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Partial Synthesis of $Co_\alpha Co_\beta$-Dicyano-176-Norcobinamide[#]

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Summary. Recent interest in norvitamin B_{12}-derivatives, homologues of complete vitamin B_{12}-derivatives, lacking the methyl group at carbon 176, stems from the identification of the corrinoid cofactor of the tetrachloroethene reductive dehalogenase of *Sulfurospirillum multivorans* as 176-nor-pseudovitamin B_{12}. Here we report the partial synthesis of the corrinoid $Co_\alpha Co_\beta$-dicyano-176-norcobinamide by condensation of cobyric acid and 2-aminoethanol. In addition, the partial synthesis of crystalline Co_α-aquo-Co_β-cyanocobyric acid by acid catalyzed hydrolysis of vitamin B_{12} is detailed, improving the method and the isolation procedure worked out earlier by *Bernhauer et al.* The solution structure of $Co_\alpha Co_\beta$-dicyano-176-norcobinamide was studied by spectroscopy and was compared with that of the homologue $Co_\alpha Co_\beta$-dicyanocobinamide. The title compound, $Co_\alpha Co_\beta$-dicyano-176-norcobinamide, represents the dicyano-form of a potential biosynthetic precursor of the 176-nor-B_{12}-derivatives, such as 176-nor-pseudovitamin B_{12}.

Keywords. Corrinoid; Dehalogenase; Synthesis; Vitamin B_{12}.

Introduction

The reductive dehalogenases of anaerobic bacteria, which couple the dehalogenation of chlorinated substrates to energy conservation (dehalorespiration) [1], use a B_{12}-derivative as cofactor [2, 3]. We recently elucidated the structure of the cyano-Co(III)-form of the corrinoid cofactor of tetrachloroethene reductive dehalogenase (PCE-dehalogenase) of *Sulfurospirillum multivorans* and identified it as 176-norpseudovitamin B_{12} (**1**, Co_β-cyano-[7-adeninyl]-176-norcobamide) [4]. This novel corrinoid is a homologue of pseudovitamin B_{12} (**2**, Co_β-cyano-[7-adeninyl]-cobamide) and is devoid of the methyl group at carbon-176 of the cobamide ligand [5] (see Fig. 1). The ''complete'' corrinoid **1** thus turned out to be the first known naturally occurring B_{12}-cofactor lacking such a characteristic peripheral methyl group of the cobamide moiety [4, 6, 7].

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[#] Dedicated to Prof. *Ulrich Schubert*

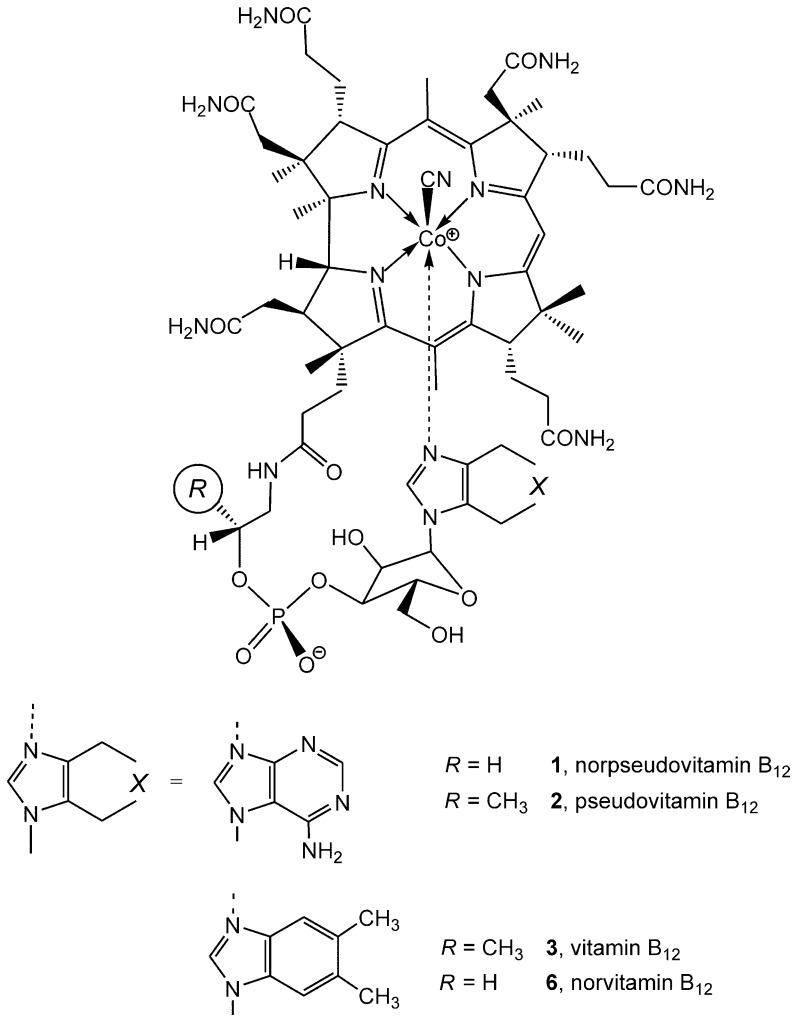


Fig. 1. Structural formulae of "complete" corrinoids, with variation of the nucleotide base and/or the substituent *R* in the nucleotide-loop: 176-norpseudovitamin B_{12} (**1**, Co_{β}-cyano-[7-adeninyl]-176-norcobamide), pseudovitamin B_{12} (**2**, Co_{β}-cyano-[7-adeninyl]-cobamide), vitamin B_{12} (**3**, Co_{β}-cyano-[1-(5,6-dimethyl)benzimidazolyl]-cobamide), and 176-norvitamin B_{12} (**6**, Co_{β}-cyano-[1-(5,6-dimethyl)benzimidazolyl]-176-norcobamide)

