X-Ray Crystal Structure Analysis of Factor A (2-Methyladeninyl-cyanocobamide), a Native Vitamin B_{12}-Analogue

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Dedicated to Professor Dorothy C. Hodgkin *on the Occasion of Her 70th Birthday*

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Vitamin B_{12}-factor A (2-Methyladeninyl-cyanocobamide), Vitamin B_{12}, X-Ray Crystal Structure Analysis

The crystal and molecular structure of 2-methyladeninyl-cyanocobamide (factor A) has been determined. This compound crystallizes in space group $P2_12_12_1$ with $a = 2630.6\,(15)$, $b = 2210.6\,(13)$ and $c = 1592.1\,(9)$ pm. The structure has been solved by the heavy-atom method and refined by least-squares methods on the basis of 3682 X-ray counter data to $R = 0.166$ and $R_w = 0.148$. As far as we know, this is the first X-ray-investigation of a purine-corrinoid, which differs from cyanocobalamin (vitamin B_{12}) by containing a purine base instead of 5,6-dimethylbenzimidazole. The structure analysis of 2-methyladeninyl-cyanocobamide shows unambiguously, that the purine base coordinates with cobalt *via* NB9. The choice of NB9 as coordinative atom can be ascribed partly (or mainly?) to steric influences, since coordination *via* NB3, which was also discussed, would presumably lead to severe distortion of the nucleotide loop.

Introduction

Shortly after vitamin B_{12} (cyanocobalamin) had first been obtained in crystalline form [1, 2], a number of substances related to B_{12} were isolated and crystallized more or less at the same time: Pseudovitamin B_{12} and "pseudovitamin B_{12b}" from an incompletely identified microorganism isolated from rumen contents by Pfiffner *et al.* [3], "vitamin B_{12m}" from pig manure by Wijmenga [4], and "factor A" from bovine gut contents and faeces by Ford and Porter [5, 6]. It was not possible at that time to clearly distinguish or further separate the above B_{12}-preparations by chromatographic methods. However, paper electrophoresis (which was probably applied for the first time to B_{12} research in these investigations) showed them to be non-homogeneous: In 0.5 M acetic acid as buffer solution in the presence of cyanide several fractions appeared, one fraction of each B_{12}-preparation, however, moving at the same velocity (3.9×10^{-5} cm^2 V^{-1} s^{-1}); it was the main fraction of "factor A" and "vitamin B_{12m}" and was then finally designated factor A [7].

Factor A has been isolated from many kinds of bacteria [8], including *Chromatium vinosum* [9], and, like pseudovitamin B_{12}, is mostly found in anaerobically fermenting substrates such as ruminal [8, 10] and intestinal [11] contents, faeces [12] and sewage sludge [13]. Factor A probably always dominates among purine-B_{12}-analogues in these media, often, in conjunction with pseudovitamin B_{12}, occurring as the main constituent of the B_{12}-family, one example being sewage sludge [14]. Its occurrence in rumen contents is remarkable (2.15 µg factor A, 0.52 µg pseudovitamin B_{12}, 2.84 µg cobalamin and 1.24 µg cobinamide in 1 g of dry substrate on average [10]).

Factor A has also been prepared by "guided biosynthesis" by means of *Escherichia coli 113-3*, starting from cobinamide and 2-methyladenine, nucleoside of factor A or nucleotide of factor A [15], as well as *via Propionibacterium arabinosum* fermentation by addition of 2-methyladenine [16, 17]. A

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partial chemical synthesis of factor A [18, 19] used cobyric acid isolated from sewage sludge [20, 21] as starting material.

It is further to be noted [22] that, like other nucleotide-containing native B_{12}-analogues, factor A in the presence of 5,6-dimethylbenzimidazole is transformed by *Propionibacterium shermanii* into cobalamin *via* nucleoside exchange.

The biological activity of factor A has been thoroughly investigated and compared with that of vitamin B_{12} (*i. e.* 5,6-dimethylbenzimidazolylcobamide, its activity taken as 100%). Activity for growth rate: *E. coli 113-3* ca. 50% [23, 24], *Lactobacillus leichmannii* 40% [23], 20% [25] or 17% [26], *Euglena gracilis* 60% [23], *Ochromonas malhamensis* 0.0% [23, 27], *Flavobacterium* 0.6% [28], chicken ca. 1.0% [29]. Propionate oxidation: *O. malhamensis* 2% [30], *Flavobacterium* 92% [28]. Antipernicious activity: 0.0% [27] or "slight" [23]. The other purine-analogues of vitamin B_{12} isolated thus far show activities similar to those of factor A [31]. Like pseudovitamin B_{12}, factor A is not, or only extremely poorly so, absorbed from human intestine (it does not inhibit the absorption of cyanocobalamin in the intestine *in vitro* [17]). This can be attributed to the very poor affinity of the purine-B_{12}-analogues for the intrinsic factor [32].

