Photolysis of Alkylcobalamins and Coenzyme B_{12}: an Electron Spin Resonance Study

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Exposure of methylcobalamin in CD_3OD–D_2O glasses at 77 K to a high-pressure mercury arc gave low yields of methyl and $\dot{C}D_2OD$ radicals and high yields of a radical-Co^{II} pair species (triplet state), which on annealing was converted into normal $Co^{II}B_{12r}$. The mean separation between cobalt and the radical was *ca.* 8.3 Å. The ethyl derivative gave broad features for $Co^{II}B_{12r}$ and alkyl radicals, suggesting weak spin–spin interaction and hence greater mean separation. In contrast, the acetyl derivative gave no detectable features at 77 K, but intense $Co^{II}B_{12r}$ features grew in on annealing above 77 K. We suggest that close random pair-trapping broadened all features beyond the detection limit at 77 K.

For the coenzyme, in agreement with Lowe *et al.*, no detectable reaction occurred on photolysis at 77 K, but normal photolysis was detected above *ca.* 200 K. Again, some random pair-trapping was evident.

Animals and man contain three major types of cobalamin: the hydroxo derivative, the methyl derivative, and the deoxyadenosyl derivative (coenzyme B_{12}).[1] The cobalt–carbon bond in all alkyl derivatives is extremely photolabile, and because of the biological importance of these compounds, their photolyses have been extensively studied.[2–9] One of the most complete recent studies is that of Giannotti and his co-workers.[7–9] Using evidence mainly from the use of various spin-traps, these workers conclude that, following light absorption, there are two major chemical processes, one being loss of a hydrogen atom from the corrin ring (see Figure 1), the other the homolytic fission of the cobalt–carbon bond. These reactions can be envisaged as in the Scheme.

The light absorption causes electron-transfer from the corrin ring to cobalt. This, in some way, must weaken the carbon–hydrogen bond at the C-10 position in the corrin ring to give a Co^{II} derivative and a hydrogen atom [process (2): we have no information about this from our work, and therefore do not discuss this process further]. Presumably, reaction (3) occurs when the acceptor orbital is the $Co(d_{z^2})$ σ^* orbital, since the antibonding character of this is relieved by bond fission. This gives, directly, $R^{\cdot} + Co^{I}$ with an electron-deficient corrin ring. Reverse electron-transfer then gives the normal B_{12r} derivative.

Despite the possibility that temperature changes may modify the course of photolyses, it is nevertheless often mechanistically helpful to study intermediate products in low temperature matrices.[10] Loew *et al.* in an e.s.r. study of coenzyme B_{12} in aqueous propane-1,2-diol found no overall reaction on photolysis at 77 K.[5] However on photolysis at higher temperatures, but well below the glass softening point, they obtained e.s.r. evidence for B_{12r} formation together with a broad ($\Delta H_{MS} \approx 30$ G) free radical signal. An important aspect of this study was that on annealing to *ca.* 300 K the radical signal was lost, but the B_{12r} signal greatly increased. Our aim was to check this study and to extend it to other alkyl derivatives in the hope of understanding the solid-state photochemistry more fully.