So far, the natural "complete" corrinoid cofactors were known to vary only according to the structure of their "nucleotide base", *e.g.* an adenine in pseudovitamin B_{12} (**2**), but a 5,6-dimethylbenzimidazole in vitamin B_{12} (**3**) [6, 7]. The major causes for this structural variability have been suggested to be the respective biosynthetic capacities of the host organisms, rather than to have a functional significance [8]. The discovery of natural 176-norcobamides has raised the question concerning the structural effect and role of the "conserved" extra methyl group at carbon 176 of the nucleotide linker of vitamin B_{12} (**3**) and of the other known "complete" corrinoids [4].

This paper reports the partial synthesis of $Co_{\alpha}Co_{\beta}$-dicyano-176-norcobinamide (**4**) as a possible preparative entry to the more complex norcobamides, such as 176-

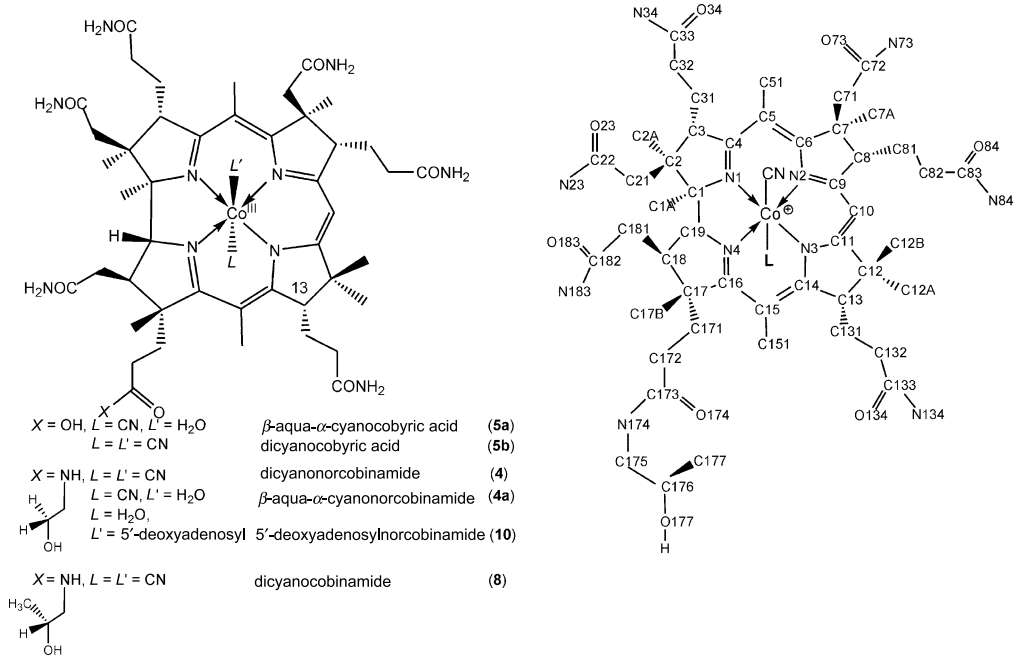


Fig. 2. Left: Structural formulae of "incomplete" corrinoids: dicyano-176-norcobinamide (**4**), β-aqua-α-cyanonorcobinamide (**4a**), β-aqua-α-cyanocobyric acid (**5a**), dicyanocobyric acid (**5b**), dicyanocobinamide (**8**), and 5′-deoxyadenosylnorcobinamide (**10**); aquocyanoneocobyric acid (**7a**) and dicyanoneocobinamide (**9**) are C(13)-epimers of **5a** and **8**, respectively; right: Atom numbering system used here for cobyric acid derivatives and cobinamides [5]

