119. Acetyl-cobalamin from Photoinduced Carbonylation of Methyl-cobalamin

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Summary

Irradiation with visible light of a deoxygenated aqueous solution of methyl-cobalamin (**1**) under an atmosphere of CO and at ambient temperature converts **1** into acetyl-cobalamin (**2**). This carbonylation has potential relevance with respect to bacterial acetate biosynthesis from CO_2.

Thauer recently suggested[1]) bacterial synthesis of CH_3COOH (or of a coenzyme A bound CH_3CO-group) from two CO_2 to proceed *via carbonylation* of methyl-Co (III)-corrinoids analogous to methyl-cobalamin (**1**). This contrasted with earlier proposals, where C–C bond formation in the course of bacterial acetate synthesis from CO_2 was considered to occur by *carboxylation* of vitamin B_{12} derivatives (reviewed in [1]). However, in recent studies with acetogenic bacteria (*Clostridium thermoaceticum* [2], *Acetobacterium woodii* [3], *Butyribacterium methylotrophicum* [4]) and with methanogenic bacteria (*Methanosarcina barkeri* [5], *Methanobacterium thermoautotrophicum* [6]), the role of a direct precursor of the acetoxy carboxyl was reassigned to a C_1-unit on the oxidation level of HCOOH by *Wood et al.* [1] [2] [8] or to ('bound') CO by *Thauer, Diekert et al.* (see *Scheme 1* [3] [6a] [7b]). Further support was also obtained [1] [5b] concerning the involvement of vitamin B_{12} derivatives as intervening CH_3-group carriers in bacterial acetate synthesis from 2 CO_2[2]), discovered earlier by way of the (intact [9]) incorporation of the cobalt-bound CH_3-group of methyl-cobalamin (**1**) during acetate biosynthesis in *Clostridium thermoaceticum* [10].

On the other hand, for the presumed participation of methyl-cobyrinates in the assembly of the CH_3CO-group from 'one-carbon' precursors, a chemical model has not yet been established (experimentally). In particular, a carboxylation of the axial CH_3-group of the organo-corrinate was considered originally [1] [11] as the C–C bond forming step, but in extensive (non-enzymatic) studies with methyl-Co(III)-corrinoids and with CO_2, formation of CH_3COOH was achieved merely as a minor, barely detec-

[1]) Private communications of Prof. Dr. *R. K. Thauer* (Marburg) to Prof. Dr. *A. Eschenmoser* (June 1983) and to the author (December 1983), who is grateful to Prof. *Eschenmoser* for drawing his attention to this problem.

[2]) Recently, acetate biosynthesis from CO_2 in *Clostridium acidiurici* has been recognized to proceed *via* a non-corrinoid ('glycine decarboxylase') pathway [1].

Scheme 1

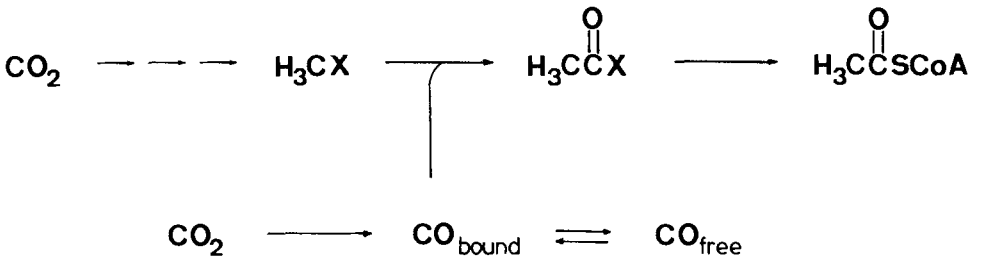
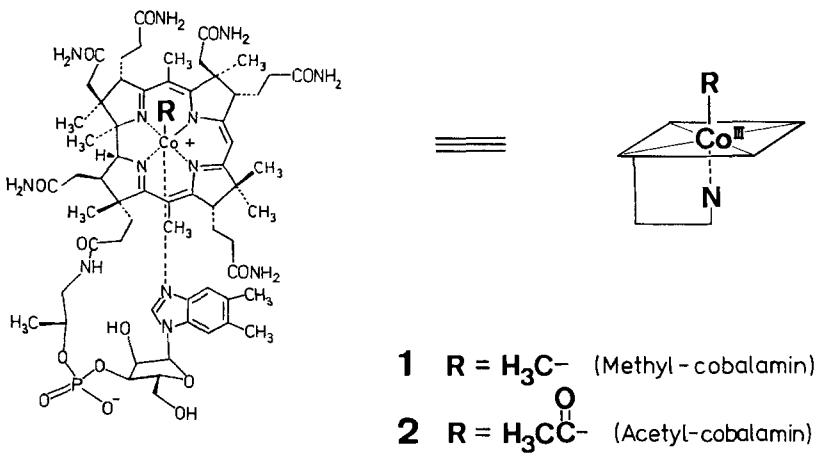


table reaction [12]. Similarly, for a carbonylation of methyl-Co(III)-corrinoids, chemical precedence is not available either. For this reason, the industrial synthesis of CH_3COOH from CH_3OH and CO [13] has served as a reference to illustrate the incorporation of CO into CH_3COOH by the bacterial synthesis [6a] [7b].

