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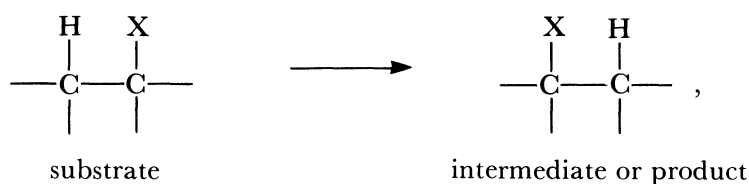
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Concerning the intermediacy of organic radicals in vitamin B₁₂-dependent enzymic reactions

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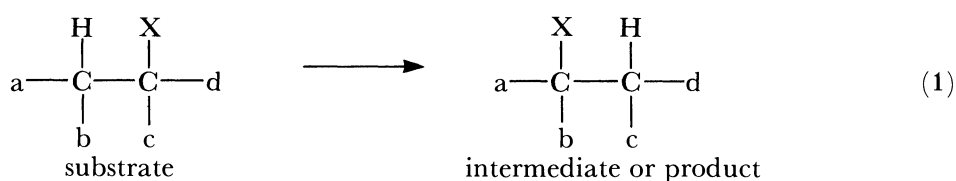
The vitamin B₁₂ coenzyme adenosylcobalamin assists the enzymic catalysis of molecular rearrangements of the type



in which the migrating group X can be OH, NH₂ or a suitable substituted carbon atom such as C(=CH₂)CO₂H. This paper discusses evidence for the participation of organic radicals as intermediates in these reactions. Theoretical and model studies supporting the intermediacy of radicals in the reactions catalysed by the enzymes diol dehydratase and α-methyleneglutarate mutase are described. For the model studies, alkyl radicals, alkylcobaloximes (alkyl represents, for example, ethoxycarbonyl substituted, but-3-enyl and cyclopropylmethyl) and also dihydroxyalkylcobalamins have been investigated. The Co–C_α–C_β angle of 125° in adenosylcobalamin is shown to be an ‘especial’ angle by analysis of the crystal structures of *R*- and *S*-2,3-dihydroxycobalamin.

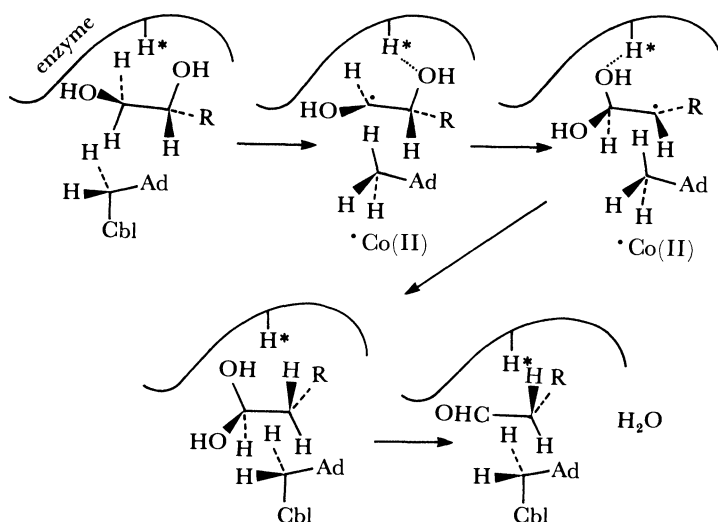
1. INTRODUCTION

The vitamin B₁₂ coenzyme adenosylcobalamin (AdoCbl, **1a**; see Appendix for structures) assists in the enzymic catalysis of molecular rearrangements of the following kind



Examples of such systems are diol dehydratase when X = OH, a = OH, b = c = H, d = Me; and α-methyleneglutarate mutase when X = C(=CH₂)CO₂H, a = CO₂H, b = c = d = H. For recent reviews of these reactions see Finke *et al.* (1984), Golding (1982) and Zagalak (1982).

Scheme 1 presents a pathway for diol dehydratase operating on propane-1,2-diol (Finlay *et al.* 1972), that is applicable in principle to all of the AdoCbl-dependent enzymic reactions. In an initiation step, homolysis of the Co–C σ-bond of AdoCbl gives cob(II)alamin (Cbl(II), **1b**) and the adenosyl radical (Ado•), a primary organic radical. This propagates the reaction



