1650 (s); mass spectrum, m/e 139 (M$^+$), 96 (100%), 68, 67, 56, 55, 39.

1-Acetyl-1-methyl-2(Z)-(methylcarboxymethylene)cyclopentane (22) **(Y = CO$_2$CH$_3$).** ^{1}H NMR 1.27 (s, 3 H), 1.40–2.0 (m, 4 H), 2.10 (s, 3 H), 2.7–3.2 (m, 2 H), 3.68 (s, 3 H), 5.65 (t, J = 2 Hz, 1 H); IR (CDCl$_3$) 1715 (vs), 1650 (s); mass spectrum, m/e 165, 154 (100%), 122, 95, 79, 77, 43.

1-Acetyl-1-methyl-2(Z)-(bromomethylene)cyclopentane (22) **(Y = Br).** ^{1}H NMR 1.30 (s, 3 H), 1.45–2.00 (m, 4 H), 2.12 (s, 3 H), 2.20–2.80 (m, 2 H), 6.05 (t, J = 2 Hz, 1 H); IR (CDCl$_3$) 3080 (w), 1705 (vs), 1635 (m); mass spectrum, m/e 175, 173, 137, 93, 91, 77, 43 (100%), 39.

1-Acetyl-1-methyl-2(Z)-(acetylmethylene)cyclopentane (22) **(Y = COCH$_3$).** ^{1}H NMR 1.25 (s, 3 H), 1.35–2.05 (m, 4 H), 2.10 (s, 3 H), 2.7–3.1 (m, 2 H), 6.05 (t, J = 2 Hz, 1 H); IR (CDCl$_3$) 1710 (vs), 1690 (s), 1615 (vs), 1250 (vs); mass spectrum, m/e 180 (M$^+$), 138, 95, 80, 43 (100%).

Purification Technique for Compound 8: After workup, the crude mixture was distilled bulb to bulb (room temperature, 0.08 torr, dry ice cooling). Enal **8** and its precursor, hept-6-ynal were collected altogether.

The Structure of a B$_{12}$ Coenzyme: Methylcobalamin Studies by X-ray and NMR Methods

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Abstract: The B$_{12}$ coenzyme methylcobalamin crystallizes in the orthorhombic space group $P2_12_12_1$, with $Z = 4$ and $a = 17.887$ (6) Å, $b = 32.68$ (1) Å, $c = 17.447$ (5) Å, $V = 10197$ (6) Å^3. The structure was determined by X-ray diffraction methods. The cobalt atom was located from the Patterson map, and lighter atoms were located from subsequent electron density maps. The final R value, after least-squares refinements, is 0.146 based on 4254 observed intensities (diffractometer data) and 538 parameters (isotropic refinement except for Co and P which were refined anisotropically). Many hydrogen atoms in the methylcobalamin molecule, including those in the methyl group attached to the cobalt atom, were located from difference electron density maps. Approximately 40 sites for water molecules (many disordered) plus one disordered molecule of acetone of crystallization were located. The structure of methylcobalamin is very similar to that of cyanocobalamin (vitamin B$_{12}$), although in methylcobalamin there is some disorder in the area of the phosphate group. The orientation of the benzimidazole group and the conformations of most side chains are remarkably similar in the two molecules, with the exception of the orientations of the amide groups at the ends of side chains. These apparently can rotate to accommodate nearby hydrogen-bonding groups. The Co–C (methyl) bond length is 1.99 (2) Å, the Co–N (Bzm) bond length is 2.19 (2) Å, and the four Co–N (equatorial) bond lengths are 1.88 (2), 1.97 (2), 1.93 (2), and 1.89 (2) Å. The corrin ring system is not folded about the Co–C(10) line as much as in 5′-deoxyadenosylcobalamin (coenzyme B$_{12}$). The ^{1}H NMR and ^{31}P NMR spectra of a series of cobalamins with H$_2$O, CN, CH$_2$CN, CH$_2$CF$_3$, CH$_3$, CH$_2$CH$_3$, CH$_2$CH$_2$CH$_3$, CH$_2$CH(CH$_3$)$_2$, and 5′-deoxyadenosyl as (upper) axial ligands are reported. The H(BC7) of the benzimidazole group, H(RC1), and ^{31}P resonances (except in the case of charged H$_2$O cobalamin) follow reasonable trends with respect to the donor ability of the alkyl group. These resonances also suggest that in the compound containing the bulky axial CH$_2$CH(CH$_3$)$_2$ group, there are some steric interactions with the corrin side chains. Thus, NMR techniques may prove useful in assessing conformational and structural changes in cobalamins in much the same way that such spectroscopic methods have proved to be useful with B$_{12}$ model compounds. The structural and NMR studies reveal no particular steric interactions involving the methyl group of methylcobalamin. Furthermore, the similarity in structure of the nonalkyl portion of coenzyme B$_{12}$ and methyl B$_{12}$, one with a bulky and one with a small alkyl ligand, suggests that the bulkiness of the alkyl substituent in coenzyme B$_{12}$ is not a main structural determinant.

According to Halpern,[1] the B$_{12}$ prosthetic group, coenzyme B$_{12}$ or 5′-deoxyadenosylcobalamin (DBC), can be considered to be an "organic radical carrier" much as the heme group in hemoglobin is a dioxygen carrier. In both types of protein systems, the conformation of the protein influences the ability of the metal center to bind the carried species (radical or dioxygen);[2–4] therefore, the relationship between the structure at the metal center and its function is both intriguing and important. In many B$_{12}$ holoenzymes, the Co–C bond in coenzyme B$_{12}$ is readily cleaved to produce, eventually, a protein-bound substrate radical. This protein-bound radical then undergoes rearrangement reactions

that are often not favored by the analogous (unbound) free radical. In a similar way, in hemoglobin the affinity for ligands other than oxygen and the irreversible oxidation of the heme groups are greatly modulated by the protein.

Recently, Finke[5–7] has critically reviewed aspects of the B$_{12}$

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Table I. Crystal Data for Methylcobalamin

formula	$CoPO_{55}N_{13}C_{62}H_{169}$
empirical formula	$CoPO_{14}N_{13}C_{59}H_{83} \cdot CH_3COCH_3 \cdot 40H_2O$
formula weight, daltons	2067 daltons
$F(000)$	4456
space group	$P2_12_12_1$
Z	4
a, Å	17.887 (6)
b, Å	32.68 (1)
c, Å	17.447 (5)
V, Å^3	10197 (6)
D_X, g cm^{-3}	1.35
λ(Mo Kα), Å	0.70926
μ(Mo Kα), cm^{-1}	2.407
crystal size, mm^3	0.5 × 0.25 × 0.25
temp., °C	25

literature and pointed out the need for a greater understanding of the structural factors that influence Co–C bond homolysis. Surprisingly, X-ray structural information is available in only one organometallic B_{12} compound, namely coenzyme B_{12} itself.[8] The determination of this structure established the presence of a Co–C bond when it was found that the 5′-deoxyadenosyl group was attached to the cobalt via the 5′-carbon atom. This was the first demonstration of the existence of a naturally occurring alkyl organometallic compound.

One other B_{12} coenzyme, methyl B_{12} or methylcobalamin, is known to be biologically active and essential for human metabolism.[3] We selected this molecule for structural and spectroscopic investigation not only because of its natural occurrence, as a cofactor for a methyltransferase enzyme, but also because it serves as a spectroscopic and structural prototype for comparative studies of alkyl cobalamins. Investigations of model B_{12} compounds, such as the cobaloximes [LCo(DH)$_2$X (DH = monoanion of dimethylglyoxime; L = neutral, two-electron donor; X = mononegative electron donor)], have revealed a wealth of information concerning the relationship of structure to reactivity or spectroscopic patterns.[9] Similar comparative spectroscopic and structural information on the biologically more relevant cobalamins will facilitate investigations into conformational changes that influence the radical-carrying ability of methyl B_{12} or coenzyme B_{12} when incorporated into B_{12} holoenzymes. This study, together with those of adenosylcobalamin coenzyme, represents the only structural characterization of an alkylcobalamin to date.