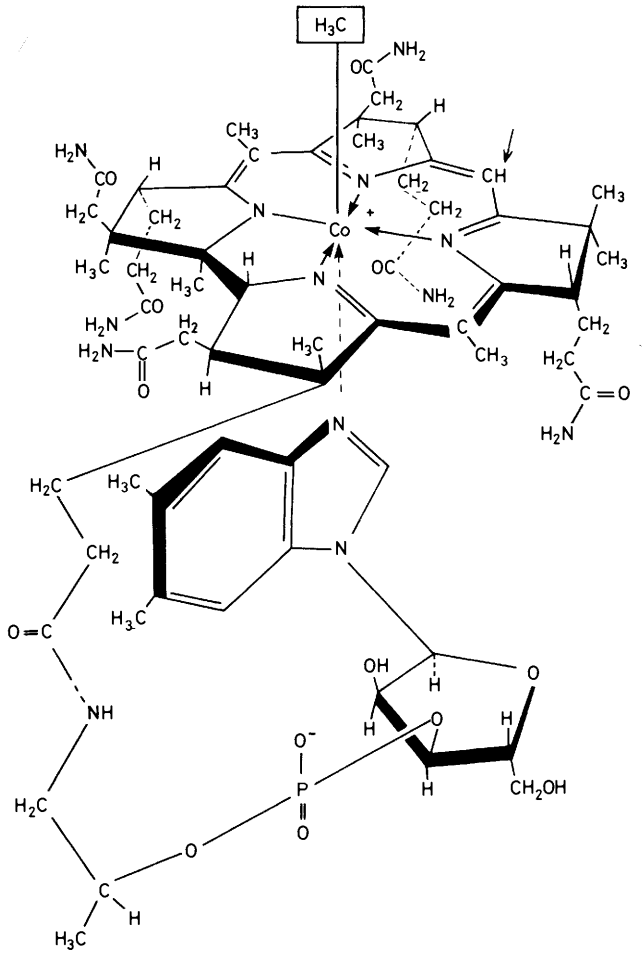


Figure 1. Structure of methylcobalamin; the arrow indicates the photo-labile hydrogen

Experimental

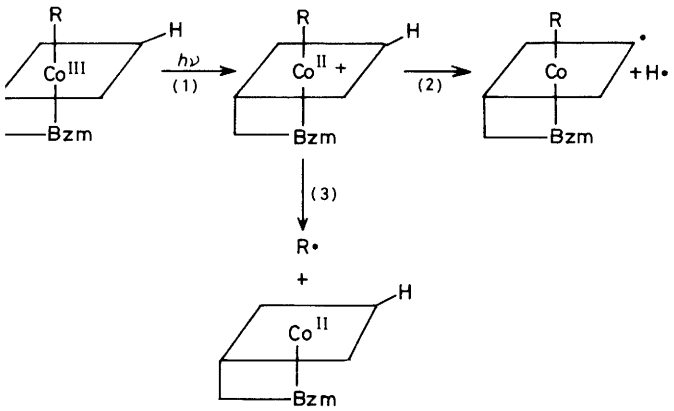
Methylcobalamin, coenzyme B_{12}, and cyanocobalamin were obtained from Sigma Chemicals. CD_3I (99 atom %) was supplied by Aldrich Chemicals and $^{13}CH_3I$ (90 atom %) was from Merck, Sharp & Dohme Ltd. All other reagents were of analytical grade. Aquocobalamin was prepared by the method of Kaczka *et al.*[11]

Methylcobalamin, CD_3-cobalamin, and $^{13}CH_3$-cobalamin were prepared as described in ref. 12, except that CD_3I was used for the preparation of CD_3-cobalamin and $^{13}CH_3I$ was used for the preparation of $^{13}CH_3$-cobalamin. Ethylcobalamin was prepared by using ethyl iodide, and acetylcobalamin was prepared by the method of Smith *et al.*[13] as described by Dolphin.[12] All products were purified by carboxymethylcellulose chromatography, purity-

Experimental e.s.r. parameters for radical species formed on photolysis of methylcobalamin

Radical	Hyperfine coupling constants (G) [a] A_z	A_x	A_y	g Values $g_{\parallel}$	$g_{\perp}$	$2D_{\parallel}$
B_{12r}	(^{59}Co) 108	NR [b]	NR [b]	2.003	*ca.* 2.32	
	(^{14}N) 18	NR	NR			
B_{12r} [c] Pair	(^{59}Co) 56	NR	NR	2.003	*ca.* 2.16	*ca.* 100 G
	(^{14}N) *ca.* 9	NR	NR			
R· Pair [d]				2.0025 (av.)		*ca.* 100 G
$D_2\dot{C}OD$ [e]	(^{2}H) *ca.* 3 G (av.)			2.0025 (av.)		
$\cdot CH_3$ [e]	(^{1}H) 22.5 (av.)			2.0025 (av.)		

[a] 1 G = 10^{-4} T. [b] NR = not resolved. [c] Data for the B_{12r} component of the radical pair (data for ^{14}N estimated from line widths). [d] Data for the radical component (? $D_2\dot{C}OD$) of the radical pair. [e] Identical with normal values for these radicals.