It appears likely that factor A (as well as the other purine-analogues of vitamin B_{12}) represents an evolutionary older form of B_{12}, since it is found almost exclusively under anaerobic conditions and possesses no or only very slight activity toward animal cells [13]. In activated sludge (aerobic fermentation) one finds only extremely small amounts of factor A (and pseudovitamin B_{12}), as compared with cobalamin and its benzimidazole analogues [14]. It is interesting to note in this connection that *P. shermanii* under anaerobic conditions produces the cobinamide portion only, while the 5,6-dimethylbenzimidazole part of vitamin B_{12} is synthesized after exposure to air [8].

Physico-chemical investigations of factor A started with the detection of 2-methyladenine after decomposition of the corrinoid with 1–2 M HCl at 100 °C [33–36]. Hydrolysis of factor A by $Ce(OH)_3$ yielded cobinamide and the crystalline nucleoside, which was identified as 2-methyl-7-D-ribofuranosyladenine [37]. Similar to pseudovitamin B_{12} [38, 39], the ribose function in factor A binds to N 7 of the purine ring (contrasting the binding to N 9 in the case of nucleic acids). This binding of the ribose portion had been predicted by D. C. Hodgkin for pseudovitamin B_{12} [40] on steric grounds, since the central cobalt atom would not be able to coordinate at N 7. Protonation of the purine portion is responsible for the significant basicity of cyano-factor A by electrophoresis in acetic acid [7] (pK-values 2.9 for the corrinoid and 4.8 for the isolated nucleoside [37]). A further contribution toward the elucidation of the structure of factor A came through partial chemical synthesis [18, 19]. UV/VIS-spectra of the aqua-factor A, Co(II)-factor A (obtained by reduction of aqua-factor A by CO in water [41]), and Co(I)-factor A [42] were reported, as well as CD-spectra of Co(I)-factor A and mono- and dicyano-factor A [42]. The coordinative bond between the purine base and cobalt ion in factor A is weak, as compared with that in cobalamin. This is evidenced by relatively high affinity toward cyanide ion [43] and rapid reduction to the Co(I)-form by $NaBH_4$ in water [42]. According to Pfiffner *et al.* [44], there exist two modifications of factor A, both containing 2-methyladenine but differing in refractive indices. The same duality has been observed with pseudovitamin B_{12} [44]. However, no confirmation of the existence of these two modifications has been reported elsewhere.

Putting these results together, the structure of factor A was essentially clear, since the cobinamide portion has long been known from the X-ray structure determination of vitamin B_{12} [45] and from partial synthesis *via* cobyric acid and 1-aminopropanol-2 [46]. However, the details of the binding of the purine base to cobalt were not determined unambiguously, since coordination could conceivably take place *via* NB 9 or NB 3. This follows from comparable electron densities at both of these nitrogen atoms in adenine [47] and the ability of the purine ring to bind to metals either *via* the imidazole-N or *via* NB 3 [48, 49]. Another possibility would be an equilibrium of coordination isomers, especially in view of the postulated modifications [44]. The X-ray crystal structure analysis [50] reported here now shows 2-methyladenine to be coordinated to cobalt *via* NB 9. Furthermore, all crystallization attempts yielded one crystal modification only. Coordination *via* NB 9 (rather than NB 3) is probably dictated by steric requirements within the molecule as a whole, since a bond between cobalt and NB 3 would lead to undue distortions of the nucleotide loop. It is known that even small changes

within this loop, *e.g.* binding of phosphate to CR 2 or CR 5 (instead of CR 3) effect in cyanocobalamin a significant weakening of the coordinative bond of the nucleotide base [51].

As to our knowledge, this is the first X-ray structure determination of a purine analogue of vitamin B_{12}.

Experimental

Reagents and chemicals

Factor A (2-methyladeninyl-cyanocobamide) was prepared from sewage sludge [13, 14]. Elemental analysis data:

$(C_{60}H_{85}O_{14}N_{17}PCo \cdot 12\ H_2O\ 1574.543)$
Calc. C 45.77 H 6.98 N 15.12 P 1.97 Co 3.74
Found C 45.6 H 7.06 N 14.9 P 2.04 Co 3.87

X-ray analysis

Large crystals of factor A grown from aqueous solution were available. Preliminary X-ray investigation indicated that "air-dried" crystals were not sufficiently stable. We therefore decided to prepare "wet" crystals together with their mother liquor, and several crystals were sealed in 0.5 mm diameter Lindemann glass capillaries.

Since we were very concerned about the possibility of crystal decomposition, no further preliminary Weissenberg and precession photographs were taken and the "best" crystal was selected and transferred directly to a SYNTEX $P2_1$ FORTRAN diffractometer. From a rotation photograph 15 reflections of varying intensities were selected and centered for automatic lattice determination, which showed the crystal to be orthorhombic P. Oscillation photographs about the three prompted axes confirmed the data supplied by the diffractometer program. Θ-2 Θ scans were recorded numerically for selected reflections along each of the reciprocal axes, in order to check on the quality of the peak profiles under standard operating conditions. All were found to be satisfactory, and intensity data were now collected *via* a Θ-2 Θ scan in 96 steps using bisecting

Table I. Experimental data for the X-ray diffraction study of factor A (2-methyladeninyl-cyanocobamide) $C_{60}H_{85}O_{14}N_{17}PCo$[a]. The structure was refined to $R = 0.166$ and $R_w = 0.148$ for 3682 reflections with $|F_o| > 3\sigma(F)$. 93 non-hydrogen atoms and 7 oxygen (water) atoms.

