Although the reaction mechanism of the enzymatic conversion of a 1,2-diol to a corresponding deoxyaldehyde is thought to be a direct hydrogen shift according to Brownstein and Abeles (11), it appears interesting to study whether the reaction proceeds by ligand exchange through the formation of a substrate-coenzyme complex as an active intermediate or not.

¹ Following abbreviations are used: DBCC, 5,6-dimethylbenzimidazolylcobamide coenzyme; OH-B₁₂, hydroxo(or aquo)cobalamin; CN-B₁₂, cyanocobalamin; CN-CA, monocyanomono-aquocobinamide; DHP-CA, Co- β,γ -dihydroxypropylcobinamide; DHP-B₁₂, Co- β,γ -dihydroxypropylcobalamin; OH-CA, dihydroxo(or diaquo)cobinamide.

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EXPERIMENTAL

*Materials and Methods**1. Materials*

CN-B₁₂ was purchased from Merck and Company, Inc. CN-CA was obtained from the bacterial cell preparations for feed supplement as a source of animal protein factor (Vitamin Inc., U.S.A.) in the same way as reported in a previous paper (12).

NaBH₄, hydroxyalkyl halide, 2,4-dinitrophenylhydrazine, and developing reagents were all commercial products.

Except glyceric acid which was prepared by oxidation of glycerol with mercuric oxide, all polyols and their derivatives used as standards on paper chromatography were also obtained from commercial sources.

2. Paper Chromatography

It was carried out on Toyo filter paper No. 50 by an ascending method except one case using a descending method in which water-saturated sec.-butanol was employed as the solvent.

Solvent systems used were as follows. S₁, *n*-butanol-isopropanol-water (100:70:100); S₂, *n*-butanol-isopropanol-acetic acid-water (100:70:1:75); S₃, water saturated sec.-butanol; S₄, *n*-butanol-acetic acid-water (40:10:30); S₅, isopropanol-pyridine-water-acetic acid (18:8:4:1); S₆, ethyl acetate-pyridine-water (70:20:10); S₇, chloroform-ethanol (30:20).

All the corrinoid compounds were spotted in such concentration as to be visible. Polyols were detected by spraying aqueous sodium metaperiodate solution (0.5%) and subsequent spraying of benzidine in a mixture of ethanol and acetic acid (40:10 v/v). Acidic compounds were detected as yellow spots in an atmosphere of ammonia after being sprayed with methanolic bromocresol purple solution (0.1%). 2,3,5-triphenyltetrazolium chloride in 0.1 N sodium hydroxide was used for the detection of reducing materials.

3. Thin Layer Chromatography

Silica Gel G suspended in water (0.5 g/ml) was layered 0.2 mm thick on a glass plate (20×20 cm) and was heat-activated at 100° for 2 hours before use. Solvent system used was a mixture of ethyl acetate-toluene (30:10 v/v).

3. Paper Electrophoresis

It was performed with the apparatus, Type II-C of Toyo Roshi Co., and Toyo filter paper No. 50. Visible amounts of corrinoid compounds were spotted.

4. Visible Absorption Spectra Measurement

Visible absorption spectra were studied using a Hitachi EPS-II Self-recording spectrophotometer in cells with an absorbing path of 1.0 cm.

5. Microbiological Activity Assay

It was performed by usual tube assay methods using *E. coli* No. 215 (vitamin B₁₂ or methionine-auxotroph) as a test organism (10).

6. Coenzyme Activity Assay

The coenzyme activity of the compounds tested was determined according to the method of Abeles and Lee (9). Apoenzyme was obtained from the cell extract of *Aerobacter aerogenes* (ATCC 8724) grown in a culture containing glycerol and yeast extract. In the presence of DBCC and its analogue the enzymatic reaction was carried out and the amount of propionaldehyde formed from propylene glycol was determined colorimetrically as 2,4-dinitrophenylhydrazone.

