The Effect of Temperature and Light on the Carbon-13 Nuclear Magnetic Resonance Spectra of Alkylcorrinoids, Selectively Enriched with Carbon-13

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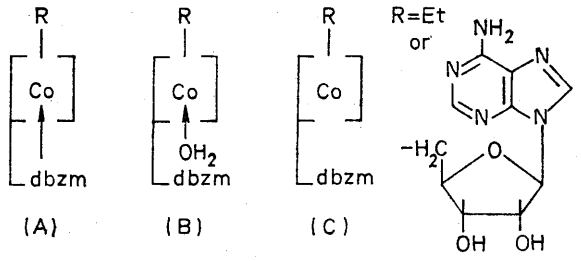
^{13}C N.m.r. spectra of aqueous (D_2O) solutions of methyl-, ethyl-, and adenoxyl-cobalamin, and methylaquocobinamide, selectively enriched with carbon-13 in the alkyl ligands attached to cobalt have been recorded at 25.2 MHz and over the temperature range 5—90 °C. The methyl resonances of [^{13}C]methylcobalamin and [^{13}C]methylaquocobinamide, as well as the methylene resonances of [1,2-$^{13}C_2$]ethylcobalamin and [5'-^{13}C]-adenosylcobalamin, exhibit small upfield shifts with increasing temperature. These small differences in chemical shift can best be accounted for by invoking a temperature-dependent conformational change involving substituents on the corrin ring, rather than a change in the co-ordination number of cobalt. ^{13}C N.m.r. spectroscopy has also been used to study homolytic cleavage of the carbon–cobalt bond of the alkylcobalamins. Ethylcobalamin undergoes thermal decomposition even at 60 °C, while methyl- and adenosyl-cobalamin are stable in aqueous solution at 94 °C for periods of up to 5 h. Methylcobalamin is very resistant to photolytic cleavage in the absence of oxygen, whereas ethyl- and adenosyl-cobalamin are photolyzed to cob(II)alamin under the same conditions.

EARLIER studies [1] from this laboratory have shown that ^{13}C n.m.r. spectroscopy is an extremely sensitive technique for the study of the *trans* effect in the corrinoids. For instance, protonation of the 5,6-dimethylbenzimidazole moiety of methylcobalamin to the 'base-off' form is accompanied by an upfield shift of the methyl resonance of 7.7 p.p.m. while the conversion of [^{13}C]-methylaquocobinamide into [^{13}C]methylcyanocobinamide is associated with a large downfield shift of 14 p.p.m. Similar large changes in the ^{13}C resonances of cobalt-bound alkyl moieties would be expected on varying the temperature if radical changes in co-ordination geometry about the cobalt atom were to occur.

Several years ago Firth *et al.*[2] reported that certain organocorrinoids show reversible changes in the visible absorption spectrum and in the chemical shifts of the H^{10} proton of the corrin ring when heated in aqueous solution. They concluded that the spectral changes observed with a series of aquocobinamides are associated with reversible removal of co-ordinated water at elevated temperatures to yield five-co-ordinate complexes. Since the high-temperature spectra of ethyl- and adenosylcobalamin resembled those of the corresponding cobinamides, they further suggested that at high temperature the 5,6-dimethylbenzimidazole base (dbzm) is no longer co-ordinated to cobalt which is five-co-ordinate, and that even at room temperature these two cobalamins are present as an equilibrium mixture of the two six-co-ordinate forms (A) and (B) and the five-co-ordinate form (C).

Since the electronic-absorption spectra of cyano- and methyl-cobalamin did not undergo similar changes when heated in aqueous solution up to 95 °C, Firth *et al.* suggested that the ease of formation of the five-co-ordinate forms depended on the nature of the ligand in the upper co-ordination position. This *trans* effect varied in the order expected from the pK_a values of the dbzm ligand: cyanide < methyl < adenosyl < ethyl. In the cobinamide series a similar *trans* effect was observed; cyanoaquocobinamide and isopropylaquocobinamide did not show significant spectral changes, whereas the cobinamides containing a vinyl, methyl, and ethyl moiety showed large changes in the optical spectra and in the chemical shift of H^{10} of the corrin ring. Firth *et al.*[2] concluded that cyanoaquocobinamide



is six-co-ordinate and isopropylaquocobinamide is five-co-ordinate at all temperatures studied, and that the other cobinamides are present as mixtures of the six- and five-co-ordinate forms.