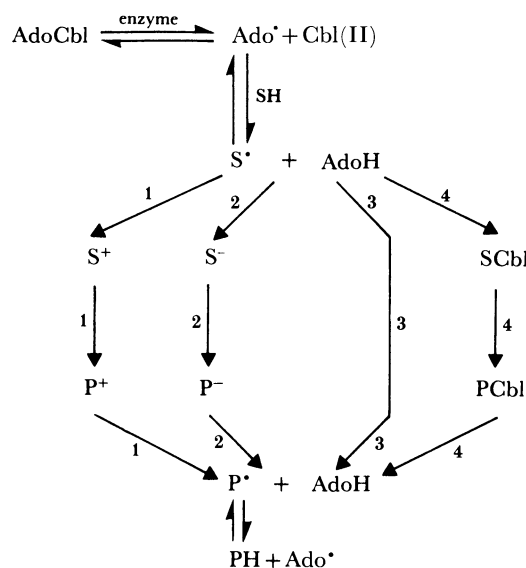
SCHEME 2. Hypothetical pathways (1–4) for AdoCbl-dependent enzymatic reactions. Pathways 1 and 2 involve electron transfer (e.g. $S^{\cdot} + \text{Cbl(II)} \rightleftharpoons S^- + \text{Cbl(III)}$); pathway 3 proceeds via protein-bound organic radicals; pathway 4 goes via σ -bonded organocorrinoids.

(c) *Does scheme 1 account for the stereochemical features of the reactions?*

In a recent comment (Rooney 1984) on the so-called ‘free-radical’ mechanism for ethanolamine ammonia lyase, it was stated that the ‘highly stereospecific’ nature of this reaction is ‘difficult to explain on the basis of a ... radical mechanism’. As first recognized by Ogston and clearly reviewed by Alworth (1972) many years ago, the discrimination between enantiotopic atoms or groups by chiral reagents (such as enzymes) is an inevitable consequence of the symmetry properties of prochiral molecules (those with lack of rotational symmetry). All of the AdoCbl-dependent enzymic reactions exhibit sharp discrimination whenever there is a choice in respect of enantiotopic atoms or groups (Retey 1982). While these results cannot define a unique mechanism, any mechanism suggested must be in accord with them. The bound radical mechanism for diol dehydratase (scheme 2, $R = \text{Me}$) is in perfect accord with the experimental evidence from studies with labelled propane-1,2-diols (as stated by Golding (1979) and fully explained by Finke *et al.* (1984). The product-related radical of scheme 2 ($R = \text{Me}$) receives a hydrogen atom at a prochiral centre and delivery is therefore to a particular face. When either *R*- or *S*-[1- ^2H , 1- ^3H]ethane-1,2-diol was used as substrate in the diol dehydratase reaction, racemic [2- ^2H , 2- ^3H]ethanal was obtained (Retey 1982). With ethane-1,2-diol as substrate, the faces of the product-related radical (scheme 2, $R = \text{H}$) are equivalent, provided that rotation about the C-1–C-2 bond is faster than transfer of a hydrogen atom from deoxyadenosine. Substitutions of the hydrogen atoms at the radical centre, one by deuterium and the other by tritium, make the faces of this centre enantiotopic. In practice it is highly unlikely that the enzyme can discriminate sufficiently between deuterium and tritium to prevent rotation about C-1–C-2 occurring at a rate similar to that with the corresponding unlabelled substrate, and so racemic [2- ^2H , 2- ^3H]ethanal results. The evidence from isotopic labelling of ethane-1,2-diol is therefore fully consistent with the bound-radical hypothesis for diol dehydratase (for detailed accounts see Finke *et al.* (1984) and Retey (1982)).

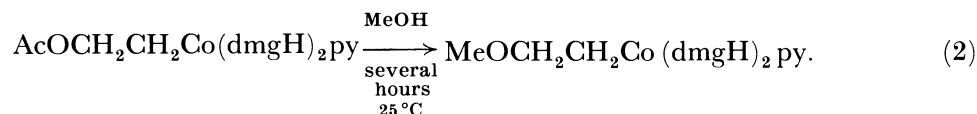
2. MODEL STUDIES

The purpose of a model study is to create a chemical system that faithfully reproduces the main features of an enzyme-catalysed transformation. A model study is most valuable with those enzymic reactions for which chemistry apparently offers no precedent and are not presently amenable to study at an enzymic level. To attempt to answer the questions posed in the introduction we have studied both the chemistry of alkyl radicals and alkylcobalt compounds. The alkyl species are structurally related to the substrate-derived and product-related entities of scheme 1. Our approach is based on the recognition that cob(II)alamin could be a 'conductor' or 'spectator' in scheme 1. By 'conductor' it is meant that for S^{\cdot} to be converted into P^{\cdot} it is necessary for Cbl(II) either to join with S^{\cdot} to give an organocorrinoid ($S-Cbl$), or to effect oxidation or reduction of S^{\cdot} (to S^+ or S^- , respectively; cf. scheme 3 (Abeles & Dolphin 1976)). One of these modified S species has the necessary reactivity to be converted into P . By 'spectator' it is meant that after homolysis of AdoCbl, Cbl(II) plays no further part until it terminates the reaction by repossessing the adenosyl radical. If this is the role of cobalt, then AdoCbl evolved as a masked primary organic radical and no more.