7. Photolysis

Samples were irradiated with a tungsten filament lamp (300 W) at 10 to 30 cm distance for 5 to 60 minutes. The vessels containing the samples were cooled in running water.

RESULTS

1. Preparation and Several Physicochemical Properties of Hydroxyalkyl Derivatives of Cobamide and Cobinamide

Hydroxyalkyl derivatives were prepared by the reaction of hydroxyalkyl halide with reduced CN-B₁₂ or CN-CA according to the method for preparation of alkyl derivatives (13).

For example, 100 ml of aqueous solution of CN-B₁₂ (1 mg/ml) was deoxygenized by bubbling hydrogen gas for 15 minutes and then 100 mg of NaBH₄ was added. After standing for 30 minutes in a hydrogen atmosphere, the red solution turned completely to dark-green of vitamin B₁₂s (14). By adding 2 ml of glycerol α -monochlorohydrin a rapid reaction occurred and a light red solution was obtained. The product was isolated by extracting into phenol, then, being washed with water, it was displaced back into water with a large amount of ether. This was found to contain a single corrinoid compound by paper chromatography and paper electrophoresis. The yield at this stage was nearly quantitative. In order to remove contaminating glycerol α -monochlorohydrin, this solution was applied on a column of cellulose powder and was washed with a mixture of ethyl acetate and pyridine (70:20 v/v). Thus isolated product is expected to have a structure as illustrated in Fig. 1 and is designated as Co- β,γ -dihydroxypropylcobalamin (I). Co- β -hydroxypropylcobalamin (II) and Co- β -hydroxyethylcobalamin (III) were synthesized in the same procedure except that propylene bromohydrin and ethylene chlorohydrin were

FIG. 1 Hydroxyalkyl Derivatives of Cobalamin and Cobinamide Tested in this Study

respectively used in place of glycerol α -monochlorohydrin.

CN-CA reduced in the same way reacted with glycerol α -monochlorohydrin to yield an orange-yellow compound. This reaction product is expected to be Co- β , γ -dihydroxypropylcobinamide (IV).

Absorption spectra of the products (I)-(III) in neutral and acidic solutions gave similar spectra to those of DBCC and Co-alkylcobalamins, having in neutral solution an absorption maximum at about 530 m μ , with a shoulder at about 380 m μ and in acidic solution a maximum at about 460 m μ (13). On the other hand, DHP-CA gave an absorption maximum at about 460 m μ similarly to cobinamide coenzyme (10) and other cobinamide derivatives such as Co-alkylcobinamides (13,15) and SB₁₂₁ of Pawelkiewicz (16). This was not changed in either acidic or alkaline solution. In Fig. 2 the spectra of DHP-B₁₂ is given as a representative of the three cobalamin derivatives, and in Fig. 3 the spectrum of DHP-CA.

These four compounds, as well as cobamide- and cobinamide-coenzymes, were very unstable in light and almost completely decomposed to hydroxo (or aquo) forms on exposure to sunlight or artificial light for a few minutes. OH-B₁₂ thus obtained was identified by paper chromatography and paper electrophoresis with an authentic sample prepared by catalytic reduction of CN-B₁₂. The formation of OH-CA from the cobinamide derivative was evident, since on paper electrophoresis in

FIG. 2 Visible Absorption Spectrum of Co- β , γ -dihydroxypropylcobalamin and its Photolysis Product (Concentration, $3.32 \times 10^{-5} M$)

—, in aqueous solution; ---, in 0.2 N HCl; ···, photolysis product.

FIG. 3 *Visible Absorption Spectrum of Co-β,γ-dihydroxypropylcobinamide and its Photolysis Product* (Concentration, $2.98 \times 10^{-5} M$)
—, in aqueous solution; ---, photolysis product.

