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Assignments in the Carbon-13 Nuclear Magnetic Resonance Spectra of Vitamin B_{12}, Coenzyme B_{12}, and Other Corrinoids: Application of Partially-Relaxed Fourier Transform Spectroscopy

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ABSTRACT **High-resolution Fourier transform NMR at 15.08 MHz was used to observe the proton-decoupled natural-abundance ^{13}C spectra of aqueous solutions of cobinamide dicyanide (0.067 M), cyanocobalamin (0.024 M), dicyanocobalamin (0.14 M), and coenzyme B_{12} (0.038 M). Assignments were made with the aid of chemical shift comparisons, off-resonance single-frequency proton decoupling, partially-relaxed Fourier transform spectra, and splittings arising from ^{13}C–^{31}P coupling.**

As expected, the ^{13}C spectra of the corrinoids were appreciably more informative than the corresponding proton spectra. Nearly all the lines in the ^{13}C spectra of the corrinoids were well-resolved single-carbon resonances, in spite of the structural complexity.

Partially relaxed ^{13}C Fourier transform NMR spectra, which yield spin-lattice relaxation times of each resolved resonance, were found to be a very useful addition to the arsenal of NMR techniques.

Proton nuclear magnetic resonance (NMR) has been useful for investigating the solution properties of vitamin B_{12} (cyanocobalamin, Fig. 1*A*) and other corrinoids (1–4). However, even at 220 MHz, proton NMR spectra of corrinoids contain many overlapping lines, as a result of a small range of chemical shifts and complex spin–spin splittings (4). For the study of complex molecules, proton-decoupled spectra of ^{13}C nuclei in natural abundance are, in general, much more resolved and simpler to analyze than the corresponding proton spectra (5). The enhanced sensitivity of the Fourier transform technique (6) relative to continuous-wave NMR has expanded the range of accessible ^{13}C spectra (7). We will show that by using the Fourier transform method it is easy to obtain high signal-to-noise ratios on single-carbon resonances in the proton-decoupled natural-abundance ^{13}C spectra of corrinoids, even when the concentration is limited to 0.02 M.

There are only two well-known generally applicable aids to the assignment of ^{13}C resonances in complex molecules: comparisons within a series of compounds with similar structures, and the use of splitting patterns arising from incomplete proton-decoupling (5). The former is often complicated by uncertainties in chemical shift changes with structure; the latter is difficult to use when there are many overlapping lines (5). We show below that ^{13}C partially-relaxed Fourier transform (PRFT) spectra (8, 9) provide additional help in making assignments. Intensities in PRFT spectra are given (10) by

$$A = A_0 [1 - 2 \exp(-\tau/T_1)], \quad (1)$$

where A and A_0 are the observed and equilibrium intensities, respectively, τ is the interval between the 180° and 90° pulse (8), and T_1 is the ^{13}C spin-lattice relaxation time. Thus, if two carbons have different T_1 values, the intensity of their resonances will have a different dependence on τ. We have shown (to be published) that ^{13}C T_1 values measured from PRFT spectra can be used to distinguish methine, methylene, and non-protonated carbons on fused ring structures, and to detect groups that have internal motion.

In this paper we present assignments in the proton-decoupled natural-abundance ^{13}C spectra of cyanocobalamin (Fig. 1*A*), 5′-deoxyadenosylcobalamin (coenzyme B_{12}, Fig. 1*B*), dicyanocobalamin (Fig. 2*B*), and cobinamide dicyanide (Fig. 2*A*).

MATERIALS AND METHODS

Cyanocobalamin was obtained from Nutritional Biochemicals, Cleveland, Ohio. Dicyanocobalamin was prepared by addition of KCN to cyanocobalamin (11). Coenzyme B_{12} and cobinamide dicyanide were kindly supplied by Dr. L. Mervyn of Glaxo Research Ltd., Great Britain. The equipment has been

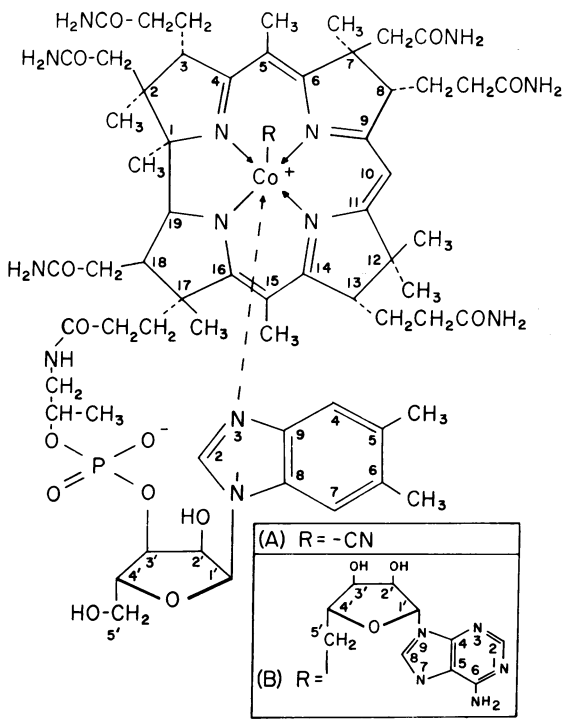

FIG. 1. Structures of corrinoids. *A*, cyanocobalamin (vitamin B_{12}). *B*, 5′-deoxyadenosylcobalamin (coenzyme B_{12}).

