162. Thermal Methyl-Group Transfer between Methylcobalt(III) Corrinates and Cobalt(II) Corrinates. Equilibration Experiments with Heptamethyl Cobyrinates and Cobalamins

by **Bernhard Kräutler***, **Mark Hughes**, and **Christian Caderas**

Laboratorium für Organische Chemie, ETH Zürich, Universitätstrasse 16, CH-8092 Zürich

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Separate neutral aqueous solutions of either a) methylcob(III)alamin (**2**) and (heptamethyl cob(II)yrinate) perchlorate (**3**) or of b) cob(II)alamin (= vitamin B_{12r}; **4**) and [*Coβ*-methyl(heptamethyl cob(III)yrinate)] perchlorate (**5**) equilibrated thermally at r.t. according to $\mathbf{2} + \mathbf{3} \rightleftarrows \mathbf{4} + \mathbf{5}$. The corresponding equilibrium constant K_e was determined ($K_e = 0.63 \pm 0.15$). This equilibration experiment indicates that the coordination of the nucleotide function in methylcob(II)alamin (**2**) hardly affects the thermodynamics of the Co–C bond homolysis in aqueous solution when compared to nucleotide-free methylcorrinoids such as **5**.

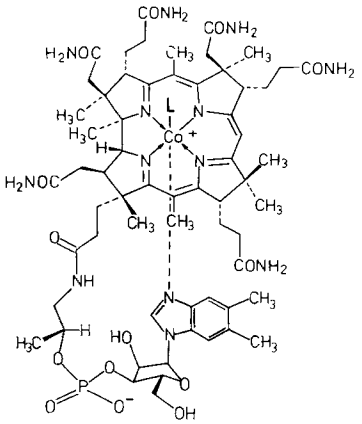
Introduction. – The biological role of the coenzyme B_{12} (= (5-deoxyadenosyl)-cob(III)alamin; **1**) [1–3] and of the methylcorrinoids [4a–c] such as methylcob(III)alamin (**2**) has been closely associated with the reactivity of the Co–C bond in organocorrinoids [2] [5] [6]. The weak organometallic bond has been found to be further labilized kinetically towards homolytic cleavage in some organocobalamins (related to **1** and **2**) as a result of the intramolecular axial coordination of the unique nucleotide function [2] [7]. It has not been clarified, however, to what extent the coordination of the dimethylbenzimidazole function in organocobalamins also affects the Co–C bond in a thermodynamic sense. We report here on a first series of equilibration experiments that point to the negligible *'trans'* effect of the nucleotide base on the homolysis of the Co–C bond in **2**.

Results. – When an equimolar solution of **2** [8a] and (heptamethyl cob(II)yrinate) perchlorate (**3**) [8b] was stored in deoxygenated CH_3OH/0.01M aq. phosphate buffer (pH = 7) 2:1 at r.t. for 16 days with protection from light (*Exper. A*)[1]), partial conversion to cob(II)alamin (**4**) [8c] and [*Coβ*-methyl(heptamethyl cob(III)yrinate)] perchlorate (**5**) [8b] [8d] occurred. Likewise, when a *ca.* equimolar solution of **4** and **5** in the same solvent mixture was stored under the same conditions for 16 days[1]) *(Exper. B)*, partial conversion to **2** and **3** was found. UV/VIS spectra of the equilibrated mixtures indicated no significant formation of nonalkylated cobalt(III) corrinates[2]). Rapid air oxidation of the Co(II) species after addition of 1% HCN in CH_3OH produced diamagnetic cobalt(III) corrinates, amenable to ^{1}H-NMR analysis[3]). The oxidized equilibrium mixtures were partitioned between CH_2Cl_2 and H_2O to separate the heptamethyl cob(III)yrinates (*i.e.*

[1]) Control experiments indicated a $t_{1/2}$ of the equilibration of *ca.* 3 days (by TLC and UV/VIS).

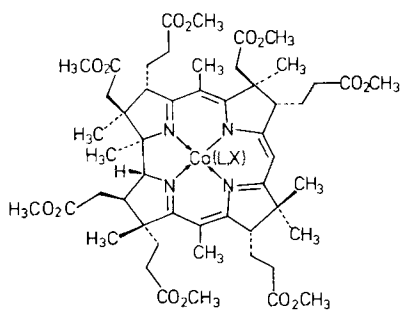
[2]) Compound **8** and (diaquo)cobalt(III) corrinates show a strong UV-absorption band near 350 nm, see [4d].

[3]) In the control experiments, the ^{1}H-NMR analyses of the entire reaction mixtures at this stage agreed with the results obtained here after the subsequent separation.