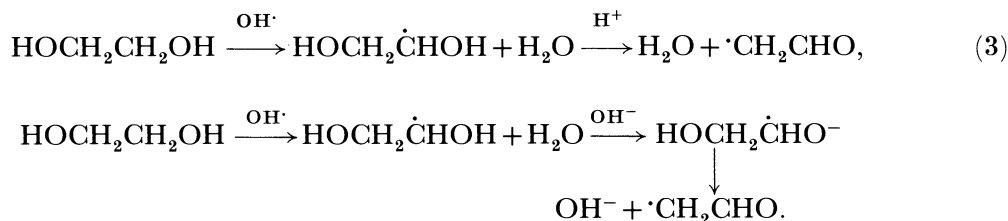
SCHEME 3. Pathway for AdoCbl-dependent diol dehydratase via protein-bound organic radicals (H^{\cdot} is an acidic group of the proteins, $R = H$ or Me).

Early attempts were made to explain the action of AdoCbl by means of known organometallic chemistry (Whitlock 1963). It was important to be aware of the properties of alkyl groups bound to cobalt. Although this knowledge is best acquired with the 'real thing' (i.e. alkylcobalamin) a valuable innovation was the development of model compounds for alkylcobalamins, the alkylcobaloximes, which are easy to prepare and characterize (Schrauzer 1968). Studies of the model compounds provide information about the reactivities of alkyl groups σ -bonded to cobalt in a corrinoid-like environment. (For a review of similarities and differences between cobalamins and cobaloximes see Elliott *et al.* 1981.) It was shown that remarkable activation of simple ester groupings could be achieved in 2-acetoxyalkylcobaloximes (Golding *et al.* 1970).



(dmgH = monoanion of dimethylglyoxime, py = pyridine).

This B_{AL1} cleavage of an ester is facilitated by σ - π hyperconjugation (electron donation from the Co-C σ -bond to the developing vacant p-orbital at C-2), leading to an intermediate ethene-Co^{III} complex that is captured by methanol. This finding strengthened the proposal of π -complexes as intermediates in certain AdoCbl-dependent enzymic reactions (Babior 1970; Silverman *et al.* 1972). However, the possibility that the enzymic reactions might proceed via organic radicals was also considered (Eggerer *et al.* 1960; Cockle *et al.* 1972). This was taken more seriously when it was noticed that non-enzymatic chemistry of 1,2-diols and vicinal amino-alcohols (see equations (3) and (4); Gilbert *et al.* 1972; Bansal *et al.* 1973), known to occur via free radicals, bore a suggestive similarity to the enzymic reactions catalysed by AdoCbl-dependent diol dehydratase and ethanolamine ammonia lyase (Golding & Radom 1973, 1976).



3. CONVERSION OF S' INTO P'

(a) Modelling diol dehydratase

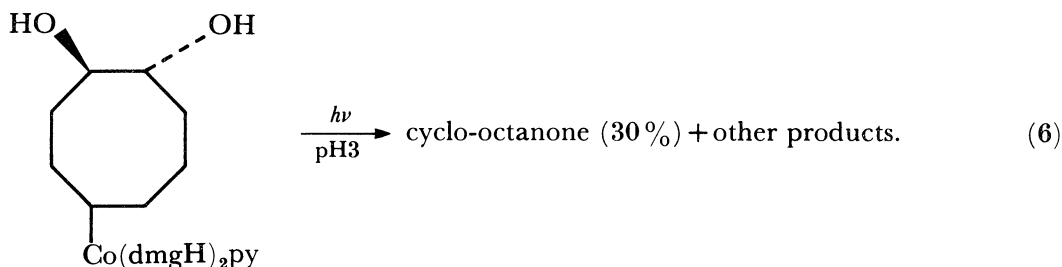
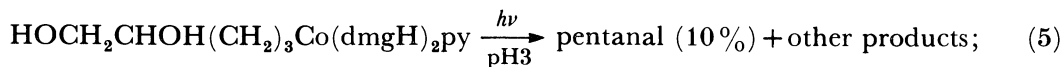
Once we had become aware of the type of non-enzymic chemistry exemplified by equations (3) and (4), we set about the design of an 'ideal model system' for diol dehydratase that would illustrate two main points.

