Diekmann, H., Englert, G., and Wallenfels, K. (1964), *Tetrahedron 20*, 281.
Haas, E. (1936), *Biochem. Z. 288*, 123.
Hope, H. (1968), *Acta Cryst.* (in press).
Johnston, C. C., Gardner, J. L., Suelter, C. H., and Metzler, D. E. (1963), *Biochemistry 2*, 689.
Karrer, P., and Warburg, O. (1935), *Biochem. Z. 282*, 157.
Maas, W., Janssen, M. J., Stamhuis, E. J., and Wynberg, H. (1967), *J. Org. Chem. 32*, 1111.
Meinhart, J. O., and Hines, M. C. (1957), *Federation Proc. 16*, 425.
Meinhart, J. O., Chaykin, S., and Krebs, E. G. (1956), *J. Biol. Chem. 220*, 821.
Rafter, G. W., Chaykin, S., and Krebs, E. G. (1953), *J. Biol. Chem. 208*, 799.
Segel, R., and Stein, G. (1960), *J. Chem. Soc.*, 5254.
Stamhuis, E. J., and Maas, W. (1965), *J. Org. Chem. 30*, 2156.
Stock, A., Saan, E., and Pfleiderer, G. (1961), *Ann. Chem. 647*, 178.
von Euler, H., Schlenk, F., Helwinkel, H., and Hogberg, B. (1938), *Z. Physiol. Chem. 256*, 208.
Wallenfels, K., and Schuly, H. (1957), *Biochem. Z. 329*, 75.
Wallenfels, K., Hofmann, D., and Schuly, H. (1959), *Ann. Chem. 621*, 188.
Warburg, O., and Christian, W. (1934), *Biochem. Z. 274*, 112.
Wiberg, K. B. (1955), *Chem. Rev. 55*, 713.

Isolation of Co-5′-Deoxyadenosylcobinamide Guanosine Diphosphate, α-(2-Methyladenyl)-Co-5′-deoxyadenosylcobamide, and Co-Methylcorrinoids from *Clostridium thermoaceticum**

Eckart Irion† and Lars Ljungdahl‡

ABSTRACT: Co-5′-Deoxyadenosylcobinamide guanosine diphosphate (DA-GDP-cobinamide) and α-(2-methyladenyl)-Co-5′-deoxyadenosylcobamide (DA-factor A) have been found in *Clostridium thermoaceticum*. It is likely that the biosynthesis of complete B_{12} factors occurs as the Co-5′-deoxyadenosyl derivatives of corrinoids. DA-GDP-cobinamide, which is formed in a reaction between DA-cobinamide phosphate and guanosine triphosphate, is probably one of the intermediates in the formation of complete B_{12} factors. The presence of DA-factor A shows that *C. thermoaceticum* synthesizes B_{12} factors with derivatives of purines as the base of the nucleotide moiety as well as of the more abundant benzimidazoles, which were reported previously. Co-Methyl-factor IIIm, Co-methylcobyric acid, Co-methylcobyrinic acid pentaamide, Co-methylcobyrinic acid tetraamide, Co-methylcobyrinic acid triamide, Co-methylcobinamide, and a Co-methyl derivative with an unknown corrinoid moiety are present in *C. thermoaceticum*.

Corrinoids play an important role in the metabolism of *Clostridium thermoaceticum*. It is likely that α-(5-methoxybenzimidazolyl)-Co-methylcobamide (methyl-factor IIIm)[1] and Co-methylcobyric acid are intermediates in the total synthesis of acetate from CO_2 as performed by this organism (Ljungdahl *et al.*, 1965). The high content (30–70 μmoles of corrinoids/100 g of wet cells) and the great variety of corrinoids in *C. thermoaceticum* provide evidence that this organism also performs the biosynthesis of B_{12} compounds. We have previously reported (Irion and Ljungdahl, 1965) the presence of the complete B_{12} factor;[2] 5-methoxybenzimidazolylcobamide (factor IIIm) and of the incomplete B_{12} factors; cobinamide phosphate, cobinamide, cobyric acid, and di- to pentaamides of cobyrinic acid in *C. thermoaceticum*. These corrinoids occur mostly as their Co-5′-deoxyadenosyl derivatives. The incomplete factors are likely intermediates in the bio-

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: factor IIIm, 5-methoxybenzimidazolylcobamide; factor A, α-2-methyladenylcobamide.