(A) Crystal Data at 20 °C	
solvent molecules:	28 H_2O
crystal system:	orthorhombic
space group:	$P2_12_12_1$
molecular weight:	1358.35[a] dalton
density:	$D_{calc} = 1.34\ g \cdot cm^{-3}$ $D_{meas} = 1.33\ g \cdot cm^{-3}$ (by flotation)
$a = 2630.6\ (15)$ pm $b = 2210.6\ (13)$ pm $c = 1592.1(9)$ pm	$V = 9258.4 \cdot 10^6\ pm^3$ $Z = 4$ $F\ (000) = 2869$[a]
(B) Intensity Data	
radiation:	Mo-K α_1 ($\lambda = 70.926$ pm)
reflections measured:	+h, +k, +l
scan type:	coupled θ (crystal)-2θ (counter) (96 steps)
2θ range:	2.0° → 40.0°
scan speed:	linear variable between 6.0°/min for 150 counts/s or less and 29.3°/min for 2500 counts/s or more.
scan width:	$[2\theta(\text{Mo-K}\alpha_1) - 0.9]° \rightarrow [2\theta(\text{Mo-K}\alpha_2) + 0.9]°$.
background measurement:	stationary-crystal, stationary-counter at beginning and end of each scan, each for one-fourth of the time taken for the scan.
reflections collected:	4960 total, yielding 4856 allowed symmetry independent data.
absorption coeff.:	$\mu = 2.36$[a] cm^{-1}; no absorption correction made (see text).

[a] Without water of crystallization.

geometry. During data collection the stability of the entire assembly was monitored by measuring two strong check reflections after every 98 data. Analysis of the check reflection showed a steady (monotonic) decrease in intensity of one reflection over the period of data collection (40 h), the final intensity being ~ 85% of the initial intensity. A linear decay correction was therefore applied.

Following the data collection, some reflections close to $\chi = 90°$ and 270° were measured at 10° intervals of rotation about their diffraction vector. Examination of these ψ scans showed that the worst variation in intensity was less than 10%. This, together with the facts that we have a relatively small value for the absorption coefficient and that we observed some amount of decay of X-ray intensity for one check reflection, induced us not to apply empirical absorption corrections. All numerical details of crystal data and data collection are summarized in Table I.

A survey of the complete data set revealed the systematic absences h00 for $h = 2n + 1$, 0k0 for $k = 2n + 1$, and 00l for $l = 2n + 1$; the noncentrosymmetric orthorhombic space group $P2_12_12_1$ (D_2^4; No. 19) is uniquely indicated. All data were converted to $|F_0|$ values following correction for Lorentz and polarization effects.

The structure determination and refinement was carried out with 3682 reflections having $|F_0| > 3\sigma(F)$ and omitting some for extinction. The position of the