(i) The regioselective attack by a primary organic radical, derived by homolysis of a Co-C σ -bond, at C-1 of a 1,2-diol giving a 1,2-dihydroxyalkyl radical (this would mimic the highly regioselective attack by the adenosyl radical on propane-1,2-diol in the diol dehydratase reaction).

(ii) The conversion of the 1,2-dihydroxyalkyl radical into an aldehyde, preferably *via* a 2,2-dihydroxyalkyl radical, formed by a 1,2-oxygen shift (the occurrence of 1,2-oxygen shifts in the conversion of propane-1,2-diols into propanal, and ethane-1,2-diol into ethanal, have been proved by oxygen-labelling studies with diol dehydratase (Retey 1982)).

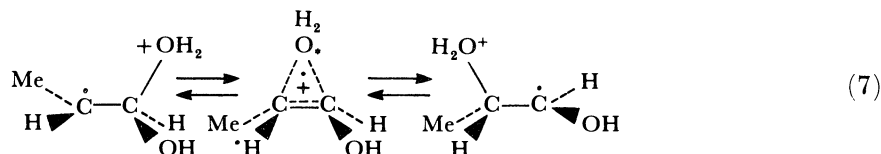
We showed initially that irradiation of methyl(pyridine)cobaloxime in aqueous ethane-1,2-diol at pH 3 gave a low yield of ethanal (Golding *et al.* 1975). This was interpreted by postulating attack of methyl radicals (from photolysis of the methyl-cobalt bond) on ethane-1,2-diol to give 1,2-dihydroxyethyl radicals. These underwent acid-catalysed conversion into the formylmethyl radical or 2,2-dihydroxyethyl radical, which then abstracted a hydrogen

atom from dimethylglyoxime to give ethanal (hydrate). More efficient models were obtained by making intramolecular hydrogen transfer from vicinal diol to radical (Golding *et al.* 1980):



Equation (5) reproduces one important feature of the diol dehydratase reaction (see (i)), and at least the overall conversion 1,2-diol \rightarrow aldehyde (see (ii)); but is the mechanism the same as that of diol dehydratase?

Ab initio molecular orbital calculations showed that conversion of, for example, the 1,2-dihydroxyethyl radical into the 2,2-dihydroxyethyl radical would be facilitated by protonation of the migrating OH, enabling the reaction to occur via a bridged transition state or intermediate (Golding & Radom 1973, 1976):



This mechanism is also applicable to the non-enzymic reactions of equations (3), (5) and (6). Recently, mass spectrometric studies have detected the protonated 2-hydroxyethyl radical $\cdot\text{CH}_2\text{CH}_2\text{O}^+\text{H}_2$, while further calculations have shown that it and its bridged form are significantly more stable than the well known ethanol radical cation (Bouma *et al.* 1983). It was recognized (Golding & Radom 1976) that the model reactions (equations (3), (5) and (6)) and diol dehydratase could proceed via an alternative acid-catalysed fragmentation, as proposed for (3) by Gilbert *et al.* (1972). However, this mechanism requires that, at least for diol dehydratase, it is necessary for water to recombine with the intermediate 2-oxoalkyl radical to give a 2,2-dihydroxyalkyl radical. It was also recognized (Golding & Radom 1976) that the fragmentation of the conjugate base of dihydroxyalkyl radicals postulated for reactions observed at higher pH (equation (4)) suggested an alternative dissociation–recombination pathway for diol dehydratase (see also discussion on this enzyme by Finke *et al.* 1984)).

Recently, we have studied a series of dihydroxyalkylcobalamins in the search for an ideal model system for diol dehydratase. When either *R*- or *S*-2,3-dihydroxypropylcobalamin (**1c** and **1d**, respectively) were photolysed or thermolysed at pH 7–8 in aqueous solution (D_2O) the main products were [H]-3-hydroxypropanone and prop-2-en-1-ol. The yields of these products depended on the dioxygen content of the system and the mode of cleavage of the Co–C bond (table 1). The formation of these products can be rationalized by scheme 4. Thus,