The present work was undertaken to examine the temperature dependence of the chemical shift in alkylcorrinoids. This report describes the effect of temperature on the ^{13}C n.m.r. spectra of a series of alkylcorrinoids, selectively enriched with carbon-13 in the axial ligands. Homolytic cleavage of the carbon–cobalt bond of a few cobalamins by light and heat has also been studied by ^{13}C n.m.r. spectroscopy.

EXPERIMENTAL

Materials.—Cyano- and aquo-cobalamin were purchased from Sigma Chemical Co.; [^{13}C]methanol and [1,2-$^{13}C_2$]-ethanol (90% enriched), were generous gifts from Dr. D. G. Ott of L.A.S.L. [^{13}C]Methyl iodide and [1,2-$^{13}C_2$]ethyl iodide were prepared from the corresponding alcohols according to the method of Murray and Ronzio.[3] [5'-^{13}C]Adenosylcobalamin (90% enriched),[4] cobinamide from [^{13}C]-

[1] T. E. Needham, N. A. Matwiyoff, T. E. Walker, and H. P. C. Hogenkamp, *J. Amer. Chem. Soc.*, 1973, **95**, 5019.

[2] R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, and R. G. Thorp, *J. Chem. Soc.* (*A*), 1967, 1013; R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *ibid.*, 1968, 2419.

[3] A. Murray and A. R. Ronzio, *J. Amer. Chem. Soc.*, 1952, **74**, 2408.

[4] T. E. Walker, H. P. C. Hogenkamp, T. E. Needham, and N. A. Matwiyoff, *Biochemistry*, 1974, **13**, 2650.

methylcobalamin,[5] methyl- and -ethyl-cobalamin from the alkyl iodides and cob(I)alamin [6] were prepared by published procedures. Cob(II)alamin was prepared from aquocobalamin by electrochemical reduction at −0.3 V *versus* a standard calomel electrode.

Procedure.—As described previously,[1,4] proton-noise decoupled ^{13}C Fourier-transform n.m.r. spectra were obtained at 25.2 MHz with a Varian XL-100-15 spectrometer interfaced to a Data General Supernova computer using the deuterium resonance (15.4 MHz) of internal D_2O as a lock. Chemical shifts are reported in p.p.m. (±0.05 p.p.m.) downfield from a neat tetramethylsilane external standard. The temperature dependence of the deuterium lock was determined using the methyl resonance of sodium 2,2-dimethyl-2-silapentane-5-sulphonate as internal standard. Linewidths and relative intensities were determined from the transformed data by least-squares line-shape analysis. ^{13}C N.m.r. spectra were obtained of solutions containing *ca.* 25 mg of cobalamin per cm^3 of D_2O in 12-mm o.d. n.m.r. tubes.

RESULTS

The proton-noise decoupled ^{13}C n.m.r. spectra of methyl- and adenosyl-cobalamin and of methylaquocobinamide (upper isomer) exhibited simple intense resonances for the ^{13}C enriched moieties, while the spectrum of [1,2-$^{13}C_2$]ethylcobalamin showed the expected AB quartet [Figure 2(*a*)]. The assignments of the methyl and methylene carbon-atom resonances were confirmed by obtaining the proton-coupled ^{13}C n.m.r. spectra, the latter exhibiting a triplet [J(C–H) 136 Hz] and the former a quartet [J(C–H) 124 Hz]. In these spectra, the resonances of the carbon atoms containing natural-abundance ^{13}C were exceedingly weak. On

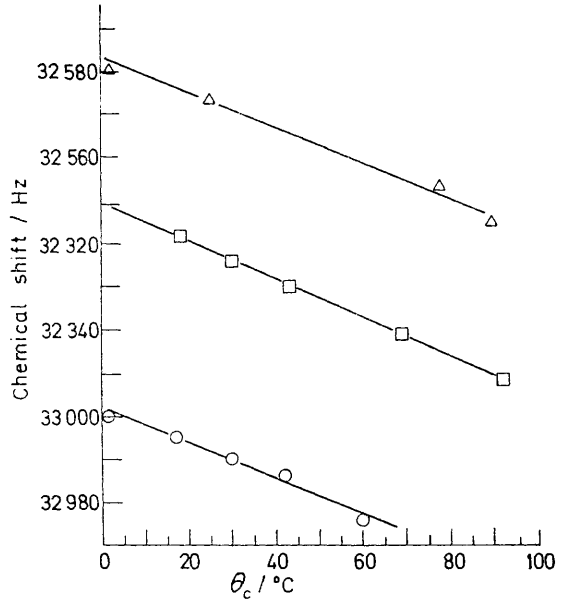


FIGURE 1 Variation with temperature of the chemical shifts of the methyl resonances of [^{13}C]methylcobalamin (Δ), and ([^{13}C]methyl)aquocobinamide (□), and of the methylene resonance of [1,2-$^{13}C_2$]ethylcobalamin (○)

acidification of the solutions, the resonances of the labelled moieties shifted upfield reflecting the displacement of 5,6-dimethylbenzimidazole (dbzm) by water at low pH.[1,7]