Scheme.

being checked by paper chromatography and optical spectroscopy.[12]

Anaerobic samples of cobalamins, prepared by dissolving cobalamins in degassed solvents (CD_3OD–D_2O or H_2O–ethylene glycol), were irradiated in Supracil e.s.r. tubes at 77 K using a 100 W high-pressure mercury lamp. All samples were irradiated for 6 h and e.s.r. spectra were recorded at 77 K on a Varian E-109 spectrometer calibrated with a Hewlett–Packard 5246L frequency counter and a Bruker B-H12E field probe, standardized with a sample of diphenylpicrylhydrazyl. Annealing experiments were carried out by decanting the liquid nitrogen from the capped insert Dewar and monitoring the e.s.r. spectra as the sample warmed up. Liquid nitrogen was added whenever significant changes were observed.

In all these studies great care was taken to avoid fortuitous photolysis in solution since the alkyl derivatives are extremely photolabile.

Results

Methylcobalamin.—At low microwave powers, well defined signals from methyl and $\cdot CD_2OD$ radicals were detected (Figure 2a). At high powers, very weak features in the low-field region indicated a minor yield of normal B_{12r} but intense features from a novel Co^{II} species dominated (Figure 2b) (species A). After some annealing above 77 K to remove the signals from $\cdot CH_3$ and $\cdot CD_2OD$ radicals, broad features in the $g = 2$ region became better defined (Figure 2c) (species B). On further annealing to *ca.* 180 K these features were replaced by those for normal $Co^{II}B_{12r}$ (Figure 2d).

Ethylcobalamin.—In this case there was no evidence for species A or B, but broadened features for $Co^{II}B_{12r}$ were detected together with a broad central component which is probably due to ethyl radicals. On annealing these radicals were lost and the B_{12r} features narrowed to their normal widths.

Coenzyme B_{12}.—Our results were similar to those of Lowe *et al.*[5] (see above).

Acetylcobalamin.—No e.s.r. features were resolved after photolysis at 77 K. However on annealing features for B_{12r} grew in at *ca.* 180 K.

Discussion

The e.s.r. parameters for species A (Table) are approximately half those for normal B_{12r}, except that, in the parallel region, there are extra features which, we suggest arise from an extra doublet splitting, indicated in Figure 2b. This implies the formation of radical pairs of the type first discovered in photolysed persulphates.[14,15] Provided the radical R· is trapped at a fixed distance from Co^{II} there should be a well defined zero-field splitting, and if some orbital overlap is retained, spin-exchange should occur, thereby reducing the coupling constants and Δg ($g_{exp} - 2.0023$) values by a factor of 2, as observed. In order to explain the extra high-field parallel features (Figure 2b) we need a fine-structure splitting of *ca.* 100 G ($2D_{\parallel}$). This is strongly supported by the fact that the centre of these two sets of cobalt parallel features is then close to 2.0023 as is normally found for Co^{II} ($d_{z^2}^1$) complexes. We stress that this value need not be a principal value of the zero-field splitting since the powder spectra are dominated by the cobalt parameters.

If this postulate is correct, there should be an equal zero-field splitting for the other component of the pair, namely, R·. We tentatively suggest that the signals assigned to species B are mainly due to this component. These features are very broad and no hyperfine coupling can be extracted; however, they are of the form expected for a zero-field splitting, provided $2D_{\parallel}$ is in the region of 100 G. The perpendicular features are poorly defined and probably split, but are largely concealed by a central free-radical component.

The fact that this value is equal to the value derived from the cobalt features means that the principal (parallel) axis for the zero-field splitting is the same as that for the parallel cobalt splitting, namely, z, the direction normal to the corrin ring. In that case, the mean separation between R· and Co^{II} must be

$$D' = 3\beta\,(3\cos^2\theta - 1)/R^3 \qquad (1)$$

ca. 8.3 Å, from the normal equation (1) where R is the mean separation between the spins.[16]