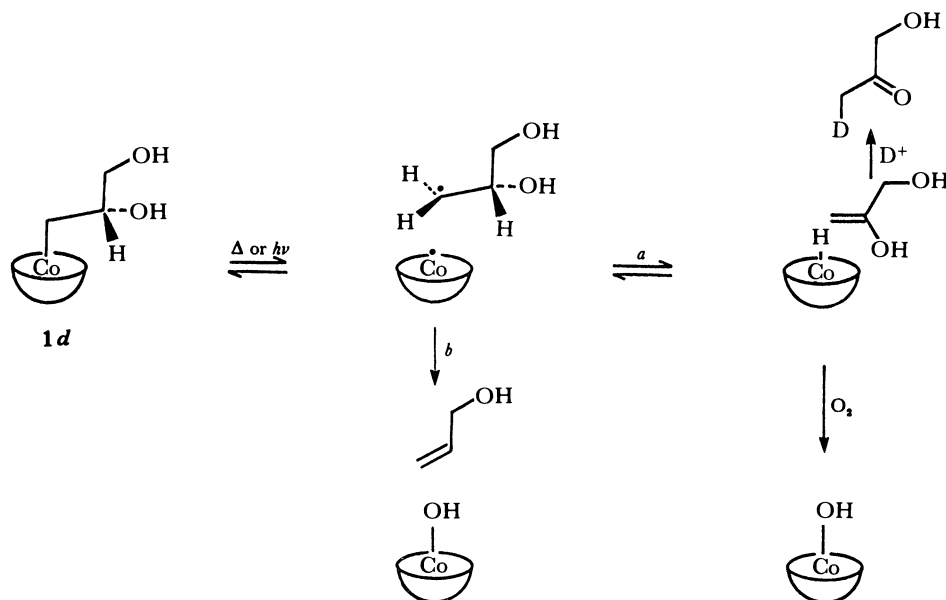
TABLE 1. PRODUCTS FROM THERMAL AND PHOTOLYTIC REACTIONS OF DIHYDROXYALKYL-COBALAMINS (RCbl)^a

R ^b	products	Δ	$h\nu$	Δ (O ₂)	$h\nu$ (O ₂)
		(anaerobic)	(anaerobic) ^c		
<i>R</i> -2,3-dihydroxypropyl	DCH ₂ COCH ₂ OH	20	65	70	45
	CH ₂ = CHCH ₂ OH	75	35	15	0
<i>S</i> -2,3-dihydroxypropyl	DCH ₂ COCH ₂ OH	5	65	70	70
	CH ₂ = CHCH ₂ OH	50	35	30	0
4,5-dihydroxypentyl	CH ₂ = CHCH ₂ CHOHCH ₂ OH	70	60	100	100
	<i>t</i> -MeCH = CHCHOHCH ₂ OH	30	40	0	0
5,6-dihydroxyhexyl	CH ₂ = CHCH ₂ CH ₂ CHOHCH ₂ OH	0	20	100	100
	<i>t</i> -MeCH = CHCH ₂ CHOHCH ₂ OH	20	80	0	0

^a All reactions were performed (normally in duplicate) in n.m.r. tubes in unbuffered D₂O (p[²H] 7–8). Thermolyses were done in darkness at 90 °C for *ca.* 8 h. Photolyses were done at 20 °C, irradiating with a 100 W lamp at a distance of 20 cm. Products were identified by ¹H n.m.r. spectroscopy (after purging the tubes with oxygen), gas chromatography, and isolation in some cases (carbonyl compounds as 2,4-dinitrophenylhydrazones). Yields are absolute and are accurate to $\pm 10\%$.

^b The 4,5-dihydroxypentyl- and 5,6-dihydroxyhexylcobalamin were mixtures of diastereoisomers.

^c One experiment was done with 4,5-dihydroxypentylcobalamin at p[²H] 3.4 in D₂O-CD₃CO₂D and did not yield detectable pentanal (less than 1%).

SCHEME 4. Pathways for thermal (Δ) or photochemical ($h\nu$) decomposition of *S*-2,3-dihydroxypropylcobalamin.

following homolysis of the Co–C bond, the derived radical pair could undergo elimination of the hydrogen β to the radical, giving hydridocobalamin (H–Cbl, **1e**), and the enol of hydroxypropanone, prop-2-ene-1,2-diol (pathway *a*, scheme 4). Alternatively, the β -hydroxyl group is eliminated giving prop-2-en-1-ol and hydroxocobalamin, OH–Cbl (**1f**) (pathway *b*, scheme 4). Under anaerobic conditions, the formation of H–Cbl and prop-2-en-1-ol is reversible and relatively more prop-2-en-1-ol is formed by pathway *b*, which is assumed to be irreversible. In the presence of oxygen, H–Cbl is scavenged (probably via its conjugate base cob(I)alamin) to give OH–Cbl irreversibly, and the prop-2-ene-1,2-diol tautomerizes to

[1-²H]3-hydroxypropanone. The different ratios of products obtained under thermal or photolytic conditions may be ascribed to one or both of the following reasons:

- (i) temperature effects on the rates of pathways *a* and *b* (table 1);
- (ii) the mechanism of cleavage may differ (for example, thermal radical pair formation (as in scheme 4) or photochemical concerted β -elimination from an excited state of R-Cbl).

