Pseudocoenzyme B_{12} and Adenosyl-Factor A: Electrochemical Synthesis and Spectroscopic Analysis of Two Natural B_{12} Coenzymes with Predominantly 'Base-off' Constitution

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Pseudocoenzyme B_{12} (= *Coβ*-(5′-deoxy-5′-adenosyl)-(adenin-7-yl)cobamide; **1**) and adenosyl-factor A (= *Coβ*-(5′-deoxy-5′-adenosyl)-(2-methyladenin-7-yl)cobamide; **3**) are two natural analogues of coenzyme B_{12} (= adenosylcobalamin-*Coβ*-(5′-deoxy-5′-adenosyl)-(5,6-dimethyl-1*H*-benzimidazolyl)cobamide; **2**), where the Co-coordinating 5,6-dimethyl-1*H*-benzimidazole nucleotide base of **2** is replaced by the purine bases adenine and 2-methyladenine. In contrast to **2**, which exists solely in the 'base-on' form, UV/VIS spectroscopy qualitatively indicates 'base-off' constitution for **1** and **3** in aqueous solution. (*cf.* the established 'base-off' form as unexpected binding mode of B_{12} cofactors in several B_{12}-dependent enzymes, such as in methionine synthase from *Escherichia coli* and in glutamate mutase from *Clostridium cochlearium*). In the present work, pseudocoenzyme B_{12} (**1**) was synthesized in 85% yield by alkylation with 5′-*O*-tosyladenosine of (adenin-7-yl)cob(I)amide, which was produced electrochemically from pseudovitamin B_{12} (*Coβ*-cyano-(adenin-7-yl)cobamide). Likewise, adenosyl-factor A (**3**) was prepared in *ca.* 70% yield from factor A (= *Coβ*-cyano-(2-methyladenin-7-yl)cobamide; **5**). All the spectroscopic properties of **1** and **3** in aqueous solution indicated that these two *Coβ*-(5′-deoxy-5′-adenosyl)-(adenin-7-yl)cobamides exist predominantly in a 'base-off' constitution, with minor but significant contributions of the 'base-on' form. From the UV/VIS spectra, the temperature-dependent equilibrium constants of the 'base-off'/'base-on' reconstitution reaction were determined as K_{on} (**1**) = 0.30 and K_{on} (**3**) = 0.48 at 25°, corresponding to a contribution of the 'base-on' forms of 23% for **1** and of 32% for **3**.

1. Introduction. – Recent crystallographic studies have given first insights into the mode of binding of B_{12} cofactors to B_{12}-dependent enzymes [1]. Methylmalonyl-CoA mutase from *Propionibacterium shermanii* was shown to bind coenzyme B_{12} in the 'base-off'/' His-on' form [2][3], first discovered in methionine synthase from *E. coli* [4][5]. The 'base-off'/' His-on' binding mode, in which a histidine residue of the protein displaces the Co-coordinating 5,6-dimethyl-1*H*-benzimidazole (DMB) base [1] was likewise found in glutamate mutase (Glm) from *Clostridium cochlearium* [6]. In this respect, it appeared particularly intriguing that pseudocoenzyme B_{12} (= *Coβ*-(5′-deoxy-5′-adenosyl)-(adenin-7-yl)cobamide; **1**; see *Fig. 1*), the natural corrinoid cofactor of Glm from *Clostridium tetanomorphum* [7], inherently exists in a 'base-off' form in aqueous solution as indicated by its UV/VIS spectra [8]. Pseudocoenzyme B_{12}, the first naturally occurring B_{12} coenzyme to be discovered and isolated [9], is an analogue of coenzyme B_{12} (= adenosylcobalamin = *Coβ*-(5′-deoxy-5′-adenosyl)-(5,6-dimethyl-1*H*-benzimidazolyl)cobamide; **2**; see *Fig. 2*) in which the Co-coordinating DMB is replaced by the less basic and decoordinated adenine. Recently, **1** and its homologue, adenosyl-factor A (= *Coβ*-(5′-deoxy-5′-adenosyl)-(2-methyladenin-7-yl)cobamide; **3**; see *Fig. 1*),

were also shown to be native corrinoids of *C. cochlearium*, identified *via* the two corresponding cyano-corrinoids pseudovitamin B_{12} (= *Coβ*-cyano-(adenin-7-yl)cobamide; **4**) and factor A (= *Coβ*-cyano-(2-methyladenin-7-yl)cobamide; **5**) from workup with addition of cyanide (see *Fig. 2*) [10]. Purinyl-cobamides, such as **4** and **5**, have been isolated from a variety of microorganisms, from anaerobes, in particular [11–14].

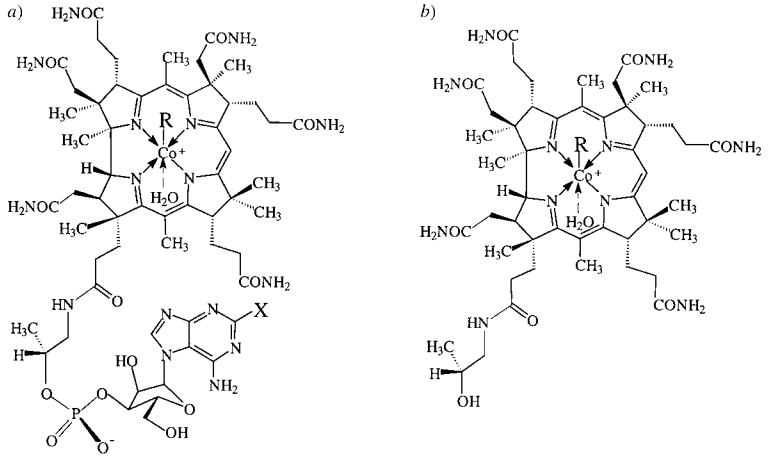


Fig. 1. *Structural formulae of 'base-off' adenosyl-corrinoids* (R = Ado = 5′-deoxy-5′-adenosyl): *a*) *the 'complete' purinylcobamides pseudocoenzyme B_{12}* (= *Coβ*-(5′-deoxy-5′-adenosyl)-(adenin-7-yl)cobamide; X=H, **1**) *and adenosyl-factor A* (= *Coβ*-(5′-deoxy-5′-adenosyl)-(2-methyladenin-7-yl)cobamide; X=Me, **3**); b) *the 'incomplete' cobamide* Co*β*-*(5′-deoxy-5′-adenosyl)cobinamide* (**7**)

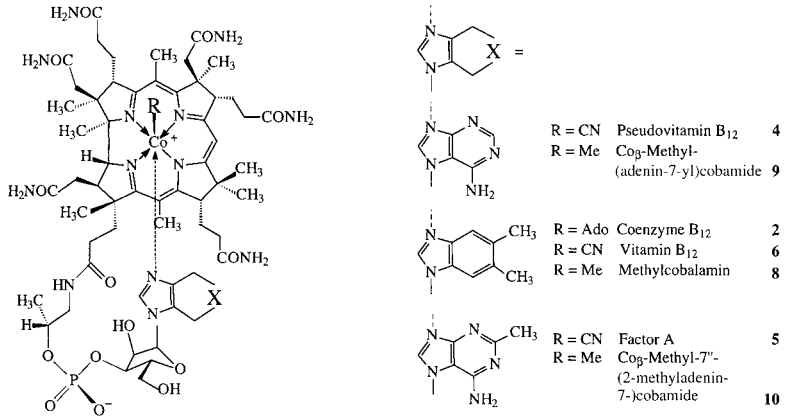


Fig. 2. *Structural formulae of 'complete' cobamides with 'base-on' constitution* (Ado = 5′-deoxy-5′-adenosyl): *coenzyme B_{12}* (= Co*β*-(5′-deoxy-5′-adenosyl)cobalamin; R=Ado, **2**), *cyano-(adenin-7-yl)cobamide* (= pseudovitamin B_{12}; R=CN, **4**), *cyano-(2-methyladenin-7-yl)cobamide* (= factor A, R=CN, **5**), *cyano-cobalamin* (= vitamin B_{12};R=CN, **6**), *methylcobalamin* (R=Me, **8**), Co*β*-*methyl-(adenin-7-yl)cobamide* (R=Me, **9**), and Co*β*-*methyl-(2-methyladenin-7-yl)cobamide* (R=Me, **10**)