[2] Complete B_{12} factors are defined as corrinoids with a heterocyclic base normally attached as ligand to the cobalt atom (*e.g.*, 5,6-dimethylbenzimidazole in vitamin B_{12}). All corrinoids lacking a base are incomplete.

synthesis of complete B_{12} factors. The present communication describes the identification of Co-5′-deoxyadenosylcobinamide guanosine diphosphate (DA-GDP-cobinamide), α-(2-methyladenyl)-Co-5′-deoxyadenosylcobamide (DA-factor A), and of several Co-methylcorrinoids. The presence of these compounds in *C. thermoaceticum* was mentioned in an earlier publication (Ljungdahl *et al.*, 1966) but details of isolation and identification were not given at that time.

Experimental Section

Materials and Methods. Most methods and procedures used during this investigation are described in previous papers (Ljungdahl *et al.*, 1965; Irion and Ljungdahl, 1965).

Samples of α-(2-methyladenyl)cobamide cyanide (factor A) and of cobinamide guanosine diphosphate monocyanide were generous gifts from Professor K. Bernhauer, Technische Hochschule, Stuttgart, Germany. All other chemicals are either commercially available or were obtained as described in the previous communications. Carboxymethylcellulose (Brown Co., Berlin, N. H.) was washed successively with 0.5 N sodium hydroxide, water, 1 N hydrochloric acid, and water until the pH was near neutrality. DEAE-cellulose (Carl Schleicher & Schuell Co.) was washed with 0.5 N sodium hydroxide and then with water until the pH was neutral.

Isolation of Co-5′-Deoxyadenosylcobinamide Guanosine Diphosphate and α-(2-Methyladenyl)-Co-5′-deoxyadenosylcobamide from C. thermoaceticum. The isolation and separation of corrinoids from *C. thermoaceticum* was done under dim light. The corrinoids were obtained from 200 to 500 g of wet cells by extraction with 70% aqueous acetone. They were purified by absorption on and elution from Celite, by phenol extraction, and finally were obtained in an aqueous solution as described by Ljungdahl *et al.* (1965). The aqueous solution was concentrated to about 5 ml in a rotary vacuum evaporator and was applied to a carboxymethylcellulose column (260 × 32 mm). The column was eluted with water to separate the corrinoids into several fractions as shown in Figure 1. The first fraction, which passed without retardation directly through the column, contained DA-GDP-cobinamide and several Co-sulfitocorrinoids. In the next 700 ml of water several minor bands were eluted, these consisted of Co-methylcorrinoids including Co-methyl-factor IIIm. DA-cobinamide phosphate was eluted next followed by DA- and Co-methylcobyrinic acid amides and several unknown corrinoids, one of which is IIa of Irion and Ljungdahl (1965). After 1800 ml of water had passed through the column two bands, one red and one brown-yellow, remained on the top of the column. The red band containing some hydroxy-B_{12} factors was eluted with 0.04% acetic acid. The brown band was then eluted with 0.1% ammonium hydroxide. The ammonium hydroxide eluate was the major corrinoid fraction and contained DA-factor A, DA-factor IIIm, DA-cobyric acid, DA-cobinamide, and several different amides of DA-cobyrinic acid, as well as Co-methylcobyric acid. The corrinoids in this fraction were extracted with phenol and then back into water. The concentrated aqueous solution was applied to a DEAE-cellulose column (80 × 14 mm) to separate the DA-cobyrinic acid amides from the other corrinoids. The latter compounds were obtained in a single fraction by elution with water. The DA-cobyrinic acid amides were then eluted with water containing 5% of acetic acid.