Experimental Section

Hydroxocobalamin (B_{12}OH·HCl) and all alkyl halides were purchased from Sigma and Aldrich, respectively, and were used without further purification. The B_{12}R complexes (R = alkyl) were prepared via a modified, published procedure[10] as follows. Hydroxocobalamin (1.0 g) was dissolved in a deaerated MeOH/H$_2$O mixture (3:1, 20 mL) under continuous stirring and N$_2$ purging. A NaBH$_4$ solution (0.4 g in 2 mL of H$_2$O) was slowly added, followed by alkyl halide (10-fold molar excess). After 15 min, the N$_2$ was removed and the solution diluted to a volume of 400 mL with acetone. After the solution was left to stand in the dark at –5 °C for 1–2 days a red precipitate formed and was collected by vacuum filtration. The unreacted B_{12}OH$_2$ was removed by column chromatography with SP-sephadex as described.[11] Yields ranged from ~50% for R = CH$_2$CN to ~90% for R = n-C$_3$H$_7$. (Values for yields are approximate since the number of molecules of water of hydration is not known.) The purity of the B_{12} derivatives was established via ^{1}H and ^{31}P NMR and by reverse-phase HPLC.[12] Recrystallization was achieved by dissolution in a minimal amount of water and addition of acetone until the solution became turbid. The solution was kept in the dark at –5 °C in a sealed container to prevent evaporation of acetone. Dark red crystalline samples were obtained in 3–10 days.

Crystallization of Methylcobalamin. MeB$_{12}$ (80 mg) was stirred in

water (5 mL) for 5 min. Then acetone (5 mL) was added and the solution filtered through glass wool. The filtrate was stored in a tightly stoppered flask at –8 to –10 °C. Crystals formed over a period of a few days. Such crystals cracked on drying. Consequently they were stored at room temperature under a filtered solution (MeB$_{12}$ (80 mg) in water (5 mL) and acetone (20 mL) in a tightly stoppered flask).

NMR Measurements. FT-NMR spectral measurements were made with Nicolet NB-360 (360 MHz, ^{1}H) and IBM WP200-SY (81.01 MHz, ^{31}P) spectrometers. Preacquisition HDO solvent suppression was employed for ^{1}H measurements. All data were obtained in D$_2$O with solvent-D lock. ^{1}H and ^{31}P NMR chemical shifts were referenced internally to trimethylsilylpropionate (TSP) and trimethylphosphate (TMP), respectively.

Crystal Data Collection. A deep red crystal of methylcobalamin of dimensions 0.5 × 0.25 × 0.25 mm^3 was mounted with mother liquor in a capillary tube. Cell parameters (Table I) were obtained by a least-squares analysis of 15 centered reflections obtained from diffractometer measurements.

Data were collected on a Nicolet $P2_1$ four-circle diffractometer with Mo Kα radiation and a highly oriented graphite monochromator. The θ–2θ scan technique (bisecting mode) was used to measure 9848 unique reflections to a 2θ limit of 60°, at a variable scan speed (2.0–29.5 deg min^{-1}) (depending upon intensity). The scan:background time ratio was 2.0, and 4254 reflections with $I > 2\sigma(I)$ (where $\sigma(I)$ was determined from counting statistics) were considered observed and included in further calculations. Three check reflections that were measured every 100 reflections showed no decay in intensity. Values of $\sigma(F)$ were calculated as $\sigma(F) = (F/2)[\sigma^2(I)/(I)^2 + \delta^2]^{1/2}$, where δ (=0.038) is an instrumental uncertainty determined from the variation in the intensity of the check reflections. The data were corrected for Lorentz and polarization factors and put on an absolute scale with a Wilson plot. An empirical absorption correction was applied by using the Ψ scan technique.

Structure Determination and Refinement. The structure was solved by locating the cobalt atom from the Patterson map and then finding the lighter atoms in subsequent electron density and difference electron density maps.

The atomic positions and thermal parameters of all non-hydrogen atoms were refined by a full-matrix least-squares computer program (cobalt and phosphorus atoms were refined anisotropically and all other atoms isotropically).[13,14] The quantity minimized was $\sum w[||F_o| - |F_c||]^2$ where the weights, w, were $1/\sigma^2(F)$. Atomic scattering factors for non-hydrogen atoms were those listed in the literature;[15,16] a correction for anomalous scattering was applied.[17] The computer programs used are part of the Crystallographic Program Library written at the Institute for Cancer Research.[18-20]

The final value of $R = \sum ||F_o| - |F_c||/\sum |F_o|$ is 0.146; the average shift in the atomic parameters is less than their standard deviations. The highest peak in the final difference Fourier map is 1.5 e Å^{-3}, indicative of the problems associated with the solvent structure. Fractional coordinates of non-hydrogen atoms are listed in Table II; temperature factors of these atoms and fractional coordinates of hydrogen atoms have been deposited in Table A, supplementary material. Observed and calculated structure factors are deposited in Table B, supplementary material.

There were many problems associated with the location of some of the solvent molecules. The B_{12} molecule itself is highly disordered; this is indicated by the high-temperature factors of certain of the side chain amide groups, certain methyl groups, part of the phosphate linkage (including the phosphorus), and part of the benzimidazole ligand. The disorder was hard to resolve because of the apparent absence of a significant amount of scattering from the solvent molecules. The solvent structure was interpreted by analyses of difference electron density maps, and the model presented here merely represents an average of several possible water (plus acetone) networks of varying occupancies. Hence

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Table II. List of Atomic Coordinates and Their Associated Standard Deviations