[5] M. E. Grant and H. P. C. Hogenkamp, unpublished work.
[6] D. Dolphin, *Methods Enzymol.*, 1971, **18**, 34.

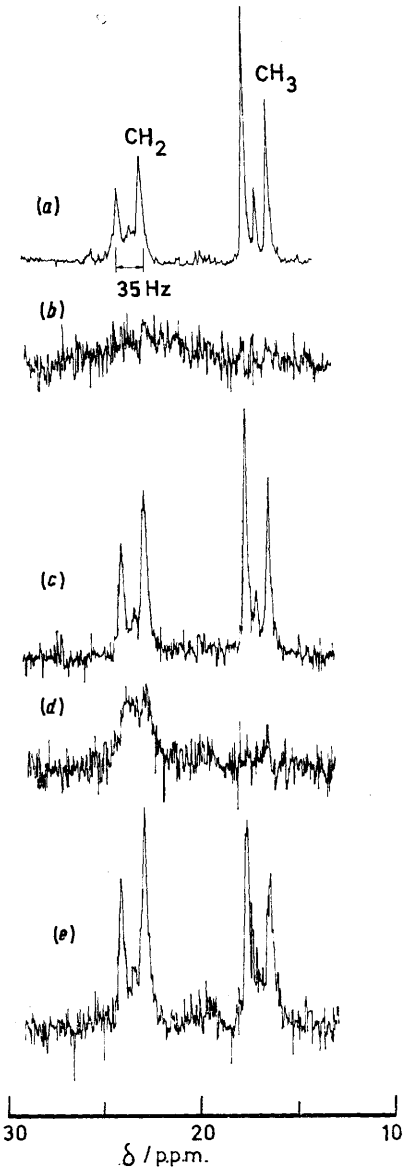


FIGURE 2 Homolytic cleavage of the carbon–cobalt bond of [1,2-$^{13}C_2$]ethylcobalamin by heat and by light. ^{13}C N.m.r. spectra of (*a*) [1,2-$^{13}C_2$]ethylcobalamin at 20 °C, (*b*) after heating at 94 °C for 2 min, (*c*) after oxidation of cob(II)alamin, (*d*) after exposure to light for 1 min, and (*e*) after treatment with oxygen (5 000 scans per spectrum).

The ^{13}C chemical shifts of the alkylcorrinoids at 32 °C are summarized in the Table. From ' titration ' plots [7] of the

^{13}C Chemical shifts for the alkylcorrinoids at 32 °C

Alkyl corrinoid		Chemical shift [a]
[^{13}C]Methylcobalamin		7.2
	(' base off ')	0.2
[^{13}C]Adenosylcobalamin		25.3
	(' base off ')	19.8
[1,2-$^{13}C_2$]Ethylcobalamin		24.4 (CH_2),[b] 17.3 (CH_3) [c]
	(' base off ')	21.7 (CH_2),[b] 18.0 (CH_3)
([^{13}C]Methyl)aquocobinamide		0.1

[a] In p.p.m. (±0.05) from external tetramethylsilane. [b] J(^{13}C — ^{13}C) 35.5, J(^{13}C — H) 136 Hz. [c] J(^{13}C — H) 124 Hz.

[7] T. E. Walker, H. P. C. Hogenkamp, T. E. Needham, and N. A. Matwiyoff, *J.C.S. Chem Comm.*, 1974, 85.

pH dependence of the chemical shifts of each component of the ^{13}C–^{13}C spin–spin doublets for the methyl and methylene carbons of [1,2-$^{13}C_2$]ethylcobalamin, a pK of 3.8 was estimated. As expected the resonance of the methylene carbon atom of [1,2-$^{13}C_2$]ethylcobalamin is more sensitive than that of the methyl carbon to changes in the *trans*-co-ordination site; displacement of the dbzm ligand by water was accompanied by an upfield shift (2.7 p.p.m.) of the CH_2 resonance and a small downfield shift (0.7 p.p.m.) of the Me resonance.