This paper describes the outcome of experiments which were carried out as a result of a suggestion made by *Thauer*[1]) to examine the possibility of obtaining CH_3COOH chemically by carbonylation of methyl-cobalamin (**1**). The central elements of the working hypothesis followed in these experiments were: *a*) the formation of the crucial C–C bond (connecting the CH_3- and the COOH-fragment of CH_3COOH) at the level of the CH_3CO-radical by the exothermic addition of a CH_3-radical to CO[3]); *b*) the possibility to achieve the envisaged radical reaction to acetyl-cobalamin (**2**) by photohomolysis of methyl-cobalamin (**1**) in the presence of CO in aqueous solution, and *c*) the consideration of the CH_3CO-group of **2** as a relevant precursor for CH_3COOH, released by **2** upon (rapid) attack of OH^--ion on the cobalt(III)-acetyl function [16] (other acetyl derivatives formed correspondingly [17])[4]).



1 R = H_3C– (Methyl-cobalamin)

2 R = $H_3CC(=O)$– (Acetyl-cobalamin)

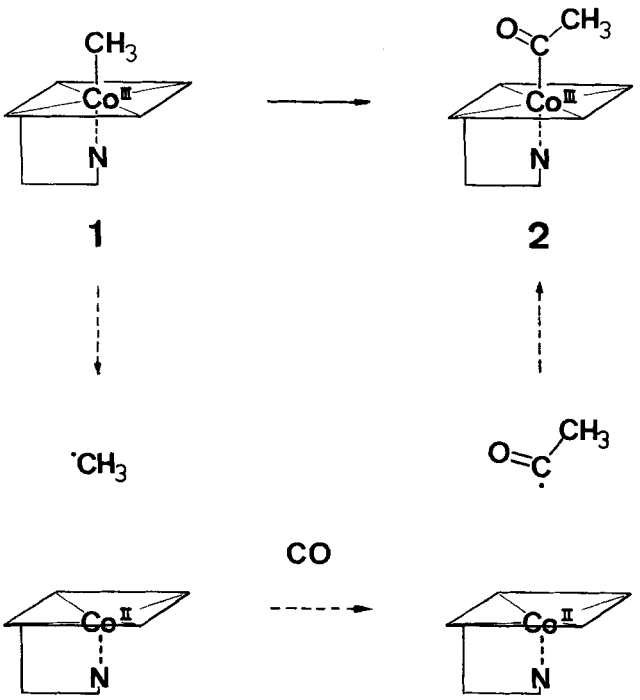
[3]) Heats of formation, ΔH_f° (298) [14]: 34.0 ± 1.2 kcal/mol for CH_3, −5.8 ± 0.4 kcal/mol for $CH_3C(O)^\cdot$, and −26.4 kcal/mol for CO (see [15] for kinetic studies on formation/cleavage of the CH_3CO free radical in the gas phase).

[4]) The corresponding reaction of **2** with a thiol (to give a thioester, analogous to acetyl-coenzyme A) apparently still remains to be demonstrated.

As a result of the restriction imposed by the corrin ring on available coordination sites at the Co-center [18], activation of the carbonylation (**1**→**2**) by proximal binding of methyl and carbonyl fragments at the metal center could not be invoked. However, in the carbonylation *via* radical intermediates, reactive and at the same time selectively acting species would be involved. Such a free radical process could be induced specifically by photohomolysis of methyl-cobalamin (**1**), shown to result in Co(II)-cobalamin and in a free CH_3-radical (in an efficient, (thermally) reversible reaction) [19–21]. Trapping of the photogenerated CH_3-radical by CO would provide a CH_3CO radical, which, in turn, could combine with Co(II)-cobalamin to give acetyl-cobalamin (**2**; *Scheme 2*).