FIG. 4 *Paper Electrophoretic Behavior of Hydroxyalkyl Derivatives of Cobalamin and Cobinamide in 0.5 N Acetic Acid* (at 13 v/cm for 2.5 hours)
A, Co-β,γ-dihydroxypropylcobalamin; B, Co-β-hydroxypropylcobalamin; C, Co-β-hydroxyethylcobalamin; D, Co-β,γ-di-hydroxypropylcobinamide.

0.5 *N* acetic acid it gave a spot migrating to the cathode about twice as fast as CN-CA. Ofen occurrence of another substance behaving like CN-CA, however, left some unsolved problem.

As shown in Fig. 4, paper electrophoretic behavior of DHP-CA in 0.5 *N* acetic acid was almost the same as that of CN-CA. The three cobalamin derivatives moved to the cathode a little more slowly than OH-B₁₂.

Paper chromatographic behaviors of these compounds are shown in Table 1.

TABLE 1
Paper Chromatographic Behaviors of Hydroxyalkyl Derivatives of Cobalamin and Cobinamide and of Reference Compounds

Substance	<i>R_f</i> CN-B ₁₂ value			
	S ₁	S ₂	S ₃	S ₃ ^a
Co-β,γ-dihydroxypropylcobalamin	0.67	—	0.49	—
Co-β-hydroxypropylcobalamin	0.92	—	1.13	—
Co-β-hydroxyethylcobalamin	1.01	0.93	0.97	—
Co-β,γ-dihydroxypropylcobinamide	1.00	0.88	1.15	1.10
CN-B ₁₂	1.00	1.00	1.00	1.00
CN-CA	1.05	0.97	—	1.22

Solvent systems are described in the text.

^a Descending method was used.

The low *R_{CN-B12}* value of DHP-B₁₂ seems to reflect the hydrophilic property of the dihydroxypropyl group. Similar relationship was observed between CN-CA and DHP-CA.

On standing in the dark for 13 hours in 0.017 *M* KCN solution, not more than 20 % of the cobalamin derivatives and not more than 50 % of the DHP-CA were converted to the dicyano form. In this property they are similar to Co-alkylcobalamins and Co-alkylcobinamides, but differ from the coenzyme forms which are very sensitive to cyanide even in the dark.

Infra-red spectrum of DHP-B₁₂ given in Fig. 5 has no absorption in 2000 cm⁻¹ region, indicating the absence of CN group in this compound. The presence of dihydroxypropyl group cannot be substantiated because of extremely complicated structure of this compound.

2. Microbiological Activity of DHP-B₁₂

In order to obtain informations of the effect of these hydroxyalkylcobalamins, microbiological activity of DHP-B₁₂ was, by way of example, examined with vitamin B₁₂-requiring *E. coli* No. 215. DHP-B₁₂ was added aseptically to the medium without heat sterilization, and all the operation was carried out in the dark in order to avoid plausible pyrolysis and photolysis. Based on the spectrophotometric determination at 368 mμ in dicyano form, DHP-B₁₂ showed a nearly equal activity with CN-B₁₂ or OH-B₁₂.

3. Behavior of DHP-B₁₂ in an Enzymatic Reaction System

Since DHP-B₁₂ was found to be active toward a vitamin B₁₂-requiring micro-organism, behavior of this compound in enzymatic reaction systems involving DBCC

FIG. 5 *Infra-red Spectrum of Co-β,γ-dihydroxypropylcobalamin (in KBr)*

interested us. The system of Abeles and Lee converting 1,2-glycol to deoxyaldehyde (9) was chosen, considering a possibility of DHP-B₁₂ to be an intermediate in the enzymatic reaction.

In a preliminary experiment adding DHP-B₁₂ in the system without the concomitant addition of DBCC, aldehyde formation was not detected. Then the influence of this compound added in the system containing DBCC was examined. As shown in Table 2, DHP-B₁₂ exerted an inhibitory effect in this reaction system.