The effect of variation of temperature on the [^{13}C]methyl resonances of methylcobalamin and methylaquocobinamide and on the [^{13}C]methylene resonance of ethylcobalamin is shown in Figure 1. The resonances exhibited upfield shifts of *ca.* 4—5 Hz per 10 °C rise in temperature. The resonance of the 5′-methylene carbon atom of adenosylcobalamin was more sensitive to variations in temperature, a rise of 10 °C causing an upfield shift of 8—9 Hz.

Thermal Decomposition of Ethylcobalamin.—Figure 2(*b*) shows the proton-noise-decoupled spectrum obtained under conditions identical to those of Figure 2(*a*) for the same solution which had been heated at 94 °C for 2 min and then cooled to 20 °C. The characteristics of the initial spectrum were recovered [Figure 2(*c*)] after the solution was shaken with oxygen for 15 h. Similar extensive broadening of both resonances was also evident after a solution of [1,2-$^{13}C_2$]-ethylcobalamin under argon was irradiated with a 100 W incandescent lamp at a distance of 10 cm for 1 min [Figure 2(*d*)], or when cob(II)alamin, generated electrochemically, was added to the solution. These results indicate that ethylcobalamin is readily decomposed at 94 °C by a homolytic mechanism to cob(II)alamin and predominantly ethylene.[8,*] It is evident from the spectra in Figure 2(*c*) and (*d*) that the resonance of the Me carbon atom is much more susceptible to the paramagnetic broadening by cob(II)alamin than is the CH_2 resonance. Indeed, on treatment of the heated solution with oxygen the CH_2 resonance was regenerated much more readily than the Me resonance. Both methyl- and adenosyl-cobalamin are extremely stable at higher temperatures; heating solutions of both cobalamins under argon at 94 °C for 5 h does not affect their ^{13}C n.m.r. spectra. Even when concentrated solutions of these two cobalamins were heated at 94 °C for 1 h in the presence of oxygen there was no ^{13}C n.m.r. evidence of decomposition. In the case of [^{13}C]methylcobalamin, the pronounced broadening of the $^{13}CH_3$ resonance in the presence of cob(II)alamin allows us to place an upper limit of *ca.* 1% decomposition during the heat treatment. The CH_2 resonance of [5′-^{13}C]-adenosylcobalamin was not affected by the presence of cob(II)alamin and, in its heat treatment, we place an *upper* limit of *ca.* 5% decomposition based on the absence of ^{13}C resonances of decomposition products in the spectra obtained after heating.

Photodecomposition of Alkylcobalamins.—As shown in Figure 2(*d*), exposure of a concentrated solution of ethylcobalamin in the absence of oxygen to a 100 W incandescent lamp at a distance of 10 cm for 1 min caused extensive broadening of both the Me and CH_2 resonances. Under the same conditions methylcobalamin is extremely stable and, indeed, no evidence for photolysis could be detected in the ^{13}C n.m.r. spectrum of a sample of aqueous [^{13}C]methylcobalamin that had been exposed to a 100 W lamp for 16 h. As shown before, methylcobalamin is readily photolyzed in the presence of oxygen.[9] Exposure of a solution of [^{13}C]methylcobalamin, saturated with oxygen, to a 200 W incandescent lamp at a distance of 10 cm for 30 min yielded