The experiments fully confirmed these expectations: Irradiation of a deoxygenated, unbuffered aqueous solution of methyl-cobalamin (**1**, $c = 2.43 \cdot 10^{-3}$ mol/l) under an atmosphere of CO (at a pressure of *ca.* 31 atm[5])) at 20 ± 2 °C with the light of a 200-W W-lamp[6]) led, after 2 hours, to an orange-red solution which, according to analysis[7]) by HPLC, TLC and UV/VIS, contained two product fractions besides some starting material. Separation of the mixture by chromatography on carboxymethyl(CM)-cellulose

Scheme 2



[5]) Estimate based on CO vapor pressure at 77 K, volumes of storage vessel and of reaction vessel[6]).

[6]) See *Exper. Part* for details. For the handling of pressurized reaction vessels, security shields were installed.

[7]) All experiments were performed in a dark room (with reduced room light) and using a well ventilated hood.

allowed the isolation of acetyl-cobalamin (**2**) as major product (raw yield *ca.* 70%), besides **1** (*ca.* 15%) and (presumably) aquo-cobalamin (*ca.* 13%). Acetyl-cobalamin (**2**) was obtained in crystalline form from aqueous acetone in 65% yield and was identified with authentic material[8]) by comparison of its spectral (^{1}H-NMR, UV/VIS, and FAB-MS) and chromatographic properties (TLC, HPLC).

Several control experiments were performed to gain information on the experimental conditions suitable for the photoinduced carbonylation of **1** and to characterize the reaction: irradiation of a deoxygenated, CO-free solution of **1** under otherwise standardized conditions (as described above) led to only *ca.* 5% decomposition of the starting material (91% of crystalline **1** recovered). Irradiation of a deoxygenated solution of **1** under 1.5 atm of CO under otherwise standardized conditions resulted in 20% decomposition of **1** and a 14% yield of **2**. Storage of a deoxygenated solution of **1** under CO (at *ca.* 31 atm) in the dark and at room temperature for 2 hours did not lead to decomposition of **1**, which was recovered quantitatively (98% crystalline).

Thus, the carbonylation of **1** at room temperature depended upon light activation. Furthermore, for the photoinduced carbonylation to proceed significantly in these experiments, CO preferentially was supplied in concentrated, pressurized form. In a deoxygenated aqueous solution (see above), **1** exhibited *high photostability,* confirming earlier information [19].

The available experimental data are in accordance with the mechanistic hypothesis (see *Scheme 2*): Light cleaves the Co(III)–C bond of **1** homolytically to produce a CH_3-radical and Co(II)-cobalamin. The CH_3-radical either recombines with the Co(II)-corrin to regenerate **1** or it is trapped by CO.

As expected, in view of the assumed competition between CO and the Co(II)-corrin for reaction with the photogenerated CH_3-radical, the concentration of CO in the aqueous phase is a crucial experimental parameter. Under the conditions described here, only at an elevated pressure of CO its concentration in the aqueous solution[9]) appears to be sufficient for efficient reaction with the CH_3-radical. In qualitative accordance with this result, formation of acetyl-cobyrinates was not noticed [24] when the (thermally and photochemically induced) interconversion of Co_α-methyl- and Co_β-methyl-cobyrinates was studied in the presence of CO at (presumably) a pressure of 1 atm. Furthermore, similar to the situation with **1**, the reported pronounced photolability [22] of **2** appears to be reduced considerably in deoxygenated (and here CO containing) aqueous solutions and in the absence of radical traps[10]).

In conclusion, a *radical reaction* of potential relevance with regard to the *C–C bond forming step of bacterial acetate synthesis* from CO_2 is presented. Corresponding to current experimental evidence [1–8], it was sought in the form of a *carbonylation of methyl-cobalamin* (**1**) which, according to the working hypothesis, was driven by an *energetically favourable addition of a CH_3-radical to CO*. Such a type of a radical reaction, apparently, has not been considered so far in biological C–C bond-forming trans-

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[8]) Prepared by the method of *Müller & Müller* [22].

[9]) At 31 atm of CO at r.t. the concentration of CO dissolved in the aqueous phase can be estimated to be about 0.03 mol/l [23].

[10]) For a recently published synthetic application of olefin trapping of a photolytically liberated CH_3CO-group from **2** (generated *in situ* electrochemically), see [25].

formations. The carbonylation product, *acetyl-cobalamin* (**2**), shown here to be formed readily under suitable experimental conditions (irradiation with visible light, elevated pressure of CO, deoxygenated aqueous solution, room temperature), *carries an electrophilic CH_3CO-group* [17], *and it appears to have chemical properties as those required for a potential biosynthetic intermediate*[11]) *in bacterial acetate metabolism*[12]) (a point to be clarified in further studies).