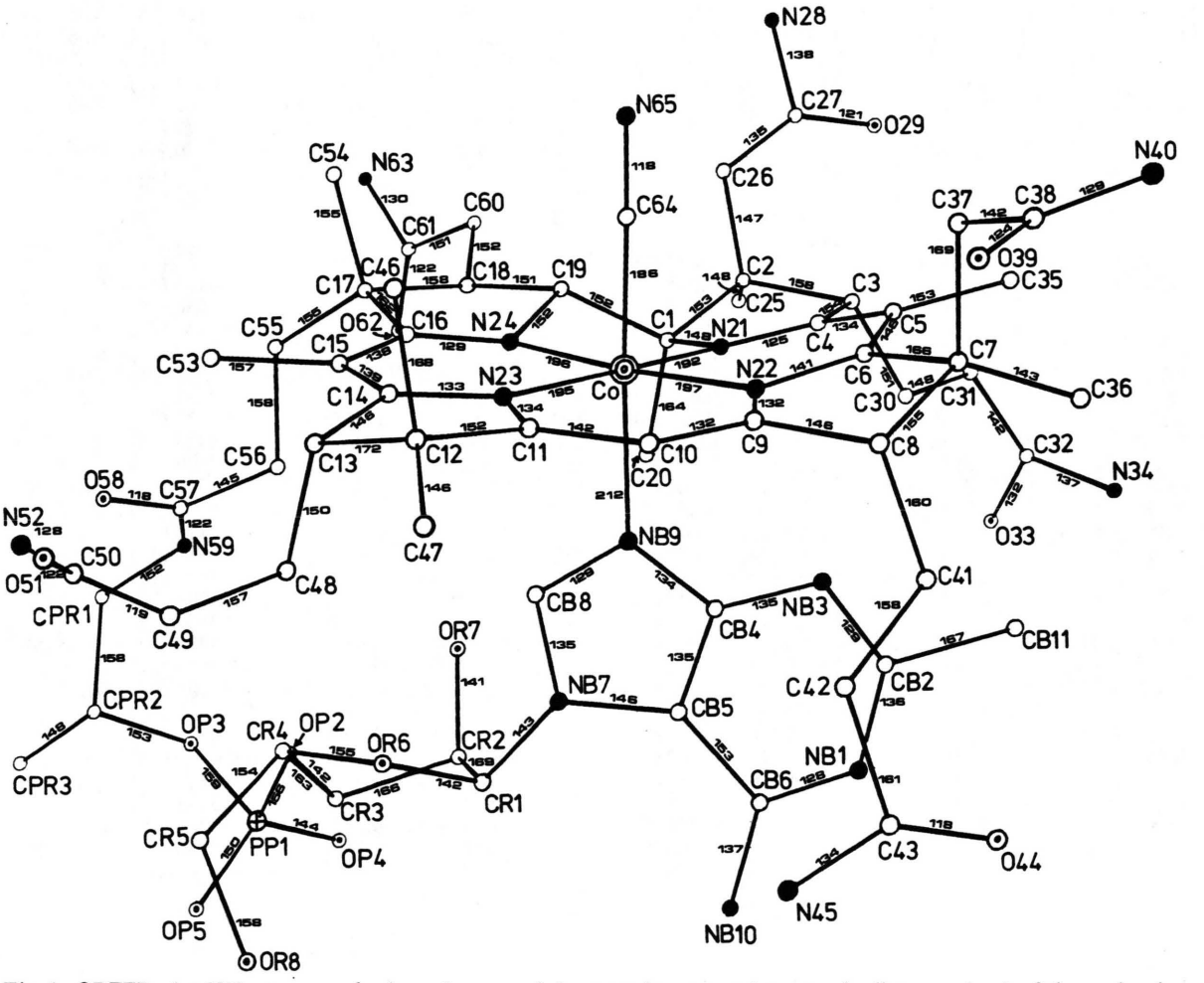

Fig. 1. ORTEP plot [52], atom numbering scheme and the most important interatomic distances (pm) of the molecular structure of factor A. The estimated standard deviations are: Co–N, 3 pm; all others ≦ 5 pm.

Table II. Atomic parameters of 2-methyladeninyl-cyanocobamide (factor A). Standard deviations are given in parentheses. Temperature factors are in the form: $T = \exp[-2\pi^2 U (2 \sin\theta/\lambda)^2]$. The Co- and P-atom were refined anisotropically. The starred values are U_{eq} with $U_{eq} = 1/3$ of the trace of the orthogonalized U_{ij} matrix. The anisotropic values are:

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Co	0.035	0.022	0.039	0.002	0.003	−0.002
P	0.064	0.029	0.062	0.011	−0.002	−0.001