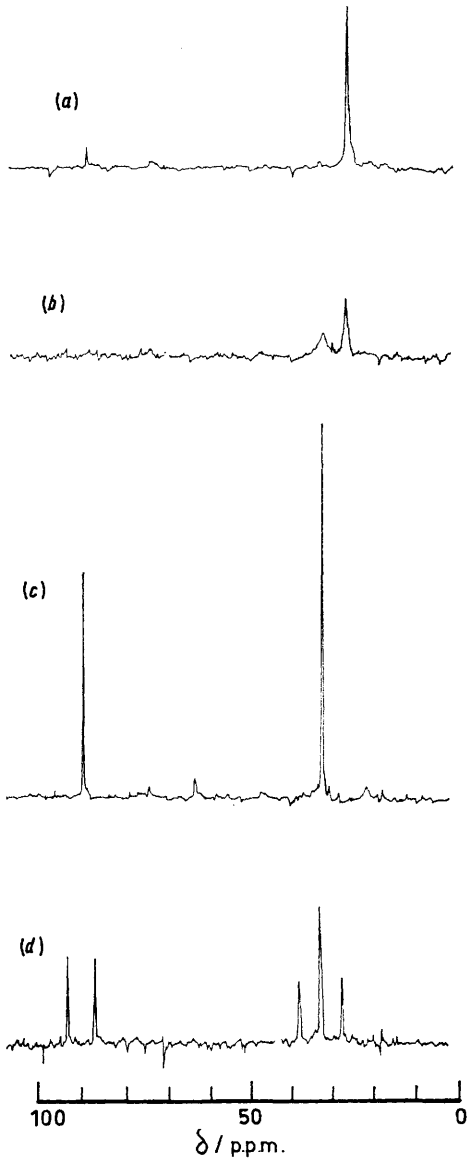


FIGURE 3 Photolysis of adenosylcobalamin. ^{13}C N.m.r. spectra (10 000 scans) of (*a*) [15′-^{13}C]adenosylcobalamin at 17 °C (δ 25.6 p.p.m.), (*b*) after anaerobic photolysis (5′,8-adenosine, δ 314 p.p.m.), (*c*) after photolysis under oxygen (adenosinecarbaldehyde, δ 89.9 p.p.m.), and (*d*) proton-coupled spectrum (138 000 scans) of cyclic [5′-^{13}C]5′,8-adenosine and of [5′-^{13}C]-adenosinecarbaldehyde

predominantly formaldehyde, detected in the ^{13}C n.m.r. spectra by the appearance of a new resonance at 82.6 p.p.m., identical to one of the most prominent absorption signals observed for authentic aqueous formaldehyde.

Homolytic cleavage of the carbon–cobalt bond of

* Mass-spectral studies were carried out on the volatile materials produced by thermolytic and photolytic decomposition of the [^{13}C]ethylcobalamin in both H_2O and D_2O. In all cases [^{13}C]ethylene (*m/e* 30) was the predominant product; however, no conclusive evidence was found to indicate that deuterium incorporation into the ethylene occurred to an extent greater than 1%.

[8] G. N. Schrauzer, J. W. Sibert, and R. J. Windgassen, *J. Amer. Chem. Soc.*, 1968, **90**, 6681.

[9] H. P. C. Hogenkamp, *Biochemistry*, 1966, **5**, 417.

[5′-^{13}C]adenosylcobalamin by light is demonstrated by the spectra in Figure 3. Exposure of a solution of [5′-^{13}C]-adenosylcobalamin under argon to a 200 W incandescent lamp for 100 min yielded cob(II)alamin and cyclic 5′,8-adenosine [10] [Figure 3(*b*)]: the resonance of [5′-^{13}C]cyclic 5′,8-adenosine at 31.4 p.p.m. broadened appreciably (full width at half height *ca.* 43 Hz) by the paramagnetic cobalamin, whereas that of the residual [5′-^{13}C]adenosylcobalamin at 25.6 p.p.m. (full width at half height = 14.7 Hz) was not. When the same solution was saturated with oxygen and exposed to the same light source for 60 min, the ^{13}C n.m.r. spectrum [Figure 3(*c*)] exhibited two intense narrow resonances (full width at half height <5 Hz) at 31.0 p.p.m. for [5′-^{13}C]cyclic 5′,8-adenosine and at 89.9 p.p.m. for [5′-^{13}C]adenosine-5′-carbaldehyde.[11] The proton-coupled ^{13}C n.m.r. spectrum of the same solution [Figure 3(*d*)] contained a triplet at 31.0 p.p.m. due to splitting by the two 5′-methylene protons [J(^{13}C–H) 134 Hz] and a doublet at 89.8 p.p.m. due to splitting by the aldehydic proton [J(^{13}C–H) 163 Hz].