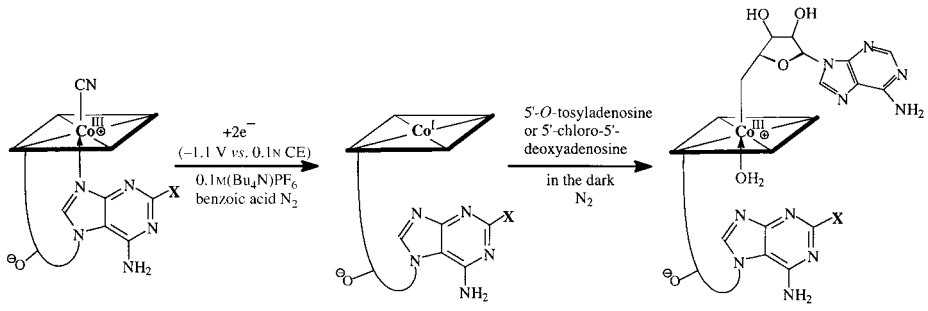
Coenzyme B_{12} (**2**), pseudocoenzyme B_{12} (**1**), and other adenosyl-cobamides are the corrinoid cofactors of about a dozen enzymes, all of which catalyse remarkable biosynthetic rearrangement reactions. X-Ray crystallographic analysis of **2** [15] revealed the unique organometallic nature of this derivative of vitamin B_{12} (=cyanocobalamin; **6**; see *Fig. 2*). The organometallic group is an intriguing feature of the coenzymes **1** and **2**, in addition to the unique structural elements of the corrin ligand and of the Co-coordinating α-nucleotide function [16–19]. The organometallic bond of adenosyl-cobamides has been shown to hold the key to their biological function: the homolytic cleavage of the Co–C bond in the protein-bound B_{12} coenzymes induces the mechanistically complex coenzyme-B_{12}-dependent enzymatic reactions [20–23]. Early on, the remarkably different UV/VIS spectra of **1** and coenzyme B_{12} (**2**) were noted [9]. This difference was rationalized later by the different coordination properties of the nucleotide bases, decoordinated ('base-off') in **1** and coordinated ('base-on') in **2** [8]. With the amazing crystallographic discovery of the 'base-off'/' His-on' forms of protein-bound B_{12} cofactors [1][2][4][6], acute current interest concerns questions about the effect of the nucleotide coordination on the binding of corrinoid cofactors to their apo-enzymes and on their reactivity [24].

Nature provides us with a spectrum of 'complete' corrinoids that differ from vitamin B_{12} (**6**) and other cobalamins by the constitution of their nucleotide base [11][24][26].

We are interested in learning about relevant structural and functional differences of coenzyme forms of benzimidazolyl-, purinyl-, and 'phenolyl'-cobamides [24]. Up to now, the structures of neither pseudocoenzyme B_{12} (**1**) nor adenosyl-factor A (**3**) have been studied in any detail. Intrigued by the early UV/VIS-spectral evidence for the existence of the 'complete' coenzyme B_{12} analogue **1** in the 'base-off' form in neutral aqueous solution [9], we have set out to study the structures of **1** and **3** and to learn about the functional role of their adenine nucleotide bases. To obtain pseudocoenzyme B_{12} (**1**) and adenosyl-factor A (**3**) in the amounts needed, an electrochemical method [25] for their synthesis from pseudovitamin B_{12} (=*Coβ*-cyano-(adenin-7-yl)cobamide; **4**) and factor A (*Coβ*-cyano-(2-methyladenin-7-yl)cobamide; **5**) was developed. The synthesis of **1** and **3** (from the cyano-(adenin-7-yl)cobamides **4** and **5**), as well as the spectroscopic characterization of the 'base-on'/'base-off' equilibria of **1** and **3** in aqueous solution are the subject of the present report.

2. Results and Discussion. – 2.1. *Syntheses.* Pseudovitamin B_{12} (**4**) and factor A (**5**) have been isolated from several natural sources [11]. The corrinoid **4** was also produced with the help of 'guided biosynthesis' [10]. The efficient synthesis of **1** from **4** and of **3** from **5** exploits a recently developed electrochemical procedure [25][27][28] for the preparation of organocobalamins from **6**. In a typical experiment (see *Scheme 1*), the Co^{III}-corrinoid pseudovitamin B_{12} (**4**) was reduced to (adenin-7-yl)cob(I)amide at −1.1 V *vs.* a 0.1M calomel electrode (CE) in a two-compartment electrolysis cell at room temperature and under N_2. The alkylating agent 5′-*O*-tosyladenosine was then added, and electrolysis was continued for another 2 h, under protection from light and air. After workup and precipitation from H_2O by addition of acetone, the crude product **1** (84.7% overall yield) was obtained in practically pure form. Similarly, adenosyl-factor A (**3**) was prepared in 69.5% yield. Attempts to crystallize **1** or **3** were not successful.

Scheme 1



4 X = H, pseudovitamin B_{12} — X = H, (adenin-7-yl)cob(I)amide — **1** X = H, pseudocoenzyme B_{12}

5 X = Me, factor A — X = Me, (2-methyladenin-7-yl)cob(I)amide — **3** X = Me, adenosyl-factor A

2.2. *UV and MS Data.* The UV/VIS spectra of aqueous solutions of **1** and of **3** at room temperature differed significantly from that of the coenzyme **2** [11], but were similar to that of *Coβ*-(5′-deoxy-5′-adenosyl)-cobinamide (**7**) [29] and to that of the protonated 'base-off' form $\mathbf{2}\cdot H^+$ of coenzyme B_{12} [11]. The spectra of **1** and **3** thus showed the known features of 'complete' adenosyl-cobamides in a 'base-off' constitution, or of 'incomplete' cobamides lacking a nucleotide function (see *Figs. 3* and *4*) [8][9]. Correspondingly, also the CD spectra of **1** and **3** were similar to the spectra of **7** and $\mathbf{2}\cdot H^+$ [11]. However, as displayed in *Fig. 3* and *4*, both types of spectra of **1** and **3** showed an unusually strong temperature dependence. The long-wavelength maxima in the UV/VIS spectra shifted bathochromically at lower temperatures. The absorbance changes were consistent with the presence of a temperature-dependent equilibrium between the 'base-off' and 'base-on' forms, where the latter was the minor contributor but was gaining in importance at lower temperatures. The temperature-dependent UV/VIS spectra of **1** and **3** were quantitatively analyzed by using experimental absorbance data from the UV/VIS spectra of **2** and $\mathbf{2}\cdot H^+$. The spectra of **1** and **3** could be simulated as a superposition of **2** (a 'base-on' adenosyl-cobamide) and $\mathbf{2}\cdot H^+$ (the corresponding 'base-off' form), and the 'base-on'/'base-off' reconstitution reaction of **1** and **3** could be characterized thermodynamically (see *Table 1*). In both cases, the 'base-off' form is clearly established to predominate at room temperature, amounting to *ca.* 77% for **1** and 68% for **3** (see *Scheme 2*).

Fast-atom-bombardment (FAB) MS of **1** exhibited signals of the intact pseudo-molecular ion at *m/z* 1569 ($[M+H]^+$) and of a fragment at *m/z* 1319, due to the loss of the 5′-deoxy-5′-adenosyl ligand, confirming the molecular formula $C_{68}H_{95}CoN_{21}O_{17}P$ deduced for **1**. The FAB-MS of homologue **3** also exhibited signals due to the intact pseudo-molecular ion at *m/z* 1582.8 ($[M+H]^+$) and of a fragment at *m/z* 1332.8) due to the loss of the 5′-deoxy-5′-adenosyl ligand, confirming the deduced molecular formula $C_{69}H_{97}CoN_{21}O_{17}P$ of **3**.