atom	x	y	z	atom	x	y	z
Co	0.3399 (2)	0.2943 (1)	0.3874 (2)	C(3P)	0.2144 (17)	0.4160 (10)	−0.2072 (18)
C(1)	0.3587 (11)	0.3812 (5)	0.3917 (12)	O(3)	0.3207 (10)	0.4101 (5)	−0.1167 (10)
C(2)	0.3779 (13)	0.4128 (7)	0.4588 (12)	O(4)	0.4536 (16)	0.3967 (9)	−0.1126 (19)
C(3)	0.4405 (14)	0.3930 (7)	0.5029 (14)	O(5)	0.3694 (13)	0.3449 (7)	−0.1751 (13)
C(4)	0.4239 (12)	0.3479 (6)	0.4901 (12)	O(2)	0.3643 (9)	0.3559 (5)	−0.0388 (9)
C(5)	0.4525 (13)	0.3152 (7)	0.5344 (13)	P	0.3850 (3)	0.3772 (1)	−0.1166 (2)
C(6)	0.4434 (13)	0.2751 (7)	0.5177 (12)	C(1R)	0.4646 (12)	0.2835 (6)	0.0883 (11)
C(7)	0.4669 (13)	0.2344 (7)	0.5625 (13)	C(2R)	0.4525 (13)	0.3281 (7)	0.0609 (13)
C(8)	0.4659 (11)	0.2023 (7)	0.5014 (11)	C(3R)	0.4065 (13)	0.3189 (7)	−0.0106 (13)
C(9)	0.4087 (12)	0.2206 (6)	0.4457 (12)	C(4R)	0.3539 (12)	0.2863 (6)	0.0127 (11)
C(10)	0.3681 (13)	0.1978 (7)	0.3961 (14)	C(5R)	0.3312 (18)	0.2597 (10)	−0.0544 (17)
C(11)	0.3107 (11)	0.2106 (6)	0.3473 (10)	O(6R)	0.3958 (9)	0.2624 (5)	0.0658 (8)
C(12)	0.2567 (14)	0.1816 (8)	0.3062 (14)	O(7R)	0.4086 (8)	0.3517 (4)	0.1151 (9)
C(13)	0.2160 (13)	0.2078 (8)	0.2526 (13)	O(8R)	0.2822 (16)	0.2316 (9)	−0.0378 (16)
C(14)	0.2328 (11)	0.2538 (6)	0.2840 (11)	N(1B)	0.4647 (10)	0.2841 (6)	0.1758 (10)
C(15)	0.1974 (12)	0.2869 (7)	0.2610 (11)	C(2B)	0.4038 (13)	0.2867 (7)	0.2228 (12)
C(16)	0.2193 (12)	0.3258 (7)	0.2882 (12)	N(3B)	0.4222 (9)	0.2907 (5)	0.2943 (9)
C(17)	0.1823 (12)	0.3690 (6)	0.2745 (11)	C(4B)	0.5471 (12)	0.2993 (7)	0.3530 (12)
C(18)	0.2445 (14)	0.3977 (7)	0.3025 (14)	C(5B)	0.6265 (13)	0.3001 (8)	0.3360 (13)
C(19)	0.2839 (12)	0.3731 (6)	0.3691 (11)	C(6B)	0.6494 (13)	0.2896 (7)	0.2602 (12)
C(20)	0.4108 (12)	0.3905 (6)	0.3160 (11)	C(7B)	0.6027 (12)	0.2849 (6)	0.1990 (11)
N(21)	0.3813 (10)	0.3429 (5)	0.4268 (10)	C(8B)	0.5273 (12)	0.2865 (6)	0.2174 (11)
N(22)	0.4053 (10)	0.2593 (6)	0.4506 (10)	C(9B)	0.4999 (12)	0.2918 (7)	0.2943 (11)
N(23)	0.2904 (10)	0.2496 (6)	0.3362 (10)	C(10B)	0.6797 (15)	0.3047 (8)	0.4026 (15)
N(24)	0.2776 (10)	0.3323 (5)	0.3365 (9)	C(11B)	0.7389 (16)	0.2882 (10)	0.2432 (17)
C(25)	0.4008 (14)	0.4576 (7)	0.4290 (13)	C(15A)	0.2646 (13)	0.2882 (8)	0.4705 (12)
C(26)	0.3102 (12)	0.4172 (7)	0.5096 (12)	O(W1)	0.9754 (13)	0.0756 (7)	0.2742 (14)
C(27)	0.3188 (15)	0.4459 (9)	0.5766 (15)	O(W2)	0.9702 (29)	0.4134 (16)	0.4376 (31)
O(28)	0.3682 (13)	0.4487 (7)	0.6212 (13)	O(W3)	0.9714 (11)	0.2207 (6)	0.2027 (12)
N(29)	0.2713 (16)	0.4751 (9)	0.5827 (15)	O(W4)	0.6337 (14)	0.2426 (8)	0.0046 (15)
C(30)	0.5219 (14)	0.3993 (8)	0.4821 (14)	O(W5)	0.3755 (14)	0.4360 (8)	0.0875 (14)
C(31)	0.5571 (19)	0.4333 (11)	0.5344 (20)	O(W6)	0.1206 (28)	0.4248 (15)	0.5132 (27)
C(32)	0.6448 (29)	0.4427 (14)	0.5191 (27)	O(W7)	0.0182 (27)	0.4788 (14)	0.3761 (29)
N(33)	0.6730 (27)	0.4260 (15)	0.4699 (29)	O(W8)	0.9628 (29)	0.3908 (16)	0.1717 (29)
O(34)	0.6842 (19)	0.4507 (10)	0.5797 (20)	O(W9)	0.2044 (24)	0.0522 (14)	0.1619 (26)
C(35)	0.4853 (13)	0.3292 (7)	0.6146 (15)	O(W10)	0.3926 (27)	0.4930 (15)	0.1898 (27)
C(36)	0.5414 (12)	0.2410 (6)	0.6065 (13)	O (W11)	0.3359 (31)	0.1068 (16)	0.5115 (29)
C(37)	0.4056 (12)	0.2263 (7)	0.6197 (14)	O(W12)	0.9777 (68)	0.3253 (33)	0.0621 (63)
C(38)	0.4191 (15)	0.1888 (8)	0.6719 (15)	O (W13)	0.1444 (27)	0.4972 (14)	0.4661 (25)
O(39)	0.4346 (11)	0.1973 (6)	0.7450 (12)	O(W14)	0.8118 (22)	0.1672 (13)	0.3183 (24)
N(40)	0.4056 (12)	0.1578 (7)	0.6478 (12)	O(W15)	0.1172 (34)	0.0430 (19)	0.2639 (34)
C(41)	0.5464 (12)	0.1982 (7)	0.4581 (12)	O(W16)	0.4949 (37)	0.0602 (19)	0.3068 (36)
C(42)	0.5478 (16)	0.1722 (9)	0.3878 (19)	O(W17)	0.5237 (63)	0.5956 (34)	0.0823 (62)
C(43)	0.6302 (21)	0.1636 (11)	0.3664 (21)	O(W18)	0.6936 (47)	0.4020 (27)	0.1015 (47)
O(44)	0.6617 (19)	0.1912 (10)	0.3224 (19)	O(W19)	0.5142 (63)	0.5339 (33)	0.5051 (55)
N(45)	0.6590 (17)	0.1372 (9)	0.3878 (17)	O(W20)	0.6338 (47)	0.4093 (25)	0.2768 (46)
C(46)	0.1988 (16)	0.1679 (9)	0.3704 (17)	O(W21)	0.9931 (54)	0.0513 (27)	0.0577 (49)
C(47)	0.2982 (15)	0.1428 (8)	0.2785 (15)	O(W22)	0.6881 (47)	0.5397 (26)	−0.1485 (47)
C(48)	0.2452 (12)	0.2081 (8)	0.1668 (12)	O(W23)	0.6470 (58)	0.5708 (30)	0.0874 (55)
C(49)	0.2166 (15)	0.1734 (8)	0.1224 (16)	O(W24)	0.8095 (54)	0.4400 (29)	0.1242 (57)
C(50)	0.1378 (16)	0.1734 (8)	0.1162 (17)	O(W25)	0.6395 (87)	0.5203 (45)	0.0484 (79)
O(51)	0.0910 (11)	0.2008 (7)	0.0998 (12)	O(W26)	0.3756 (102)	0.4185 (50)	0.3555 (97)
N(52)	0.1023 (18)	0.1360 (10)	0.1435 (18)	O(W27)	0.5434 (47)	0.2174 (27)	0.1053 (53)
C(53)	0.1362 (13)	0.2845 (7)	0.2104 (13)	O(W28)	0.5463 (60)	0.5534 (38)	−0.3039 (67)
C(54)	0.1120 (14)	0.3677 (8)	0.3218 (14)	O(W29)	0.0783 (56)	0.2591 (31)	0.1317 (59)
C(55)	0.1572 (13)	0.3773 (6)	0.1884 (11)	O(W30)	0.5066 (100)	0.5198 (47)	0.2709 (87)
C(56)	0.2253 (14)	0.3674 (7)	0.1319 (14)	O(W31)	0.5139 (67)	0.6295 (36)	0.3345 (65)
C(57)	0.1878 (18)	0.3783 (10)	0.0489 (18)	O(W32)	0.6690 (74)	0.2700 (46)	0.0340 (82)
O(58)	0.1563 (17)	0.3494 (9)	0.0187 (16)	O(W33)	0.6211 (51)	0.4057 (29)	−0.2122 (50)
N(59)	0.2257 (14)	0.4086 (8)	0.0160 (14)	O(W34)	0.6565 (64)	0.5342 (35)	0.3022 (64)
C(60)	0.2187 (15)	0.4426 (8)	0.3282 (15)	O(W35)	0.3282 (44)	0.1461 (24)	−0.0656 (42)
C(61)	0.2046 (15)	0.4708 (8)	0.2587 (15)	O(W36)	0.6280 (69)	0.5759 (36)	0.4334 (66)
N(62)	0.1438 (16)	0.4916 (8)	0.2601 (15)	C(A2)	0.4403 (42)	0.1541 (23)	0.0727 (42)
O(63)	0.2506 (12)	0.4718 (6)	0.2107 (12)	C(A3)	0.7082 (107)	0.1962 (55)	0.1654 (117)
C(1P)	0.1960 (17)	0.4243 (9)	−0.0639 (17)	C(A1)	0.5310 (37)	0.1726 (21)	0.1344 (40)
C(2P)	0.2492 (14)	0.3997 (8)	−0.1261 (15)	O(A1)	0.5092 (46)	0.1921 (24)	0.2167 (46)

O⋯O distances may be apparently abnormally short if they involve water molecules in different networks.