DISCUSSION

Thermolysis.—As indicated in the Table, on protonation the methyl resonance of [^{13}C]methylcobalamin shifts 7.0 p.p.m. upfield, whereas the methylene resonances of [5′-^{13}C]adenosylcobalamin and of [1,2-$^{13}C_2$]ethylcobalamin shift 5.5 and 2.7 p.p.m., respectively. The magnitude of this *trans* effect on the ^{13}C resonances of [^{13}C]methylcorrinoids increases in the order: water < pyridine < 5,6-dimethylbenzimidazole (dbzm) < cyanide.[1] Thus similar or larger upfield shifts of the alkyl resonances are expected if co-ordinated water or dbzm is detached from the central cobalt atom to form five-co-ordinate complexes. Firth *et al.*[2] concluded from their spectral studies that, in aqueous solution at 20 °C, ethylaquocobinamide is 100%, methylaquocobinamide is 90%, methylcobalamin is 0%, ethylcobalamin is 15%, and adenosylcobalamin is 10% in the five-co-ordinate form. They further concluded that at 94 °C ethyl- and adenosyl-cobalamin are predominately five-co-ordinate, whereas methylcobalamin remains six-co-ordinate. It is clear from Figure 1 that the Me resonances of ([^{13}C]methyl)aquocobinamide and [^{13}C]-methylcobalamin as well as the CH_2 resonance of [1,2-$^{13}C_2$]-ethylcobalamin show very similar, if not identical, upfield shifts with increasing temperature. An increase in temperature from 10 to 60 °C is associated with an upfield shift of 20—22 Hz (*ca.* 0.8 p.p.m.).

Ethylcobalamin in the ' base-off ' form should exhibit properties similar to ethylaquocobinamide. If, as suggested by Firth *et al.*,[2] the latter complex exists predominantly in the five-co-ordinate form, so should ethylcobalamin in the ' base-off ' form. Accordingly, we might have expected the $^{13}CH_2$ resonance of ethylcobalamin to shift 2.7 p.p.m. upfield and the $^{13}CH_3$ resonance to shift 0.7 p.p.m. downfield as the temperature was raised (*cf.* the shifts of the ' base-on ' forms in the Table). Instead, both resonances shifted a small amount *upfield*. This observation, together with the similarities in the temperature dependences of the chemical shifts of the carbon bound to Co^{III} in the three complexes, suggests an origin for the ^{13}C shifts other than simple ' base-on ' $\rightleftharpoons$ ' base-off ' or six-co-ordinate $\rightleftharpoons$ five-co-ordinate temperature-dependent equilibria. The observed shifts may be due to a temperature dependence of the corrin ring flexing and/or the conformation of the acetamide side-chains. In this context we note that the ^{13}C shifts of the cobalt alkyl group are sensitive to remote effects, *i.e.* the conversion of the *c*-acetamide side-chain to the γ-lactam or γ-lactone [12] shifts the $^{13}CH_3$–Co resonance by more than 1 p.p.m.[3] Similar temperature-dependent perturbations may affect the electronic-absorption spectra and the ^{1}H n.m.r. shifts of H[10] of the corrin ring. However, we note here that one of the complexes extensively studied by Firth *et al.*,[2] ethylcobalamin, does not survive heating above 60 °C, even for short periods (<2 min) in anaerobic solutions (see below).

The 5′-methylene resonance of [5′-^{13}C]adenosylcobalamin (not shown) is more sensitive to variations in temperature; in the range 10—90 °C it exhibits an upfield shift of 2.6 p.p.m. Assuming that a ' base-on ' $\rightarrow$ ' five-co-ordinate ' transition yields an upfield shift of the CH_2 resonance at least as large as the shift corresponding to ' base-on ' $\rightarrow$ ' base-off ' transition (5.5 p.p.m.), the measured shift over this temperature range corresponds to *ca.* 50% [(2.6/5.5) × 100] conversion, at most, of the coenzyme to a five-co-ordinate form at 90 °C. Again, however, this shift is probably due to temperature-dependent conformational equilibria involving not only the acetamide side-chains but also the configuration of the bulky adenosyl moiety.

The ethyl ligand of ethylcobalamin is surprisingly labile at elevated temperatures. Schrauzer *et al.*[8] showed that solid methyl- and ethyl-cobalamin decompose between 215 and 225 °C: pyrolysis of the former under argon yields approximately equal amounts of methane and ethane, whereas ethylene is the main product of ethylcobalamin pyrolysis. In contrast to the work of Firth *et al.*[2] who reported *reversible* changes in the spectra of ethylcobalamin, this cobalamin decomposes in anaerobic aqueous solution at elevated temperatures; decomposition is slow at 60 but rapid at 80 °C. Sufficient cob(II)alamin is formed on heating an anaerobic aqueous solution of ethylcobalamin at 60 °C for 45 min to cause broadening of the Me resonance from 6 to 35 Hz. Heating a concentrated solution of ethylcobalamin at 94 °C for 2 min causes extensive homolysis and the formation of sufficient cob(II)alamin to ' obliterate ' both the Me and CH_2 resonances (half widths $\gg$ 100 Hz). In contrast, heating similar solutions of methyl- or adenosyl-cobalamin in the absence of oxygen at the same temperature for 5 h does not cause significant detectable decomposition.