Abbreviation: PRFT, partially-relaxed Fourier transform.

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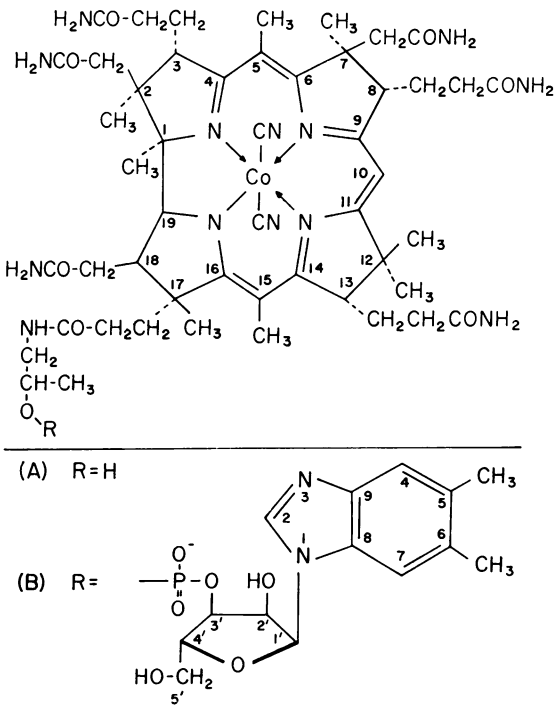

FIG. 2. Structures of corrinoids. *A*, Cobinamide dicyanide. *B*, Dicyanocobalamin.

described previously (7, 9). When using the 180°-τ-90° pulse sequence (10) needed to obtain PRFT spectra (8, 9), we chose the recycle time between sequences to be at least three times the longest T_1 value of interest.

RESULTS

Completely proton-decoupled ^{13}C spectra of cobinamide dicyanide, cyanocobalamin, and coenzyme B_{12} are shown in Fig. 3. The completely proton-decoupled and the off-resonance single-frequency decoupled (5) spectra of dicyanocobalamin are shown in Fig. 4. Cyanocobalamin (0.024 M) and coenzyme B_{12} (0.038 M) were studied as nearly saturated aqueous solutions at 65°C and 56°C, respectively. Cobinamide dicyanide and dicyanocobalamin were examined at 56°C, in 0.067 M and 0.14 M aqueous solutions, respectively.

Chemical shifts of the resonances that were assigned are given in Tables 1–3. As expected (12), some carbons, up to three bonds removed from phosphorus, exhibited resolved splitting arising from ^{13}C–^{31}P scalar coupling. Observed coupling constants are given in Table 2. Carbons directly bonded to cobalt are expected to show the effect of scalar coupling to ^{59}Co (100% abundance, spin $^7/_2$). It is likely that the quadrupolar contribution to $1/T_1$ of the cobalt in corrinoids is sufficiently great so that $2\pi J_{C-Co}T_{1Co} \ll 1$. In this case no resolved splittings of the carbon resonances should be observed, but appreciable residual line broadening may still occur (13). Only in the spectrum of the relatively concentrated dicyanocobalamin did we detect a resonance assignable to the cyano carbons.

Spin-lattice relaxation times of some carbons in dicyanocobalamin, obtained from intensities in a series of PRFT spectra, are given in Tables 1 and 2. A few representative PRFT spectra are shown in Fig. 5.

ASSIGNMENTS

We have used several of the following five types of evidence in making each assignment: (*1*) Chemical shift comparisons with model compounds such as benziminazole (Table 3), nucleotides (12), and porphyrins (D. Doddrell and W. S. Caughey, unpublished results). (*2*) Spectral comparisons within the corrinoids. (*3*) Off-resonance single-frequency decoupling (5) for dicyanocobalamin; not attempted on the more dilute samples. (*4*) Scalar coupling to ^{31}P. (*5*) Partially-relaxed spectra and ^{13}C spin-lattice relaxation times. We believe this is the first successful application of ^{13}C PRFT spectra in assignments of resonances.