Refinement proceeded in the following manner: after isotropic refinement of the B₁₂ molecule (92 heavy atoms), a difference electron density map was calculated to determine the solvent structure (which is made up mainly of water molecules and a disordered acetone). The next few cycles of isotropic refinement were done on these heavy atoms. Hydrogen atoms in the methylcobalamin molecule were located from the difference map or their positions were calculated; if the positions were calculated they were not refined. Least-squares calculations were alternated with the computation of difference electron density maps which were examined for additional solvent peaks. At this stage it became clear that portions of the methylcobalamin molecule itself were disordered. Attempts to refine and interpret this disorder were hampered by the disorder of many of the solvent molecules. It is concluded that the structure has been refined to the extent warranted by the data (high

Table III

(a) Bond Distances[a] to Co (Å) and Bond Angles[b] (deg)

	MeB$_{12}$	adenosyl coenzyme (ref 8)	vitamin B$_{12}$ (wet) (ref 71)	vitamin B$_{12}$ (dry) (ref 72)
Co–N(21)	1.88	1.92	1.80	1.86
Co–N(22)	1.97	1.91	1.92	1.89
Co–N(23)	1.93	1.97	1.86	1.91
Co–N(24)	1.89	1.98	1.87	1.95
Co–N(3B)	2.19	2.24	1.97	2.06
Co–C(A15)	1.99	2.05	1.92	2.02

	MeB$_{12}$	adenosyl coenzyme	vitamin B$_{12}$ (wet)	vitamin B$_{12}$ (dry)
N(21)–Co–N(22)	93	91	89	91
N(21)–Co–N(23)	172	170	175	174
N(21)–Co–N(24)	81	81	84	81
N(21)–Co–C(15A)	95	95	87	89
N(22)–Co–N(23)	95	98	96	94
N(22)–Co–N(24)	173	172	171	170
N(23)–Co–C(15A)	86	86	87	87
N(23)–Co–N(24)	90	90	92	94
N(23)–Co–C(15A)	87	89	91	90
N(24)–Co–C(15A)	90	93	89	87
N(21)–Co–N(3B)	93	93	96	94
N(22)–Co–N(3B)	89	88	88	90
N(23)–Co–N(3B)	86	85	87	88
N(24)–Co–N(3B)	95	95	97	97
C(15)–Co–N(3B)	171	170	174	175

(b) Folding of the Corrin System

	MeB$_{12}$	adenosyl coenzyme	vitamin B$_{12}$ (wet)	vitamin B$_{12}$ (dry)
angle between planes 1 and 2,[c] deg	15.8	14.6	17.7	18.7
rms deviation, Å				
plane 1	0.05	0.08	0.11	0.10
plane 2	0.02	0.03	0.04	0.03
upper substituent	methyl	adensoyl	CN	CN
lower substituent	Bzm	Bzm	Bzm	Bzm

(c) Orientation of Benzimidazole

	MeB$_{12}$	adenosyl coenzyme	vitamin B$_{12}$ (wet)	vitamin B$_{12}$ (dry)
angle between planes[d] through 6- and 5-membered rings of the benzimidazole, deg	4.2	1.8	3.8	2.7
rms deviation				
plane 1	0.01	0.02	0.065	0.01
plane 2	0.03	0.01	0.012	0.04
torsion angles, deg				
N(21)–Co–N(3B)–C(9B)	53	50	48	53
N(22)–Co–N(3B)–C(9B)	−40	−40	−40	−38
N(23)–Co–N(3B)–C(9B)	−136	−139	−136	−132
N(24)–Co–N(3B)–C(9B)	134	132	132	134
N(21)–Co–N(3B)–C(2B)	−127	−125	−124	−132
N(22)–Co–N(3B)–C(2B)	140	143	147	136
N(23)–Co–N(3B)–C(2B)	44	45	51	42
N(24)–Co–N(3B)–C(2B)	−46	−44	−40	−52
C(15A)–Co–N(3B)–C(9B)	−100	−89	−70	−75
C(15A)–Co–N(3B)–C(2B)	81	95	117	99

(d) Distances from C(20) Methyl to Corrin Nitrogens and Benzimidazole Atoms (Å)

	MeB$_{12}$	adenosyl coenzyme	vitamin B$_{12}$ (wet)	vitamin B$_{12}$ (dry)
C(20)–N(21)	2.54	2.47	2.57	2.62
C(20)–N(22)	4.89	4.68	4.70	4.87
C(20)–N(23)	5.09	5.10	4.99	5.14
C(20)–N(24)	3.07	3.17	3.05	3.05
C(20)–N(3B)	3.29	3.19	3.20	3.32
C(20)–N(1B)	4.36	4.30	4.34	4.37
C(20)–C(4B)	3.90	3.84	3.91	3.90
C(20)–C(7B)	5.28	5.04	5.03	5.23
C(20)–C(10B)	5.77	5.57	5.74	5.90
C(20)–C(11B)	6.87	6.49	6.57	6.95

[a] Standard deviations for MeB$_{12}$ are 0.02 Å, higher for other entries. [b] Standard deviations for MeB$_{12}$ are 2°, higher for other entries. [c] Plane 1: N(21), C(4), C(5), C(6), N(22), C(9), C(10). Plane 2: N(24), C(16), C(15), C(14), N(23), C(11), C(10). [d] Plane 1: N(1B), C(2B), N(3B), C(8B), C(9B). Plane 2: C(4B), C(5B), C(6B), C(7B), C(8B), C(9B).
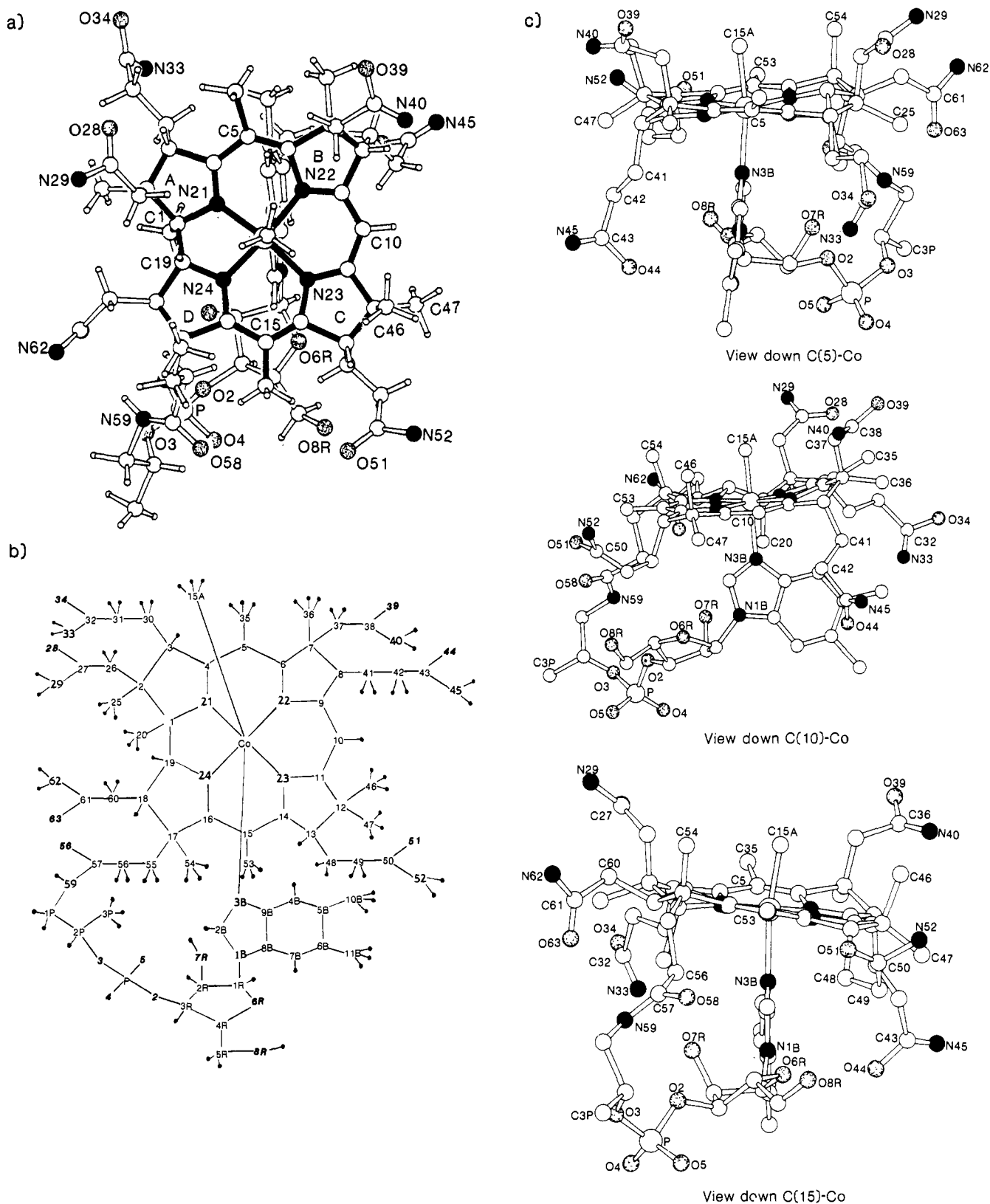
Figure 1. (a) Molecular structure of methylcobalamin looking down the C(A15) (the axial methyl ligand)–cobalt bond. (b) Atomic numbering of methylcobalamin. Atom types are differentiated by font type: oxygen atoms are bold and slanted, nitrogens are the largest size, and the most prevalent font type represents carbon atoms. (c) Views looking down the bond between C(5)–Co, C(10)–Co, and C(15)–Co.

mosaicity and considerable disorder). Further work is planned to try to define this more clearly.