TABLE 2
Inhibitory Effect of Co-β,γ-Dihydroxypropylcobalamin in the Diol-Dehydrase System

Cobamide in the reaction system		Relative amount of pro- pionaldehyde formed
DBCC	Co-β,γ-dihydroxy- propylcobalamin	
<i>mμmoles</i>	<i>mμmoles</i>	
0	0	0
4	0	1.00
4	2.4	0.82
4	12	0.65
4	240	0

4. Photolysis of DHP-B₁₂

As described above, hydroxyalkyl derivatives of cobalamin and cobinamide are evidently sensitive to light like their coenzyme forms. Of these compounds DHP-B₁₂ was chosen as a model compound of DBCC in photolytic reactions for the

convenience of detection of photolytic products. Since the photolysis product derived from the cobalamin moiety was known to be OH-B_{12} as described above, the fate of the dihydroxypropyl group was mainly followed. Irradiation of aqueous solution of DHP-B_{12} was carried out under following conditions: (a) under aeration, (b) containing dissolved air, (c) in a nitrogen atmosphere, (d) containing NaBH_4 .

Photolysis under Aeration—20 ml of the aqueous solution ($0.4 \mu\text{moles/ml}$) was irradiated at a distance of 30 cm from the lamp for five minutes under vigorous aeration. The color of the solution immediately turned from light red to somewhat dark red, which showed the formation of OH-B_{12} . The material thus irradiated was applied on a column of CM-cellulose ($2 \times 40 \text{ cm.}$) and was washed with 100 ml of water-ethanol mixture (1:1). OH-B_{12} was retained on the column. The effluent was concentrated under reduced pressure in a nitrogen atmosphere, and then examined by paper chromatography. On paper chromatogram the main product of photolysis was positive to bromocresol purple and periodate-benzidine reagent and negative to triphenyl tetrazolium chloride. From these results it was concluded that the compound was periodate-consuming acidic substance containing no reducing functional group, and was presumed to be glyceric acid. As shown in Table 3, R_f value of this compounds coincided with that of authentic glyceric acid, which also behaved similarly toward the developing reagents.

TABLE 3
Comparison of Paper Chromatographic Behavior of the Photolysis Product under Aeration with Several Reference Compounds

Substance	R_f value	
	S_4	S_5
Ethylene glycol	0.63	—
Propylene glycol	0.78	—
Glycerol	0.47	0.72
Glyceraldehyde	0.54	0.61
Glycolic acid	0.69	—
Glyceric acid	0.45	0.50
"Photolysis product"	0.45	0.50

Solvent systems are described in the text.

Photolysis in an Air-Containing Aqueous Solution—10 ml of aqueous solution of DHP-B_{12} ($0.6 \mu\text{mole/ml}$) was irradiated for 20 minutes at a distance of 20 cm from the lamp. The color change of the solution was similar to the case of aeration. The solution thus irradiated was applied on a column of P-cellulose ($1.5 \times 20 \text{ cm.}$) and was washed with 600 ml of the mixture of water and ethanol. The effluent was concentrated under reduced pressure in a nitrogen atmosphere. Paper chromatographic behavior of this material detected with periodate-benzidine reagent is shown in Table 4. Judging from these results, this material consists of glyceraldehyde as a major component and a little amount of glycerol as a minor component.

Glyceraldehyde was further verified in a form of its 2,4-dinitrophenylhydrazone and of its 2,4-dinitrophenylosazone after reacting with 2,4-dinitrophenylhydrazine in ethanol solution and in acidic aqueous solution, respectively. These derivatives gave

TABLE 4
Comparison of Paper Chromatographic Behavior of the photolysis Product in the Presence of Dissolved Oxygen with Several Reference Compounds

Substance	<i>Rf</i> value	
	S ₁	S ₆
Ethylene glycol	0.55	0.58
Propylene glycol	0.69	0.74
Glycerol	0.41	0.38
Glyceraldehyde	0.53	0.14
"Photolysis product"	0.52, 0.41 ^a	0.38 ^a , 0.14

Solvent systems are described in the text.