2.3. *NMR Data. Preamble.* The NMR spectra of aqueous solutions **1** and of **3** (see *Fig. 5*) provided rather precise and complementary experimental information for the dynamic structures of **1** and **3**. One-dimensional and two-dimensional homo- and heteronuclear NMR experiments and techniques for water suppression have greatly

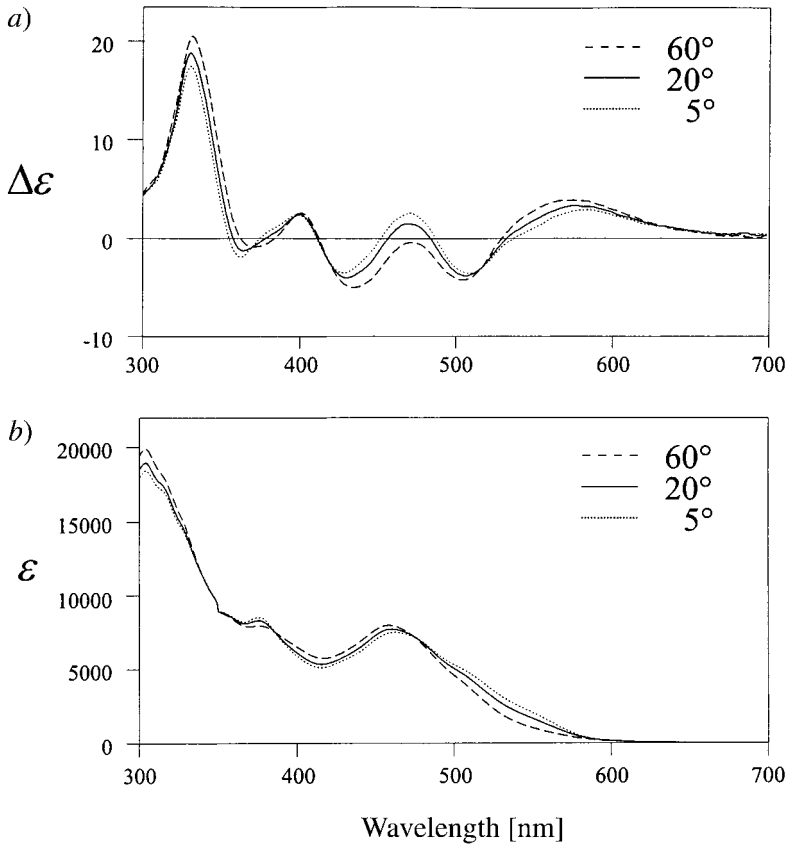


Fig. 3. a) *CD Spectra and* b) *UV/VIS spectra of aqueous solutions of pseudocoenzyme* B_{12} (**1**) *at 5, 20, and 60°.* c(**1**) = 1.09 mM, 20 mM potassium phosphate buffer, pH 7.0.

enhanced the possibilities of ^{1}H-NMR spectroscopy of vitamin B_{12} derivatives in aqueous solution [30]. As the position of the signals in the ^{1}H-NMR spectra of **1** showed insignificant sensitivity to the solution pH in the range pH 7–4, most measurements were carried out at pH 4.2, where the signals of all protons were identified, except for those of the exchangeable labile OH groups at the ribose units HO(2R), HO(5R), HO(2RL), HO(3RL)) and NH_2 groups at the adenine units (HN(61N), HN(61L)) (see *Fig. 6* for the atom numbering). The signals of all amide protons and of all diastereotopic methylene protons, except for those at C(5R), could individually be assigned from analysis of NOE-derived distance constraints as well as of homo- and heteronuclear ^3J couplings. Complete assignment of both ^{1}H- and ^{13}C-NMR signals was achieved by two-dimensional gradient-enhanced heteronuclear experiments (PFG-HSQC, PFG-HMBC) and homonuclear experiments, such as total correlation spectroscopy ('watergate'-TOCSY) and rotating-frame *Overhauser* enhancement spectroscopy ('watergate'-ROESY). ^{1}H,^{15}N PFG-HSQC and ^{1}H,^{15}N PFG-HMBC experiments were carried out to achieve complete assignment of the amide

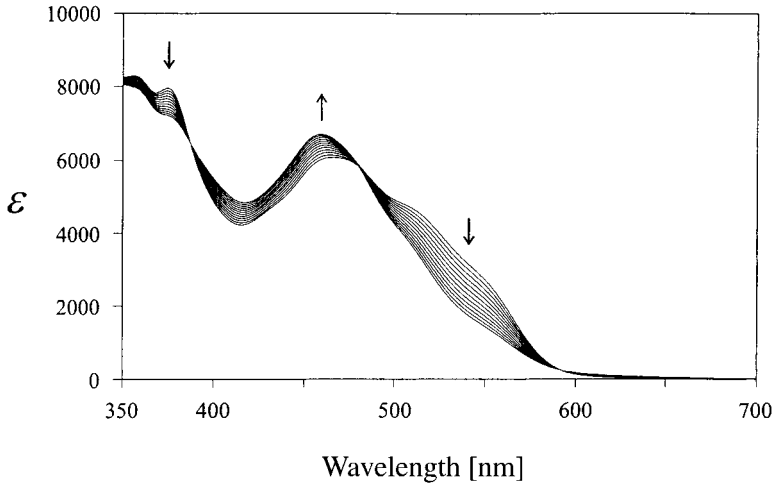


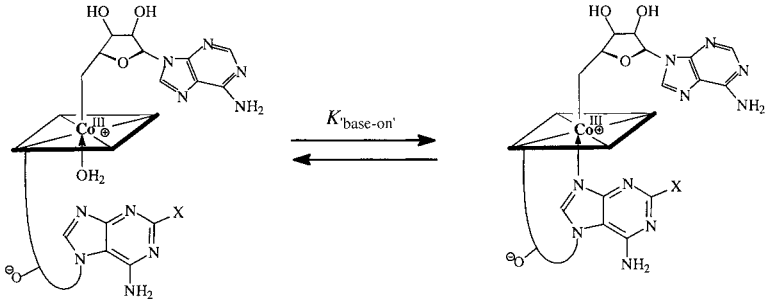
Fig. 4. *UV/VIS Spectra of adenosyl-factor A* (**3**) *in aqueous solution in the temperature range 5–60° and at 5° intervals.* Arrows indicate change of spectra with increasing temperature; $c(\mathbf{3}) = 1.00$ mM, 20 mM potassium phosphate buffer, pH 7.0.

Table 1. *Thermodynamic Data*[a]) *for 'Base-off'/'Base-on' Equilibria of* **1** *and* **3**

	ΔH^0 [kJ mol^{-1}]	ΔS^0 [J K^{-1} mol^{-1}]	ΔG^0 [kJ mol^{-1}]	$K_{\text{'base-on'}}$ (25°)
1	– 20.5	– 78.8	2.9	0.30
3	– 21.1	– 76.9	1.8	0.48

[a]) Standard enthalpy (ΔH^0), standard entropy (ΔS^0), standard free enthalpy (ΔG^0), and equilibrium constant ($K_{\text{'base-on'}}$) at 25° for the 'base-off'/'base-on' equilibria of **1** and **3**. Data were extracted from the temperature dependence of the absorbance at 524 nm of **1** and **3**. The extinction coefficients at 524 nm of 'base-on' ($\log \varepsilon = 3.82$; at pH 7) and protonated 'base-off' forms ($\log \varepsilon = 3.29$; at pH 1) of coenzyme B_{12} (**2**) were used as reference values for the 'base-on' and 'base-off' forms, respectively, of **1** and **3**.