Results and Discussion

The molecular structure is illustrated in Figure 1 which also contains the numbering system of the molecule as used in ref 4.

A stereo view of the molecule is deposited (Figure A, supplementary material). A full list of bond distances has been deposited in Table C. It should be noted that there is an approximate mirror plane with respect to bond distances through the Co–C(10) direction. This indicates considerable resonance in the corrin system.

The general conformation of the molecule of methylcobalamin

(solid bonds) is compared with those of B_{12} coenzyme (5'-deoxyadenosylcobalamin) and wet and air-dried vitamin B_{12} (cyanocobalamin) (dashed lines) in Figure 2 and Table III. The similarity is remarkable. The orientation of the benzimidazole group, Table IIIc,d, and the general disposition of the ribose, phosphate, and side chains are the same in each structure. This constant orientation of the benzimidazole group is not unexpected in view of the presence of axially oriented atoms C(20), C(41), C(48), and C(55) which control this orientation.

The conformation of the corrin ring in B_{12} derivatives may be described by the fold angle between the planes of the conjugated system of double bonds.[4] This is probably caused by steric hindrance between H(BC2) and C(6) and between H(BC4) and C(14) as shown in Figure 3. The planes are defined as follows: atoms N(21), C(4), C(5), C(6), N(22), C(9), and C(10) in plane 1 and N(24), C(16), C(15), C(14), N(23), C(11), and C(10) in plane 2. The least-squares planes are calculated through these atoms, Table IIIb. In methylcobalamin the fold angle is 15.8° The corrin ring in methylcobalamin is made up of more planar regions (root mean square = 0.05, 0.02 Å) than are either the adenosylcobalamin (root mean square = 0.08, 0.05 Å) or cyanocobalamin (root mean square = 0.11, 0.04 Å) (note that in all cases the esd for plane 1 is higher than that for plane 2). The fold angle between these planes for the two B_{12} derivatives is 14.6° (adenosylcobalamin) and 17.7–18.7° (wet and air-dried vitamin), respectively. This is illustrated in Figure 4. When the benzimidazole group coordinated to the cobalt is replaced by a smaller group (such as cyanide) this fold angle is markedly decreased.[4]

Another estimate of the effect of the folding about the Co–C-(10) line in the corrin ring can be seen from the deviation of various atoms from the least-squares plane through the four nitrogen atoms equatorially coordinated to cobalt.[4] It is found for methylcobalamin that the deviations from this plane are the following: C(1) −0.26 Å, C(5) 0.50 Å, C(10) −0.16 Å, C(15) 0.08 Å, and C(19) 0.36 Å. Thus C(5), C(15), and C(19) are above the plane and C(1) and C(10) below it. These atoms deviate in the same direction from the plane of the four nitrogen atoms in both adenosylcobalamin and cyanocobalamin.[4]

An important result of this structure determination is the information it gives on the trans influence (measured by the distances from the cobalt atom to its two axial ligands). An advantage in studying methylcobalamin is that these effects can be compared easily to model compounds (such as the cobaloximes) that contain methyl axial ligands, whereas no such model exists for adenosylcobalamin. A comparison of these parameters in methylcobalamin with the measured values for related compounds is listed in Table IV. The distances for methylcobalamin are Co–N(Bzm) = 2.19 Å and Co–C(methyl) = 1.99 Å, with approximate estimated standard deviations of 0.02 Å. These values may be compared with Co–N(adenine) = 2.12 (3) Å in factor A,[21] and Co–N(Bzm) = 2.230 (1) Å in a dimethylphosphito derivative.[21] In the adenosyl coenzyme the Co–N(Bzm) distance is 2.24 Å and the Co–C(adenosyl) distance is 2.03 Å.[4,8] Thus these axial distances are marginally shorter in methylcobalamin than in 5'-deoxyadenosylcobalamin. This is evident in the benzimidazole position shown in Figure 2. In contrast, there appears to be little difference in Co–C bond lengths in cobalamins and cobaloximes for compounds with axial alkyl groups of similar bulk.[9]

The hydrogen bonding system is described in Table V and Figure 5; the surroundings of various amide groups and solvent molecules are diagrammed in this figure.

Trends in NMR Data. In addition to revealing the connectivity between atoms, NMR spectral data can provide information concerning conformational changes, bond length variations, etc., in metal complexes. For example, as the length of the bond between Co and L increases in cobaloximes, there are well-defined trends in ^1H, ^{13}C, and ^{31}P NMR shifts and coupling constants.

Table IV. Distances and Angles from the Cobalt Atom to Its Two Axial Ligands

models[a]	Co–C, Å	Co–N, Å	C–Co–N, deg
(a) Cobaloximes			
(py)Co(DH)$_2$CH$_3$[b]	1.998 (5)	2.068 (3)	178.0 (2)
1-CH$_3$IMDCo(DH)$_2$CH$_3$[b]	2.009 (7)	2.058 (5)	178.0 (3)
(C$_6$H$_5$)(CH$_3$)CHNH$_2$Co-(DH)$_2$CH$_3$[c]	1.988 (19)	2.087 (9)	
(b) Cobalamins			
MeB$_{12}$	1.99 (2)	2.19 (2)	171 (1)
adenosyl coenzyme	2.03 (3)	2.24 (3)	170 (2)

[a]Abbreviations: py = pyridine, 1-CH$_3$IMD = 1-methylimidazole. [b]Bigotto, A.; Zangrando, E.; Randaccio, L. *J. Chem. Soc., Dalton Trans.* **1976**, *96*. [c]Ohashi, Y.; Sasada, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1710.

In a series of eleven structurally characterized complexes of the type (pyridine)Co(DH)$_2$X, there is found to exist a linear relationship between the Co–N(pyridine) bond length and the $\gamma^{13}C$ NMR shift of the closely related compounds (4-*tert*-butyl-pyridine)Co(DH)$_2$X with the same X ligand.[9] From rate and spectroscopic data for cobaloximes, we rank the negatively charged axial ligands used in this study as follows in order of increasing trans influence and trans effect: $CN < CH_2CN < CH_2CF_3 < CH_3 < CH_2CH_3 \sim CH_2CH_2CH_3 \sim CH_2CH(CH_3)_2$. Unfortunately, inadequate information is available for the placement of 5'-deoxyadenosine within this list.

Cobalamins are, obviously, much more complex molecules than are cobaloximes and, furthermore, steric factors may be more evident in cobalamins than they are in cobaloximes. Nevertheless, it is just these steric factors which we are trying to understand, since they should be important in Co–C bond cleavage. Furthermore, cobalamins provide an additional spectroscopic probe in the ^{31}P NMR resonance of the phosphodiester group. It is well established that ^{31}P NMR shifts can reveal structural information since these shifts respond to changes in bond and torsion angles at P.[22-29]

In contrast to the extensive spectroscopic and rate correlations with structure that are available for B_{12} models,[9] the few published correlations of ^1H,[30-40] ^{31}P,[41-44] and ^{13}C[32,45-56] NMR data on