We have thus built up the following mechanism. On light

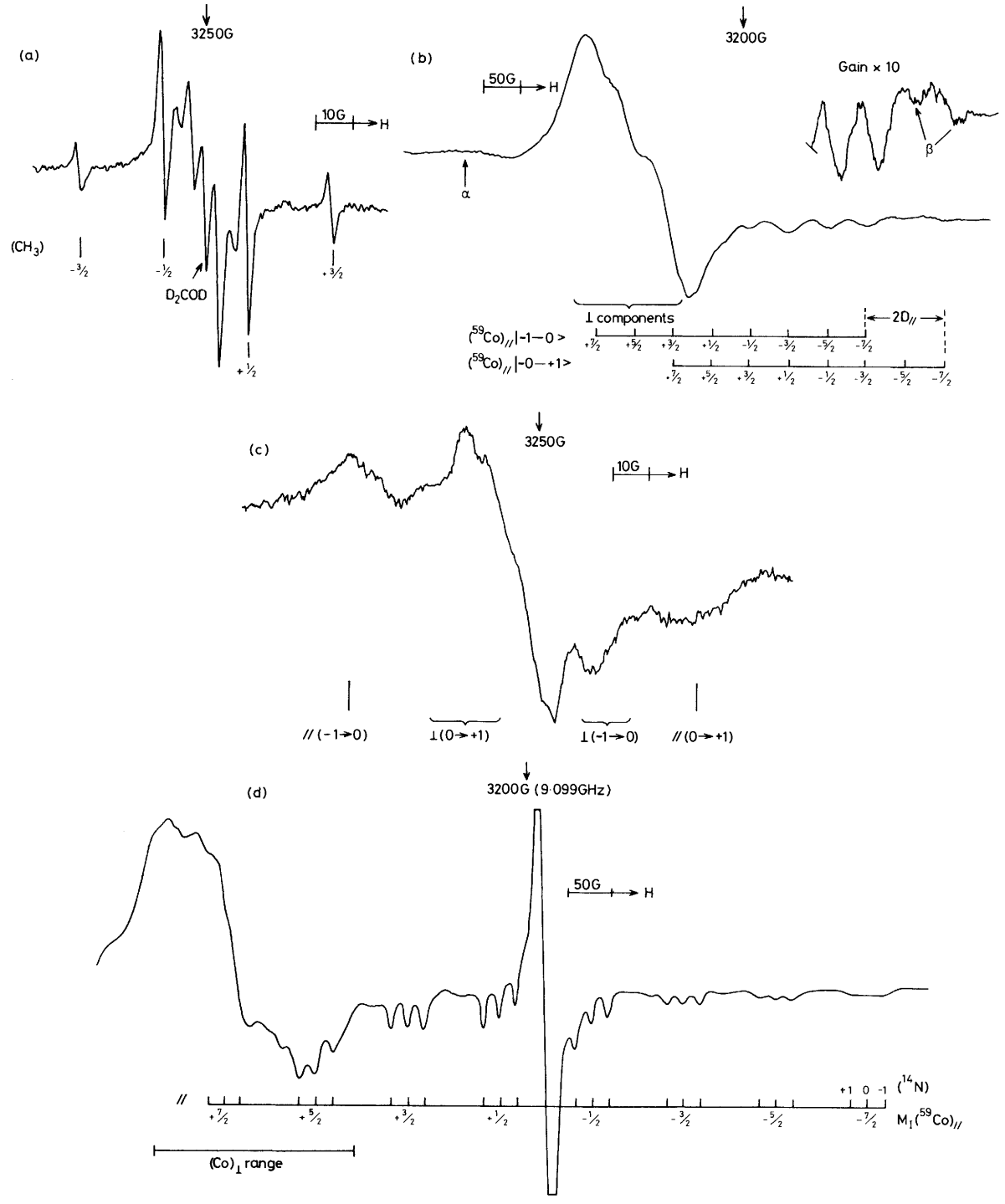


Figure 2. First derivative *X*-band e.s.r. spectrum for methylcobalamin in D_2O–CD_3OD solution after exposure to u.v. light, (a) at low microwave power showing features assigned to $\dot{C}H_3$ and $\cdot CD_2OD$ radicals; (b) at high power, showing features assigned to $B_{12}(\alpha)$ ($\perp$ features only) and species A [note the extra pair of parallel features at high field (β)]; (c) after annealing, showing features for species B; (d) a typical e.s.r. spectrum for $Co^{II}B_{12r}$, as obtained, for example, on annealing the above system to *ca.* 180 K