The effect of cob(II)alamin on the spectrum of the ethylcobalamin which remains in solution following

[10] H. P. C. Hogenkamp, *J. Biol. Chem.*, 1963, **238**, 477.
[11] H. P. C. Hogenkamp, J. N. Ladd, and H. A. Barker, *J. Biol. Chem.*, 1962, **237**, 1950.
[12] R. Bonnett, *Chem. Rev.*, 1963, **63**, 573.

homolysis at 94 °C [*ca.* 80 and *ca.* 55% on heating for 2 and 4 min, respectively, estimated by comparing signal intensities with methanol contained in a concentric capillary after the oxidation of cob(II)alamin] results from the fact that the Co^{II} in the former complex possesses one unpaired electron which is localized mainly on the cobalt.[14] Because the e.s.r. signal for this species is detectable over a wide temperature range up to and including 30 °C,[15] it must possess a long electron-spin relaxation time, a condition which leads to effective ^{13}C nuclear-spin relaxation (broad n.m.r. lines) *via* electron–nuclear dipolar interactions.[16] In addition, the efficacy of the ^{13}C nuclear relaxation is enhanced because the unpaired electron resides in a molecule having a long rotational correlation time, τ_c. A good approximation for τ_c in cob(II)alamin can be obtained from the long rotation correlation times of cyano- and adenosylcobalamin which have been determined as 4.8×10^{-10} (ref. 17) and 9.6×10^{-10} s,[18] respectively, from ^{13}C spin-lattice relaxation times (T_1).

Without making assumptions [19] about the association constant for 'aggregation' of cob(II)alamin and ethylcobalamin, and the relative values of τ_c and the electron T_1 and T_2 times, we can estimate the 'average' distance between the paramagnetic centre of cob(II)alamin and the ethyl-group carbons of [1,2-$^{13}C_2$]ethylcobalamin from the relative paramagnetic broadening of the $^{13}CH_3$ and $^{13}CH_2$ resonances (3 : 1) whose T_2 values (inversely proportional to linewidth) depend, in the dipolar approximation, on the inverse sixth power of the average distance between the carbon atoms of interest and the paramagnetic centre.[16] Inspection of molecular models of ethylcobalamin (based on crystal structures of B_{12} derivatives [20–22]) indicates that cob(II)alamin can approach ethylcobalamin at an oblique angle in the 'upper' co-ordination site [23] with little steric interference in a configuration in which the distance of closest approach of the two cobalt atoms is *ca.* 8 Å and in which the distances between Co^{II} and $^{13}CH_3$ and $^{13}CH_2$ of the Et group of ethylcobalamin are in the approximate ratio, $r : (r + 1)$. Using the latter ratio and the relative linewidths of the $^{13}CH_3$ and $^{13}CH_2$ resonances, an 'average' distance of 6 Å between the Co^{II} paramagnetic centre and the $^{13}CH_3$ group can be calculated, a reasonable value in terms of the models employed. Alternatively, if we assume that the line broadening occurs in a 1 : 1 ethylcobalamin–cob(II)alamin complex having an association constant 10 l mol^{-1} and that the correlation time for the relaxation has a lower limit of *ca.* 10^{-9} s (the estimated rotational correlation time for cobalamin), the distance of closest approach, $^{13}CH_3$–Co^{II} is calculated to be *ca.* 8.5 Å. The extreme limits implicit in both calculations confirm that a dipolar relaxation mechanism is reasonable for these systems.

In this context, we note that the $^{13}CH_2$–Co resonance of [5′-^{13}C]adenosylcobalamin is not broadened in the presence of cob(II)alamin. The explanation for this observation is that the paramagnetic corrinoid cannot approach the CH_2 carbon atom to a distance less than 15 Å without experiencing serious steric constraints. A straightforward calculation [16] demonstrates that dipolar broadening of a ^{13}C nucleus by a paramagnet with a correlation time for relaxation of *ca.* 10^{-9} s [or less, which should apply to cob(II)alamin] is negligible (<2 Hz) when the distance of closest approach is >15 Å. However, in this context we note also that cob(II)alamin by virtue of its large values of T_{1e} and T_{2e} and its long rotational correlation time (which would be lengthened by its incorporation into a high-molecular-weight enzyme) should provide an effective, but innocuous, spin label of corrinoid-dependent enzymes for the study of the mode of bonding of ^{13}C-labelled substrates and inhibitors.