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a) MeB12(solid) vs. Vitamin B12 (dry)

b) MeB12(solid) vs. Vitamin B12(wet)
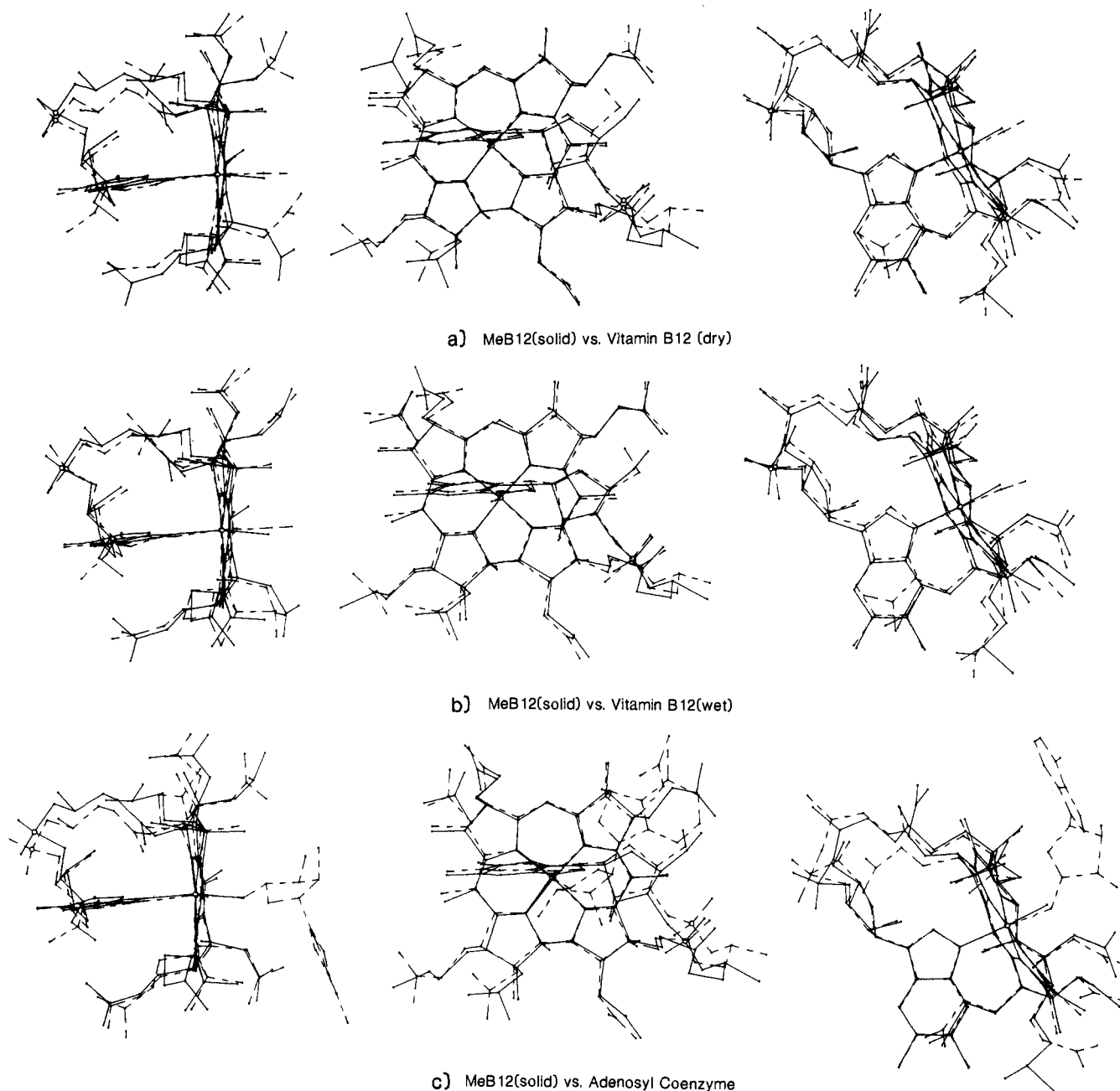

c) MeB12(solid) vs. Adenosyl Coenzyme

Figure 2. General comparison of the conformation of methylcobalamin vs. cyanocobalamin (dry), cobalamin (wet), and the adenosylcobalamin. The two molecules being compared are superimposed and illustrated with the MeB_{12} in solid lines and the other in dashed lines. Views are (left) perpendicular to the corrin ring, (center) a projection onto the plane of the corrin ring, and (right) a projection onto the plane of the benzimidazole ligand.

cobalamins have, of necessity, not involved structural comparisons. However, extensive information is available on chemical shift and

coupling constant assignments in the NMR spectra of cobalamins.[31]

Comparisons of NMR and X-ray Results. This determination of the three-dimensional structure of methylcobalamin has made it possible for us to compare the results of the X-ray diffraction study with those obtained by NMR techniques. Some NMR data are presented in Table VI. We have chosen the alkylcobalamins listed with two preliminary predictions in mind. First, we felt that the intrinsic electron donor ability of the axial ligand would prove to be similar to that of simple model compounds. Second, we felt that resonances not confounded by several factors that influence

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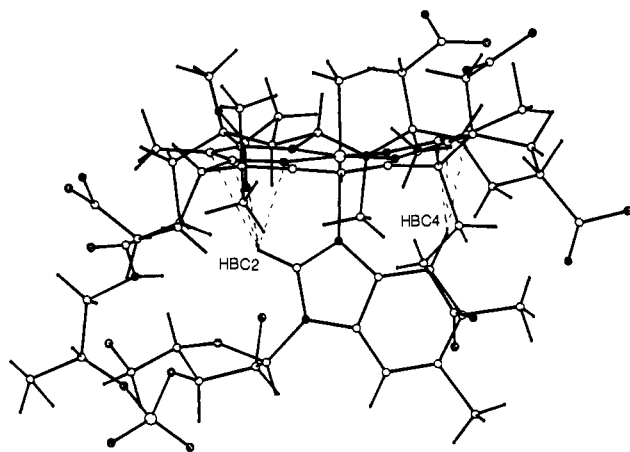
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Figure 3. Short distances from H(BC2) and H(BC4) to C(6), C(14), and their neighboring atoms (dashed lines).

Table V. Hydrogen Bonding and Close Contacts Involving Methylcobalamin (See also Figure 3)

A	B	A···B, Å	symmetry operation relating B to A
O(28)	H ← O(W1)	2.76 (3)	$-1/2 + x, 1/2 - y, 1 - z$
O(28)	H ← N(62)	3.12 (3)	$1/2 - x, 1 - y, 1/2 + z$
N(29) → H	O(63)	2.86 (3)	$1/2 - x, 1 - y, 1/2 + z$
N(29) → H	O(W13)	3.13 (5)	x, y, z
N(33) → H	O(W11)	3.12 (7)	$1/2 + x, 1/2 - y, 1 - z$
N(33) → H	O(W20)	3.48 (9)	x, y, z
O(34)	H ← O(W15)	2.99 (7)	$1/2 + x, 1/2 - y, 1 - z$
O(34)	H ← O(W23)	3.10 (11)	$3/2 - x, 1 - y, 1/2 + z$
O(39)	H ← O(W3)	2.90 (3)	$-1/2 + x, 1/2 - y, 1 - z$
O(39)	H ← O(W8)	3.26 (5)	$-1/2 + x, 1/2 - y, 1 - z$
N(40) → H	O(W2)	2.99 (6)	$-1/2 + x, 1/2 - y, 1 - z$
N(40) → H	O(W11)	3.16 (6)	x, y, z
O(44)	H ← O(W14)	2.80 (5)	x, y, z
N(45) → H	O(W6)	2.75 (6)	$1/2 + x, 1/2 - y, 1 - z$
N(45) → H	O(W14)	3.15 (5)	x, y, z
O(51)	H ← O(W3)	2.87 (3)	$-1 + x, y, z$
O(51)	H ← O(W4)	2.71 (3)	$-1/2 + x, 1/2 - y, -z$
O(51)	H ← O(W32)	2.88 (14)	$-1/2 + x, 1/2 - y, -z$
N(52) → H	O(4)	2.92 (4)	$-1/2 + x, 1/2 - y, -z$
N(52) → H	O(W9)	3.31 (5)	x, y, z
O(58)	H ← O(W4)	3.06 (4)	$-1/2 + x, 1/2 - y, -z$
N(59) → H	O(3)	2.87 (3)	x, y, z
N(59) → H	O(W5)	3.09 (3)	x, y, z
N(62) → H	O(W7)	3.05 (6)	x, y, z
O(63)	H ← O(W10)	2.66 (5)	x, y, z
O(63)	H ← N(29)	2.85 (3)	$1/2 - x, 1 + y, -1/2 + z$
O(4)	H ← O(W1)	2.98 (4)	$-1/2 + x, 1/2 - y, -z$
O(5)	H ← O(W3)	2.86 (3)	$-1/2 + x, 1/2 - y, -z$
O(5)	H ← O(W14)	2.73 (5)	$-1/2 + x, 1/2 - y, -z$
O(2)	H ← O(7R)	2.80 (2)	x, y, z
O(6R) → H	O(W27)	3.10 (9)	x, y, z
O(7R)	H ← O(W5)	2.86 (3)	x, y, z
O(8R) → H	O(W4)	2.84 (4)	$-1/2 + x, 1/2 - y, -z$
O(8R)	H ← O(W35)	2.95 (8)	x, y, z
N(1B)	H ← O(W27)	2.87 (9)	x, y, z
N(1B)	H ← O(7R)	2.65 (2)	x, y, z

shifts simultaneously would prove to correlate reasonably well between the compounds chosen. With some minor exceptions, these predictions seem to be borne out by the data. We can divide the experimentally measured proton and ^{31}P NMR resonances into two groups. Those in the first class involve atoms distant from the corrin; these data correlate between themselves and also to some extent with cobaloximes. The second group contains those resonances involving either in the corrin or near the corrin; these are not well correlated. Table VII contains a listing computed from the X-ray structure of distances from the cobalt atom to other atoms that have or have been proposed to have[31] resonances sensitive to structural changes. The short distances from hydrogen atoms H(BC2) and H(BC4) (to C(14) and C(6), re-

spectively) are shown in Figure 3. In order to investigate Co–H distances in a uniform manner, hydrogen atom coordinates for all the C–H bonds were calculated at distances of 1.08 Å from the carbon atom. This was done because bond lengths involving hydrogen atoms found in X-ray analyses are usually shorter than accepted values since the technique, which measures electron density, gives peaks that are concentrated along the C–H bond rather than symmetrically around the hydrogen atom position. Neutron diffraction studies give more accurately determined nuclear positions and show that internuclear C–H distances lie in the region of 1.08 Å.