Scheme 2



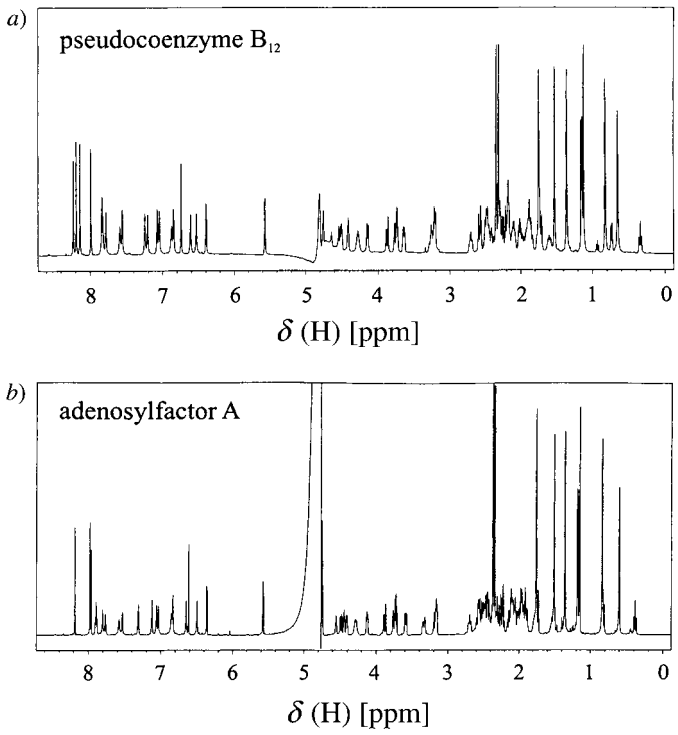


Fig. 5. *500-MHz ^{1}H-NMR spectrum* a) *of pseudocoenzyme B_{12}* (**1**), *and* b) *of adenosyl-factor A* (**3**) *in aqueous solution*. **1**: pH 4.2, H_2O/D_2O 9:1, 26°; **3**: pH 6.5, H_2O/D_2O 19:1, 26°

protons and to obtain the chemical-shift data of the amide N-atoms and of some of the N-atoms of the heterocycles. In this way, all the C- and N-atoms, except for N(1) and N(4) of the corrin ring system and of N(1N)/N(1L) and N(3N)/N(3L) of the adenine units, could be assigned (see *Tables 2–4*). A similar set of data was collected from corresponding spectra of an aqueous solution of **3**(at pH 6.5).

2.3.2. *Qualitative Analysis of Chemical-Shift Data* (see *Tables 2–4*). In this section, chemical-shift data of pseudocoenzyme B_{12} (**1**) and of adenosyl-factor A (**3**) are compared to data of the 'base-on' corrinoids pseudovitamin B_{12} (**4**) [10] and coenzyme B_{12} (**2**) [31][32], as well as to data of the protonated ('base-off') form of coenzyme B_{12} (**2**·H^+) [33] and of *Coβ*-(5′-deoxy-5′-adenosyl)-cobinamide (**7**) [29], two models for 'base-off' corrinoids.

A comparison of the chemical-shift data of **1** and **3** reveals that the three sets of δ(H), δ(C), and δ(N), of **1** and of **3** are very similar: The largest $\Delta\delta$(H) of 0.27 ppm and 0.15 ppm are observed for H–C(8N) and H–C(10), respectively. Likewise, the δ(N) also differ only marginally, with a maximal upfield shift of N(9N) of 1.6 ppm in the spectrum of **3**. The small upfield shifts in the spectra of **3** are all consistent with a somewhat more important contribution of its 'base-on' form. The ^{13}C-NMR data of **1** and **3** differ insignificantly, but only for the C(2N) position of the adenine base, whose Me substituent in the spectrum of **3** gives rise to a signal at 27.1 ppm. The NMR data of

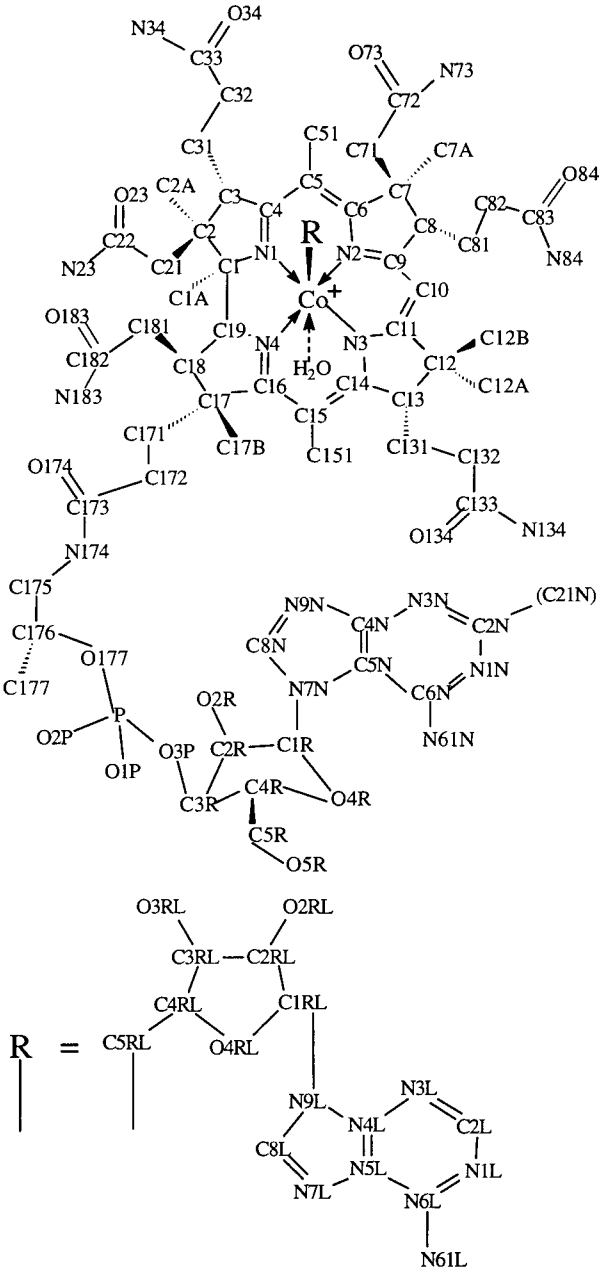


Fig. 6. *Atom numbering of pseudocoenzyme B_{12}* (**1**) *and adenosyl-factor A* (**3**) *used for the description of NMR results* (from [10][19])

Table 2. *^{1}H-NMR Data of* **1** *and* **3**, *Compared with Those of* **2** *and* **2**·H^+[a]). δ(H) in ppm.

	δ(**1**)[b])	δ(**3**)[b])	δ(**2**)[c])	δ(**2**·H^+)[d])	$\Delta\delta$(**1**,**2**)[e])
Me(1A)	0.66	0.59	0.47	0.81	0.19
Me(2A)	1.37	1.34	1.36	1.48	0.01
CH_2(21)	2.60(*R*); 2.27(*S*)	2.54(*R*); 2.28(*S*)	2.41	2.60; 2.46	0.19; −0.14
H−C(3)	4.15	4.11	4.10	4.23	0.05
CH_2(31)	1.99(*R*); 1.90(*S*)	1.95	2.06; 1.96	2.11; 1.97	−0.07; −0.06
CH_2(32)	2.48	2.45	2.50	2.55	−0.02
Me(51)	2.35	2.35	2.45	2.43	−0.10
Me(7A)	1.76	1.74	1.70	1.82	0.06
CH_2(71)	1.73(*R*); 2.26(*S*)	1.73(*R*); 2.23(*S*)	1.72; 2.19	2.14; 2.61	0.01; 0.07
H−C(8)	3.65	3.57	3.29	3.73	**0.36**
CH_2(81)	2.13(*R*); 1.60(*S*)	2.06(*R*); 1.51(*S*)	1.75; 0.81	2.21; 1.75	**0.38**; **0.79**
CH_2(82)	2.19	2.12; 2.02	0.88; 1.73	2.35	**1.31**, **0.46**
H−C(10)	6.76	6.61	5.93	6.97	**0.83**
Me(12A)	1.54	1.48	1.32	1.67	0.22
Me(12B)	0.84	0.82	0.87	1.00	−0.03
H−C(13)	3.22	3.15	2.89	3.43	0.33
CH_2(131)	1.89(*R*); 2.11(*S*)	1.89(*R*); 2.06(*S*)	2.00; 2.22	1.92; 2.21	0.11; −0.11
CH_2(132)	2.19	2.21; 1.98	2.54	2.21; 1.86	−**0.35**
Me(151)	2.32	2.33	2.43	2.46	−0.11
Me(17B)	1.13	1.13	1.36	1.40	−0.23
CH_2(171)	2.41(*R*); 1.76(*S*)	2.42(*R*); 1.74(*S*)	2.45; 2.06	2.51; 1.85	−0.04; −0.30
CH_2(172)	1.87(*R*); 2.31(*S*)	1.89(*R*); 2.31(*S*)	1.78	1.85; 2.31	0.09; **0.53**
H−C(18)	2.71	2.68	2.65	2.85	0.06
CH_2(181)	2.57(*R*); 2.49(*S*)	2.56(*R*); 2.49(*S*)	2.65	2.78	−0.08; −0.16
H−C(19)	4.54	4.48	4.24	4.7	0.30
CH_2(175)	3.25	3.32; 3.17	3.54; 3.16	3.38; 3.27	−0.29; 0.09
H−C(176)	4.29	4.27	4.33	4.36	−0.04
Me(177)	1.17	1.16	1.21	1.23	−0.04
H−C(1R)	6.40	6.35	6.26	6.56	0.14
H−C(2R)	4.65	4.54	4.23	4.97	**0.42**
H−C(3R)	4.75	[f])	4.72	4.83	0.03
H−C(4)	4.51	4.39	4.10	4.79	**0.41**
CH_2(5R)	3.76; 3.87	3.74; 3.86	3.74; 3.88	3.84; 3.94	0.02; −0.01
H−C(2N) or Me−C(2N)	8.12	2.35	–	–	–
H−C(8N)	8.23	7.96	6.95[g])	9.16[g])	**1.28**
H−C(1RL)	5.59	5.56	5.56	5.61	0.03
H−C(2RL)	4.42	4.43	4.54	4.34	−0.12
H−C(3RL)	3.74	3.71	3.74	3.90	0.00
H−C(4RL)	2.03	2.09	2.54	1.98	−**0.51**
CH_2(5RL)	0.34(*R*); 0.73(*S*)	0.36(*R*); 0.79(*S*)	0.57; 1.55	0.38; 1.46	−**0.23**; −**0.82**
H−C(2L)	8.18	8.18	8.19	8.43	−0.04
H−C(8L)	7.98	7.98	8.00	8.21	−0.02