norpseudovitamin B_{12} (**1**, see Figs. 1 and 2). Cobyric acid is a natural B_{12}-derivative which represents the corrin nucleus of vitamin B_{12} (**3**) [6] and was selected as the corrinoid starting material. In cobyric acid, the six side-chains terminating with amide groups are distributed identically about the periphery as in **3**, but in the *f*-position there is a free carboxylate group. β-Aqua-α-cyanocobyric acid (**5a**) and dicyanocobyric acid (**5b**) were first isolated by the groups of *Bernhauer et al.* in 1960 [9] and the structure was elucidated by the conversion of **5a** to known corrinoid compounds, including vitamin B_{12} (**3**) [10]. Cobyric acid was also analyzed by X-ray crystallography by *Venkatesan* and co-workers and was thus shown to crystallize in its β-aqua-α-cyano form **5a** [11]. Subsequently, cobyric acid (either as **5a** or **5b**) has been prepared by the degradation of vitamin B_{12}, with concentrated hydrochloric acid by *Bonnett et al.* [12], or with hydrogen fluoride or zinc chloride to form the ester of cobinamide, followed by acylation of the amino group and hydrolysis to give cobyric acid, by *Müller* and *Müller* [13]. In spite of being available by partial degradation of vitamin B_{12} (**3**), as described [12, 13], both the published methods of preparing cobyric acid from **3** have the disadvantages of low yields, long and tedious purification methods, large number of other hydrolysis products, and the use of phenol for the extraction of the corrinoids.

Crystalline β-aqua-α-cyanocobyric acid (**5a**) was prepared in the 1970s by the outstanding synthesis efforts of *Eschenmoser* [14] and *Woodward* [15] in the course of their (formal) total syntheses of vitamin B_{12} (**3**). We have set out to gain an improved preparative access to β-aqua-α-cyanocobyric acid (**5a**) from vitamin B_{12} (**3**) by still

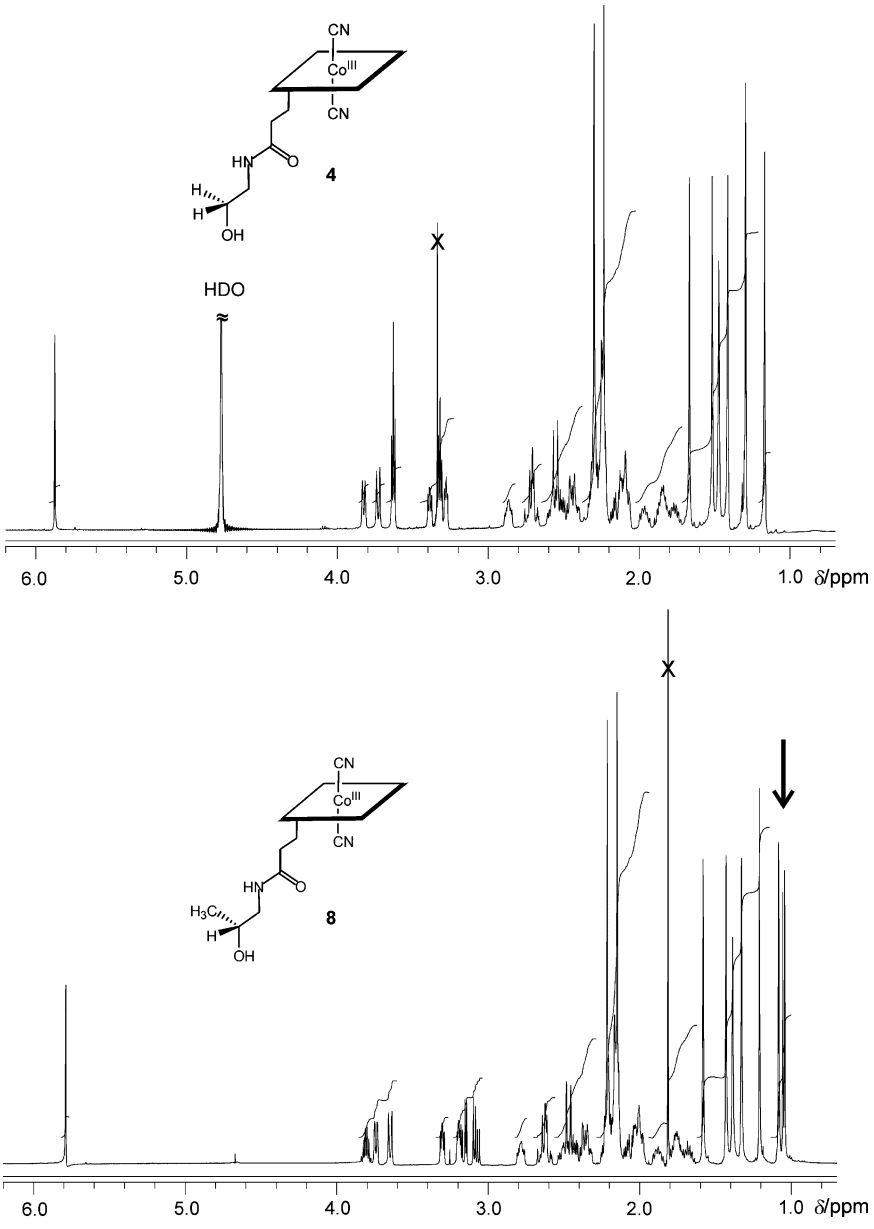


Fig. 3. 500 MHz ^{1}H NMR spectra of aqueous solutions of dicyano-176-norcobinamide (**4**, top) and dicyanocobinamide (**8**, bottom); the arrow in the spectrum of dicyanocobinamide (**8**) points to the doublet signal due to the methyl group $H_3C(177)$ which is missing in dicyano-176-norcobinamide (**4**); solvent signals are marked by an "x"