Let us consider the first group of resonances. This group includes H(BC7), H(RC1), and ^{31}P. They are in quite different environments but share the property of being in the benzimidazole–ribose–phosphate side chain, distant from the corrinoid moiety. The H(RC1) resonance follows a similar trend to the others in this group but shows a concentration dependence (Table D, supplementary material). We will not discuss it further. The phosphorus atom is at least eight bonds removed from the cobalt atom. An inductive effect resulting from a change in the axial ligand is improbable, especially since only two of the intervening bonds are unsaturated.[9] The resonance of C(1P), which is also eight bonds away, is insignificantly influenced by changing the axial group from cyanide to deoxyadenosyl.[31] These ^{31}P shifts possibly reflect changes in torsion angles at the phosphorus atom,[22-29] although differences in solvation cannot be ruled out as a cause. Torsion angles around the phosphorus atom, obtained from the X-ray diffraction analysis, are listed in Table VIII. It was found that there is no significant trend in the O(3)–P–O(2) bond angle as a function of the ^{31}P shift (Table VIII), but the C(2P)–O(3)–P–O(2) torsion angle does reflect the ^{31}P NMR data. However the Co–P distance correlates nicely with the ^{31}P chemical shift.

The shift of the H(BC7) resonance correlates fairly well with the ^{31}P shift, as shown in Figure 6 and Table VI. The dependence of the H(BC7) resonance on the nature of the axial alkyl ligand probably involves a "through-bond effect" transmitted through the unsaturated benzimidazole. The origins of the changes in shifts for H(BC7) and ^{31}P in different alkylcobalamins are probably different, but since both are responding to the trans influence of the axial ligand (the only chemical change), the parallel trend is understandable. This relationship is reflected in the structural data in Table VII. The position of isobutylcobalamin resonances in Figure 6 indicate that the isobutyl group is somewhat less electron donating than one would expect. This may reflect steric effects. Such steric effects have been noted for model systems but only when bulkier ligands than isobutyl are used (for example, isopropyl or neopentyl).[57-59]

Of the second group of resonances, that for H(BC4) is most like the first class. H(BC4) is on the six-membered ring of benzimidazole and faces the corrinoid, but it is further removed from the corrinoid than H(BC2) which is on the five-membered ring of benzimidazole and only three bonds from the cobalt atom (Table VII). For the H(BC4) resonance, the greatest deviation from the expected trend is found when R = isobutyl, and again, this may reflect steric effects. The H(BC2) and H(C10) resonances shift in an erratic manner, possibly reflecting counteracting influences of changes in electron density and in anisotropy of both the cobalt atom and the corrin macrocycle. This means that previous correlations of H(C10) with electronic properties[31] should be reexamined. In addition, the suggestion that the methyl group on C(1) (H$_3$C(20)) might be a sensitive indicator of the position[31] of the benzimidazole is not borne out by the H(C20) shifts (Table VII). This resonance does not shift in a systematic fashion, and there appears to be no great difference in the position of C(20)

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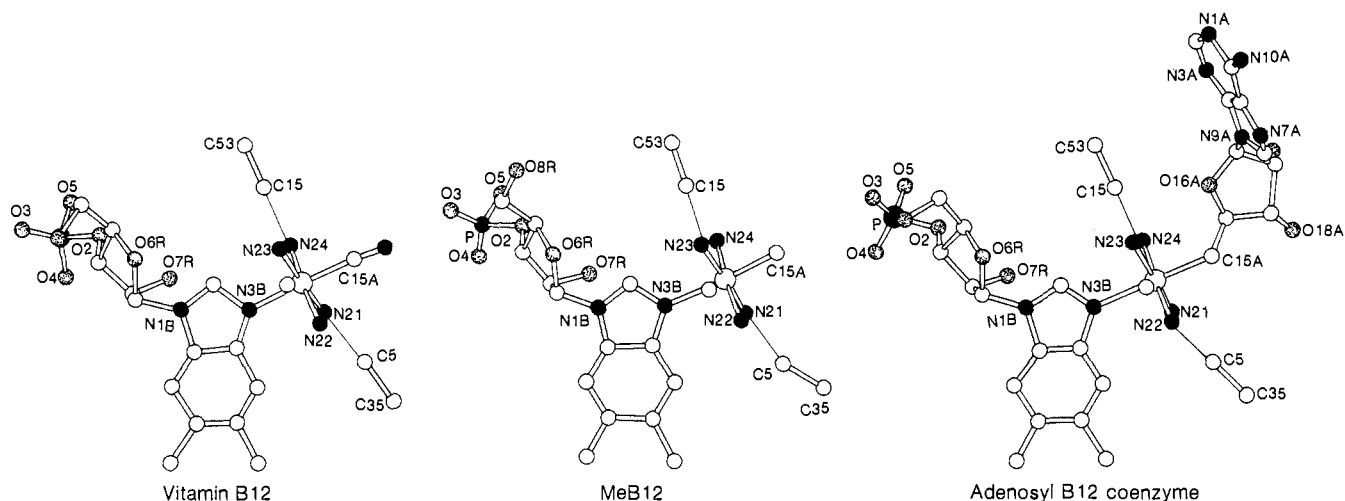
Figure 4. View of vitamin B$_{12}$ and its two coenzymes, illustrating the similarity in structure for the three compounds. Most of the corrin rings have been removed. This diagram shows the relationship of the benzimidazole group to the C(5)–C(35) bond.
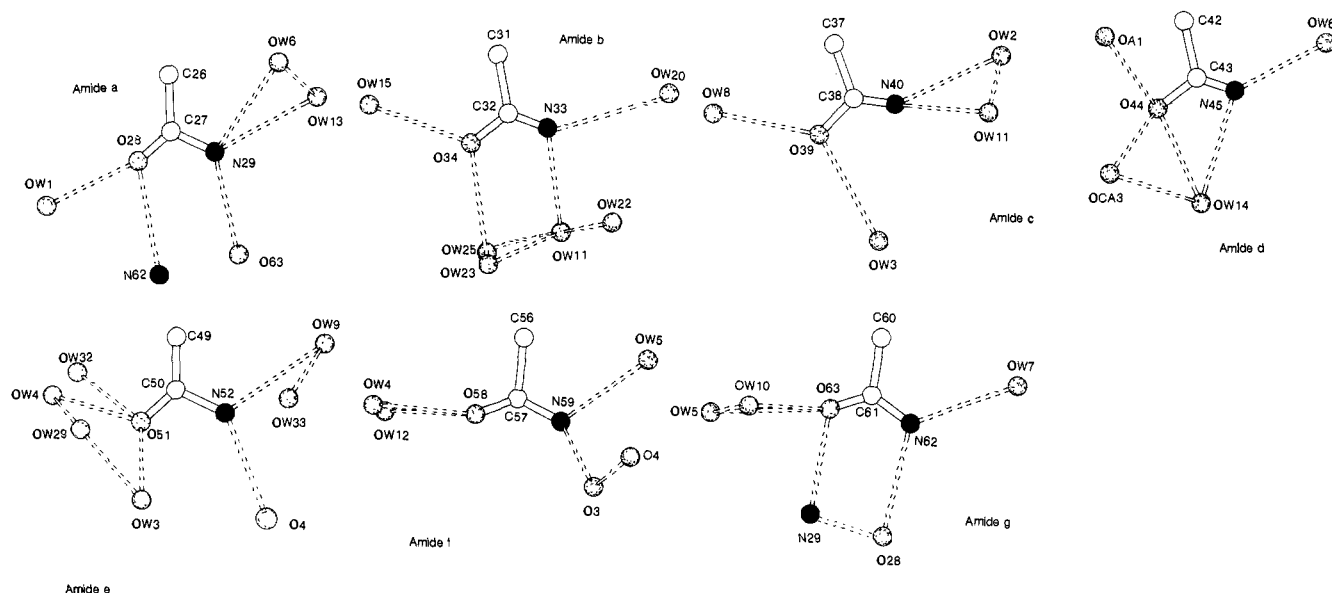

Figure 5. Hydrogen bonding evident around each of the seven amide groups. The solvent molecules shown are among the better resolved atoms in the structure.