[a]) *R* and *S* are short forms of $H_{pro\text{-}R}$ and $H_{pro\text{-}S}$, resp.; δ in ppm. [b]) With external DSS (sodium 3-(trimethylsilyl)propane-1-sulfonate) as reference. [c]) From [32], with TSP (sodium 3-(trimethylsilyl)(D_4)propanoate) as reference. [d]) From [33], with TSP as reference. [e]) Shift differences of more than 0.3 ppm are highlighted in bold face (DSS is 0.01 ppm downfield compared to TSP). [f]) Could not be detected due to spectral overlap with the water signal. [g]) Indexed as H−C(B2) in coenzyme B_{12}.

Table 3. *^{13}C-NMR Data of* **1** *and* **3**, *Compared with Those of* **2** *and* **2**·H^+. δ(C) in ppm.

	δ(**1**)[a])	δ(**3**)[a])	δ(**2**)[b])	δ(**2**·H^+)[c])	$\Delta\delta$(**1**,**2**)[d])
C(1)	89.2	89.4	88.5	89.8	0.7
C(1A)	26.0	25.6	23.5	26.7	**2.5**
C(2)	48.4	48.5	49.5	47.4	– 1.1
C(2A)	19.2	19.3	19.9	19.4	– 0.9
C(21)	45.7	45.8	46.2	46.2	– 0.8
C(22)	179.3	179.3	179.3	179.1	0
C(3)	57.7	58.0	58.5	58.1	– 0.7
C(31)	28.6	28.7	29.2	29.0	– 0.9
C(32)	37.7	38.0	38.3	37.9	– 0.7
C(33)	181.3	[4])	181.1	181.1	0.2
C(4)	179.3	179.0	178.7	179.4	0.6
C(5)	110.8	111.0	108.4	111.2	**2.4**
C(51)	18.1	18.2	18.3	18.4	– 0.1
C(6)	166.3	166.2	166.6	166.2	– 0.3
C(7)	52.6	53.1	53.1	53.2	– 0.5
C(7A)	21.5	21.7	21.7	21.8	– 0.4
C(71)	45.5	45.6	45.3	45.6	0.1
C(72)	178.1	178.2	177.9	177.4	0.2
C(8)	57.7	57.8	57.5	58.1	0.3
C(81)	29.0	29.0	28.7	29.4	0
C(82)	34.9	34.9	34.8	35.2	0.1
C(83)	181.3	181.3	180.3	180.9	**1.0**
C(9)	174.6	174.5	173.1	175.1	**1.5**
C(10)	99.5	99.2	97.7	100.4	**1.1**
C(11)	178.8	178.3	177.6	179.0	**1.2**
C(12)	49.1	49.6	49.5	49.5	– 0.4
C(12A)	22.3	22.5	23.9	22.6	– **1.8**
C(12B)	34.0	34.1	34.2	34.4	– 0.1
C(13)	55.1	55.4	55.8	55.4	– 0.7
C(131)	28.6	28.7	30.3	28.9	– **2.0**
C(132)	34.9	35.3	38.1	35.0	– **3.2**
C(133)	181.4	181.4	181.1	181.0	0.3
C(14)	166.2	166.7	167.2	166.7	– **1.0**
C(15)	109.1	109.1	106.9	109.8	**2.2**
C(151)	18.1	18.2	18.8	18.4	– 0.6
C(16)	179.5	178.8	178.7	179.4	0.8
C(17)	61.6	61.7	60.8	61.8	0.8
C(17B)	20.3	20.3	19.6	20.8	0.5
C(171)	34.6	34.9	34.4	35.2	0.1
C(172)	34.2	34.5	34.6	34.4	– 0.5
C(173)	178.5	178.5	178.2	178.3	0.3
C(18)	41.5	41.9	42.5	42.2	– **1.0**
C(181)	35.0	34.9	34.8	35.2	0.1
C(182)	179.1	179.2	179.0	179.1	0.1
C(19)	77.2	77.2	76.8	77.6	0.4
C(175)	47.4	47.7	47.8	48.9	– 0.5
C(176)	75.0	75.3	76.0	75.2	– **1.1**
C(177)	21.3	21.4	21.7	21.5	– 0.4
C(1R)	90.5	90.6	89.4	90.2	**1.1**
C(2R)	74.5	74.1	72.0	74.5	**2.5**
C(3R)	77.0	[e])	76.2	77.6	0.7
C(4R)	87.3	86.8	84.6	89.2	**2.7**

Table 3 (cont.)

	δ(**1**)[a])	δ(**3**)[a])	δ(**2**)[b])	δ(**2**·H^+)[c])	$\Delta\delta$(**1**,**2**)[d])
C(5R)	63.7	63.6	63.4	64.1	0.3
C(2N)	154.5	27.1 (Me)	–	–	–
C(4N)	160.9	[e])	–	–	–
C(5N)	114.4	[e])	–	–	–
C(6N)	155.1	[e])	–		–
C(8N)	147.8	147.5	–	–	–
C(1RL)	90.3	90.4	91.0	91.1	0.5
C(2RL)	75.4	75.3	75.6	76.0	– 0.3
C(3RL)	75.1	75.3	76.6	74.9	– **1.7**
C(4RL)	88.5	88.5	88.6	88.9	– 0.1
C(5RL)	22.0	22.8	27.3	22.3	– **5.3**
C(2L)	155.4	155.9	156.0	148.4	– 0.2
C(4L)	151.8	[e])	151.8	151.0	0
C(5L)	122.1	[e])	121.8	121.5	0.3
C(6L)	158.6	[e])	158.7	153.5	– 0.1
C(8L)	143.1	143.2	143.8	145.5	– 0.2

[a]) With external DSS as reference. [b]) From [32], with TSP as reference. [c]) From [33], with TSP as reference. [d]) Shift differences of more than 1 ppm are highlighted in bold face (DSS is 0.16 ppm downfield compared to TSP. [e]) Could not be detected due to spectral overlap or due to low intensity.

Table 4. *^{15}N-NMR Data of* **1**, **3**, *and* **4**. δ in ppm.