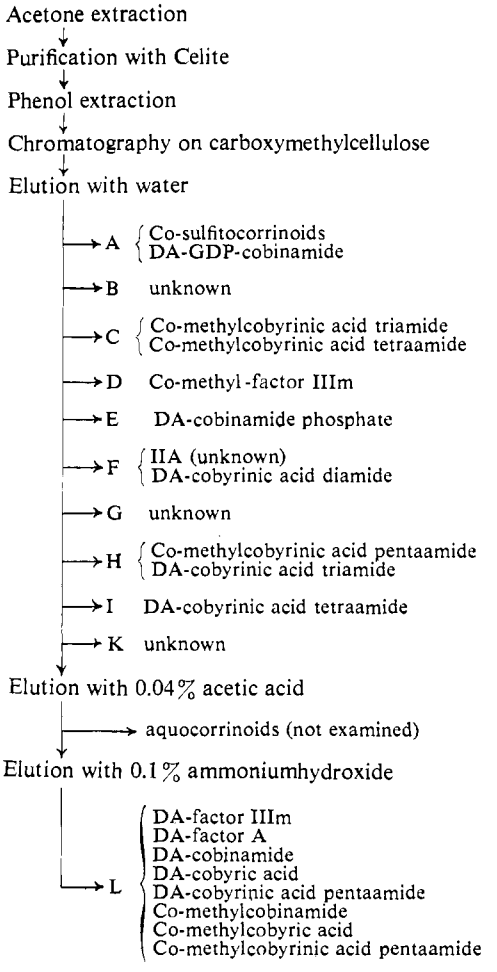

FIGURE 1: Scheme for purification and chromatography on carboxymethylcellulose of corrinoids obtained by acetone extraction from *C. thermoaceticum*. Fraction L can be eluted with 0.5 N acetic acid instead of 0.1% ammonium hydroxide.

Further purification of DA-GDP-cobinamide and DA-factor A was achieved by using paper electrophoresis and paper chromatography in solvents A and B according to Irion and Ljungdahl (1965). The DA-GDP-cobinamide fraction from the carboxymethylcellulose column was subjected to paper electrophoresis in 0.5 M acetic acid at pH 2.7 to separate the weakly cationic DA-GDP-cobinamide from the anionic Co-sulfitocorrinoids. Pure DA-GDP-cobinamide was finally obtained by paper chromatography using solvent B. Paper chromatography with solvent B was also used for the separation of DA-factor A from the other corrinoids present in the aqueous eluate of the DEAE-cellulose column. DA-factor A, which moves very slowly (R_F 0.05) with solvent B, was found together with an un-

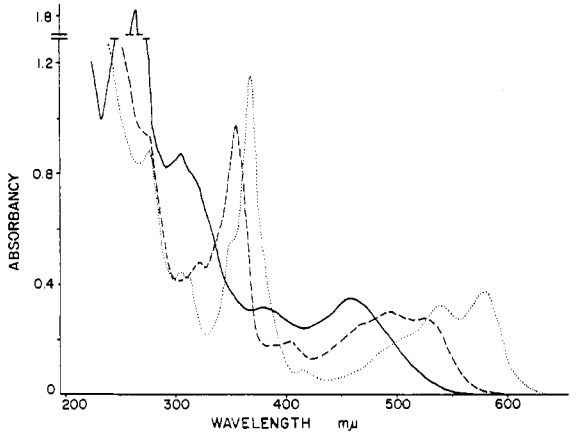

FIGURE 2: Absorption spectra. (—) DA-GDP-cobinamide in water: maxima at 264, 304, and 458 mμ; (----) monocyano-GDP-cobinamide in water: shoulder at 273 and maxima at 320, 354, 404, 495, and 527 mμ; (·····) dicyano-GDP-cobinamide in 0.1 M KCN: maxima at 275, 304, 367, 538, and 578 mμ. The concentration of the corrinoid for each spectrum was about 3.72×10^{-5} M.

known corrinoid in the band closest to the origin of the chromatogram. DA-factor A was then separated from the unidentified corrinoid IIa by the use of solvent A. With this solvent DA-factor A moved somewhat more rapidly than the unknown compound.