TABLE 1. *^{13}C chemical shifts of the corrin ring carbons*

Assignment[b]	Chemical shift[a] Cobinamide dicyanide	Coenzyme B_{12}	Cyanocobalamin	Dicyanocobalamin	T_1[c] (sec)
5,15	88.0(10)	87.3(21)	85.9(18)	87.6(18)	$\gtrsim$1.0
	90.1(11)	88.7(22)	89.3(19)	89.7(19)	$\gtrsim$1.0
10	102.0(12)	98.4(23)	98.5(20)	101.7(20)	0.11
1	109.8(13)	107.5(26)	108.1(22)	109.6(23)	$\gtrsim$1.0
19	117.7(14)	118.5(29)[d]	118.3(25)	117.5(24)	0.10
2	134.1(16)	134.7(35)	134.1(31)	133.8(32)	$\gtrsim$1.0
3,8,13	136.3(17)	136.9(36)	136.7(32)	136.1(33)	0.082
	137.5(18)	138.0(37)	137.5(33)	137.2(34)	0.099
	139.5(19)	139.8(38)	139.3(34)	139.3(35)	0.11
7,12,17	143.7(20)	142.7(39)	142.0(35)	143.5(36)	$\gtrsim$1.0
	146.0(21)	146.2(40)	145.1(36)	146.0(37)	$\gtrsim$1.0
	146.5(22)	146.2(40)	145.9(37)	146.5(38)	$\gtrsim$1.0
18[e]	162.3(31)	[f]	[f]	162.2(49)	~0.2

[a] In parts per million upfield from carbon disulfide. Estimated accuracy ±0.3 ppm. Carbons 4, 6, 9, 11, 14, and 16, which resonate below 30 ppm, could not be assigned (see text). Numbers in parentheses are peak designations in Figs. 3–6. [b] Carbons written on the same line could not be assigned on a one-to-one basis. [c] ^{13}C spin-lattice relaxation time in 0.14 M aqueous dicyanocobalamin at 56°C and pH 9.8. Estimated accuracy ±25%. [d] This is the most likely assignment, but 119.0 ppm (peak 30) cannot be excluded as a possibility. [e] Tentative assignment (see text). [f] Could not be identified, because of closely spaced resonances in this region.

It is safe to assume that at 15.08 MHz, ^{13}C relaxation of protonated carbons in large molecules is overwhelmingly dominated by dipolar interactions with the directly attached protons (14) with T_1 given by

$$1/T_1 = (h/2\pi)^2\gamma_C^2\gamma_H^2 N r_{CH}^{-6}\tau_{eff}, \qquad (2)$$

where γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^1H, r_{CH} is the CH distance, N is the number of attached hydrogens, and τ_{eff} is an effective correlation time for rotational reorientation. Eq. **2** is valid when τ_{eff} is much smaller than the inverse of the proton and carbon resonance frequencies, a condition which holds for the solutions studied here, as can be shown by means of linewidth measurements (9). In a rigid fused-ring system, τ_{eff} is about the same for all ring-carbons.‡ Thus, T_1 of protonated ring-carbons is inversely proportional to the number of attached hydrogens. Non-protonated carbons have much longer relaxation times than protonated ring-carbons. Carbons of methyl groups and other side-chains capable of undergoing fast internal reorientation may have appreciably shorter τ_{eff} values than ring-carbons. The net result is that protonated side-chain carbons may have T_1 values much

‡ Evidence for all the statements in the rest of this paragraph is to be published elsewhere.