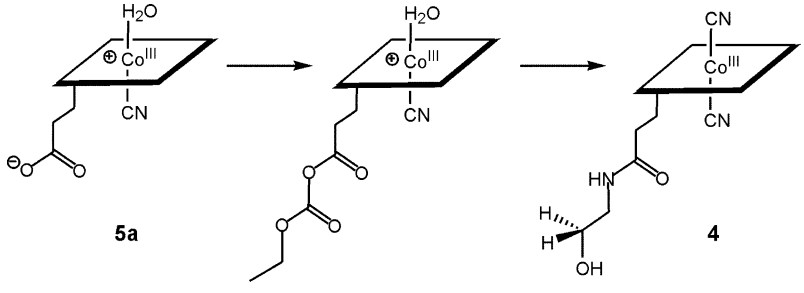
applying the procedure of *Bonnett et al.* [12, 16] for the hydrolysis of **3**, and to focus on improving the method for isolation and purification. The synthesis of $Co_\alpha Co_\beta$-dicyano-176-norcobinamide (**4**) is thus based on the known procedure for the synthesis of β-aqua-α-cyanocobyric acid (**5a**) [12] and using a more efficient method for the purification and isolation of **5a**. The title compound **4** and dicyanocobyric acid (**5b**) were subjected to extensive spectroscopic characterization, as also reported here.

Results and Discussion

Synthesis

To obtain pure β-aqua-α-cyanocobyric acid (**5a**), the procedure of *Bonnett et al.* [12, 16] was carried out, as originally outlined where cyanocobalamin (vitamin B_{12}, **3**) is stirred in concentrated HCl for 18.5 min at 65°C. The use of column chromatography with "reversed phase" adsorbent (RP-18) allowed the development of a much easier and quicker purification procedure to isolate dicyanocobyric acid (**5b**), where no phenol-ether extraction was required any more. Instead, the diluted acidic reaction mixture was neutralised with $NaHCO_3$ and concentrated to near dryness before the corrinoid material was dissolved in methanol, and the remaining salt was filtered off. This material, in its dicyano form, could then be purified by RP-18 column chromatography giving pure fractions of dicyanocobyric acid (**5b**), dicyanoneocobyric acid (**7b**) [16, 17], dicyanocobinamide (**8**) [18], dicyanoneocobinamide (**9**) [16, 17], along with more polar fractions of the dicyanomonocarboxylic acids of cobinamide, and dicyanomonocarboxylic acids of neocobinamide. Acetic acid was then added to each pure fraction of the dicyano-isolates **5b** and **7b**, to convert these isolates into their respective crystallizable aqua/cyano forms **5a** and **7a** [17].

By our method a higher yield of β-aqua-α-cyanocobyric acid (**5a**) has routinely been obtained (generally 17–20% compared to 10% previously [12]). This improvement appears to be the consequence of the more efficient purification, which causes less loss of material. However, the required acidic reaction conditions still result in the formation of corrinoids belonging to the so called neo-series, which are (C13)-epimers of the "normal" corrinoids [16]. As a side product, therefore, aquacyanoneocobyric acid (**7a**) was obtained, in 15–17% yield [17]. This disadvantage could be compensated (with some additional effort), as it has been shown that equilibration between neo- and "normal"-cobyric acid can be achieved by stirring in trifluoracetic acid at room temperature [16]. The other hydrolysis products were dicyanocobinamide (**8**) and dicyanoneocobinamide (**9**), which were isolated in 17% and 18% yields (besides about 7% each of more polar corrinoid fractions, tentatively identified as aquacyanomonocarboxylic acids of cobinamide and of neocobinamide). It should be noted, all these yields could vary if the procedure of *Bonnett et al.* [12] was not accurately followed. The cobyric acid derivatives **5a** and **7a** [17] were obtained as isomerically pure, crystallized samples using water/acetone.



Scheme 1

Table 1. ^{1}H and ^{13}C NMR data (ppm) for dicyano-176-norcobinamide (**4**) and dicyanocobyric acid (**5b**)