Moreover, the photoinduced carbonylation of methyl-cobalamin (**1**), assumed to proceed in predictable form *via* free radicals, leads to the expectation that further investigations on the carbonylation of suitable alkyl-Co(III)-cobyrinates will uncover this new type of reaction of a corrinoid cobalt complex to be also of preparative utility.

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Experimental Part

1. *General.* TLC: on *'opti-up' C_{12}* reversed-phase silica gel plates, *Antec AG,* Bennwil, Switzerland; HPLC: on *RP-8* silica gel column (7 μm/34 cm/0.9 cm). Reagents and solvents: methylcobalamin (**1**) and authentic acetyl-cobalamin (**2**), prepared form vitamin B_{12} by the method [22], crystallized from H_2O/acetone, dried (high vacuum/r.t./ 5 d), and stored at 0° under protection from light; H_2O: 'nano-pure', prepared with ultrafilter, *Barnstead,* USA; CO: > 99.9%, Pyrogallol: *Fluka, purum;* KOH: 'rein', *Siegfried AG,* Zofingen; $Na_2Cr_2O_7$: techn.; Na_2CO_3: *Merck, p.a.;* CM-Cellulose: *Serva,* Heidelberg, FRG; acetone: *Fluka, puriss.;* solvents for TLC and HPLC: techn. grade, redistilled; *Pyrex* ampules: inner diameter: 5.5 mm; wall thickness 3.25 mm, volume 7 ± 0.3 ml. UV/VIS: *Perkin-Elmer PE-555,* in H_2O; λ_{max} (log ε) in nm; min. = λ_{min}; ^{1}H-NMR: *Bruker WM-300* (300.14 MHz), in D_2O; chemical shifts in ppm with δ(HDO) = 4.71; MS(FAB (fast atomic bombardment)) [29]: *Kratos AEI MS-50,* fitted with M-scan FAB-system; Xe-bombardment at 8.3 kV.

2. *Experimental Procedures.* 2.1. *Acetyl-cobalamin* (**2**) *from Methyl-cobalamin* (**1**).[6][7]) A solution of crystalline **1** (10.0 mg; *ca.* 7.3 μmol) in 3 ml of H_2O was introduced into a *Pyrex* tube, where it was deaerated by freezing (with liq. N_2), pumping (till $< 10^{-2}$ torr), and thawing (to r.t.) it 3 times. CO (purified by passing through a solution of pyrogallol (1.1 g) and of KOH (3.0 g) in 30 ml of H_2O, followed by a cold trap (dry ice/acetone)) was then condensed twice from a 100-ml storage vessel, at a pressure of 1.5 atm, into the *Pyrex* tube (cooled in liq. N_2), to give a layer of colourless liquid on top of the red, frozen cobalamin solution. The ampule was sealed off carefully and then allowed to warm to r.t. overnight in the dark (in a covered, precooled *Dewar* vessel). The ampule then was mounted concentrically in a glass tube (∅ = 3 cm), filled with a $Na_2Cr_2O_7$/Na_2CO_3-filter solution (with $OD > 1$ for $\lambda < 485$ nm) [30], which was immersed into a H_2O-bath, cooled to 20 ± 2° with running H_2O. The ampule was irradiated through the cooling and filter solutions from its side with a 200-W W-lamp, at a distance of *ca.* 10 cm. The irradiation was stopped after 120 min and the ampule cooled by immersing it slowly into liq. N_2. CO was observed to condense and the ampule was opened while cold (in a hood!). CO was allowed to evaporate off while the content of the ampule warmed to r.t. The mixture was examined by UV/VIS, TLC and HPLC, then transferred to a column of CM-cellulose (l = 5 cm, ∅ = 1 cm; washed with 10 ml of 0.1M aq. HCl, then with H_2O, until neutral) and eluted with H_2O. A red fraction (35 ml) eluted first, uniform by TLC and HPLC, apparently recovered **1**, *ca.* 1.1 μmol (15%), as estimated from its UV/VIS. The second, orange fraction (150 ml) was analoguously analyzed to be **2**, *ca.* 5.1 μmol (70%). Finally,

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[11]) The 'complete' corrinoids differ from the cobalamins by their axial base (*e.g.* in *Methanosarcina barkeri* by a 5-hydroxybenzimidazole [26a, b] and in *Clostridium thermoaceticum* by a 5-methoxybenzimidazole [26]).