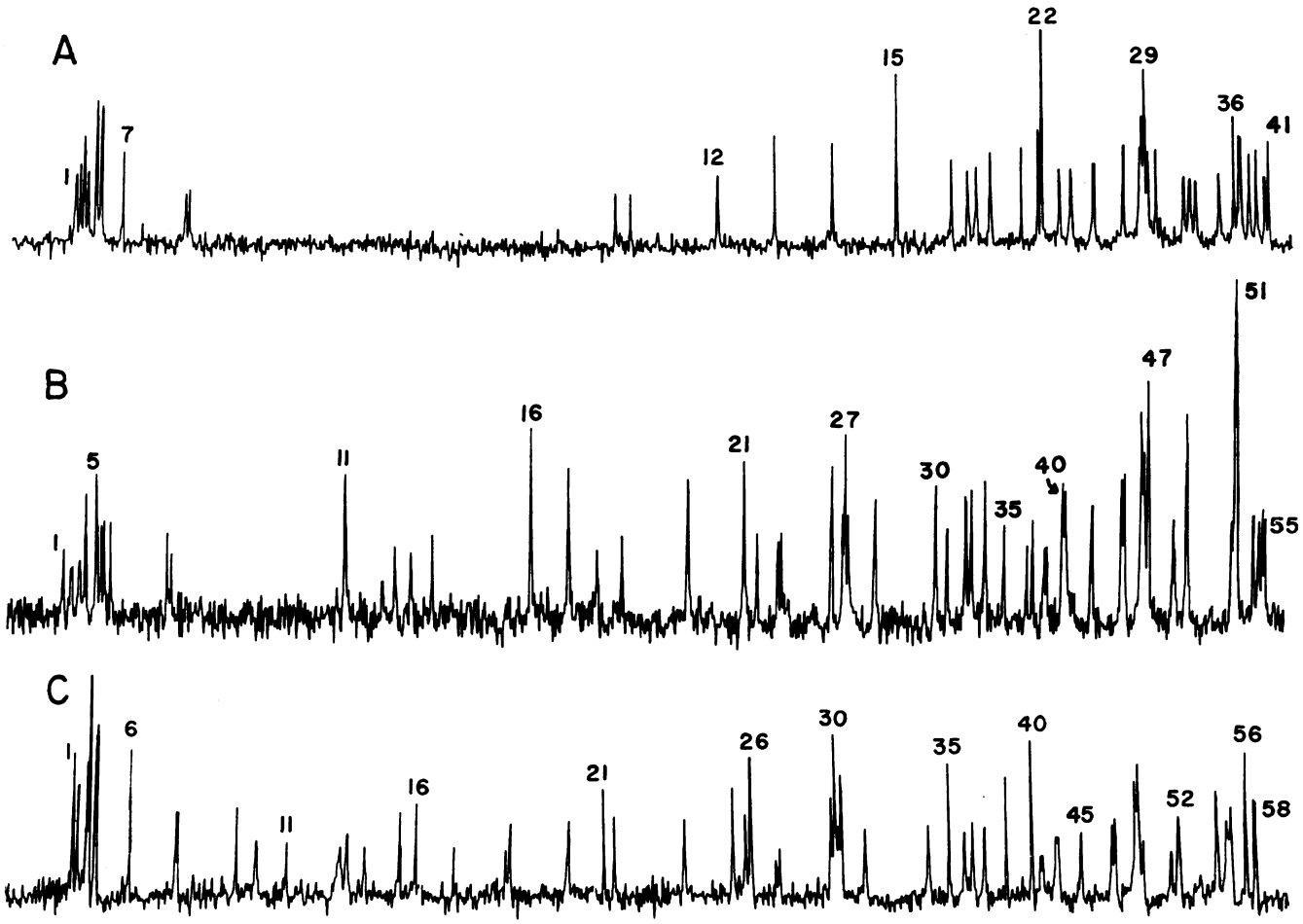

FIG. 3. Proton-decoupled natural-abundance ^{13}C NMR spectra of some corrinoids at 15.08 MHz, obtained by the Fourier transform method, with 4096 points in the time domain, and 250 ppm sweep widths. Only the range 6–181 ppm upfield from CS_2 is shown. Peaks are numbered consecutively from right to left. *A*, 0.067 M aqueous cobinamide dicyanide at 56°C and pH 7.3, using 10,720 scans with a recycle time of 2.72 sec (total time 8.1 hr). *B*, 0.024 M aqueous cyanocobalamin at 65°C, using 17,560 scans with a recycle time of 1.36 sec (total time 6.6 hr). *C*, 0.038 M coenzyme B_{12} at 56°C and pH 7.5; 14,216 scans with a recycle time of 2.72 sec (total time 10.7 hr).

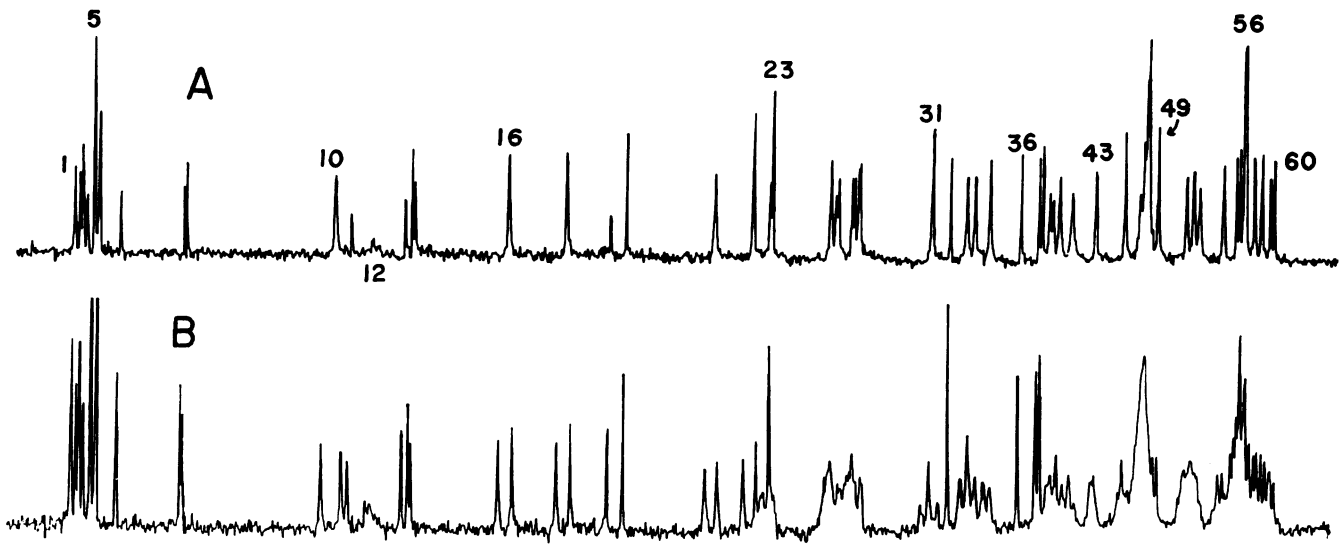

FIG. 4. Natural-abundance ^{13}C NMR spectra of 0.14 M aqueous dicyanocobalamin at 56°C and pH 9.8, obtained at 15.08 MHz by the Fourier transform method, with 4096 points in the time domain and 250 ppm sweep widths. Only the range 6.4–186.4 ppm upfield from CS_2 is shown. *A*, Completely proton-decoupled spectrum, using a noise-modulated decoupling radio-frequency field, 16,384 scans, and 0.68-sec recycle time (total time 3.1 hr). Peaks are numbered consecutively from right to left. *B*, Partially proton-decoupled spectrum, using single-frequency off-resonance decoupling, 23,217 scans, and 1.36 sec recycle time (total time 8.8 hr). (The full intensity of peaks 5 and 6 is not shown).