Assignment*	^{1}H for dicyano-norcobinamide (**4**)	^{1}H for dicyano-cobyric acid (**5b**)	^{13}C for dicyano-norcobinamide (**4**)	^{13}C for dicyano-cobyric acid (**5b**)
C(1)			85.7	86.1
CH_3(1A)	1.47	1.49	24.3	24.4
C(2)			48.8	49.2
CH_3(2A)	1.52	1.53	19.1	19.1
CH_2(21)	2.25, 2.32	2.25, 2.31	44.8	44.9
C(22)				
CH(3)	3.84	3.82	59.0	58.9
CH_2(31)	1.97, 2.27	1.99, 2.28	27.6	27.5
CH_2(32)	2.48, 2.54	2.43, 2.53	37.6	37.6
C(33)				
C(4)			179.3	179.5
C(5)			107.6	107.8
CH_3(51)	2.24	2.24	17.9	17.8
C(6)			165.5	165.7
C(7)			51.7	52.1
CH_3(7A)	1.67	1.67	21.3	21.4
CH_2(71)	2.59, 2.26	2.56, 2.27	46.4	46.5
C(72)				
CH(8)	3.39	3.38	57.9	58.0
CH_2(81)	1.76, 2.11	1.77, 2.11	29.3	29.2
CH_2(82)	2.25	2.26	34.6	34.5
C(83)				
C(9)			174.6	174.6
CH(10)	5.87	5.85	93.8	93.5
C(11)			180.1	180.4
C(12)			49.4	49.7
CH_3(12A)	1.42	1.42	21.2	21.3
CH_3(12B)	1.17	1.17	33.1	32.9
CH(13)	3.28	3.28	56.0	55.8
CH_2(131)	1.84, 2.09	1.82, 2.12	28.3	28.6
CH_2(132)	2.18, 2.29	2.12, 2.35	34.4	34.5
C(133)				
C(14)			165.9	165.9
C(15)			105.7	106.5
CH_3(151)	2.30	2.30	17.4	17.6
C(16)			179.9	180.8
C(17)			61.3	62.0
CH_3(17B)	1.29	1.29	20.0	20.1
CH_2(171)	1.84, 2.43	1.84, 2.38	35.2	36.0
CH_2(172)	2.07, 2.58	2.01, 2.50	34.2	36.2
C(173)				
CH_2(175)	3.32		44.3	
CH_2(176)	3.63		62.8	
CH(18)	2.87	2.88	41.8	41.8
CH_2(181)	2.72	2.70	35.0	35.4
C(182)				
CH(19)	3.73	3.73	77.9	78.0

* ^{1}H and ^{13}C signal assignments from N*O*E-, ^{1}H,^{13}C-HSQC-, and ^{1}H,^{13}C-HMBC-spectra [20]

With a method for producing β-aqua-α-cyanocobyric acid (**5a**) in hand the target compound, dicyanonorcobinamide (**4**), could now be synthesized. Using a slightly modified version of the procedure of *Bernhauer et al.* [9], **4** was obtained from crystalline **5a** in a yield of over 95% by *in-situ* formation of a mixed anhydride, using triethylamine and ethyl chloroformate, followed by the addition of ethanolamine (Scheme 1). In one type of experiment, after work-up, the red product was directly precipitated using water/acetone to give over 95% of the aquacyanonorcobinamide **4a**, which was uniform (TLC). Addition of stoichiometric amounts of cyanide during the work-up gave the purple dicyano form **4**, which was isolated in over 90% yield also.

Spectroscopic Properties

As a detailed solution structural analysis for cobyric acid was not available, dicyanocobyric acid (**5b**) was subjected to full spectroscopic analysis, including UV-Vis-, CD-, FAB-MS-, and ^{1}H NMR spectroscopy [19, 20]. The signals of 56 non-exchangeable H-atoms were assigned, as well as the signals of 38 of its 46 carbons (all, except for the seven carbonyl carbons of the amide side chains; from indirect detection *via* HSQC- and HMBC-spectra [20], see Table 1). The singlet of the vinyl H(C10) occurs at $\delta = 5.85$ ppm, characteristic of a dicyanocobyrinate [19, 21]. For practical reasons, an extensive spectral analysis was only carried out for **5b**, although β-aqua-α-cyanocobyric acid (**5a**) was the original crystalline isolation form. Indeed, when **5a** was dissolved in D_2O for ^{1}H NMR spectroscopy, it equilibrated to a mixture of the two isomeric aqua/cyano forms. The positive ion FAB-MS of crystallized **5a** showed two major signals at $m/z = 958.5$ and 932.3, corresponding to the ions $[M\text{-}H_2O]^+$ and $[M\text{-}H_2O\text{-}CN]^+$.

For 176-norcobinamide, both in its aquacyano- (**4a**) and dicyano-form (**4**), the UV-Vis spectra show the typical pattern [6]. A detailed spectroscopic analysis (including ^{1}H and ^{13}C NMR) was again carried out only for the dicyano-form **4**. When the spectrum of **4** was compared to those of dicyanocobyric acid (**5b**) and of dicyanocobinamide (**8**, see Fig. 3), there were only the expected substituent effects on the chemical shift values of the side chain extending from C17. In the ^{1}H NMR spectrum, the two CH_2-groups (CH_2(175) and CH_2(176)) gave two triplets at 3.34 and 3.63 ppm. As was the case with norpseudovitamin B_{12} (**1**) [4, 22] and norvitamin B_{12} (**6**) [23], a high field doublet of a methyl group at C(176) was lacking. The remarkable similarity of the ^{1}H NMR spectra of **4** and **5b** (except for the mentioned differences in the *f*-side chain) is in line with a conformationally rigid corrin ring in dicyanocobalt corrins [19, 21, 24]. The EI-MS for **4a** shows a major signal at 1001.7 $[M + H\text{-}H_2O]^+$ which is 14 mass units lower than the major signal obtained for dicyano-cobinamide (**8**) (1016, $[M + H\text{-}CN]^+$) [25].