The anaerobic thermolyses and photolyses of the dihydroxyalkylcobalamins **1g** and **1h** gave mixtures of dihydroxyalkenes (table 1), but no aldehyde (for example, pentanal from **1g**) or ketone (such as hexan-2-one from **1h**) under either neutral or acidic conditions. Aerobically, the terminal alkene was the sole organic product. The behaviour described can be rationalised in a manner similar to the explanation for *R*- and *S*-2,3-dihydroxypropylcobalamin. In the presence of oxygen, H-Cbl is scavenged leaving 4,5-dihydroxypent-1-ene [from **1g**] or 5,6-dihydroxyhex-1-ene (from **1h**) as the exclusive organic product. In the absence of oxygen, β -elimination to H-Cbl and terminal alkene is followed by the recombination of these species to afford, for example, either **1g** or the secondary alkylcobalamin **1i**. The latter dissociates to H-Cbl and an internal alkene (mainly *t*-4,5-dihydroxypent-2-ene). The 4,5-dihydroxypent-1-ene obtained from **1g** by anaerobic thermolysis is partly deuterium labelled, whereas the alkene obtained in aerobic thermolysis is unlabelled. These observations support the postulated reversibility of the dissociation of **1g** to H-Cbl and 4,5-dihydroxypent-1-ene (N.B. $\text{H-Cbl} + \text{D}_2\text{O} \rightleftharpoons \text{D-Cbl} + \text{HOD}$ via cob(I)alamin).

The failure of cobalamin (**1g**) to produce pentanal under any of the conditions described (table 1) is in contrast to the corresponding cobaloxime (equation (5)); see also Golding *et al.* 1980). For the formation of pentanal a 1,5-shift of a hydrogen atom is obligatory in the 4,5-dihydroxypentyl radical from homolysis of the Co-C bond of **1g**. The 4,5-dihydroxypentyl radical can undergo, *inter alia* a 1,5-H shift or β -elimination (to 4,5-dihydroxypent-1-ene) or recombination with Cbl(II). When this radical is generated from 4,5-dihydroxypentyl(pyridine)cobaloxime (equation (5)), two of these processes (β -elimination involving cobaloxime(II), or recombination of a radical with cobaloxime(II)) are less likely because of the rapid degradation of cobaloxime(II) in acidic solution to aquated Co^{II} . The behaviour observed with the cobaloxime is therefore that of the mobile 4,5-dihydroxypentyl radical (i.e. radical surrounded by solvent molecules and not influenced by cobalt ions), which rearranges to the 1,2-dihydroxypentyl radical, the precursor of pentanal. To observe analogous chemistry with cobalamins obviously requires that both β -elimination and recombination are blocked. To achieve this we are synthesizing 4,5-dihydroxy-2,2-dimethylpentylcobalamin.

Our studies to date of dihydroxyalkylcobalamins have not provided a better model for diol dehydratase than that developed with cobaloximes (Golding *et al.* 1980). However, we believe that they support the premise that the function of deoxyadenosine in the mechanism for diol dehydratase of scheme 2 is to prevent the interaction of substrate-derived radical with Cbl(II), which would lead to non-productive elimination of either H or OH β to the radical centre.

(b) *Modelling α -methylglutarate mutase*

Following the model studies of Dowd *et al.* (1975) and Chemaly & Pratt (1976) concerning the possible intermediacy of dicarboxy-substituted but-3-enyl- and cyclopropylmethylcobalamins in the rearrangement catalysed by α -methylglutarate mutase (equation (1)), we undertook the synthesis and study of but-3-enyl- and cyclopropylmethylcobaloximes. We have reported thermally induced and trifluoroacetic acid-catalysed interconversions of these

compounds, which were rationalized by pathways in which the organic moiety never left cobalt (the 'conductor' role) (Atkins *et al.* 1980). To improve the correspondence of the model with the 'real thing' we synthesized the ethoxycarbonyl-substituted cobaloximes **2a–2d**. In contrast to the methyl-substituted series, the ethoxycarbonyl-substituted compounds were much less readily interconverted (either thermally or by acidic catalysis), perhaps because intermediate η^3 homoallyl species are destabilized by replacing methyl by ethoxycarbonyl (Golding & Mwesigye-Kibende 1983).