TABLE 2. *^{13}C chemical shifts of the α-ribazole and isopropanolamine moieties*

Assignment[b]	Chemical shift[a] Coenzyme B_{12}	Cyanocobalamin	Dicyanocobalamin	T_1[c] (sec)
	51.4(12)			
2 or	52.4(13)	51.6(11)	50.1(10)	0.22
9	54.7(14)	56.7(12)	52.2(11)	≳1.0
8	59.5(15)	58.4(13)	59.7(13)	≳1.0
5,6	61.7(16)	60.5(14)	60.5(14)	≳1.0
	67.0(17)	63.5(15)	60.9(15)	≳1.0
4	74.6(19)	76.9(16)	73.8(16)	0.25
7	82.5(20)	82.0(17)	81.6(17)	0.25
1′	105.0(24)	106.2(21)	107.0(21)	0.19
4′	111.1(27)[d]	111.2(23)[e]	109.4(22)[f]	
2′,3′	[g]	120.0(26)[f]	118.4(25)[e]	∼0.2
		120.3(27)[e]	120.6(27)[h]	∼0.2
5′	131.9(34)	132.5(30)	131.5(31)	0.18
CH[i]	123.2(33)	124.3(29)	121.4(29)[j]	0.24
CH_2[k]	147.6(41)[j]	147.8(38)[f]	147.6(39)[e]	∼0.1

[a] In parts per million upfield from carbon disulfide. Estimated accuracy ±0.3 ppm. Numbers in parentheses are peak designations in Figs. 3–6. In the case of doublets arising from coupling to ^{31}P, the number in parentheses refers to the downfield peak of the doublet. [b] Carbons written on the same line could not be assigned on a one-to-one basis. The methyl carbon of the isopropanolamine moiety in cobinamide dicyanide resonates at 173.2 ppm (peak 36). The methyl carbons of the benziminazole moiety in the cobalamins resonate in the range 173–174 ppm (see text). In coenzyme B_{12}, C-2 of the benziminazole ring and C-8 of the 5′-deoxyadenosyl group could not be assigned on a one-to-one basis. [c] T_1 value in 0.14 M aqueous dicyanocobalamin at 56°C and pH 9.8. The methyl carbons of the benziminazole and isopropanolamine moieties have T_1 values of about 0.8 sec. [d] Doublet, $J_{PC} \sim 8$ Hz. [e] Doublet, $J_{PC} \sim 6$ Hz. [f] Doublet, $J_{PC} \sim 5$ Hz. [g] In the range 118–120 ppm. Overlap with 5′-deoxyadenosyl resonances. [h] Doublet, $J_{PC} \sim 4$ Hz. [i] Methine carbon of isopropanolamine moiety. Resonates at 126.6 ppm (peak 15) in cobinamide dicyanide. [j] Doublet, $J_{PC} \sim 3$ Hz. [k] Methylene carbon of isopropanolamine moiety. Resonates at 146.5 ppm (peak 22) in cobinamide dicyanide.

TABLE 3. *Some ^{13}C chemical shifts*[a]

5′-Deoxyadenosyl group of coenzyme B_{12} Assignment[d,e]	Chemical shift	Benziminazole[b] Assignment	Chemical shift	Adenosine 3′-monophosphate[c] Assignment[d]	Chemical shift
6	37.3(9)	2	53.4	1′	104.2
2	39.9(10)	8,9	57.5	4′	107.6[f]
4	44.1(11)	5,6	62.2	2′,3′	118.5[f]
8[g]	51.4(12)	4,7	78.9		119.0[h]
or	52.4(13)	CH_3	174.6	5′	130.8
5	74.0(18)				
	106.7(25)				
1′,4′	107.5(26)[i]				

[a] In parts per million upfield from carbon disulfide. Estimated accuracy ±0.3 ppm. [b] Saturated methanol solution, at about 42°C. Carbon numbering system as in Fig. 1. [c] 1 M aqueous solution, pH 13.3, at about 42°C. [d] Carbons written on the same line could not be assigned on a one-to-one basis. Numbers in parentheses are peak designations in Fig. 3*C*. [e] The resonance of C-5′ was not observed, probably because of broadening from interaction with ^{59}Co (see text). The resonances of C-2′ and C-3′ overlapped with those of the ribose moiety at 118–120 ppm. [f] Doublet, $J_{CP} \sim 5$ Hz. [g] See assignment of C-2 of the benziminazole moiety in Table 2. [h] Doublet, $J_{CP} \sim 4$ Hz. [i] Coincides with C-1 of the corrin ring (see text).

longer than those on the ring backbone. The effect of internal motion on $1/T_1$ is most pronounced for carbons at, or near the free end of a side-chain. These principles are quite useful in making spectral assignments in complex molecules.

