Light-induced Anaerobic Co^{III}–C Homolysis of Aqueous Vitamin B_{12} Coenzyme or of Ethylcobalamin; Spin-trapping of the 5′-Deoxyadenosyl or Et Radical

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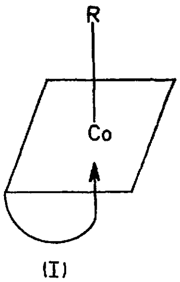
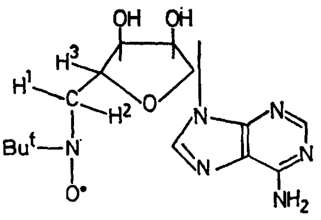
Summary Irradiation of vitamin B_{12} coenzyme or ethylcobalamin (LCo^{III}–R) in the presence of a nitroso compound R′NO (R′ = Bu^t or 2,3,5,6-Me_4C_6H) under anaerobic conditions in H_2O (R′ = Bu^t), Me_2SO, or PhOH–$CHCl_3$, affords the spin-trapped nitroxide $R'N(\dot{O})R$.

We report e.s.r. results on the photolysis of vitamin B_{12} coenzyme (Ia) [and on the related compound ethylcobalamin (Ib)] in an aqueous medium which demonstrate unequivocally that light-induced homolysis of the Co^{III}–R bond occurs with formation of the 5′-deoxyadenosyl (or Et) free radical as well as vitamin B_{12r} (a Co^{II} corrinoid). These have implications for the mechanism of B_{12}-dependent biological isomerisations,[1] and studies using similar techniques are in hand to ascertain whether there is a parallel with the substrate-induced enzymatic reactions.

Irradiation (Pyrex-filtered light from a 250 W high-pressure mercury lamp) of a deoxygenated mixture of (Ia) (10^{-3} M) and Bu^tNO (10^{-2}M) in water at 50° in the cavity of an e.s.r. spectrometer produces the signal of spin-trapped 5′-deoxyadenosyl radical $Bu^tN(\dot{O})R$ (R = 5′-deoxyadenosyl), (II) (see Figure and Table). Ethylcobalamin (Ib)



(I)



(II)

a, R = 5′-deoxyadenosyl
b, R = Et

behaves similarly, giving $\mathrm{Bu^tN(\dot{O})Et}$. The mode of Co–C cleavage appears to be solvent independent; thus irradiation of (Ia) or (Ib) in either $\mathrm{PhOH–CHCl_3}$ (1 : 1) or the aprotic solvent $\mathrm{Me_2SO}$, in the presence of nitrosodurene as spin-trap, similarly afforded the nitroxide $\mathrm{ArN(\dot{O})R}$ (see Table). In each experiment, the $\mathrm{B_{12r}}$ moiety was characterised by a broad e.s.r. signal at *g ca.* 2·2, upon freezing the irradiated sample to 173 K.

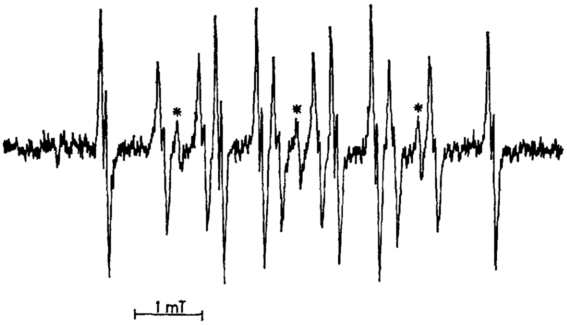


FIGURE. E.s.r. spectrum of $\mathrm{Bu^tN(\dot{O})R}$ (R = 5′-deoxyadenosyl) in $\mathrm{H_2O}$ at 50°. Peaks marked* are due to $\mathrm{Bu^t{}_2N\dot{O}}$.

TABLE

E.s.r. parameters[a] of the nitroxides $\mathrm{R'N(\dot{O})}R$

R′	R	a(N)	$a(\mathrm{H}^1)$	$a(\mathrm{H}^2)$	$a(\mathrm{H}^3)$
$\mathrm{Bu^t}$[b]	5′deoxyadenosyl	1·64	1·41	0·81	0·06
Ar[c]	,,	1·49	1·49	0·76	[e]
Ar[d]	,,	1·38	1·59	0·56	[e]
$\mathrm{Bu^t}$[b]	Et	1·71	1·13[f]		
Ar[c]	,,	1·51	1·26[f]		

[a] At 50°C, coupling constants in mT. [b] In $\mathrm{H_2O}$. [c] In $\mathrm{CHCl_3}$–phenol (1 : 1). [d] In $\mathrm{Me_2SO}$. [e] Not resolved. [f] $\mathrm{H^1 \equiv H^2}$, giving 1 : 2 : 1 triplet.

The nitroxides derived from (Ia) were unambiguously identified by the form and line-width variations in the e.s.r. spectrum (see Table). It has been shown that α-methylene protons ($\mathrm{H^1}$ and $\mathrm{H^2}$) of a nitroxide with a β-optically active chiral centre $\mathrm{R'N(\dot{O})CH_2CXYZ}$ ($X \neq Y \neq Z$) are magnetically non-equivalent and thus give rise to a **1 : 1 : 1 : 1** quartet, with selective broadening of the inner pair,[2] rather than the 1 : 2 : 1 triplet observed when the β-carbon atom is not chiral. Furthermore, the spectrum of $\mathrm{Bu^tN(\dot{O})R}$ (R = 5′-deoxyadenosyl) shows a small splitting attributable to hyperfine coupling with the hydrogen ($\mathrm{H^3}$) on the β-carbon atom.

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[1] *Cf.* D. Dodd and M. D. Johnson, *J. Organometallic Chem.*, 1973, **52**, 1; H. A. O. Hill, in 'Inorganic Biochemistry,' ed. G. I. Eichhorn, Elsevier, Amsterdam, 1973, Vol. 2, 1067; D. J. Cardin, K. N. Joblin, A. W. Johnson, G. Lang, and M. F. Lappert, *Biochem. Biophys. Acta*, **1974**, **371**, **44**, and references therein.

[2] C. Lagercrantz and M. Setaka, *Acta Chem. Scand.*, 1974, **B28**, 619 and references therein.