Conclusions

The title compound, dicyano-176-norcobinamide (**4**), was synthesized and its spectroscopic properties in solution were characterized (an NMR characterization of cobyric acid was carried out also in its dicyano-form, **5b**). The norcobinamide **4** was obtained *via* condensing ethanolamine with β-aqua-α-cyanocobyric acid (**5a**).

The NMR comparison with cobinamide showed no unexpected differences except for the local substituent effects from the lack of CH_3(177) [4, 23]. The starting material for this work, β-aqua-α-cyanocobyric acid (**5a**), can now be more easily prepared from the hydrolysis of cyanocobalamin. The dicyano-176-norcobinamide (**4**) is a rational starting material for the synthesis (by electrochemical means [26]) of Co_β-adenosylnorcobinamide (**10**) [27]. This organometallic norcobinamide is a likely, direct biosynthetic intermediate for 176-nor-B_{12} derivatives [4, 28], such as 176-norvitamin-B_{12} (**6**) [23] and 176-norpseudovitamin B_{12} (**1**) [4]. Initial tests for the take-up of dicyano-176-norcobinamide (**4**) and the biosynthetic incorporation of **4** [28] with formation of 176-norvitamin-B_{12} (**6**), 176-norpseudovitamin B_{12} (**1**), and other 176-nor-B_{12} derivatives by suitable organisms are now to be undertaken.

Experimental Part

Cyanocobalamin, Hoffmann-La Roche; water purified using Epure, Barnstead Co.; acetone, CH_2Cl_2, CH_3OH, CH_3COOH, KCN, MeCN, ethanolamine, triethylamine, hydrochloric acid fuming 37%, all Fluka puriss pa, or Fluka MicroSelect; *DMF*, Fluka, puriss, absolute, over molecular sieves ($H_2O < 0.01\%$); ethyl chloroformate, Fluka *purum*. The *pH* values were measured with a WTW SenTix 41 electrode connected to a WTW inoLab digital pH *meter*. TLC: RP18 F254s TLC plates 0.25 mm (Merck No. 115389). UV-Vis Spectra: Hitachi-U3000. CD Spectra: Jasco J715. ^1H, and ^{13}C NMR spectra: Varian Unity 500plus; δ(H) in ppm referenced to $\delta(HDO) = 4.76$ ppm and $\delta(^{13}C)$ in ppm referenced to external *TSP*, spectra were recorded at 26°C. Mass spectra: Fast atom bombardment FAB-MS: Finnigan MAT 95S, positive-ion mode; glycerine; Cs gun.

β-Aqua-α-cyanocobyric acid (**5a**)

Cyanocobalamin (**3**, 0.5 g, 0.37 mmol) was dissolved in 37% aqueous hydrochloric acid (69 cm^3). The reaction flask was immersed in a water bath at 65°C for 18.5 min with slow stirring. The reaction mixture was then poured into a large conical flask containing about 350 cm^3 water and neutralized by the addition of $NaHCO_3$ (70.5 g). The reaction mixture was concentrated on the rotatory evaporator. A whitish precipitate (salt) was removed by filtration and was washed with methanol. The methanol solution and the liquid reaction mixture were combined, and re-evaporated to dryness. The residue was dissolved in 2 cm^3 H_2O (to which 0.1 cm^3 0.1 *M* KCN were added) and was subjected to a column (RP-18, 3×20 cm) for chromatography. Initially, the column was washed with 20 cm^3 water (to which 20 mm^3 0.1 *M* KCN were added) only, to remove any of the remaining salt followed by a solvent gradient of 95% H_2O : 5% *Me*CN (0.1 cm^3 0.1 *M* KCN were added to 100 cm^3 of solvent mixture), raising to 70% H_2O : 30% *Me*CN. The corrinoid hydrolysis products came off in the following order: acidic products (*i.e.* unidentified tri- and diacids), dicyanoneocobyric acid (**7b**), dicyanocobyric acid (**5b**)[a], monoacids of neocobinamide, monoacids of cobinamide, dicyanoneocobinamide (**9**) and dicyanocobinamide (**8**).

[a] The initial identification of cobyric acid from the reaction mixture was achieved by direct comparison to an authentic sample, obtained from Prof. *R. Bonnett*. This was done by co-spotting both the authentic sample and material obtained from the reaction mixture using RP-18 TLC analysis. In the dicyano-forms only one purple spot could be seen as was originally the case with β-aqua-α-cyanocobyric acid (one red spot), however if the samples of β-aqua-α-cyanocobyric acid were allowed to stand in solution for a long enough time, the alternative isomer also formed. This led to two red spots appearing by TLC, with both compounds having the main cobyric acid structure. Further analysis and comparisons were then made using FAB-MS and finally NMR spectroscopy to confirm the structure as cobyric acid (see below)

To each product fraction a drop of acetic acid was added, to convert the purple dicyanocorrinoids into their respective red aqua-cyano forms. The red fractions were concentrated and dried to give solid red residues. The raw yields for each of the products were aquacyanocobyric acid (**5a**) 72 mg (20%), aquacyano-neocobyric acid (**7a**) 61 mg (17%), aquacyano-cobinamide 65 mg (17%), aquacyanoneo-cobinamide 69 mg (18%), a mixture of aquacyanomonocarboxylic acids of cobinamide 26 mg (7%), and a mixture of aquacyanomonocarboxylic acids of neocobinamide 26 mg (7%).