All the above techniques, when taken together, were sufficient to make the assignments given in Tables 1–3, which are justified below. Three types of carbons should resonate below 105 ppm: carbonyls, unsaturated carbons on the corrin ring, and the carbons of the benziminazole ring. The benziminazole ring carbons were identified by comparison of the spectra of cobinamide dicyanide and the cobalamins. PRFT spectra were used to identify all non-protonated carbons in this region. In the case of dicyanocobalamin, off-resonance single-frequency decoupling (Fig. 4) confirmed the identifications of non-protonated and methine carbons. Specifically, C-10 was assigned to the resonance at about 100 ppm, because it is the only protonated unsaturated carbon on the corrin ring, and because its chemical shift is comparable to that of similar carbons in porphyrins (D. Doddrell and W. S. Caughey, unpublished results). Carbons 5 and 15 of the corrin ring resonate about 10–15 ppm downfield from C-10, as a result of methyl substitution (15). The remaining unsaturated carbons of the corrin ring are strongly deshielded as a result of direct bonding to nitrogen, and they resonate in a range which overlaps partially with the amide carbonyl region (16). Position 2 of the benziminazole ring was assigned to the resonance at about 51 ppm. The remaining six benziminazole ring carbons can be divided into three pairs. The assignments for C-2 and the three pairs were made from PRFT spectra, and on the basis of chemical shifts in benziminazole itself (Table 3). Differentiation within the pairs was done for C-4,7 and C-8,9 by comparison of the spectra of dicyanocobalamin and cyanocobalamin, on the assumption that the resonances that shift appreciably upon coordination are closer to the coordinating nitrogen of the benziminazole ring than resonances that are not shifted. The base carbons of the 5′-deoxyadenosyl group in coenzyme B_{12} (Table 3) were assigned (17) by comparison of the spectra of cyanocobalamin, coenzyme B_{12}, and adenosine. The methylene carbon directly attached to cobalt in coenzyme B_{12} was not detected, probably as a result of line broadening arising from interaction with ^{59}Co (see above). The weak but reproducible broad resonance centered at about 55.5 ppm in the spectrum of dicyanocobalamin (peak 12 in Fig. 4*A*) was attributed to the CN carbons.

The five saturated non-protonated carbons of the corrin ring in all the corrinoids were identified from PRFT spectra. Fig. 6 illustrates the intensity changes in the resonances of 0.024 M aqueous cyanocobalamin when one goes from the normal spectrum (Fig. 6*A*) to a PRFT spectrum with $\tau = 1.02$ sec (Fig. 6*B*). At this τ value, the resonances of all the non-protonated carbons are nulled ($\tau \approx T_1 \ln 2$), while the relaxation times of all the protonated carbons are sufficiently short to yield positive signals. Carbon 1 was assigned to the furthest downfield resonance in this group, because of its direct attachment to nitrogen. Of the remaining four, carbons 7, 12, and 17 are