^a Weak spot

equal *Rf* values to those of the derivatives obtained from the reaction of authentic glyceraldehyde with 2,4-dinitrophenylhydrazine (Table 5).

TABLE 5
Thin Layer Chromatography of the Reaction Products of DNPH with the Photolysis Product in the Presence of Dissolved Oxygen and with Authentic Glyceraldehyde

Reaction product	<i>Rf</i> value
Glyceraldehyde + DNPH in ethanol	0.71
Glyceraldehyde + DNPH in aq. H ₂ SO ₄	0.76
"Photolysis product" + DNPH in ethanol	0.71
"Photolysis Product" + DNPH in aq. H ₂ SO ₄	0.76

Solvent system: toluene-ethyl acetate (30:10 v/v)

DNPH, 2,4-dinitrophenylhydrazine.

Photolysis in a Nitrogen Atmosphere—To remove dissolved oxygen 2 ml of aqueous solution of DHP-B₁₂ (3 μ moles/ml) was shaken under reduced pressure (25 mm Hg) and then nitrogen was flushed. This operation was repeated ten times. The solution thus deoxygenated was exposed to illumination from the lamp at a distance of 20 cm. The color of the solution turned gradually to brown, indicating the formation of vitamin B_{12r}, and after 30 minutes the color change was almost complete. After further illumination for 30 minutes the solution was exposed to air, then B_{12r} immediately turned to OH-B₁₂. The solution was put on a column of P-cellulose (1 $\times$ 10 cm) and was washed with 250 ml of water-ethanol mixture. The effluent was concentrated and was examined by paper chromatography. For the detection of the photolysis products the periodate-benzidine reagent was used.

As shown in Table 6, glycerol was the main component. The other substance giving a weak spot was not further examined.

Photolysis in a NaBH₄-Containing Aqueous Solution—To 10 ml of aqueous solution of DHP-B₁₂ (1.5 μ moles/ml), 10 mg of NaBH₄ was added, and the surface of the solution was covered with a toluene layer. The solution was irradiated for 60 minutes at a distance of 20 cm from the lamp. The color of the solution first turned to brown, showing the formation of vitamin B_{12r}, then gradually changed to

TABLE 6
Comparison of Paper Chromatographic Behaviors of the Photolysis products in a Nitrogen Atmosphere and in the Presence of NaBH₄ with Several Reference Compounds

Substance	Rf value		
	S ₄	S ₈	S ₇
Ethylene glycol	0.60	0.52	0.59
Propylene glycol	0.69	0.67	0.75
Glycerol	0.43	0.29	0.30
Glyceraldehyde	0.51	0.15	0.00
"Photolysis product" in N ₂	0.43, 0.09 ^a	0.27, 0.14 ^a	0.32, 0.10 ^a
"Photolysis product" in the presence of NaBH ₄	0.47 ^b , 0.42	0.28, 0-0.01 ^b	0.49 ^b , 0.29

Solvent systems are described in the text.

^a Weak spot, ^b fluorescent spot.

dark brown by reducing with NaBH₄. Toward the end of the irradiation faint orange color emerged. To remove the remaining NaBH₄ and its degraded product, boric acid, this irradiated material was concentrated to dryness under reduced pressure and then dissolved in methanol containing a few drops of concentrated HCl and was again concentrated to dryness. This process was further twice repeated, and then a small quantity of water was added. When this solution was applied on a column of P-cellulose (1.5×20 cm) and was washed with 200 ml of water-ethanol mixture, cobinamide-like substance was retained besides red OH-B₁₂. The effluent was concentrated and was examined by paper chromatography.