Attempts to synthesize the cobaloxime **2c** by reacting cobaloxime(I) with the *cis* iodo compound **3c** were unsuccessful, the predominant product of this reaction being the but-3-enylcobaloxime **2a**. With the *trans* iodo compound **3d**, cobaloxime(I) gave a mixture of cobaloximes **2d** and **2a**, the latter usually predominating. For the reaction of alkyl halides with cobaloxime(I), two competing mechanisms have been established: S_N2 displacement, and a stepwise pathway via an intermediate organic radical produced by electron transfer from one reactant to another (Okabe & Tada 1982). Which pathway is preferred depends on the nature of the alkyl halide, the stepwise route being favoured with hindered alkyl iodides. The results described for iodides **3c** and **3d** can be rationalized by an electron-transfer pathway leading to cyclopropylmethyl radicals (**4c** from **3c**, **4d** from **3d**). These can either be trapped by cobaloxime(II), or ring-open primarily to radical **4a**, which reacts with cobaloxime(II) to yield cobaloxime **2a**. For steric reasons the *cis* radical **4c** may open more rapidly than the *trans* radical **4d**. Thus, the *cis* cobaloxime **2c** is not observed, in contrast to the *trans* isomer **2d**. Spectroscopic characterization of the radicals **4a** and **4b**, and preliminary information on the reactivity of radicals **4a** and **4d**, has been obtained. After γ -radiolysis the halides **3a** and **3b** gave the corresponding radicals at 77 K (**4a** and **4b** respectively), characterized by e.s.r. spectroscopy (see figure 1). However, the cyclopropylmethyl iodides **3c** and **3d** were converted into butenyl radical **4a** by γ -radiolysis at 77 K. Irradiations (visible light) of cobaloximes **2a** and **2b** in deuteriochloroform, followed by exposure to air, gave the peroxyalkylcobaloxime **2e** (Alcock

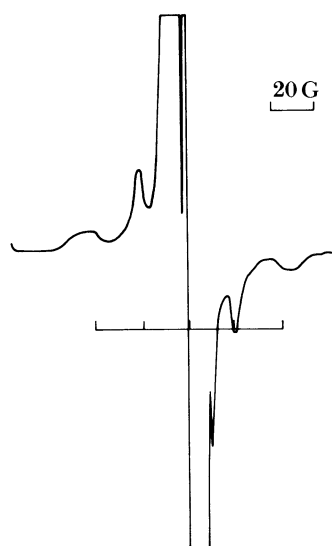


FIGURE 1. First-derivative X-band e.s.r. spectrum for a solution of methyl 2-(iodomethyl)but-3-enoate in CD_3OD after exposure to ^{60}Co γ -rays at 77 K and annealing to *ca.* 130 K, showing features assigned to the radical $CH_2 = CHCH(CO_2Et)\cdot CH_2$. The intense central absorption is due to the solvent. $1\text{ G} = 10^{-4}\text{ T}$.

et al. 1985). These observations can be explained by postulating light-induced homolysis of the Co–C bond in each cobaloxime, to give a radical (**4a** from **2a**, **4b** from **2b**, etc.), which equilibrates with its isomers (**4a** \rightleftharpoons **4c** or **4d** \rightleftharpoons **4b**). At equilibrium, the predominant radical is **4a**. This reacts with cobaloxime(II) to give hydrido(pyridine)cobaloxime and 1-ethoxycarbonylbuta-1,3-diene. Recombination of these affords allylcobaloxime **2f**, which by oxygen insertion into its Co–C bond gives the peroxyalkylcobaloxime **2e**.

Three independent pieces of evidence have shown the ability of radicals **4a–4d** to be interconverted readily. On the contrary, the corresponding alkylcobaloximes **2d–3d** are not interconverted easily. It is inferred that α -methylene-glutarate mutase operates via the protein-bound radicals **4e–4g**, the butenyl species being formed by reaction between the adenosyl radical and a substrate molecule. The deoxyadenosine formed in this process prevents the combination of an organic radical with cob(II)alamin. This is undesirable because the resulting alkylcobalamins are presumed *not* to be readily interconverted. Dowd *et al.* (1984) have reported the conversion (in darkness, 230 h, pH 8.4, 25°) of the alkylcobalamin **1j** into α -methylene-glutaric acid (*ca.* 30%), amongst other products. This result was interpreted in support of a mechanism for α -methylene-glutarate mutase via organocorrinoid intermediates. The possibility of rearrangement via radicals, either *before* (by the electron-transfer pathway in the formation of alkylcobalt compounds) or *after* formation of alkylcobalamin **1j** (by homolysis of the Co–C σ -bond) was not excluded.