1 Co–L = 5-deoxyadenosyl-Co(III)
2 Co–L = CH_3–Co(III)
4 Co–L = Co(II)
7 Co–L = CN–Co(III)
8 Co–L = H_2O–Co(III)

dicyano(heptamethyl cob(III)yrinate) (= 'cobester'; **6**) [8d] and **5**) from the cob(III)alamins (*i.e.* **2** and vitamin B_{12} (**7**)). The dried CH_2Cl_2 extract from *Exper. A* contained the cobyrinates **5** and **6** with **5**/**6** = (0.81 ± 0.1):1 according to ^{1}H-NMR analysis (300 MHz, $CDCl_3$; see *Fig. b*)[4])[5]). Correspondingly, the material from the aqueous phase of *Exper. A* was identified by its 300-MHz ^{1}H-NMR spectrum (in D_2O) as **2** and **7** only with **2**/**7** = (1.23 ± 0.1):1[6]). In the oxidized mixture of *Exper. A*, the corrins were, therefore, present in a ratio [**7**]·[**5**]/[**2**]·[**6**] = 0.66 ± 0.15. Analogous analysis of the equilibrated mixture of *Exper. B* (starting with **4** and **5**) gave [**7**]·[**5**]/[**2**]·[**6**] = 0.52 ± 0.15. From this



3 Co(L,X) = Co(II), X = ClO_4
5 Co(L,X) = Co(III), L = CH_3, X = ClO_4
6 Co(L,X) = Co(III), L = X = CN

[4]) Taken from the integral of the vinyl-proton *s*.

[5]) The ^{1}H-NMR spectrum also indicates the presence, besides **5**, of a trace of its isomer with an α-bound CH_3 group at the Co(III) center [8b], in a ratio of *ca.* 15:1.

[6]) Taken as an average of the integrals of the low-field ^{1}H-NMR signals of the mixture **2**/**7** (see *Fig. a*).

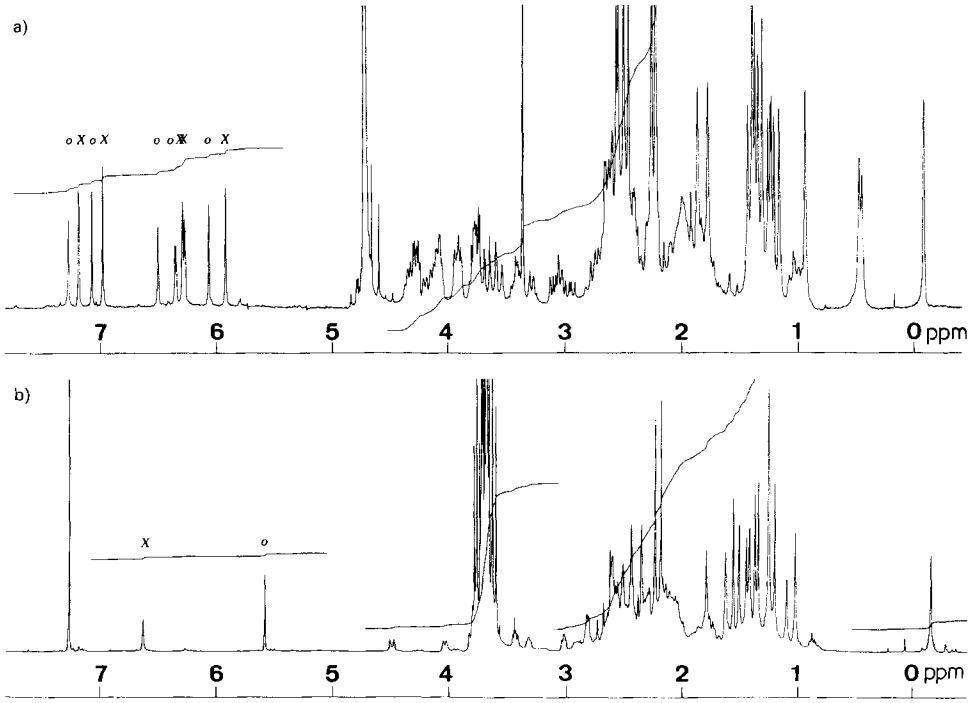


Fig. *300-MHz-^{1}H-NMR spectra of the separated corrinoid fractions from* Exper. A. a) Spectrum of cob(III)alamin mixture in D_2O (*X* marks selected signals due to **2**, *o* those of **7**). b) Spectrum of heptamethyl-cob(III)yrinate mixture in $CDCl_3$ (*X* marks selected signals due to **5**, *o* those of **6**).

and a second pair of similar experiments, the equilibrium constant K_e was determined to be 0.63 ± 0.15.

$$\mathbf{2} + \mathbf{3} \underset{\longleftarrow}{\overset{K_e}{\longrightarrow}} \mathbf{4} + \mathbf{5} \qquad (1)$$

Discussion. – These experiments document the operation of a thermal CH_3-group transfer between vitamin-B_{12}-derived methylcobalt(III) corrinates and cobalt(II) corrinates consistent with *Eqn. 1*[7]). In analogy to studies by *Endicott et al.* [9] on 'methyl-bridged electron-transfer reactions' and by *Johnson et al.* [10], a CH_3 transfer not involving free CH_3 radicals probably also operates here[8]). Such a mechanism could presumably also account for the equilibration of *Coα*- and *Coβ*-methylated nucleotide-free cobyrinic-acid derivatives in CO-containing aqueous solution, as observed by *Friedrich et al.* [12].