	δ (H) (**1**)[a])	δ (H) (**3**)[a])	δ (N) (**1**)[b])	δ (N) (**3**)[b])	δ (N) (**4**)[b])
N(1)	–		[c])	[c])	[c])
N(2)	–		**193.6**	**192.7**	**162.1**
N(3)	–		**202.4**	**201.3**	**168.6**
N(4)	–		[c])	[c])	[c])
NH_2(23)	7.83; 7.04	7.81; 7.04	110.5	110.5	110.8
NH_2(34)	7.59; 6.87	7.59; 6.86	105.0	104.9	104.7
NH_2(73)	7.55; 6.84	7.54; 6.84	109.2	109.3	109.5
NH_2(84)	7.21; 6.53	7.14; 6.50	102.9	102.8	102.6
NH_2(134)	7.24; 6.60	7.31; 6.65	104.2	104.4	105.3
H–N(174)	7.82	7.89	**109.8**	**110.2**	**113.4**
NH_2(183)	7.77; 7.07	7.78; 7.07	106.4	106.2	106.3
N(1N)	–		[c])	[c])	[c])
N(3N)	–		[c])	[c])	[c])
N(7N)	–		153.8	154.2	154.8
N(9N)	–		**224.5**	**222.9**	**182.8**
N(1L)	–		[c])	[c])	–
N(3L)	–		[c])	[c])	–
N(7L)	–		224.5	224.8	–
N(9L)	–		163.1	163.1	–

[a]) Values at higher field correspond to H_{cis} and values at lower field to H_{trans} of the amide group (external DSS as ref.). [b]) Large shift differences from comparison between the spectra of **1** and **3** with **4** are highlighted in bold face; signals are referenced to NH_3 (liq.) [c]) Signals are not detectable.

1 and **3** and the derived conclusions for their basic structural properties are so similar that the following discussion of the NMR-spectroscopic properties is mainly limited to those of **1**.

Comparison of the δ(C) values from spectra of **1** and **2** reveals similarity between the two sets of data, with the exception of several diagnostic signals. Comparing the signals of the corrin C-atoms of **1** and **2**, major shift differences can be observed for the signals of C(5), C(10), and C(15), *i.e.*, of the methine bridges. The downfield shift of the signals of most of the unsaturated C-atoms of the corrinato ligand in **1** (compared to those of **2**) indicate a reduced electron density in the π-system of **1** [34]. Similar shifts were also observed in the spectra of $\mathbf{2}\cdot H^+$ [33] and **7** [29], where the DMB base is decoordinated or absent. The observed differences would be consistent with the absence of a Co-coordinating base in **1**. A further striking difference between the spectra of **1** and **2** concerns the C(5RL), which is directly attached to the Co-center. The 5.3 ppm upfield shift of this signal from 27.3 ppm in the spectrum of **2** [32] to 22.0 ppm (for **1**) again reflects the (practical) absence of an intramolecular nucleotide coordination at the Co-center, as also documented in the spectra of the adenosyl-corrinoids $\mathbf{2}\cdot H^+$ [33] and **7** [29]. In addition, the δ(C) of the corrin-bound Me(1A), of the methylene groups of the propanoic acid side chain extending from C(13), and of some of the C-atoms of the ribose attached to the nucleotide base are significantly different in the spectra of **1** and **2**. Presumably, these differences are largely a consequence of different conformational properties of the two corrinoids.

The δ(C) of corresponding C-atoms in the spectra of **1** and of the 'incomplete' adenosyl-corrinoid **7** match closely, with the exception of those of the terminal C-atoms of the side chain extending from C(17) (carrying the nucleotide function in **1**). Likewise, the δ(C) in the spectrum of the (di)protonated form $\mathbf{2}\cdot H^+$ of coenzyme B_{12} (**2**) differ little from those of the spectrum of **1**, except for those of some C-atoms of the protonated adenine ligands [33].

The use of water suppression in ^{1}H-NMR spectra of aqueous solutions of vitamin B_{12} derivatives has made δ(N) values accessible systematically *via* 2D ^{1}H,^{15}N-heteronuclear correlations [30][34]. Comparison of the δ(N) of **1** with those of pseudovitamin B_{12} (**4**) [10] (*Table 4*) reveals the most significant shift difference for N(9N), the Co-coordinating N-center in **4**. A downfield shift of about 42 ppm compared to the signal of N(9N) in the spectrum of **4** [10] can be observed. This is consistent with a 'base-off' form of **1**, where the adenine base is decoordinated and a downfield shift results from the absence of an anisotropic effect of the corrin π-system.

The observed differences between the δ(H) of corresponding protons in the ^{1}H-NMR spectra of **1**, **2**, $\mathbf{2}\cdot H^+$, and **7** are all consistent with a predominant 'base-off' nature of **1** in aqueous solution. The δ(H) of H–C(10), which is directly attached to the corrin π-system, is rather sensitive to changes in electron density. The δ(H) of H–C(10) in the spectrum of **1** is rather close to the ones in the spectra of $\mathbf{2}\cdot H^+$ and **7** but is shifted downfield by nearly 1 ppm with respect to that in the spectrum of **2**. The δ(H) of H–C(8N) of the adenine nucleotide base is also shifted downfield by *ca.* 1 ppm in the spectra of **1** compared to that of **4**. This shift is largely due to the absence in (the 'base-off' form of) **1** of the powerful shielding effect of the corrinatocobalt system on the Co-coordinated nucleotide base unit [35]. A similar situation exists in the spectrum of $\mathbf{2}\cdot H^+$, where δ(H) of H–C(2B) (corresponding to H–C(8N) in **1**) occurs at

a more than 1 ppm lower field than that for the equivalent signal in the spectrum of **2** [33].

A further probe for the 'base-on' nature of 'complete' corrinoids are the diagnostic high-field shifts of $H_{pro\text{-}R}$–C(81) and H–C(82), methylene protons of the propanoic acid side chain extending from C(8), whose time-averaged position is within the shielding region of the Co-coordinated heteroaromatic base [30]. The δ(H) assigned to H–C(81) and H–C(82) of **2** [32][33] experience large upfield shifts with respect to those of **1**, which occur at similar positions as the δ(H) of the corresponding protons in the spectrum of **7** [29]. The upfield shift of H–C(81) and H–C(82) in the spectra of **2**, **4** [10], and **6** [36] is similarly not observed in the spectrum of **2**·H^+ [33], due to the absence of a Co-coordinating nucleotide base. Accordingly, in the spectrum of **1**, all δ(H) of diagnostic signals of protons at the corrin and nucleotide moieties consistently indicate the (predominance of the) 'base-off' constitution of pseudocoenzyme B_{12} (**1**) in aqueous solution.

The δ(H) of protons attached at the upper face of the corrin ring system are sensitive to the (time averaged) positions of the organometallic 5′-deoxy-5′-adenosyl ligand. Significant high-field shifts of several methyl or methylene protons of the corrin moiety in adenosyl-cobamides (compared to cyano- and methyl-cobamides) have been observed. From this and further NOE evidence, the solution structure of the coenzyme **2** was deduced to exhibit two relevant orientations of the 5′-deoxy-5′-adenosyl ligand [32][33]. In the spectra of **1** (and of **3**) the δ(H) of Me(12B) as well as (the mean position of the δ(H) of CH_2(71) occur at significantly higher field than in the spectra of the corresponding cyano-corrinoids pseudovitamin B_{12} (**4**) and factor A (**4**) [10], or of methyl-cobalamin (**8**) [37][38]. In the spectra of **1** and **3**, also the δ(H) of Me(17B) and of CH_1(181) are upfield compared to those of **2** and, therefore in time average, all of these protons are likely to be located within the shielding region of the ring-current field of the 5′-deoxy-5′-adenosyl ligand of **1** and **3**. The available δ(H) values from the spectra of **1** and **3** thus indicate a remarkable dynamic distribution of the Co-bound organometallic group. A rather similar situation with respect to these signals was reported for the ^{1}H-NMR spectrum of neocoenzyme B_{12}, the C(13)-epimer of **2**, for which the existence of several relevant orientations of the 5′-deoxy-5′-adenosyl ligand relative to the corrin ring system was established [28].