As shown in Table 6, glycerol and an unknown periodate-consuming substance were recognized. The latter also gave fluorescence. It was therefore considered to be a nucleotide or nucleoside containing 5,6-dimethylbenzimidazole. This fact agrees with the observation that a cobinamide-like substance was retained on the column. Presumably some degradation of cobalamin occurred during the treatment with methanol containing HCl. Only glycerol was thus demonstrated as a photolysis product from dihydroxypropyl moiety of DHP-B₁₂ under the experimental condition.

DISCUSSION

Although several hypotheses have been proposed, the mechanism of enzymatic reactions involving DBCC, has not yet been completely elucidated. It seems most likely that DBCC acts as a hydrogen carrier. In this case the cobalt-carbon bond of enzyme-bound DBCC may be cleaved, forming cobalt atom with a lone pair electrons or an odd electron may participate as a hydrogen carrier. Methine bridge in the corrin nucleus is also proposed as an active site. (14,17)

Deamino-DBCC and alkyl analogues of DBCC is known to be inhibitors in the reaction catalyzed by DBCC (18). Furthermore, Lee and Abeles (19) reported that DBCC in the diol-dehydrase system is degraded to yield OH-B₁₂-like substance in the absence of the substrate. These facts suggest a possibility that cobalt is an active site in the reaction, and formation of an intermediate in which substrate is bound to the cobalt atom may be presumed.

FIG. 6 Plausible Structures of Intermediates of the Enzymatic Conversion of 1,2-Diol to Deoxyaldehyde

Based on this assumption, DHP-B₁₂, a plausible intermediate in the conversion of glycerol to β -hydroxypropionaldehyde or propylene glycol to propionaldehyde, was synthesized and tested in the diol-dehydrase reaction system. The result, however, showed that this derivative did not stimulate the reaction, but acted as an inhibitor similarly to the Co-alkyl derivatives. Occurrence of such compounds as the intermediates seems therefore unlikely.

As shown in Fig. 6, compounds of different structure may also be possible as intermediates, which could not be examined in the present work by the limitation of reagents and reaction methods. DHP-B₁₂ was active toward the vitamin B₁₂-requiring *E. coli* strain. In this case dihydroxypropyl group would be eliminated at any step before being utilized by the microorganism. Zagalak *et al.* (15) have reported that various Co-alkylcobinamides inhibited the diol-dehydrase reaction. We also have wanted to test DHP-CA, but did not carry it out, considering the possible contamination of carboxylic acid analogues.

It was shown that dihydroxypropyl group of DHP-B₁₂ yielded by photolysis (a) glyceric acid under aeration, (b) glyceraldehyde and glycerol when the solution

contained dissolved air, (c) glycerol in a nitrogen atmosphere, and (d) glycerol in a NaBH_4 -containing solution. Comparison of these results with the photolysis of DBCC shows that adenosine-5'-carboxylic acid and adenosine-5'-aldehyde obtained from the latter under aerobic conditions correspond to glyceric acid and glyceraldehyde from the former, respectively. From our results it is obvious that carboxylic acid is formed under more vigorous aerobic conditions. It is probable that glyceric acid was formed from glyceraldehyde once formed by autoxidation. Considering that OH-B_{12} catalyzes the autoxidation of thiols and iodide (20, 21, 22), it is also possible that OH-B_{12} catalyzed the oxidation of the aldehyde to the carboxylic acid.

On the other hand, under anaerobic conditions glycerol was obtained in our experiments, while the photolysis of DBCC did not give adenosine but 8,5'-cyclo-adenosine. Weissbach *et al.* (23) obtained methanol and formaldehyde by photolysis of Co-methylcobalamin, which is similar to our results. Although Johnson *et al.* (5) reported the formation of acetic acid by a photolysis of Co-carboxymethylcobalamin under anaerobic condition, the similar reaction, which should result in the formation of propylene glycol, was not observed in our experiments.