Moreover, the equilibration experiments show that the Co–C bond in **2** is not destabilized towards homolysis by the coordination of the dimethylbenzimidazole base[7]), but rather that this bond is slightly more stable under the reaction conditions ($K_e = 0.63$) compared to **5**[9]). This result suggests that the earlier found (kinetic) weakening of the

[7]) The ^{1}H-NMR and UV/VIS spectra confirmed the intact nucleotide coordination to the Co center in **2** and **4** under the conditions of the equilibration.

[8]) According to preliminary experiments, air does not prevent the thermal equilibration in toluene solution (and in the presence of **3**) of **5** and of its isomer bearing the Co-bound CH_3 group on the α-face [8b] [11].

[9]) This result, obtained here with the lipophilic nucleotide-free cobyrinates **3** and **5**, meanwhile has been similarly reproduced also with the corresponding natural cobinamide derivatives (*B. Kräutler*, unpublished).

Co–C bond by the intramolecular nucleotide coordination in organocobalamins [2] [7] depends upon the steric bulk of the organoligand (see also [13]).

The thermodynamic *'trans'* effect of the nucleotide on the Co–C bond homolysis in **2** can also be derived independently from the nucleotide basicity in **2** ($pK_a = 2.7$) [7c] and in the homolysis product **4** ($pK_a \approx 2.9$) [14], the similarity of the two pK_a values being consistent with our result. However, in **2** the base is bonded considerably weaker than in aquocob(III)alamin (**8**; $pK_a \approx -2.4$) [15], and, as reported by *Hogenkamp et al.* [16], **2** donates its CH_3 group to a nucleotide-free aquocobalt(III) corrinate with formation of **8**. Clearly, the heterolytic CH_3 loss **2**→**8** [16] is subject to a 'normal' thermodynamic *'trans'* effect of the nucleotide base [17]. This is not the case, however, for the homolysis **2**→**4**, where the oxidation state of the corrin-bound metal center changes from Co(III) to Co(II) (see also [2]).

These observations are in agreement with the interpretation, that the strength of the nucleotide coordination in competition with solvent (as expressed by the pK_a) in methylated and demethylated 'complete' corrinoids controls the direction of the CH_3-group transfer in an equilibrium with analogous corrinoids lacking the base. As a consequence, (in a thermodynamic sense) cob(I)alamin (= vitamin B_{12s}) should be able to trap the CH_3 group from nucleotide-free methylcobalt(III) corrinates, since the transition from the cobalt(I) corrinate vitamin B_{12s} (nucleotide: $pK_a \approx 5.6$) [18] to the cobalt(III) corrinate **2** is accompanied and driven by the coordination of the nucleotide base. Such effects could be relevant to enzymatic CH_3-group transfer reactions involving protein-bound **2**, where the ability of the nucleotide or of other ligands to coordinate would be subject to control by the enzyme.

Further studies on thermally induced alkyl-group transfers between alkylcobalt(III) corrinates and nonalkylated cobalt corrinates should allow the assessment of the effects of the nucleotide coordination on the Co–C bond strengths and of the Co-bound alkyl groups on the nucleotide coordination.

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Experimental Part

1. *General.* Solvents and reagents: Methylcob(III)alamin (**2**), crystalline [8a]; (heptamethyl cob(II)yrinate) perchlorate (**3**), crystalline, from dicyano(heptamethyl cob(III)yrinate) (= 'cobester' [8d]; **6**) by reduction with HCOOH [8b]; cob(II)alamin (= vitamin B_{12r}; **4**), crystalline, by catalytic reduction [8c]; [*Coβ*-methyl(heptamethyl cob(III)yrinate)] perchlorate (**5**), powder [8b]; CH_3OH, *Fluka, puriss. p.a.;* H_2O, 'nanopure', *Ultrafilter* (Barnstead, USA); formic acid, *Fluka, puriss. p.a.;* KCN, *Merck, p.a.;* $NaClO_4$, *Fluka, p.a.* TLC: CH_2Cl_2, Et_2O, tetrahydrofuran (THF), and CH_3OH, all practical grade, redistilled; silica gel plates, *Merck* Art. *5721*; reversed plates, C_{12}-'opti-up', *Antec AG,* Bennwil, Switzerland. ^{1}H-NMR: 300.14 MHz, *Bruker WM-300,* $CDCl_3$ (δ (TMS) = 0 ppm) or D_2O (δ (HDO) = 4.71 ppm); sample preparation in the dark room.