To crystallize cobyric acid the homogenous fraction was first precipitated using H_2O/acetone. The resulting red precipitate was then re-dissolved in H_2O and acetone was added until the point of turbidity. The mixture was then left at about 5°C for 48 h. One or two more drops of acetone were then added (to the point of turbidity) over the next few days and left at about 5°C, until no more crystalline product seemed to appear. The mother liquor was then removed and the β-aqua-α-cyano-cobyric acid (**5a**) was first washed with a 90% acetone 10% H_2O mixture followed by 100% acetone and dried. UV-Vis ($c = 1.0\times10^{-4}\,M$, methanol): λ_{max} (log ε) = 274.4 (4.01), 351.6 (4.32), 490.6 (3.85), 521.8 (3.82) nm; CD ($c = 1.0\times10^{-4}\,M$, methanol): λ_{max}, λ_{min} ($\Delta\varepsilon$) 256.5 (−43.5), 301.5 (−10.7), 352.5 (14.0), 371.5 (−5.5), 429 (28.3), 485 (−14.8) nm (mol^{-1} cm3 cm^{-1}); $\lambda_0 = 240$, 329, 363, 393, 464, 531 nm; FAB-MS: $m/z(\%) = 959.39$ (43), 958.50 ($C_{46}H_{64}N_{11}O_8Co$, 60, $[M + H\text{-}H_2O]^+$), 934.39 (35), 933.40 (64), 932.29 (100, $[M + H\text{-}H_2O\text{-}CN]^+$).

Potassium dicyanocobyrate (**5b**)

Crystalline β-aqua-α-cyanocobyric acid (**5a**) (2.0 mg) was dissolved in 0.1 *M* KCN (*ca.* 0.1 cm^3) followed by the addition of acetone until the purple product precipitated. The solvent was then removed by a pipette and the product washed again in acetone. After removal of the acetone washings the product (2.0 mg) was dried under high vacuum. UV-Vis ($c = 1.0\times10^{-4}\,M$, methanol): λ_{max} (log ε) = 276.4 (3.99), 312.4 (3.92), 365.6 (4.41), 416.4 (3.32), 539.6 (3.88), 578.2 (3.96) nm; CD ($c = 1.0\times10^{-4}\,M$, methanol): λ_{max}, λ_{min} ($\Delta\varepsilon$) = 250.5 (−37.6), 308.5 (−34.3), 344.5 (−31.9), 393.5 (63.9), 422.5 (41.3), 575 (−9.3) nm (mol^{-1} cm^3 cm^{-1}); $\lambda_0 = 224$, 232, 371, 456 nm; ^{1}H NMR and ^{13}C NMR: see Table 1.

Aquacyano-176-norcobinamide chloride (**4a**)

Crystalline β-aqua-α-cyanocobyric acid (**5a**) (18 mg, 18.4 μmol) was dissolved in 7.5 cm^3 dry *DMF* and cooled to about −10°C in an ice-salt-H_2O bath, before 11.1 mg (109.9 μmol) triethylamine and 6.0 mg (55.2 μmol) ethyl chloroformate were added. After 20 min 11.2 mg (184 μmol) ethanolamine were added and the reaction was stirred for a further 40 min. H_2O (10 cm^3) was then added and the colored aqueous phase was washed three times with 20 cm^3 CH_2Cl_2, before the solvents were removed. The red residue was then dissolved in H_2O and was precipitated with acetone. Further material could be obtained from precipitating from the mother liquor, to give (after removal of the solvent and drying) in total 18 mg (96%) of the red product **4a**. UV-Vis ($c = 1.96\times10^{-4}\,M$, H_2O): λ_{max} (log ε) = 526.5 (3.42), 494.5 (3.45), 353 (3.91), 317.5 (3.61), 273 (3.67) nm; ESI-MS: m/z (%) = 1003.7 (18), 1002.7 (57), 1001.70 ($C_{48}H_{70}N_{12}O_8Co$, 100, $[M\text{-}H_2O\text{-}Cl]^+$), 975.7 (7, $[M\text{-}H_2O\text{-}CN\text{-}Cl]^+$).

Dicyanonorcobinamide (**4**)

Crystalline aquacyanocobyric acid (**5a**) (9.8 mg, 10 μmol) was dissolved in 0.7 cm^3 distilled methanol, followed by the addition of 1.5 cm^3 distilled, dry *DMF*. The red solution was dried under high vacuum and the red solid residue was then re-dissolved in 2 cm^3 *DMF*, followed by drying the mixture again under high vacuum over night. The dried solid residue was dissolved in 1.5 cm^3 distilled, dry *DMF* under inert gas again and cooled to 0–5°C in an ice-salt-H_2O bath, before 8.3 mm^3 (60 μmol) of Et_3N and 3.0 mm^3 (31 μmol) of ethyl chloroformate were added. After 5 min a sample (0.1 cm^3) of the reaction mixture was taken out and mixed with ethanolamine to check if the mixed anhydride had formed. Ethanolamine (3.6 mm^3, 60 μmol) was then added with protection from air and the reaction mixture was stirred at room temperature.