Atom	X/A	Y/B	Z/C	U	Atom	X/A	Y/B	Z/C	U
Co	0.4907 (2)	0.3615 (2)	−0.0163 (3)	0.032 (2)*	C50	0.4847 (13)	0.3639 (17)	−0.4742 (21)	0.050 (5)
C1	0.5368 (12)	0.4438 (14)	0.1016 (19)	0.027 (5)	O51	0.4522 (10)	0.3331 (11)	−0.5086 (16)	0.076 (5)
C2	0.5295 (13)	0.4595 (15)	0.1942 (20)	0.029 (5)	N52	0.4957 (11)	0.4203 (12)	−0.4817 (18)	0.062 (5)
C3	0.5262 (12)	0.3960 (15)	0.2388 (20)	0.038 (5)	C53	0.4791 (12)	0.4896 (14)	−0.2722 (19)	0.038 (5)
C4	0.5060 (12)	0.3587 (15)	0.1643 (17)	0.024 (5)	C54	0.4500 (13)	0.5719 (15)	−0.0926 (20)	0.043 (5)
C5	0.4820 (12)	0.3066 (14)	0.1792 (18)	0.028 (5)	C55	0.5382 (12)	0.5801 (14)	−0.1567 (19)	0.031 (5)
C6	0.4712 (13)	0.2674 (15)	0.1077 (20)	0.037 (5)	C56	0.5903 (13)	0.5520 (15)	−0.1860 (20)	0.044 (5)
C7	0.4474 (13)	0.1979 (15)	0.1139 (20)	0.037 (5)	C57	0.6102 (13)	0.5808 (14)	−0.2606 (21)	0.031 (5)
C8	0.4573 (11)	0.1784 (13)	0.0217 (19)	0.027 (5)	O58	0.5891 (9)	0.5839 (10)	−0.3258 (14)	0.049 (5)
C9	0.4588 (13)	0.2339 (15)	−0.0275 (21)	0.031 (5)	N59	0.6501 (10)	0.6070 (11)	−0.2466 (16)	0.036 (5)
C10	0.4533 (13)	0.2410 (15)	−0.1094 (20)	0.034 (5)	C60	0.5190 (12)	0.5988 (14)	0.0552 (19)	0.039 (5)
C11	0.4572 (13)	0.2938 (16)	−0.1593 (20)	0.035 (6)	C61	0.5506 (13)	0.6530 (15)	0.0308 (20)	0.041 (5)
C12	0.4395 (14)	0.2917 (16)	−0.2504 (22)	0.052 (6)	O62	0.5952 (9)	0.6485 (11)	0.0106 (15)	0.066 (5)
C13	0.4651 (12)	0.3597 (16)	−0.2802 (19)	0.041 (5)	N63	0.5271 (11)	0.7045 (13)	0.0324 (18)	0.062 (5)
C14	0.4717 (12)	0.3875 (13)	−0.1976 (18)	0.023 (5)	C64	0.4237 (12)	0.3814 (13)	0.0091 (20)	0.036 (5)
C15	0.4833 (12)	0.4486 (14)	−0.1919 (18)	0.028 (5)	N65	0.3824 (12)	0.3982 (14)	0.0274 (19)	0.068 (5)
C16	0.4956 (13)	0.4727 (15)	−0.1144 (20)	0.036 (5)	NB1	0.6715 (12)	0.2248 (13)	0.0602 (18)	0.051 (5)
C17	0.5041 (13)	0.5439 (14)	−0.0954 (18)	0.034 (5)	CB2	0.6258 (14)	0.2363 (16)	0.0975 (21)	0.050 (6)
C18	0.5222 (13)	0.5432 (15)	−0.0006 (21)	0.052 (6)	NB3	0.5886 (11)	0.2698 (12)	0.0730 (18)	0.046 (5)
C19	0.5087 (13)	0.4828 (14)	0.0377 (18)	0.030 (5)	CB4	0.5962 (12)	0.2951 (14)	−0.0033 (20)	0.030 (5)
C20	0.5970 (12)	0.4415 (14)	0.0758 (19)	0.030 (5)	CB5	0.6383 (13)	0.2848 (15)	−0.0493 (20)	0.041 (5)
N21	0.5134 (10)	0.3833 (11)	0.0944 (15)	0.027 (5)	CB6	0.6811 (14)	0.2456 (15)	−0.0132 (23)	0.048 (5)
N22	0.4740 (10)	0.2790 (12)	0.0206 (17)	0.037 (5)	NB7	0.6311 (10)	0.3223 (12)	−0.1235 (16)	0.035 (5)
N23	0.4719 (10)	0.3490 (13)	−0.1332 (16)	0.039 (5)	CB8	0.5856 (12)	0.3498 (14)	−0.1153 (18)	0.031 (5)
N24	0.5052 (11)	0.4464 (11)	−0.0436 (15)	0.035 (5)	NB9	0.5654 (10)	0.3325 (11)	−0.0454 (15)	0.027 (5)
C25	0.5670 (12)	0.5013 (14)	0.2327 (19)	0.033 (5)	NB10	0.7251 (10)	0.2327 (12)	−0.0556 (15)	0.038 (5)
C26	0.4804 (14)	0.4898 (16)	0.2066 (21)	0.046 (6)	CB11	0.6177 (14)	0.2098 (16)	0.1947 (22)	0.065 (6)
C27	0.4678 (15)	0.4958 (17)	0.2881 (23)	0.059 (6)	CR1	0.6693 (13)	0.3357 (14)	−0.1849 (20)	0.040 (5)
N28	0.4327 (13)	0.5396 (15)	0.3096 (20)	0.083 (6)	CR2	0.6997 (13)	0.3995 (16)	−0.1551 (21)	0.046 (5)
O29	0.4829 (11)	0.4827 (13)	0.3573 (17)	0.093 (5)	CR3	0.7096 (13)	0.4222 (15)	−0.2532 (21)	0.042 (5)
C30	0.5763 (12)	0.3688 (14)	0.2661 (18)	0.034 (5)	CR4	0.6566 (14)	0.4088 (16)	−0.3032 (22)	0.057 (6)
C31	0.5898 (14)	0.3838 (16)	0.3542 (22)	0.057 (6)	CR5	0.6741 (15)	0.3921 (18)	−0.3928 (24)	0.081 (6)
C32	0.6342 (13)	0.3535 (17)	0.3825 (21)	0.057 (6)	OR6	0.6425 (8)	0.3482 (10)	−0.2606 (13)	0.044 (5)
O33	0.6811 (10)	0.3724 (12)	0.3697 (15)	0.084 (5)	OR7	0.6641 (9)	0.4387 (10)	−0.1177 (13)	0.043 (5)
N34	0.6332 (11)	0.2937 (13)	0.4044 (18)	0.071 (5)	OR8	0.7126 (10)	0.3377 (12)	−0.4001 (16)	0.092 (5)
C35	0.4779 (12)	0.2872 (14)	0.2712 (19)	0.042 (5)	PP1	0.7727 (4)	0.5115 (5)	−0.2711 (7)	0.052 (5)*
C36	0.4646 (12)	0.1590 (13)	0.1795 (18)	0.029 (5)	OP2	0.7194 (9)	0.4854 (11)	−0.2481 (14)	0.049 (5)
C37	0.3833 (13)	0.2050 (15)	0.1176 (21)	0.046 (5)	OP3	0.7565 (9)	0.5802 (10)	−0.2823 (14)	0.047 (5)
C38	0.3584 (13)	0.1479 (16)	0.1125 (22)	0.047 (5)	OP4	0.8050 (10)	0.5052 (11)	−0.1986 (15)	0.066 (5)
O39	0.3529 (9)	0.1269 (11)	0.0412 (15)	0.072 (5)	OP5	0.7901 (9)	0.4849 (10)	−0.3529 (14)	0.050 (5)
N40	0.3445 (11)	0.1246 (13)	0.1833 (17)	0.054 (5)	CPR1	0.6823 (13)	0.6397 (17)	−0.3120 (20)	0.050 (5)
C41	0.5101 (12)	0.1430 (14)	0.0249 (18)	0.042 (5)	CPR2	0.7238 (13)	0.5997 (16)	−0.3569 (21)	0.049 (5)
C42	0.5320 (14)	0.1260 (16)	−0.0641 (21)	0.060 (6)	CPR3	0.7577 (12)	0.6292 (15)	−0.4181 (19)	0.043 (5)
C43	0.5853 (15)	0.0906 (17)	−0.0632 (23)	0.058 (6)	OW1	0.5882 (9)	0.4948 (11)	−0.4398 (15)	0.078 (5)
O44	0.5930 (11)	0.0597 (12)	−0.0045 (18)	0.097 (5)	OW2	0.3003 (9)	0.1247 (11)	−0.4212 (15)	0.078 (5)
N45	0.6125 (11)	0.1103 (13)	−0.1291 (18)	0.062 (5)	OW3	0.2824 (9)	0.0140 (11)	−0.4787 (15)	0.066 (5)
C46	0.3790 (14)	0.3154 (16)	−0.2396 (23)	0.063 (6)	OW4	0.6788 (9)	0.5132 (11)	−0.5323 (15)	0.082 (5)
C47	0.4441 (14)	0.2339 (16)	−0.2937 (21)	0.056 (6)	OW5	0.8343 (11)	0.2513 (12)	−0.3143 (17)	0.109 (5)
C48	0.5145 (13)	0.3460 (15)	−0.3227 (19)	0.051 (5)	OW6	0.0792 (10)	0.2977 (11)	−0.4241 (16)	0.097 (5)
C49	0.5085 (15)	0.3394 (16)	−0.4205 (22)	0.065 (6)	OW7	0.3315 (10)	0.1183 (11)	0.3686 (15)	0.081 (5)