2. *Experimental Setup.* The equilibration experiments and workup were carried out with strict protection from light (equilibration: homogeneous solutions in tightly stoppered flasks, stored at r.t. in a dry box *(Mecaplex GB-80)*; workup: dark room with minimal exposure to white light).

3. *Equilibration Exper. A.* In a soln. of **2** (15.9 mg, 11.8 μmol) in CH_3OH (1 ml) and 0.01M aq. phosphate buffer (pH 7; 0.5 ml), 13.7 mg of **3** (11.8 μmol) were dissolved with protection from light and under an inert atmosphere (*Mecaplex* box, < 10 ppm of O_2). The soln. was allowed to equilibrate for 16 d, prior to removal of the flask from the dry box and addition of 4 ml of 1% HCN/CH_3OH in the presence of air. The mixture was taken into a

dark room, and the solvents were evaporated at r.t. The residue was partitioned between 20 ml each of H_2O and of CH_2Cl_2. The aq. phase was evaporated (40°), the residue dried for 2 h at r.t./0.5 Torr and then analyzed by ^{1}H-NMR (300 MHz, D_2O; see *Fig. a*). The spectrum exhibited signals due to **2** and vitamin B_{12} (**7**) only, ratio (1.23 ± 0.1):1 as determined from the integrals of the low-field signals. The org. phase was washed with 30 ml of 0.01M phosphate buffer (pH 7) containing *ca.* 10 mg of KCN and *ca.* 50 mg of $NaClO_4$, dried by filtration through a plug of dried cotton wool and evaporated at r.t. The residue was again dried for 2 h at r.t./0.5 Torr and subjected to ^{1}H-NMR analysis (300 MHz, $CDCl_3$, see *Fig. b*). The spectrum exhibited signals due to **5** and **6**[5]), ratio (0.81 ± 1):1 as taken from the integral of the vinyl *s* at 6.62 and 5.57 ppm.

4. *Equilibration Exper. B.* A soln. of **4** (8.5 mg, 6.4 μmol) and **5** (7.7 mg, 6.7 μmol) in CH_3OH (0.5 ml) and 0.01M aq. phosphate buffer (pH 7, 0.25 ml) was stored for 16 d and subsequently analyzed in the same manner and in parallel to the above described *Exper. A*. Analysis of the samples of this equilibration by ^{1}H-NMR gave spectra that were similar to the ones from *Exper. A* and from which the aq. phase was calculated to contain **2** and **7** in a ratio of (1.25 ± 0.2):1 and the org. phase **5** and **6** in a ratio of (0.65 ± 0.1):1.

5. *Control Experiments. Exper. A* and *B* were repeated using 12 mg of **2** (9.1 μmol)/10 mg of **3** (8.8 μmol; *Exper. CA*) and 8.6 mg of **5** (7.5 μmol)/10 mg of **4** (7.5 μmol; *Exper. CB*), resp., dissolved in 1.5 ml of CH_3OH/phosphate buffer 2:1. The course of the reaction was followed by UV/VIS and TLC (carried out with protection from light): Samples (30-μl) were removed periodically and diluted with 400 μl of CH_3OH anaerobically to record the UV/VIS. The sample was then oxidized by addition to *ca.* 0.1 ml of 1% HCN/CH_3OH in the presence of air and evaporated to dryness. The residue was partitioned between *ca.* 1 ml each of H_2O and CH_2Cl_2 and the org. phase shaken with *ca.* 1 ml of aq. phosphate buffer (0.1M, pH 7) containing a trace of KCN. The 2 phases were analyzed by UV/VIS and (after concentration) by TLC. TLC of a sample of the org. phase on silica-gel plates (Et_2O/CH_2Cl_2/THF 2:2:1) separated **5** (R_f 0.48) and **6** (R_f 0.40), while TLC of a sample of the aq. phase on reversed-phase plates (CH_3OH/H_2O 1:2) separated **2** (R_f 0.2) and **7** (R_f 0.35). As expected, the original equilibration mixtures showed the starting materials, while, over 14 d, the product ratios estimated by TLC qualitatively approached the values of the subsequent ^{1}H-NMR analysis (300 MHz, CD_3OD) of the oxidized equilibrated mixtures (*Exper. CA:* **2**/**5**/**6**/**7** = 1.29:1:1.25:1.15 (K_c = 0.71 ± 0.2); *Exper. CB:* **2**/**5**/**6**/**7** = 1.27:1:1.18:1.0 (K_c = 0.66 ± 0.2)).

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