Isolation of Co-Methylcorrinoids from C. thermoaceticum. Radioactive [^{14}C]Co-methylcorrinoids were isolated from 650 g of wet cells of *C. thermoaceticum*. The cells were exposed to 0.375 mmole of [^{14}C]sodium bicarbonate (1.38×10^9 cpm) during 15 sec as described by Ljungdahl *et al.* (1965). The corrinoids then were extracted from the cells with acetone. A concentrated aqueous solution of the corrinoids was prepared as indicated above and it was chromatographed on carboxymethylcellulose (260 × 32 mm). The column was eluted with water and ten fractions, colored red-yellow, were collected separately. They contained corrinoids and were named A–K according to the sequence of elution as indicated in Figure 1. After elution of fraction K, the column was eluted with a gradient of water to 0.5 N acetic acid to obtain the remaining corrinoids in fraction L. Fractions A, C, D, H, and L contained radioactive compounds. The radioactivity in fraction A was associated with leucine and other amino acids, which apparently remained as contaminants in the corrinoid fraction after purification in Celite and phenol extraction. Ljungdahl and Wood (1965) have shown that radioactive amino acids are produced in pulse-labeling experiments with *C. thermoaceticum*. The other radioactive fractions contained [^{14}C]Co-methylcorrinoids, this being demonstrated by the loss of radioactivity on exposure to light and the formation of [^{14}C]-methyl iodide in the reaction with iodine (Ljungdahl *et al.*, 1965).

The Co-methylcorrinoids in the radioactive fractions from the carboxymethylcellulose column were purified by paper chromatography. With solvent A, fraction D separated into a faster moving band, D1, and a slower moving band, D2. Both bands contained radioactivity. Band D2 consisted of overlapping material from fraction C and was combined with this fraction, which was chromatographed using solvent B. Two ^{14}C compounds, C1 and C2, were obtained. Fraction H was chromatographed with solvent B and the fastest moving corrinoid H1 was radioactive. Fraction L was also chromatographed with solvent B and the three fastest moving compounds L1, L2, and L3 were radioactive. The Co-methylcorrinoids D1, C1, C2, H1, L1, L2, and L3 were further purified by paper electrophoresis using 0.5 M acetic acid at pH 2.7 and by rechromatography with solvent B.

Results

Identification of Co-5'-Deoxyadenosylcobinamide Guanosine Diphosphate in C. thermoaceticum. The identification of DA-GDP-cobinamide is based on the following observations. Adenine and a cyanocorrinoid were formed in a reaction between the compound and KCN when the reaction was performed in the absence of light, indicating a 5'-deoxyadenosyl moiety, which was attached to the cobalt atom. The cyanocorrinoid was identical with C1C4 previously isolated from *C. thermoaceticum* by Irion and Ljungdahl (1965). The absorption spectra of the isolated DA-GDP-cobinamide and of its mono and dicyano derivatives are shown in Figure 2. The spectrum of DA-GDP-cobinamide, which is unchanged between pH values 1 and 10, is similar to spectra of incomplete DA-corrinoids. Therefore, it can be concluded that the compound lacks the base of the nucleotide moiety which is coordinated with the cobalt atom in complete B_{12} factors. The cyano derivatives of the isolated compound have high absorptivity around 275 mμ which is due to the presence of the guanidine moiety. The spectra of the cyano compounds were identical with corresponding spectra of authentic GDP-cobinamide and the absorption maxima were those found by Ronzio and Barker (1967) for mono- and dicyano-GDP-cobinamide. These investigators also synthesized a compound which they tentatively considered to be DA-GDP-cobinamide and the spectrum of their compound is the same as for the DA derivative isolated from *C. thermoaceticum*.

The cyano derivatives of the isolated DA-GDP-cobinamide were compared with corresponding GDP-cobinamide cyanides by coelectrophoresis and co-chromatography on paper. The isolated compound was in all instances identical with the authentic GDP-cobinamide cyanides. The R_F values obtained during paper chromatography are listed in Table I.

The isolated DA-GDP-cobinamide moved during paper electrophoresis in 0.5 N acetic acid slightly toward the cathode (+0.1 relative to cobinamide monocyanide +1). In 0.02 M phosphate buffer (pH 7.3) the isolated compound was anionic with a migration of −0.8. The R_F values in solvents A–C for DA-GDP-cobinamide were all less than 0.03. The electrophoretic mobility of the monocyano derivative of the isolated GDP-cobinamide was −0.2 in 0.5 N acetic acid compared with cobinamide monocyanide (+1) and for the dicyano

TABLE I: Paper Chromatography of GDP-cobinamide and Factor A from *C. thermoaceticum* Compared with B_{12}.[a]

Compound	R_F Values in Solvent System			
	D	E	F	G
GDP-cobinamide[b]	0.04	0.03	0.04	0.14
Factor A[b]	0.12	0.11	0.15	
B_{12}	0.21	0.23	0.24	0.41

[a] Ascending chromatography on Schleicher & Schuell paper no. 2043a at room temperature. Solvents: (D) 2-butanol saturated with 0.01% HCN, (E) 2-butanol saturated with 0.01% HCN–acetic acid (100:1), (F) 2-butanol saturated with 0.01% HCN–25% NH_3 (100:0.5), and (G) 1-butanol–0.05% HCN–isopropyl alcohol–acetic acid (100:100:70:1). [b] Authentic GDP–cobinamide cochromatographed with the isolated GDP-cobinamide as did authentic factor A with the isolated factor A.