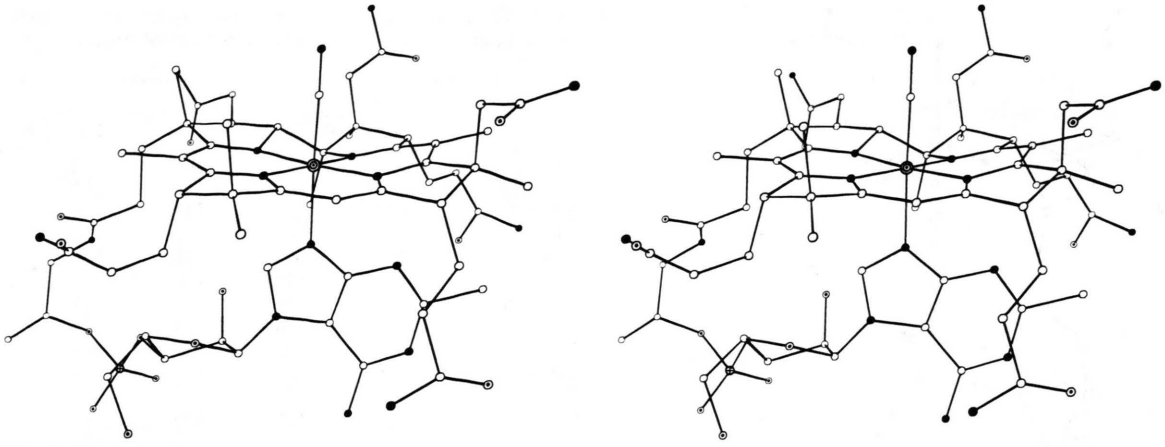

Fig. 2. Stereoview of factor A.

cobalt atom was found from a Patterson synthesis. Successive difference Fourier maps coupled with least-squares refinement (SHELX 76 [58]) showed the positions of all other non-hydrogen atoms. From 28 water molecules which were calculated according to the observed density of the crystal, only 7 could be found clearly in difference Fourier maps. Isotropic block-diagonal least-squares methods [58] refined the 93 non-hydrogen and the 7 oxygen (water) atoms to a final conventional $R = 0.166$ and $R_w = 0.148$.

Results and Discussion

Fig. 1 shows an ORTEP plot [52] of factor A. The atom numbering scheme of the molecular structure which is taken from D. C. Hodgkin [53], and the most important interatomic bond distances are also given. The final positional and thermal parameters are listed in Table II. A stereoview of factor A is presented in Fig. 2. This stereo diagram may be viewed either with stereoglasses [54] or, better, with a stereomonokel (stereomirror) [55]. Fig. 3 and 4 show two alternative views of the molecule.