It has been shown that a homolysis of cobalt-carbon bond occurs in these photolytic reaction, which yields vitamin B_{12r} and an organic radical (24). The formation of 8,5'-cycloadenosine shows that a free radical on 5'-carbon atom attacks the

FIG. 7 Photochemical Decomposition Route of Co-β,γ-dihydroxypropylcobalamin under Aerobic or Anaerobic Condition

carbon at 8 position with an elimination of hydrogen atom. Thus it is considered that glycerol from DHP-B₁₂ and methanol from Co-methylcobalamin were produced by similar free radical substitution reactions with water (Fig. 7). The similarity of these reactions to the formation of methionine by an anaerobic photolysis of Co-methylcobalamin in the presence of homocysteine (6) seems to support this mechanism (Fig. 8). The whole photolytic reaction routes of DHP-B₁₂ may be summarized as shown in Fig. 9.

In the present work we could not indicate the stoichiometric relationships between DHP-B₁₂ used and photolysis products obtained, but the report by Weissbach *et al.* (23) that the yield of methanol and formaldehyde from Co-methylcobalamin was only about 30 % suggests that the problems remain to be solved.

SUMMARY

1. In the present work, several analogues of 5,6-dimethyl benzimidazolyl cobamide coenzyme (DBCC), containing hydroxyalkyl group linked to the cobalt atom, were synthesized as the possible intermediates in the diol-dehydrase system on the assumption of active participation of the cobalt atom, and their properties, above all, behaviors in photolysis and in the enzymatic system were examined.

2. Co- β,γ -dihydroxypropyl-, Co- β -hydroxypropyl-, and Co-hydroxyethylcobalamins were prepared by reacting glycerol α -monochlorohydrin, propylene bromohydrin and ethylene chlorohydrin with vitamin B₁₂s. Co- β,γ -dihydroxypropylcobinamide was also synthesized by the reaction of glycerol α -monochlorohydrin with a reduced product of monocyano mono-aquocobinamide with NaBH₄.

3. Absorption spectra of the three hydroxyalkylcobalamins were similar to those of DBCC or Co-alkylcobalamins, and the spectrum of the hydroxyalkylcobinamide was analogous to those of cobinamide coenzyme or Co-alkylcobinamide. These four compounds, alike cobamide- and cobinamide coenzymes, were very unstable in light and easily decomposed to hydroxo(or aquo) forms. When they were allowed to stand in the dark in 0.017 *M* KCN solution, they were converted only partly to dicyano forms like the alkyl derivatives. Paper chromatographic behavior showed their higher hydrophilicity than alkyl derivatives as expected.

4. Hydroxyalkylcobalamin, for example Co- β,γ -dihydroxypropyl cobalamin, showed an almost equal activity on vitamin B₁₂-requiring *E. coli*. However, this compound did not exhibit the coenzyme activity, and, moreover, exerted an inhibitory effect, like the alkyl derivatives, in the diol-dehydrase system. From the result it would be concluded that the hydroxyalkyl derivatives of DBCC so far tested are not the active intermediate of the diol-deoxyaldehyde conversion system.

5. Co- β,γ -dihydroxypropylcobalamin was irradiated (a) under bubbling of air, (b) in the presence of dissolved oxygen, (c) in a nitrogen atmosphere, (d) in the presence of NaBH₄. The photolysis products formed from the dihydroxypropyl group were as follows: (a) glyceric acid, (b) glyceraldehyde and glycerol, (c) glycerol and a small amount of an unidentified substance and, (d) glycerol. The decomposition routes of this compound are considered to be analogous to those of DBCC. The formation of glycerol is not consistent with the formation of 8,5'-cycloadenosine from DBCC. It is similar to the formation of methanol in the photochemical

decomposition of Co-methylcobalamin reported by Weissbach *et al.* The reaction mechanism may be interpreted on the formation of vitamin B₁₂^r and dihydroxypropyl free radical by the homolytic fission of cobalt-carbon bond.

ACKNOWLEDGEMENT

This research was supported in part by the grant from Japan Vitamin B Research Committee.

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