After 20 min 5 cm^3 brine were added and the red mixture was washed three times with 15 cm^3 CH_2Cl_2. The organic phase was separated and the solvents were removed from the aqueous mixture, using a rotaroy evaporator at room temperature. The solid red residue was dissolved in H_2O (2 cm^3) and applied to a Sep-pak column, that was eluted with H_2O (6 cm^3) to remove the salt. The red residue was then obtained from Sep-pak by eluting it with methanol. The solvents (H_2O + methanol) were evaporated to dryness on a rotatory evaporator. The dried red solid was dissolved in H_2O and applied to a column (RP-18) for chromatography. Polar impurities in their dicyano-form eluted with methanol:H_2O (1:8, containing 0.2 cm^3 0.1 *N* KCN/100 cm^3). The product **4** was then eluted with methanol:H_2O (1:5, containing 0.2 cm^3 0.1 *N* KCN/100 cm^3). Pure fractions of **4** were analysed by TLC before removing the solvents on a rotatory evaporator. The purple solid (mostly dicyano- form) was dissolved in H_2O (1 cm^3) and applied to a Sep-pak column, which was first washed with H_2O : 0.1 *M* acetic acid (2 cm^3 : 100 mm^3) to remove basic impurities (the colour changed to red, indicating the conversion of **4** into the aquocyano-form **4a**). The Sep-pak column was then washed with H_2O (containing 0.1 cm^3 0.1 *N* KCN/100 cm^3) to convert the red aquocyano-form into the purple dicyano-form, followed by washing with H_2O to remove excess of KCN. The purple corrinoid was eluted with methanol from the Sep-pak column. The solvents were evaporated, the purple solid was dissolved in about 0.1 cm^3 H_2O (shown by TLC, to be a single corrinoid) and was precipitated by adding acetone. The purple precipitate was dried overnight under high vacuum and 9.1 mg (91%) of pure dicyano-176-norcobinamide (**4**) were obtained. UV-Vis ($c = 4.84 \times 10^{-5}$ *M*, H_2O): λ_{max} (log ε) = 277.5 (3.88), 309 (3.85), 367.5 (4.33), 539 (3.80), 579.5 (3.84) nm; CD ($c = 4.84 \times 10^{-5}$ *M*, H_2O): λ_{max}, λ_{min} ($\Delta\varepsilon$) = 222 (6.9), 250 (−12.0), 307 (−12.9), 347 (−9.8), 367 (−7.5), 397 (21.9), 425 (15.4) nm (mol^{-1} cm^3 cm^{-1}); λ_0 = 235, 373, 470, 538, 569, 584 nm; ESI-MS: m/z(%) = 1068.6 (11), 1067.6 (23), 1066.6 (28, $[M+K]^+$), 1052.6 (12), 1051.6 (30), 1059.6 (38, $[M+Na]^+$), 1013.5 (9), 1012.5 (13, [M-2HCN + K]$^+$), 1004.6 (8), 1003.6 (31), 1002.6 (76), 1001.6 (100, $C_{48}H_{70}N_{12}O_8Co$ [M-CN]$^+$), 997.6 (10), 996.6 (16, [M-2HCN + Na]$^+$), 976.6 (6), 975.6 (9), 918.5 (6), 917.5 (15), 916.5 (25, [M-2CN-C_2H_5ON]); ^{1}H NMR (9.1 m*M* in D_2O, 500 MHz, see Fig. 3 and Table 1): δ = 1.17 (s, CH_3(12B)), 1.29 (s, CH_3(17B)), 1.42 (s, CH_3(12A)), 1.47 (s, 3H, CH_3(1A)), 1.52 (s, CH_3(2A)), 1.67 (s, CH_3(7A)), 1.72–1.92 (m, 3H, CH_2(81,131,171)), 1.91–2.01 (m, 1H, CH_2(31)), 2.04–2.20 (m, 4H, CH_2(81,131,132,172)), 2.20–2.35 (m, 13H, CH_2(21,31,71,82,132, superimposed by 2.24 (s, CH_3(51)) and 2.30 (s, CH_3(151))), 2.38–2.63 (m, 5H, CH_2(32,71,171,172)), 2.65–2.77 (m, 2H, CH_2(181)), 2.82–2.92 (m, 1H, CH(18)), 3.28 (dd, *J* = 6.0, 3.5 Hz, 1H, CH(13)), 3.32 (t, 2H, CH_2(175)), 3.39 (dd, *J* = 7.0, 5.0 Hz, 1H, CH(8)), 3.63 (t, 2H, CH_2(176)), 3.73 (d, *J* = 10.5 Hz, 1H, CH(19)), 3.84 (d, *J* = 8.5 Hz, 1H, CH(3)), 5.87 (s, 1H, CH(10)) ppm; ^{13}C-NMR (9.1 m*M* in D_2O, 125 MHz): see Table 1.

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