TABLE II: Electrophoretic Properties on Paper of Co-Methylcorrinoids Isolated from an Acetone Extract of *C. thermoaceticum*.

	0.5 N Acetic Acid, pH 2.7[a]	0.02 M Phosphate, pH 7.3
I. Complete factor α-(5-Methoxybenzimidazolyl)-Co-methylcobamide (D1)	+0.45	0 (neutral)
II. Incomplete factors		
Co-Methylcobyric acid (L2)	+1	0 (neutral)
Co-Methylcobyrinic acid pentaamide (H1)	+1	−1
Co-Methylcobyrinic acid tetraamide (C1)	+1	−2
Co-Methylcobyrinic acid triamide (C2)	+1	−3
Co-Methylcobinamide (L3)	+1	+1
Unknown (L1)	+1	0 (neutral)
III. Reference compound		
Co-Methylcobalamin	+0.30	0 (neutral)

[a] Values relative to the migration of monocyanocobinamide = +1.

derivative −1.7 in 0.2 M phosphate buffer (pH 7.3) compared with cobyric acid dicyanide (−1).

Identification of α-(2-Methyladenyl)-Co-5'-deoxyadenosylcobamide (DA-Factor A) in C. thermoaceticum. The isolated DA-factor A was found to be identical with the unknown IIax previously isolated from *C. thermoaceticum* (Irion and Ljungdahl, 1965). The cyanocorrinoid C1C3, also previously isolated by cyanide extraction from *C. thermoaceticum*, was obtained together with adenine by cyanide cleavage of either IIax or of the isolated DA-factor A, showing the presence of a deoxyadenosyl moiety substituted on the cobalt atom. The absorption spectrum of the isolated DA-factor A resembles that of DA-adenylcobamide (Barker, 1962). The absorption maxima in water at pH 6.5 were at 512, 373, 318 (sh), 304, and 263 mμ. The absorption at 512 mμ shifted to 460 mμ when the pH was lowered. This shift found in complete B_{12} factors is due to the dissociation of the base from the cobalt atom. The shift occurred at about pH 5.5. The high p*K* value for the dissociation indicated that the base is a derivative of a purine rather than of a benzimidazole (Ladd *et al.*, 1961). The cyano derivative of the isolated factor A cochromatographed on paper with authentic factor A with three solvent systems (Table I). The electrophoretic mobility on paper of the monocyanide of the isolated factor A was +0.7 compared with +1 for cobinamide monocyanide in 0.5 M acetic acid. This basic property is also indicative of a B_{12} factor having a purine rather than a benzimidazole base. Finally the isolated factor A was deaminated with nitric acid according to Brown *et al.* (1955). The product was chromatographed on a carboxymethylcellulose column, through which it moved with the solvent front. Addition of acetone to the concentrated aqueous eluate yielded a crystalline product which had all the properties of factor H (α-2-methylhypoxanthylcobamide).

Identification and Properties of Co-Methylcorrinoids from C. thermoaceticum. Co-Methylcorrinoids are present in very small amounts in *C. thermoaceticum* and were it not for the radioactive Co-methyl group formed from $^{14}CO_2$, they probably would not have been detected. The relative specific ^{14}C activities of the corrinoids were reported previously (Ljungdahl *et al.*, 1966) together with a discussion of their role in the total synthesis of acetate from CO_2.