[12]) Acetate catabolism to CH_4 and to CO_2 in *Methanosarcina barkeri* [27] and in *Methanothrix soehngenii* [28] has been proposed to proceed *via* CO (in a 'bound' form) and a CH_3X-intermediate. A corrinoid is probably involved, suggesting consideration of an acetyl-Co(III)corrin as a potential CH_3CO-carrier in these processes also.

a red compound, adsorbed at the column head was washed out with dil. aq. $NaHCO_3$, tentatively analyzed (TLC, HPLC, UV/VIS) as aquo-cobalamin (*ca.* 0.9 μmol, 13%). Fractions **1** and **2** were evaporated to dryness (r.t.) and dried (high vacuum r.t., 4 h): 1.4 mg (14%) of **1**; 6.8 mg (68%) of **2**. The raw **2** was dissolved in 0.5 ml of H_2O and crystallized, after addition of *ca.* 5 ml of acetone (refrigerator, overnight), to yield, after drying (high vacuum/r.t., 6 h), bright red needles: 6.5 mg (65%) of **2**, identified by comparison (UV/VIS, ^{1}H-NMR, MS(FAB), HPLC, TLC) with a sample of **2** prepared according to [22]. TLC (H_2O/MeCN/MeOH/AcOH 8:3:2:0.05): R_f 0.25; m.p. > 165° (dec.). UV/VIS ($c = 5.45 \cdot 10^{-5}$ mol/l): 250 (4.36), 277 (sh, 4.25); 289 (sh, 4.18), 322 (4.13), 359 (4.11), 422 (sh, 3.69), 458 (sh, 3.73), 512 (3.90); min 234, 300, 344, 404. ^{1}H-NMR: 0.45 (*s*, 3 H; CH_3(1)); 1.27 (*d*, $J = 7$, CH_3(Pr3)) 1.29, 1.37 (double int.), 1.39 and 1.50 (4 *s*, 5 CH_3) superimposed by 1.1–1.4 (*m*, CH_2(8')), in total *ca.* 20 H; 1.87, 2.24, 2.25, 2.58, 2.59 (5 *s*, 5 CH_3) overlapped by 1.7–2.9 (*m*) and 2.27 (*s*, acetone), in total *ca.* 38 H; 3.16 (*dd*, $J = 14$, 8, 1 H, Prl); 3.41 (*d*-like, $J \approx 6$, 1 H, HC(13)?); 3.52 (*dd*, $J = 10$, 4, 1 H, HC(8)); 3.60 (*d*-like, $J \approx 14$, 1 H, Prl); 3.81 (*dd*, $J = 12$, 4, 1 H, R5); 3.95 (*d*-like, $J \approx 11$, 1 H, R5); 4.1–4.3 (*m*, 3 H); 4.34 (*t*, $J = 4$, 1 H, R2); 4.35–4.45 (*m*, 1 H, Pr2); 4.75–4.9 (*m*, *ca.* 1 H); 6.22 (*s*, 1 H); 6.31 (*d*, $J = 3$, 1H, R1); 6.38 (*s*, 1H); 7.19 (*s*, 1H); 7.23 (*s*, 1 H)[13], MS (FAB): 1375 (11), 1374 (34), 1373 (72), 1372 (100, $(M + 1)^+$); 1332 (10), 1331 (32), 1330 (58), 1329 (65, $(M + 1)^+ - 43$ (CH_3CO)), 1328 (13), 1327 (10); 1183 (8, $(M + 1)^+ - 43 - 146$(5,6-dimethylbenzimidazole)); 1070 (6), 1069 (10, $(M + 1)^+ - 43$–260($C_{14}H_{16}N_2O_3$)); 973 (5), 972 (9), 971 (15, $(M + 1)^+ - 43$–358($C_{14}H_{19}N_2O_7P$ = ribosyl(dimethylbenzimidazole)-phosphate)); *etc.*

2.2. *Control Experiment A (Effect of CO Pressure)* [7]. Same procedure as in *2.1*, but at *ca.* 1.5 atm of CO only: recovery of **1** (*ca.* 80%), formation of **2** (*ca.* 14%) and of aquo-cobalamin (*ca.* 5%; tentatively).

2.3. *Control Experiment B (Effect of Light)* [6][7]: Same procedure as in *2.1*, but omitting irradiation: **1** recovered quantitatively (UV/VIS, TLC, HPLC), crystallized from H_2O/acetone: 9.8 mg (98%); **2** not detected (less than 3%).

2.4. *Control Experiment C (Irradiation of* **1** *in Absence of CO)* [7]. Same procedure as in *2.1*, but deaerated solution of **1** sealed under high vacuum (without introducing CO): **1** recovered to *ca.* 95% (UV/VIS, TLC, HPLC), crystallized from H_2O/acetone: 9.1 mg (91%); **2** not detected (less than 3%), but aquo-cobalamin (*ca.* 4%; tentatively).

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