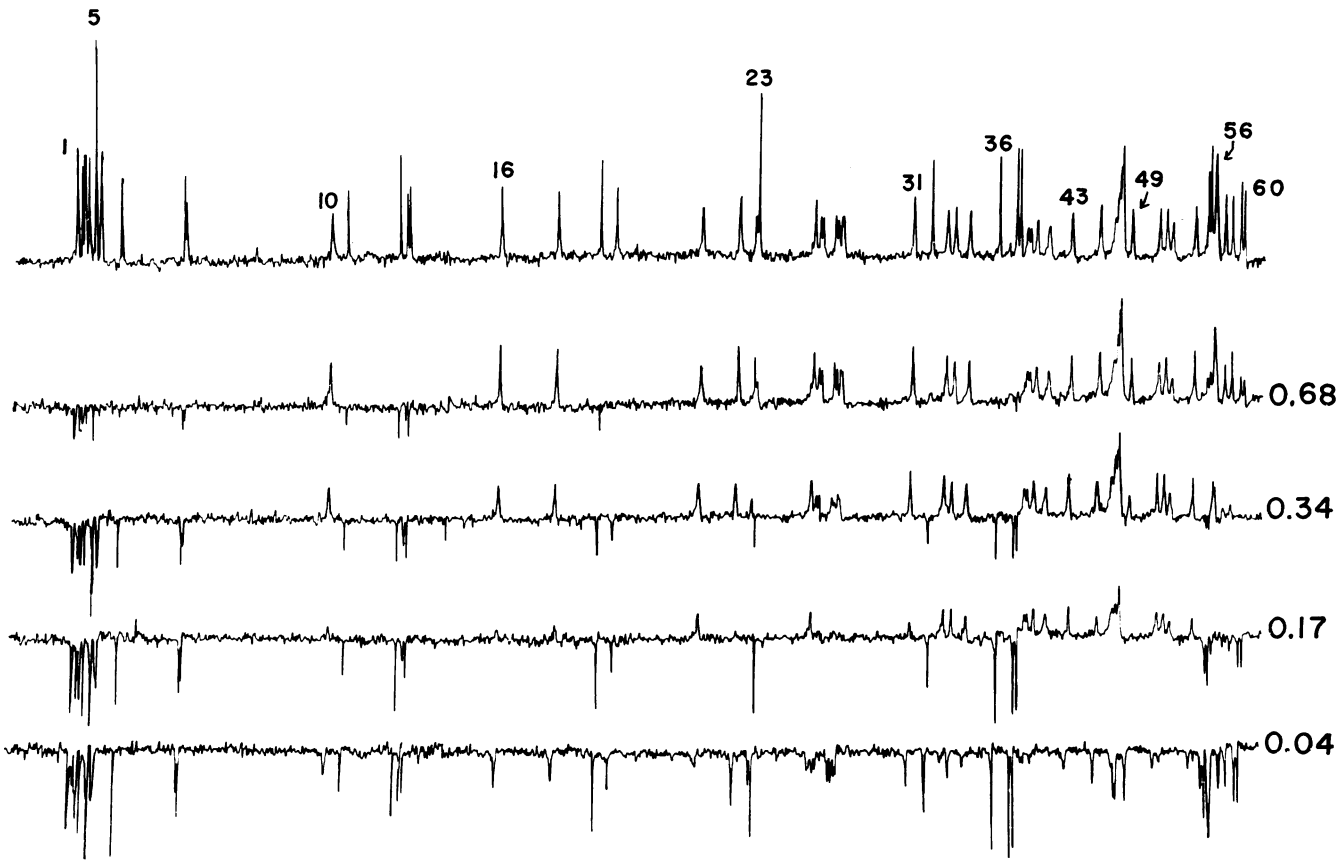

FIG. 5. Proton-decoupled natural-abundance ^{13}C Fourier transform NMR spectra of 0.14 M aqueous dicyanocobalamin (56°C, pH 9.8) obtained at 15.08 MHz using 4096 points in the time domain, 250 ppm sweep widths, 2.72 sec recycle times, and 4096 scans (3.1 hr) per spectrum. Only the range 6–181 ppm upfield from CS_2 is shown in each case. The top spectrum is the normal one, with the same peak numbering system as in Fig. 4*A*. The others are PRFT spectra, with τ values given in seconds. (For τ = 0.17 and 0.04, the full depth between peaks 5 and 6 is not shown).

structurally similar and were thus assigned to the three non-protonated resonances grouped upfield at about 142–147 ppm (Table 1). Carbon 2 should be deshielded because of a steric interaction (18) with the methyl group attached to C-1. It was assigned to the resonance at about 134 ppm.

The ribose resonances were identified by comparing the spectra of cobinamide dicyanide and the cobalamins (Fig. 1), and by the presence of resolved splittings caused by ^{31}P–^{13}C coupling. Specific assignments were made by comparison with the spectra of adenosine 3′-monophosphate (Table 3), which