To identify the corrinoid moiety of the Co-methylcorrinoids, they were dissolved in 0.1 ml of 0.1% HCN and exposed to light. The resulting cyano-factors were identified by spectra, paper chromatography using solvents D–F, and by paper electrophoresis in 0.5 M acetic acid and in 0.2 M phosphate buffer (pH 7.3) as described by Irion and Ljungdahl (1965). It was found that D1 is Co-methyl-factor IIIm and that L2 is Co-methylcobyric acid. Both compounds have already been found in *C. thermoaceticum* (Ljungdahl *et al.*, 1965). The other [^{14}C]corrinoids are Co-methylcobyrinic acid triamide (C2), Co-methylcobyrinic acid tetraamide (C1), Co-methylcobyrinic acid pentaamide (H1), and

TABLE III: Paper Chromatography of Co-Methylcorrinoids Isolated from an Acetone Extract of *C. thermoaceticum*.[a]

Compound	R_F Values[b]			
	A	B	C	
I. Complete factor				
α-(5-Methoxybenzimidazolyl)-Co-methyl-cobamide (D1)	0.22	0.20	0.18	
II. Incomplete factors				
Co-Methylcobyric acid (L2)	0.18	0.22	0.20	0.14[c]
Co-Methylcobyrinic acid pentaamide (H1)	0.10	0.28	0.12	0.09[c]
Co-Methylcobyrinic acid tetraamide (L1)	0.04	0.32	0.08	
Co-Methylcobyrinic acid triamide (C2)	0.02	0.46	0.04	
Co-Methylcobinamide (L3)	0.26	0.25	0.28	0.15[c]
Unknown (L1)	0.15	0.18	0.13	
III. Reference compound				
Co-Methylcobalamin	0.25	0.23	0.24	

[a] Ascending chromatography on Schleicher & Schuell paper no. 2043a, 20–22 hr at 22°. [b] A = water-saturated 2-butanol; B = water-saturated 2-butanol–acetic acid (100:1); C = 2-butanol–water–25% NH_3 (100:36:14). [c] These values are for red amino complexes, which are formed reversibly between incomplete factors and ammonia, when high concentration of the corrinoid is used.

Co-methylcobinamide (L3). The identification of the cobyrinic acids is not completely certain because these compounds may also be derivatives of cobinic acid amides. Finally L1 was found to be the Co-methyl derivative of a still unknown B_{12} factor in *C. thermoaceticum*. The electrophoretic migrations on paper of the isolated Co-methylcorrinoids in relation to monocyanocobinamide are listed in Table II and R_F values with three different solvent systems are given in Table III.

Discussion

Practically all corrinoids (98%) in *C. thermoaceticum* exist as Co-5′-deoxyadenosyl derivatives (Irion and Ljungdahl, 1965). This form is apparently the predominant derivative of corrinoids in nature (Barker, 1962). It is likely that the biosynthesis of complete B_{12} factors starting with cobyrinic acid or one of its amides occurs in the form of the deoxyadenosyl derivatives of the corrinoids. This was discussed in a recent review by Wagner (1966). The final steps in the synthesis of complete B_{12} factors may involve reactions 1 and 2.

DA-cobinamide phosphate + GTP ⟶ DA-GDP-cobinamide + PP_i (1)

DA-GDP-cobinamide + α-nucleoside ⟶ DA-B_{12} factor + GMP (2)

The α-nucleoside is probably formed by a specific *trans-N*-glycosidase demonstrated in *Propionibacterium shermanii* (Friedmann and Harris, 1965). GDP-cobinamide has been demonstrated in *Nocardia rugosa* (Barchielli *et al.*, 1957, 1960) and in *P. shermanii* (Pawelkiewicz *et al.*, 1959). Barchielli *et al.* (1960) suggested that it is an intermediate in the biosynthesis of complete B_{12} factors. Our demonstration of DA-GDP-cobinamide in *C. thermoaceticum* supports this hypothesis. Strong evidence for this was also recently obtained by Ronzio and Barker (1967), who found that crude extracts of *P. shermanii* convert DA-cobinamide phosphate and GTP into DA-GDP-cobinamide. Diaquocobinamide, which lacks the deoxyadenosyl moiety, was not a substrate in this conversion. This again suggests that the biosynthesis of B_{12} factors normally occurs as the Co-5′-deoxyadenosyl derivatives. Pawelkiewicz *et al.* (1964) have also obtained evidence for the formation of DA-GDP-cobinamide in cell-free extracts of *P. shermanii*.