All these figures clearly show the molecular structure and the axial ligands on cobalt (cyanide ion and the purine base 2-methyladenine) as well as the corrin ring system (C1 to C19) which is very roughly planar. As in cyanocobalamin the short side chains (methyl groups C46, C54 and the acetamide side chains) extend above the plane of the corrin ring while the long side chains (the propionamide side chains) extend below. The most important feature of the structure of factor A is the coordination of the purine base 2-methyladenine *via* NB9. Although the nature of the axial ligand bases of vitamin B_{12} (5,6-dimethylbenzimidazole) and factor A (2-methyladenine) indicates almost the same kind of bonding, there are small differences in the folding of the corrin ring about the Co–C10 line. This is attributed to the different contact of C5 and C35 with the bases: *via* the hydrogen atom on CB4 of 5,6-dimethylbenzimidazole and *via* the free electron pair on NB3 of 2-methyladenine in cobalamin and factor A, respectively. Table III gives the deviations

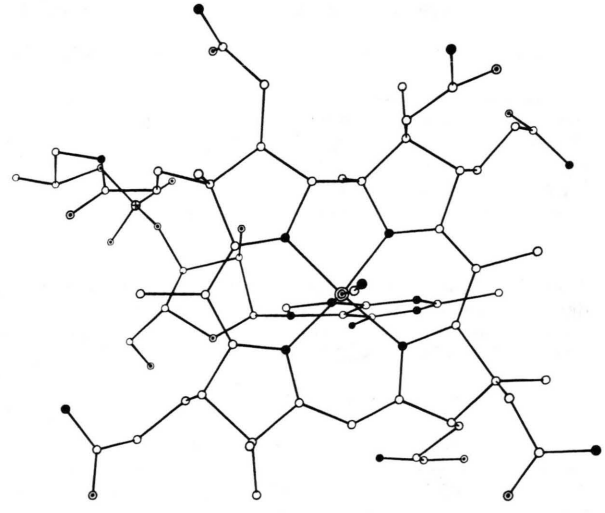

Fig. 3. View of factor A from above the corrin ring system. This figure is obtained from Fig. 2 by turning the molecule of factor A around the C15–C5 – axis by 75°.

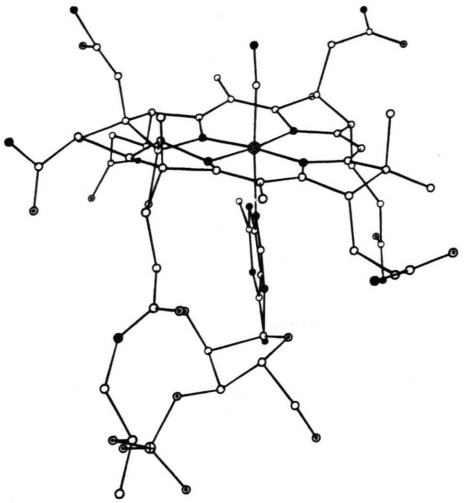

Fig. 4. View of factor A with the C1–C19 – bond to the left of the diagram along the base.

Table III. Deviations from best mean plane through equatorial nitrogen atoms N21–N24 (pm).

	C5	C35	C10	C15	C53	C1	C19
Dry B_{12}	57	105[a]	−32	8	25[a]	−29	46
Factor A	57	89	−14	7	13	−23	47

[a] According to own best mean plane calculations from atomic parameters given in [53].

Table VI. Bond lengths (pm) in corrin system. The estimated standard deviations in the structure of factor A are ≦ 5 pm.

Bond	Corrinoid			
	Ado-Cbl	Dry B_{12}	E_2	Factor A
C1–C2	160	158	169	153
C1–C19	154	153	159	152
C1–N21	153	148	147	148
C1–C20	155	167	152	164
C2–C3	160	168	138	158
C3–C4	151	149	156	154
C4–C5	146	149	152	134
C4–N21	130	126	124	125
C5–C6	133	142	129	146
C5–C35	154	150	151	153
C6–C7	159	155	165	166
C6–N22	134	136	149	141
C7–C8	154	158	162	155
C8–C9	148	144	159	146
C9–C10	143	141	133	132
C9–N22	134	137	127	132
C10–C11	136	140	142	142
C11–C12	151	163	141	152
C11–N23	133	135	126	134
C12–C13	153	156	145	172
C13–C14	149	152	150	146
C14–C15	132	135	137	139
C14–N23	141	144	136	133
C15–C16	144	143	136	138
C15–C53	158	166	157	157
C16–C17	154	135	162	162
C16–N24	129	140	131	129
C17–C18	156	144	144	158
C18–C19	150	157	155	151
C19–N24	148	145	139	152

Table IV. Distances (pm) around the cobalt atom.

Corrinoid	Upper ligand	Lower ligand	e.s.d. C–C	Deviation from best mean plane through the four N					Distances from cobalt					
				N21	N22	N23	N24	Co	Upper ligand	Lower ligand	N21	N22	N23	N24
Ado-Cbl	Ado	Bzm	2	−6	6	−5	6	1	203	224	187	194	191	191
Dry B_{12}	CN	Bzm	10	−4	4	−4	4	−7	202	206	186	189	191	195
E_2	CN	Bzm	4	−5	4	−4	5	−3	188	203	186	188	201	192
Factor A	CN	A2	5	−4	4	−4	4	−1	186	212	192	197	195	196

Table V. Bond angles (°) around the cobalt atom. X = upper ligand atom, Y = lower ligand atom. The estimated standard deviations in the structure of factor A are ≦ 1 °.