2.3.3. *NOE Contacts between the Nucleotide Base and the Corrinato Ligand of* **1**. A dominating 'base-off' constitution was deduced from the δ values presented above for the structures of **1** and **3** in aqueous solution, consistent with the qualitative conclusions from the earlier work [8]. However, the NMR data are not consistent with an exclusive 'base-off' structure for **1** and **3**: Eight diagnostic NOE contacts between protons attached to the nucleotide unit and to the lower face of the corrin ring system are observed in the ROESY spectrum of **1**. Contacts of medium intensity can be observed between H–C(8N) and the methylene protons $H_{pro\text{-}S}$–C(131), and $H_{pro\text{-}R}$–C(131) and/or $H_{pro\text{-}R}$–C(172) (due to signal overlap, the NOE cannot be assigned specifically either to $H_{pro\text{-}R}$–C(131) or to $H_{pro\text{-}R}$–C(172); the crystal structure of 'base-on' coenzyme B_{12} (**2**) shows similar distances of the corresponding two pairs of H-atoms [15]). Weak NOE contacts are also observed between H–C(8N) and Me(1A), and Me(151) and/or $H_{pro\text{-}S}$–C(172), as well as between H–C(2N) and Me(1A), $H_{pro\text{-}S}$–C(31) and/or $H_{pro\text{-}R}$–C(31), Me(7A), and $H_{pro\text{-}S}$–C(81) (see *Table 5*). These NOE-contacts point to

a spatial vicinity, with time averaging, of H–C(2N) and H–C(8N), the two H-atoms of the adenine base, and of specific protons attached to the lower face of the corrinato ligand. Such a set of NOE contacts is diagnostic of the 'base-on' constitution of the corrinoid and has been observed – with about three times higher intensities – in the ROESY-spectra of the cyano-(adeninyl)cobamide **4** [10]. All of the observed NOE contacts between the adenine unit and the corrin moiety are best explained by a Co-coordinated ('base-on') constitution, where the nucleotide base is positioned directly below the corrin ring system and in a north-south orientation [16][39].

Table 5. *NOE-Contacts between the Nucleotide Moiety and the Corrin Ring System of* **1** *and* **4**[a])

	Rel. intensity (**1**)[b])	Rel. intensity (**4**)[b])
H–C(2N)/Me(1A)	1.5	2.2
H–C(2N)/H_R–C(31)	} 4.0[c])	17.2
H–C(2N)/H_S–C(31)		6.3
H–C(2N)/Me(7A)	1.3	8.6
H–C(2N)/H_S–C(81)	2.6	9.9
H–C(8N)/Me(1A)	2.3	7.2
H–C(8N)/H_R–C(131)	} 6.5[c])	} 43.5[c])
H–C(8N)/H_R–C(172)		
H–C(8N)/H_S–C(131)	4.9	
H–C(8N)/Me(151)	} 3.1[c])	5.4
H–C(8N)/H_S–C(172)		1.5

[a]) H_R and H_S are short forms of $H_{pro\text{-}R}$ and $H_{pro\text{-}S}$, resp. [b]) % of NOE intensity of cross-peak H–C(8)/H–C(10). [c]) Assignment unspecific due to signal overlap.

2.3.4. *Dynamic 'Base-on'/'Base-off' Equilibrium*. The coordination tendency of the corrin-bound Co^{III} center for the adenine bases in **1** and **3** is clearly indicated to be significantly less than that in the cyano analogues **4** and **5**, consistent with related findings for the corresponding pair **2** and **6** [40]. Taking all of the available spectroscopic data into consideration, the structures of **1** and **3** are thus best described as representing a dynamic equilibrium between minor 'base-on' and dominant 'base-off' constitutions. From the temperature dependence of the UV/VIS spectra, as well as from chemical-shift values of pseudocoenzyme B_{12} (**1**) and from the relative intensities of the NOE signals, the predominating 'base-off' form of **1** can be deduced to represent *ca.* 80% of all the populated structures at room temperature. Likewise, adenosyl-factor A (**3**) is also indicated to exist predominantly in a 'base-off' form, which represents *ca.* 70% of the populated structures at room temperature. Consequently, the spectroscopic information points to the presence of a flexible and dynamic nucleotide tail in **1** and **3**, where an adenine heterocycle can hardly achieve the intramolecular Co-coordination of the nucleotide base with its weakly nucleophilic N(9N). The further characterization of the dynamic structures of **1** and **3** in aqueous solution by extensive NMR analyses and NMR-constrained calculations of their molecular structures is the subject of ongoing work in our laboratory.

The coordination pattern at the corrin-bound cobalt(III) center of organometallic 'base-off' forms of 'complete' cobamides and of 'incomplete' cobamides is still a matter of current investigation (see, *e.g.*, [24][40][41]). Such corrinatocobalt(III) compounds

have been considered to exist in aqueous solution in equilibrium between aquocobalt(III) forms with a hexacoordinated Co^{III} center and pentacoordinated species, lacking an axially bound H_2O ligand. At this stage, our data do not provide insights into the question of whether the 'base-off' forms of **1** and **3** exist as aquocobalt^{III} forms with a hexacoordinated Co^{III} center or with a pentacoordinated Co^{III} center (in aqueous solution at room temperature).

3. Conclusions and Outlook. – The unexpected finding of 'base-off' forms of corrinoid cofactors (such as coenzyme B_{12} (**2**)) in a variety of B_{12}-dependent enzymes has renewed the interest in vitamin B_{12} derivatives with unusual coordination properties. One important consequence of the constitutional difference at the pseudo-nucleotide base of the naturally occurring adenosyl-cobamides is their significantly differing tendency to exist in aqueous solution in the 'base-on' or 'base-off' form: the known structures of coenzyme B_{12} (**2**) represent the 'base-on' form, where the DMB base is coordinated in an intramolecular fashion, while those of **1** and **3** predominantly exist as the 'base-off' form. The natural predominance of the 'base-off' constitution of corrinoid cofactors in solution may be helpful for their recognition and binding as cofactors in B_{12}-dependent enzymes. Indeed, adenosyl-corrinoids derived from the 'base-off' '*p*-cresolyl'- and 'phenolyl'-cobamides have been found to bind strongly to those coenzyme-B_{12}-dependent enzymes that bind their corrinoid cofactor in a 'base-off'/' His-on' mode [42]. Investigations of binding to GlmS of *C. cochlearium* are underway and will be reported in due course.

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Experimental Part

1. *Materials.* Pseudovitamin B_{12} (=*Coβ*-cyano-(adenin-7-yl)cobamide; **4**) was prepared by 'guided biosynthesis' [10]; factor A (=*Coβ*-cyano-(2-methyladenin-7-yl)cobamide; **5**) was a gift of Prof. *P. Renz* (Stuttgart) [11]; 5′-*O*-tosyladenosine from *Sigma*; 5′-chloro-5′-deoxyadenosine was prepared according to [43]; benzoic acid *purum*, mercury *p.a.*, MeOH *puriss. p.a.*, tetrabutylammonium hexafluorophosphate ($(Bu_4N)PF_6$) *p.a.*, acetone puriss. *p.a.*, MeCN *purum*, isopropyl alcohol *purum*, dipotassium hydrogen phosphate *puriss. p.a.*, and potassium dihydrogen phosphate *puriss. p.a.*; DSS (sodium 3-(trimethylsilyl)propane-1-sulfonate) from *Fluka*; *Lichroprep RP-18* (25–40 μm) and pre-coated TLC plates *RP-18* F_{254S} both from *Merck* D_2O 99.9% from *Cambridge Isotope Laboratories*, water was purified by mixed-bed deionization, carbon adsorption, and filtration (*Barnstead NANOpure* system).

2. *Instrumentation*. Electrolysis: *Amel-550* potentiostat, *Amel-721* integrator. Glovebox: *Mecaplex GB-80*, N_2 atmosphere < 10 ppm O_2, determined with *Systech-Instruments EC-90-M* instrument. UV/VIS: in H_2O; *Varian Cary-100-Scan*; λ_{max} (log ε) in nm. CD: in H_2O; *Jasco J-715* spectropolarimeter; $\lambda_{max/min}$ ($\Delta\varepsilon$) in nm. NMR: *Varian 500-Unity-Plus* spectrometer; at 499.876 (^1H), 125.715 (^{13}C), and 50.658 MHz (^{15}N); soln. of **1**: conc. 6 mM, sample size 0.7 ml (H_2O/D_2O 9:1), pH 4.2, 100 mM phosphate buffer, 26°; soln. of **3**: conc. 6 mM, sample size 0.53 ml (H_2O/D_2O 19:1), pH 6.5, 26°; δ in ppm. FAB-MS: *Finnigan MAT 95S*, nitrobenzyl alcohol (NOBA) matrix, Cs bombardment, positive-ion spectrum; *m/z* (rel. int.). Model building: 'Sybyl' software (Version 6.3A) by *Tripos* [44].