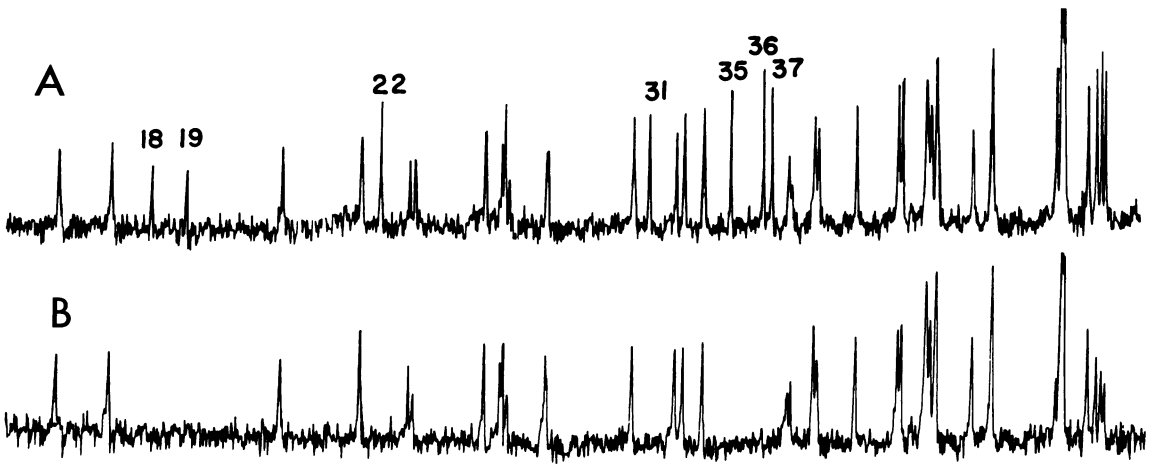

FIG. 6. Upfield portion in the proton-decoupled natural-abundance ^{13}C Fourier transform NMR spectra of 0.024 M cyanocobalamin at 61°C, obtained at 15.08 MHz using 4096 points in the time domain, 125 ppm sweep widths, 2.72 sec recycle times, and 16,384 scans (12.4 hr) per spectrum. Only the range 71.2–181.2 ppm upfield from CS_2 is shown. To avoid reflection of the downfield resonances not covered in the 125 ppm sweep width, we used a four-pole sharp cutoff filter, and set the carrier frequency upfield. *A*, Normal spectrum, with the non-protonated carbons indicated by the peak numbers of Fig. 3*B*. The resolution is better than in Fig. 3*B* because of the narrower sweep width. (The full intensity of peak 56 is not shown.) *B*, PRFT spectrum with τ = 1.02 sec. The resonances of the non-protonated carbons are nulled, while all the resonances of the protonated carbons are already quite positive.

was chosen because its sugar ring is a reasonable model for that of α-ribazole. The latter compound was unavailable. In the case of coenzyme B_{12}, carbons 1′, 4′, and 5′ of the α-ribazole moiety were specifically assigned (Table 2), but carbons 2′ and 3′ were not, because they overlapped with the corresponding carbons of the 5′-deoxyadenosyl group (Table 3). Carbons 1′ and 4′ of this group were assigned to the resonances at 106.7 and 107.5 ppm, but not on a one-to-one basis. The PRFT behavior of the resonance at 107.5 ppm in coenzyme B_{12} was indicative of the coincidence of a protonated and a non-protonated carbon. The latter was identified as C-1 of the corrin ring, as discussed above. After the above assignments were complete, the methine and methylene carbons of the isopropanolamine moiety in the cobalamins were tentatively assigned to the resonances which exhibited what appeared to be coupling to ^{31}P. These assignments were then confirmed by consideration of the spectrum of cobinamide dicyanide, which still contained these resonances, but as singlets, and with the methine signal being slightly shifted downfield (Table 2).

At this point, the only unidentified resonance below 125 ppm was one at about 118 ppm in all the corrinoids. Its relaxation time in dicyanocobalamin was 0.1 sec, a value comparable to that of C-10 of the corrin ring (Table 1). The chemical shift and short relaxation time were compatible only with an assignment to C-19. It should be noted that off-resonance single-frequency decoupling could not be used to identify C-19 as a methine carbon, because of the close proximity of other resonances (Fig. 4). The three resonances at 136–140 ppm were assigned to methine carbons on the corrin ring on the basis of their T_1 values and off-resonance single-frequency decoupling. These resonances were assigned to the structurally similar carbons 3, 8, and 13, but not on a one-to-one basis. At this point, all resonances below 147 ppm had been identified. The remaining unassigned carbons were all the methyl carbons, the methylene carbons of the corrin ring side-chains, and only one methine carbon, namely C-18. In the off-resonance proton-decoupled spectrum of dicyanocobalamin (Fig. 4*B*), the resonance at about 162 ppm appeared to be a doublet, with the downfield component partially buried under other resonances. On this basis, it was assigned to C-18. This assignment is only tentative, because we could not rule out the possibility that the apparent doublet was actually a methyl quartet, with the outer lines too weak to be observed. In addition, the T_1 value of this resonance was about twice that of all the other methine carbons on the corrin ring (Table 1).

The two resonances at about 149–151 ppm and the one at about 158 ppm were assigned to methylene carbons (see Fig. 4). It is likely that the downfield resonances correspond to methylene groups that are directly attached to the corrin ring. On the basis of their chemical shifts, they were tentatively assigned to the structurally similar methylene carbons attached at C-2 and C-7. The carbon resonating at about 158 ppm probably belongs to one of the β-methylene groups at C-3, C-8, or C-13 of the corrin ring. The only other well-resolved methylene carbons in the spectrum of dicyanocobalamin resonated at 166.1, 167.0, and 167.8 ppm. They were tentatively assigned to the three structurally similar methylenes directly attached to C-3, C-8, and C-13 of the corrin ring. No further methylene assignments could be made.

If the resonance at about 162 ppm is indeed C-18 (see above), then the line at about 154 ppm must arise from a methyl carbon (peak 43 in Fig. 4*A*). Its chemical shift is consistent with that expected for the methyl group at C-1. A comparison of the spectra of cobinamide dicyanide and the cobalamins proves that the methyl groups attached to the benziminazole ring must be located in the relatively unresolved region at 173–174 ppm (peaks 52 and 53 in the spectrum of cyanocobalamin, Fig. 3*B*). These chemical shifts are in agreement with those in benziminazole itself (Table 3), and the T_1 values of these resonances in dicyanocobalamin (Table 2) are indicative of methyl groups undergoing fast internal reorientation. All spectral lines above 170 ppm were easily identified as methyl resonances, but no further specific assignments were attempted.

Completion of the assignments should be feasible if additional derivatives are studied.

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