Corrinoid	N21–Co–N22	N22–Co–N23	N23–Co–N24	N24–Co–N21	X–Co–N21	X–Co–N22	X–Co–N23	X–Co–N24	Y–Co–N21	Y–Co–N22	Y–Co–N23	Y–Co–N24	X–Co–Y
Ado-Cbl	89	97	91	83	94	84	91	94	92	89	85	94	171
Dry B_{12}	92	94	94	81	89	87	90	87	94	90	88	97	175
E_2	93	93	89	86	89	87	92	88	92	93	87	92	179
Factor A	92	96	89	84	92	87	90	90	89	90	89	94	176

Table VII. Important angles (°) in corrin system.

Corrinoid	C1 N21 C4	N21 C4 C5	C4 C5 C6	C5 C6 N22	C6 N22 C9	N22 C9 C10	C9 C10 C11	C10 C11 N23	C11 N23 C14	N23 C14 C15	C14 C15 C16	C15 C16 N24	C16 N24 C19	N24 C19 C1	C19 C1 N21
Ado-Cbl	112	123	121	126	111	126	125	126	111	127	124	121	113	108	102
Dry B_{12}	116	125	122	116	103	127	119	129	113	123	135	111	109	102	104
E_2	117	128	125	118	107	126	128	119	108	123	128	121	115	111	101
Factor A	113	127	118	130	115	121	130	126	110	125	120	131	121	107	105

Table VIII. Some important torsion angles in the nucleotide loop.

	Torsion angles			
	Ado-Cbl	Dry B_{12}	E_2	Factor A
C16–C17–C55–C56	–46	–47	–48	–45
C18–C17–C55–C56	67	75	73	76
C17–C55–C56–C57	166	170	167	162
C56–C57–N59–CPR1	–177	–173	174	177
C57–N59–CPR1–CPR2	–82	–96	–81	–90
N59–CPR1–CPR2–OP3	–62	–69	–52	–60
CPR1–CPR2–OP3–PP1	128	137	137	132
CPR2–OP3–PP1–OP2	–74	–60	–83	–70
OP3–PP1–OP2–CR3	172	157	158	163
PP1–OP2–CR3–CR2	131	129	132	113
PP1–OP2–CR3–CR4	–114	–127	–126	–132

of some corrin atoms from the best mean plane through N21–N24. It can be seen that the distance of C35 is 105 pm for dry B_{12} and only 89 pm for factor A. Furthermore, the intramolecular distance of 351 pm between C5 and CB4 in dry B_{12} is contracted to 337 pm between C5 and NB3 in factor A; note the different atom numbering in the bases.

A surprising feature is found in the difference of $1000 \cdot 10^6$ pm^3 in the unit cell volumes of factor A and vitamin B_{12} (air-dried) [53]. This difference can be rationalized by the different chemistry of the bases. In factor A, as in vitamin B_{12}, the base does not have any freedom of rotation about the Co–N bond. It is constrained by the axial substituents of the corrin ring (methyl group C20 and methylene groups C30, C41, C48 and C55 of the propionamide side chains). Indeed, these groups form a hydrophobic pocket and so serve a protective function for the purine base against water. 2-Methyladenine is, though, much more hydrophilic than 5,6-dimethylbenzimidazole in the corrinoid molecule. This may explain the high water content of wet factor A (28 H_2O as compared with 22 H_2O in wet B_{12} [56]).

In general we can say that – despite the different chemistry of the bases – there are only small differences in bond lengths and bond angles as well as in molecular conformation between factor A and B_{12}. Computing all interatomic distances, interbond angles, torsion angles, and deviations from the best mean planes through portions of each molecule and comparing them in detail one obtains the values given in Tables IV to VIII. The compounds which were used for comparison with factor A are Ado-Cbl*, dry B_{12} and B_{12}-monocarboxylic acid E_2. The values of these compounds have been taken from the review article on "X-ray Crystallography of B_{12} and Cobaloximes" by J. P. Glusker [56]. The reason for taking dry B_{12} instead of wet B_{12} was dictated by availability of data. According to Glusker [56], there are only small differences in the molecular structure between dry B_{12} and wet B_{12}.

Table IV lists the distances around the cobalt atom and the deviations of these atoms from the best mean plane through N21 to N24. Some of

* Abbreviations used: Ado, adenosyl; Cbl, cobalamin; E_2, B_{12}-monocarboxylic acid E_2; Bzm, 5,6-dimethylbenzimidazole; A2, 2-methyladenine.

these deviations are small and may not be significant (e.s.d. ≦ 5 pm). This table also clearly shows the transeffect of the axial ligands. In addition, bond angles around the cobalt atom are listed in Table V. The distortions in the octahedral coordinates are evident. Bond distances in the corrin system are listed in Table VI, important bond angles in the corrin system are listed in Table VII. Finally in Table VIII some important torsion angles in the nucleotide loop are given. Again, this table shows the similarity of factor A in comparison with the three other compounds of interest.

Another method of comparing similar X-ray structures has been suggested by Liebman and Glusker [57]. Their method employs a partitioned distance matrix analysis and enables one to see exactly what parts of the structure can be superimposed without any prior bias. Several examples of such comparisons of B_{12} derivatives are given in the paper by Glusker [56].

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