Table VI. ^{31}P and ^{1}H NMR Spectral Data for Cobalamins with Different Upper Axial Ligands (B$_{12}$–X) in D$_2$O[a]

	group I			group 2			
	NMR δ			^{1}H NMR δ[b]			
X	^{31}P[d]	H(BC7)	H(RC1) (^3J, Hz)	H(BC4)	H(BC2)	H(C10)	H(C20)[c]
CN	−3.77	7.277	6.355 (2.9)	6.504	7.085	6.092	0.461
CH$_2$CN	−4.06	7.219	6.296 (3.1)	6.360	7.041	6.049	0.486
CH$_2$CF$_3$	−4.16	7.201	6.292 (3.1)	6.328	6.998	5.983	0.429
CH$_3$	−4.20	7.192	6.279 (3.0)	6.300	6.993	5.932	0.475
CH$_2$CH(CH$_3$)$_2$	−4.29	7.174	6.265 (3.0)	6.340	7.069	6.144	0.451
CH$_2$CH$_2$CH$_3$	−4.39	7.171	6.257 (2.9)	6.275	7.000	6.076	0.533
CH$_2$CH$_3$	−4.36	7.170	6.255 (3.2)	6.259	7.000	6.068	0.538
5′-Ado	−4.34	7.166	6.259 (3.0)	6.237	6.942	5.941	0.481
H$_2$O	−3.66	7.173	6.248 (3.1)	6.463	6.527	6.302	0.526

[a] [Cobalamin] ~ 2.0 mM. Internal references: TSP for ^{1}H, TMP for ^{31}P; negative values to higher field. [b] IUPAC assignments; see p 465 of ref 3. [c] Methyl hydrogens. [d] The ^{31}P shift is not concentration dependent. The ^{1}H shifts are concentration dependent (supplementary material, Table D), but the H(BC7) shift is only slightly affected. Low concentrations should be employed.

Table VII. Relevant Distances in Å to Co from Atoms Having Significant ^{31}P and ^{1}H Resonances[a]

|	Co–P	Co–H-(BC2)	Co–H-(BC7)	Co–H-(BC4)	Co–H-(C10)
Vit B$_{12}$	9.13	3.0	6.5	3.5	4.3
Me B$_{12}$	9.24	3.2	6.6	3.4	4.3
Coenzyme	9.38	3.3	6.7	3.6	4.4

[a] In each case hydrogen atom positions were computed so that C–H = 1.08 Å.

relative to the benzimidazole, Table IIId. In conclusion, some of the NMR resonances appear to be promising handles for evaluating changes in cobalamin structure, but further work is obviously needed.

Relationship to B$_{12}$ Biochemistry. One of the prevalent themes in B$_{12}$ biochemistry is that repulsive interactions between the bulky 5′-deoxyadenosyl group and the corrin macrocycle occur in the holoenzyme, thereby facilitating cleavage of the Co–C bond.[3,58–66] The similarity in structure between the two alkylcobalamins

Table VIII. Selected Bond Lengths and Angles and Torsion Angles Involving the Phosphate Group for Various B_{12} Derivatives (from Ref 4)

	MeB_{12}	vitamin B_{12} (dry)	vitamin B_{12} (wet)	monocarboxylic acid	factor A	adenosyl coenzyme
bond lengths, Å						
P–O(2)	1.57	1.67	1.60	1.51	1.56	1.58
P–O(3)	1.57	1.58	1.56	1.56	1.59	1.62
P–O(4)	1.38	1.52	1.65	1.47	1.44	1.44
P–O(5)	1.49	1.38	1.53	1.47	1.50	1.37
bond angles, deg						
O(3)–P–O(4)	109	108	101	103	110	108
O(3)–P–O(5)	110	90	113	119	111	110
O(3)–P–O(2)	97	102	100	101	98	100
O(4)–P–O(5)	122	126	122	108	119	117
O(4)–P–O(2)	112	103	108	110	108	109
O(5)–P–O(2)	103	121	111	115	110	111
C(P2)–O(3)–P	122	120	121	119	121	116
C(3R)–O(2)–P	121	121	114	121	121	115
torsion angles, deg						
C(P2)–O(3)–P–O(2)	–69	–60	–61	–83	–70	–70
C(P2)–O(3)–P–O(4)	175	–169	–172	163	178	175
C(P2)–O(3)–P–O(5)	38	62	55	44	44	47
O(3)–P–O(2)–C(3R)	176	157	174	158	163	173
O(4)–P–O(2)–C(3R)	–69	–91	–81	–94	–83	–74
O(5)–P–O(2)–C(3R)	63	59	55	28	47	56
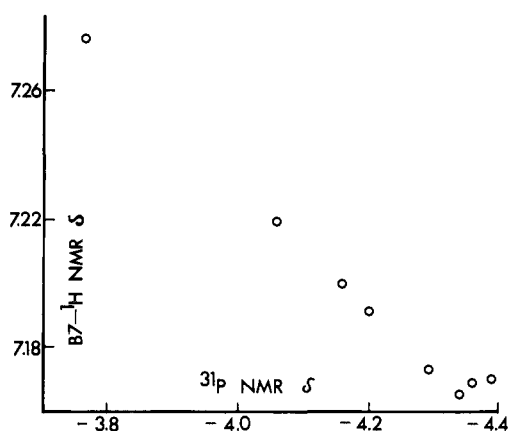
Figure 6. ^1H NMR δ for the H(B7) proton vs. ^{31}P NMR δ for B_{12}–X complexes; X = (left to right) CN, CH_2CN, CH_2CF_3, CH_3, i-C_4H_9, 5′-ado, C_2H_5, n-C_3H_7.

(methylcobalamin, adenosylcobalamin), one with a small upper axial ligand and the other with a bulky one, suggests that the bulkiness of this ligand is not a main structural determinant. Unfortunately, little information is available on the expected electron donor ability of the 5′-deoxyadenosyl group in cobaloximes or other models; such information would facilitate an assessment of the donor ability of the 5′-deoxyadenosyl in coenzyme B_{12}. Possibly methylcobalamin, with its powerful methyl transfer activity,[67] may act in a different way from adenosylcobalamin (coenzyme B_{12}).

More recently, our structural studies with model compounds, closely related to those for which estimates exist for Co–C bond energies, reveal that the only clear structural correlation to Co–C bond energies is the length of the trans Co–N bond.[68,69] This

bond is longer when the Co–C bond is weaker. This correlation can be understood when we recognize that a good electron donor will stabilize the Co(III) oxidation state and prevent homolysis as shown by the work of Halpern.[1,70] Nature is limited in that it cannot readily alter the ligand trans to the alkyl group. However, a change in protein conformation which results in an increase in the Co–N bond length could have the effect of decreasing electron donation by the benzimidazole group, thereby facilitating bond cleavage.

In comparison to typical model compounds (Table IV), the Co–N bond trans to the alkyl group in methyl B_{12} is unusually long. This distance is less accurately determined than that in the model compounds, but it suggests that Co–C bond energies in cobalamins should be lower than those in cobaloximes. No accurate estimates of Co–C bond energies in cobalamin have been reported, and such information would be most welcome.[73]

Supplementary Material Available: Table A, temperature factors of the atoms in Table I and fractional coordinates of H atoms; Table B, observed calculated structure factors; Table C, a full list of bond distances; Table D, selected ^1H NMR chemical shifts for methylcobalamin; Figure A, a stereo view of methylcobalamin; and Figure B, a ^1H NMR spectrum of B_{12}–C_2H_5 (36 pages). Ordering information is given on any current masthead page.

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