An alternate pathway is possible for the formation of a DA-B_{12} factor from DA-GDP-cobinamide. Renz (1967) has isolated cyanocobalamin 5′-phosphate from extracts of *P. shermanii*, and its deoxyadenosyl derivative may be involved in the biosynthesis of DA-B_{12} factors (reactions 3 and 4).

DA-GDP-cobinamide + α-nucleoside 5′-phosphate ⟶ DA-B_{12} factor 5′-phosphate + GMP (3)

DA-B_{12} factor 5′-phosphate ⟶ DA-B_{12} factor + P_i (4)

Although deoxyadenosyl derivatives of incomplete B_{12} factors appear to be the normal intermediates in the biosynthesis of the complete factors, corrinoids having other alkyl groups on the cobalt may also be involved

in the biosynthesis of complete factors. This was suggested by Ronzio and Barker (1967), who reported that Co-2,3-isopropylidene 5′-deoxyadenosylcobinamide phosphate was a better substrate than DA-cobinamide phosphate in reaction 1 above. Müller and Müller (1963) have shown that Co-butylcobinamide is converted into Co-butylcobalamin by cells of *P. shermanii*. The presence of seven different Co-methylcorrinoids in *C. thermoaceticum* is an indication that Co-methyl derivatives also may be intermediates in the formation of complete factors.

We reported previously (Irion and Ljungdahl, 1965) that purine cobamides were absent in *C. thermoaceticum*. However, with the identification of α-(2-methyladenyl)-Co-5′-deoxyadenosylcobamide as an acetone extract of *C. thermoaceticum* it is obvious that this organism also can synthesize purine cobamides. Brown *et al.* (1955) first isolated cyano-factor A from rumen intestinal bacteria and from sewage sludge.

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The authors wish to thank Dr. Harland G. Wood for his interest in this work, which was performed in his laboratory.

Added in Proof

Since this article was submitted Friedmann (1968) published data showing that vitamin B_{12} 5′-phosphate is formed by intact cells of *P. shermanii*. This compound was proved kinetically and by pulse-labeling experiments to be a precursor of vitamin B_{12}. Friedmann showed also that α-ribazole 5′-phosphate and GDP-cobinamide were utilized in the formation of vitamin B_{12} 5′-phosphate. These findings support the pathway shown in reactions 3 and 4 suggested in the present paper.

References

Barchielli, R., Boretti, G., DiMarco, A., Julita, P., Migliacci, A., Minghetti, A., and Spalla, C. (1960), *Biochem. J. 74*, 382.

Barchielli, R., Boretti, G., Julita, P., Migliacci, A., and Minghetti, A. (1957), *Biochim. Biophys. Acta 25*, 452.

Barker, H. A. (1962), *in Vitamin B_{12} und Intrinsic Faktor, 2nd European Symp., Hamburg*, 82.

Brown, F. B., Cain, J. C., Gant, D. E., Parker, L. F. J., and Smith, L. E. (1955), *Biochem. J. 59*, 82.

Friedmann, H. (1968), *J. Biol. Chem. 243*, 2065.

Friedmann, H. C., and Harris, D. L. (1965), *J. Biol. Chem. 240*, 406.

Irion, E., and Ljungdahl, L. (1965), *Biochemistry 4*, 2780.

Ladd, J. N., Hogenkamp, H. P. C., and Barker, H. A. (1961), *J. Biol. Chem. 236*, 2114.

Ljungdahl, L., Irion, E., and Wood, H. G. (1965), *Biochemistry 4*, 2771.

Ljungdahl, L., Irion, E., and Wood, H. G. (1966), *Federation Proc. 25*, 1642.

Ljungdahl, L., and Wood, H. G. (1965), *J. Bacteriol. 89*, 1055.

Müller, O., and Müller, G. (1963), *Biochem. Z. 337*, 179.

Pawelkiewicz, J., Bartosinski, B., and Walerych, W. (1964), *Ann. N. Y. Acad. Sci. 112*, 638.

Pawelkiewciz, J., Walerych, W., and Bartosinski, B. (1959), *Acta Biochem. Polon. 6*, 431.

Renz, P. (1967), *Angew. Chem. 79*, 311.

Ronzio, R. A., and Barker, H. A. (1967), *Biochemistry 6*, 2344.

Wagner, F. (1966), *Ann. Rev. Biochem. 35*, 405.