absorption a ligand electron moves into the Co(d_{z^2}) σ^* orbital, and the methyl radical moves away along the *z*-axis. This pathway is clear of organic substituents so the major opposition to this motion will be from solvent molecules. Some of these 'hot' radicals react with the first CD_3OD molecule encountered to give $\dot{C}D_2OD$, trapped by hydrogen-bonds to the rigid lattice. Alternatively, some methyl radicals may slow down and stop in the 8 Å region. Unfortunately, the lines are so broad (Figure 2c) that we cannot rule out the presence of an 11 G (1 : 3 : 3 : 1) splitting from such trapped $\cdot CH_3$ radicals, though we feel this is unlikely. Some $\cdot CH_3$ radicals escape this initial barrier and are either trapped (giving the normal $\cdot CH_3$ features) or react with solvent giving normal $\cdot CD_2OD$ features (Figure 2a).

Although we can think of no alternative explanation, we treat this conclusion with caution for two reasons: one is that

it is surprising that there should be such precise trapping always in the 8.3 Å region, and the other is that, for such a large separation, there should be fast spin-exchange. The latter observation surely requires that there are no intervening solvent molecules which would provide an efficient barrier for electron exchange. Another reason for preferring the idea that the R· component of the pair $\cdot CD_2OD$ rather than $\cdot CH_3$ is that this will be held away from cobalt by hydrogen bonding whereas the latter, with no intervening solvent, would surely be able to return to its original site.

For ethylcobalamin, the ethyl radical, being less reactive, is unable to extract deuterium atoms from proximal solvent, but escapes and remains trapped in the 15—20 Å region, thereby giving only spin–spin broadening. On annealing the Et· radicals diffuse away and react, leaving normal B_{12r}.

For the acetyl derivative, escape also occurs, but the mean trapping distance is shorter, so that line-broadening is so extensive that no signals are detected. Again, on annealing, the $R\dot{C}O$ radicals move away and react, leaving normal B_{12r}.

For coenzyme B_{12}, we agree with Lowe *et al.*[5] At 77 K bond fission probably occurs, but the large adenosyl radical cannot escape, and back-reaction is complete. At higher temperatures, these radicals can move away but, as with the ethyl derivative, the separation varies from molecule to molecule and the spectra are broadened. Ultimately, on annealing complete separation occurs and the spectrum of B_{12r} becomes clear.

Finally, we consider the significance of these results in relation to our studies of electron-addition to alkylcobalamins induced by ionizing radiation.[17] Two alternative mechanisms are currently considered for electron-addition, one giving $Co^{I} + R\cdot$ and the other giving $Co^{II} + R\bar{:}$. Since we were unable to detect any trace of $\cdot CH_3$ radicals for the methyl derivative, and since the present results clearly establish that good signals from methyl radicals can be obtained from these systems, we can firmly exclude the former mechanism. In fact, our results favour initial electron-addition into the corrin (π^*) orbital followed ultimately by loss of 'R^-', though we suggest that protonation occurs in the transition state.

Conclusions

In all the systems studied, photolysis results in homolysis of the Co–R bond, but our results suggest that the direct consequences vary markedly with the size of the resulting alkyl radicals. For R = CH_3 some radicals can escape and become trapped, with unequivocal e.s.r. detection of both $\cdot CH_3$ and $Co^{II}B_{12r}$. However, they also react with suitably placed solvent molecules (CD_3OD) and a precise $Co^{II}\cdots\dot{C}D_2OD$ radical-pair is a dominant species. Ethyl radicals seem to be less reactive and also less mobile so that a range of radical-pairs are formed with relatively large average separation so that broadened signals are obtained. The same occurs for the acetyl derivative except that the mean separation is reduced, and the e.s.r. features are excessively broadened. For the coenzyme, however, separation at 77 K is not large enough to prevent efficient cage-back reaction, and no photolysis is observed. At higher temperatures photolysis does occur, but pairwise trapping again broadens the e.s.r. features.

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