Photolysis.—Ethylcobalamin is very susceptible to photolysis. Exposure of a concentrated solution (25 mg cm^{-3}) to a 100 W lamp at a distance of 10 cm for 1 min causes extensive broadening of both the $^{13}CH_3$ and $^{13}CH_2$ resonances indicative of generation of significant quantities (>10%) of cob(II)alamin. Anaerobic photolysis of ethylcobalamin may cause appreciable substitution of the corrin ring, as shown in Figure 2(*e*) where it is evident that, even after oxidation of the paramagnetic cob(II)alamin, the $^{13}CH_3$ and $^{13}CH_2$ resonances are much broader than those for the parent ethylcobalamin [Figure 2(*a*)]. The increased breadth of the resonances may be due to small and unresolved chemical-shift inequivalencies for the remaining ethylcorrinoids, the substitution processes being initiated by light-induced formation of Et radicals. We estimate that photolysis under the conditions described above decomposes >10% of the ethylcobalamin, the initial products being the Et radical and cob(II)alamin. In contrast, we note that thermolysis, which generates these radicals more slowly, is probably accompanied by fewer side reactions [note the relative linewidths and peak intensities in Figures 2(*a*), 2(*c*), and 2(*e*)].

As shown before,[8,9] methylcobalamin is resistant to photolysis in the absence of oxygen and this was confirmed in our studies; exposure of anaerobic aqueous solutions of methylcobalamin to radiation from a 100 W lamp at a distance of 1 cm for 16 h resulted in no change

[13] H. P. C. Hogenkamp, unpublished work.
[14] D. G. Brown, *Progr. Inorg. Chem.*, 1973, 177.
[15] J. H. Bayston, F. D. Looney, J. R. Pilbrow, and M. E. Winfield, *Biochemistry*, 1970, **10**, 2164.
[16] A. Abragam, 'The Principles of Nuclear Magnetism,' Oxford University Press, London, 1961.
[17] D. Doddrell and A. Allerhand, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1083; A. Allerhand and R. A. Komorowski, *J. Amer. Chem. Soc.*, 1973, **95**, 8228.
[18] H. P. C. Hogenkamp, R. Fuentes, and N. A. Matwiyoff, unpublished work.
[19] R. A. Dwek, 'NMR in Biochemistry,' Clarendon Press, Oxford, 1973, ch. 9.
[20] D. C. Hogkin, J. Lindsey, R. A. Sparks, R. N. Trueblood, *Proc. Roy. Soc.* 1962, **A226**, 494.
[21] C. Brink-Shoemaker, D. W. Cruickshank, D. C. Hogkin, M. J. Kamper, and D. Pilling, *Proc. Roy. Soc.*, 1964, **A278**, 1.
[22] P. G. Lenhert, *Proc. Roy. Soc.*, 1968, **A303**, 45.
[23] J. M. Pratt, 'Inorganic Chemistry of Vitamin B_{12},' Academic Press, New York, 1972.

in the ^{13}C n.m.r. spectrum (<1% decomposition, *see above*). In contrast, although adenosylcobalamin (like methylcobalamin) does not undergo thermal decomposition readily, facile homolysis of the cobalt–carbon bond of the coenzyme does occur when the complex is exposed to light. As shown in Figure 3(*b*), exposure of an anaerobic concentrated solution (25 mg/cm^{-3}) of adenosylcobalamin to white light (100 W lamp at a distance of 10 cm) for 1 min causes extensive (*ca.* 50%) decomposition of the coenzyme to cyclic 5′,8-adenosine; photolysis in an oxygen atmosphere for 60 min induces complete homolysis of the carbon–cobalt bond of the coenzyme to cyclic 5,8-adenosine and adenosinecarbaldehyde [Figure 3(*c*)].

In summary, the following points are reflected in the ^{13}C n.m.r. spectra reported here: (*i*) methylcobalamin is surprisingly resistant to thermolysis and photolysis; (*ii*) adenosylcobalamin is extremely resistant to thermolysis but undergoes photolysis readily; (*iii*) ethylcobalamin undergoes thermolysis and photolysis readily, so readily in fact that previous reports of the temperature dependence of the absorption spectrum of the complex must be interpreted with caution; and (*iv*) the correlation of observed ranges in chemical shifts of carbon attached to cobalt with changes in temperature can best be accounted for by invoking a temperature-dependent conformational change involving the corrin ring.

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