3. *NMR Experiments. 2D TOCSY* [45]: Time-domain data size 256 × 1984 (complex); 16 scans per t_1 increment; mixing time: 70 ms (MLEV-17 [46][47], bracketed by 2 ms trim pulses, RF power 4.5 kHz); H_2O suppression with watergate echo [48]; squared shifted sine-bell functions in t_1 and t_2 dimensions; *States*

quadrature in F_1 [49]. *2D ROESY* [50][51]: Time-domain data size 256×1984 (complex); 64 scans per t_1 increment; mixing time: 200 ms (CW spin lock, RF power 2.3 kHz); H_2O suppression with watergate echo [52]; squared shifted sine-bell functions in t_1 and t_2 dimensions; *States* quadrature in F_1 [49]. *$^1H,^{13}C$ PFG-HSQC* [53][54]: Time-domain data size 512×1024 (complex); 16 scans per t_1 increment; a pair of z gradients (18.3 G/cm 2 ms and -18.1 G/cm 0.5 ms) was used for coherence selection; GARP decoupling [55] during acquisition (RF power 2.8 kHz); squared shifted sine-bell functions in t_1 and t_2 dimensions. *States* quadrature in F_1 [49]. *$^1H,^{15}N$ PFG-HSQC* [53][54]: Time-domain data size 64×1024 (complex); 512 scans per t_1 increment; a pair of z gradients (27.5 G/cm 2.5 ms and -13.7 G/cm 0.5 ms) was used for coherence selection; GARP decoupling [55] during acquisition (RF power 1 kHz); squared shifted sine-bell functions in t_1 and t_2 dimensions. *States* quadrature in F_1 [49]. *$^1H,^{13}C$* PFG-*HMBC* [53][31]: Magnitude-mode spectra were obtained by using the standard HMQC pulse sequence with an additional delay for the evolution of long-range heteronuclear coupling; time-domain data size 256×1408 (complex); 128 scans per t_1 increment; z gradients (2×9.2 G/cm 2 ms and 4.6 G/cm 2 ms) used for coherence selection and suppression of axial peaks; no decoupling during acquisition; squared sine-bell functions in both t_1 and t_2 dimensions. *$^1H,^{15}N$ PFG-HMBC* [53][31]: Time-domain data size 128×1984 (complex): 256 scans per t_1 increment; z-gradients (2×18.3 G/cm 2 ms and 3.7 G/cm 2 ms); no decoupling during acquisition; squared sine-bell functions in both t_1 and t_2 dimensions.

4. *Preparative Electrochemistry. General* [28]. The electrolysis cell consisted of two compartments connected by a salt bridge, separated by a medium-porosity glass frit. The electrolyte soln. was 0.1M $(Bu_4N)PF_6$ in MeOH. As electrodes, a Hg-pool working electrode, a Pt-wire counter electrode, and an aq. 0.1N calomel electrode (0.1N CE) as reference electrode were used. All solvents and liquids were degassed prior to the synthesis, which was carried out in a glovebox under N_2 (partial pressure of $O_2 < 10$ ppm) at r.t. All handling of organometallic B_{12} derivatives was carried out with protection from light.

Pseudocoenzyme B_{12} (=Co*β*-*(5′-Deoxyadenosin-5′-yl)cobinamide* f-*(Dihydrogen Phosphate) Inner Salt 3′-Ester with 7-α-*D-*Ribofuranosyl-7*H-*purin-6-amine*; **1**). The salt bridge and the anodic compartment of the electrolysis cell were filled with electrolyte soln.; the bottom of the cathodic compartment was covered with Hg. Pseudovitamin B_{12} (50 mg, $3.72 \cdot 10^{-5}$ mmol; **4**), from 'guided biosynthesis' with a culture of *Propionibacterium acidi-propionici* [10], and benzoic acid (5.9 mg, $4.84 \cdot 10^{-5}$ mol) were dissolved in electrolyte soln. (4 ml) and were placed into the cathodic compartment. The reduction was performed at -1.1 V referring to the 0.1N CE.After 2 h, when 6.386 C had been consumed, 5′-*O*-tosyladenosine (31.3 mg, $7.44 \cdot 10^{-5}$ mol) in a minimum amount of MeOH was added, and the soln. was stirred for another 2 h in the dark while electrolysis continued. The reaction was monitored by UV/VIS spectroscopy and by TLC (*RP18* plates, H_2O/MeCN/MeOH 9:4:2). The mixture was transferred to a separatory funnel, and after the addition of H_2O (5 ml), it was extracted with CH_2Cl_2 (3×10 ml). The aq. phase was evaporated. The red residue was dissolved in a minimum amount of H_2O and crude **1** was precipitated by dropwise addition of acetone followed by incubation at 4° overnight. Crystallization did not occur. The solid was washed with acetone and dried under vacuum: 31.5 mg (54%) of chromatographically pure **1**. A second precipitate was obtained by further addition of acetone to the mother liquor, which was contaminated with unreacted **4**. The product could be purified by column chromatography. For this purpose, *RP18* (10 g) was soaked in i-PrOH and then equilibrated to H_2O in the column. The red residue in a minimum amount of H_2O was applied and **1** eluted with H_2O/MeCN/MeOH 18:1:1 increasing successively the portion of MeCN and MeOH up to a ratio of 4:1:1. The red product was dried under vacuum: 17.9 mg (30.7%) of additional **1**. Total yield: 49.4 mg (84.7%) of **1**. This material was used for spectral analysis by MS and UV/VIS, CD and NMR spectroscopy.

Adenosyl-Factor A (=Co*β*-*(5′-Deoxyadenosin-5′-yl)cobinamide* f-*(Dihydrogen Phosphate) Inner Salt 3′-Ester with 2-Methyl-7-α-*D-*ribofuranosyl-7*H-*purin-6-amine*; **3**). As described above for **1**, factor A (40 mg, $2.95 \cdot 10^{-5}$ mol; **5**) and benzoic acid (4.7 mg, $3.83 \cdot 10^{-5}$ mol) in electrolyte soln. (4 ml) were reduced at -1.1 V *vs.* 0.1N CE. After 3 h 40 min, when 5.78 C had been consumed, 5′-chloro-5′-deoxyadenosine (17.0 mg, $5.89 \cdot 10^{-5}$ mol) [43] in a minimum amount of MeOH was added, and the soln. was stirred for another 21/2 h in the dark while electrolysis continued. Reaction monitoring, workup, and isolation as described for gave **3**. A chromatographically uniform orange-red solid was obtained by precipitation and was dried under vacuum: 32.4 mg (69.5% of **3**. This material was used for spectroscopic analysis.

5. *Spectroscopic Analysis. Pseudocoenzyme B_{12}* (**1**). UV/VIS ($c = 1.09$ mM, 20 mM potassium phosphate buffer, pH 7, 20°: 460(3.89), 376(3.92), 304(4.28). CD ($c = 1.09$ mM, 20 mM potassium phosphate buffer, pH 7, 20°): 579(3.30), 507(−3.81), 471(1.47), 430(−4.04), 400(2.42), 364(−1.26), 330(18.8); λ_0: 533, 484, 457, 411, 378, 357, 293 (*Fig. 3*). FAB-MS: 1570.9(29), 1569.7(60), 1568.6(100, $[M+H]^+$), 1320.8(15), 1319.8(47), 1318(84, $[M+H-\text{adenosyl}]^+$), 1183.2(24). ^{1}H-, ^{13}C-, and ^{15}N-NMR: *Tables 2–5*, *Fig. 5*,*a*.

Adenosyl-Factor A (**3**). UV/VIS ($c = 1.00$ nM, 20 mM potassium phosphate buffer, pH 7, 20°): 463(3.80), 374(3.89) (*Fig. 4*). CD ($c = 1.00$ mM, 20 mM potassium phosphate buffer, pH 7, 20°): 591(2.16), 510(−3.26), 471(2.45), 429(−2.26), 399(2.30), 363(−2.26), 331(13.51); λ_0: 552, 489, 451, 413, 375, 354, 293. FAB-MS: 1584.8(35), 1583.8(75), 1582.8 (100, $[M+H]^+$); 1334.8(35), 1333.8(75), 1332.8(80, $[M+H-\text{adenosyl}]^+$); 1183.6(30.0), 1069.6(33). ^{1}H-, ^{13}C-, and ^{15}N-NMR: *Tables 2–4* and *Fig. 5*,*b*.

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