4. CLEAVAGE OF THE Co–C BOND OF AdoCbl

The Co–C $_{\alpha}$ –C $_{\beta}$ bond angle was measured as $125^{\circ} \pm 3$ in the crystal structure analysis of AdoCbl (Lenhert 1968; Glusker 1982). This has been taken to imply strain in the molecule, making the Co–C bond intrinsically weak and ready to be cleaved on demand by an enzyme (Glusker 1982; Pratt 1982). To assess whether or not 125° is an especial angle, relevant to the mode of action of AdoCbl, it is necessary to have data for other alkylcobalamins and model compounds. We have therefore determined the crystal structures of the diastereoisomeric 2,3-dihydroxypropylcobalamins (**1c** and **1d**). The *S* isomer **1d** has a Co–C $_{\alpha}$ –C $_{\beta}$ angle of $113.6^{\circ} \pm 2.1$, whereas that for the *R* isomer **1c** is $119.6^{\circ} \pm 1.7$. Cobalamins **1c** and **1d** have Co–C bond lengths that do not differ significantly from Co–C for AdoCbl [2.03 (6) Å]. A very interesting feature of the structures of **1c**, **1d** and AdoCbl is that in each case the group σ -bonded to Co lies in a channel between rings C and D defined by the sentinel groups at C-12 (β -methyl) and C-17 (C-54 methyl). For **1d** this is a ‘comfortable’ arrangement that benefits from an intramolecular hydrogen bond (C $_{\beta}$ –OH...O=C (of *c*-acetamido group)). AdoCbl is isostructural with **1d** (same chirality at C $_{\beta}$), but adopts a different rotamer about the C $_{\alpha}$ –C $_{\beta}$ bond (N.B. it cannot form an analogous H-bond to **1d**). This enables the ribose of adenosyl to slot perpendicularly into the C–D channel, while the adenine is roughly parallel to the corrin and partly over ring C. The β -face of ring C is probably the sterically least demanding region of the corrin and the arrangement described may profit from hydrophobic bonding, providing the adenine does not come too close to the β -methyl group at C-12 (N-9 ... C-46 = 3.71 Å). Minimization of steric interactions may necessitate an increase in the Co–C $_{\alpha}$ –C $_{\beta}$ angle. This may weaken the Co–C σ -bond and make AdoCbl intrinsically strained. Nevertheless, although the bond dissociation energy of AdoCbl is only 132 kJ mol $^{-1}$, it has been estimated that diol dehydratase must accelerate the rate of cleavage of the Co–C bond by at least 10^{10} -fold (Finke

& Hay 1984). The crystal structure of cobalamin **1c** indicates that too close contact between C_β-OH and H-19 is avoided by increasing the angle Co-C_α-C_β. Cobalamin **1c** cannot adopt the same conformation as **1d**, with a hydrogen bond from C_β-OH to the *c*-acetamido because this would press the CH₂OH group of the side chain into ring C. For further discussion of these results see Alcock *et al.* (1985).

CONCLUSIONS

The claim (Golding & Radom 1976) that a mechanistic scheme in the reactions catalysed by diol dehydratase, ethanolamine ammonia lyase, the other aminomutases and methylmalonyl CoA mutase 'is consistent with all experimental data to date' still holds true today. Our solution to the 'B₁₂ mystery' is based on what we regard as the best assessment of the available evidence from experiments with the enzymes, theoretical calculations and model studies. We have been especially influenced by theory and models and trust that we do not suffer from what Henry James called the 'perversity (of) an innate preference for the represented subject over the real one'.

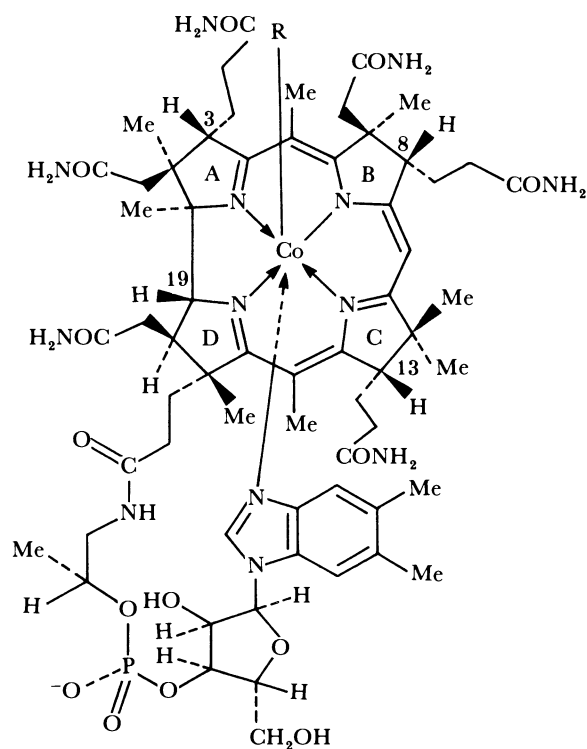
We thank Dr N. W. Alcock (University of Warwick) for collaboration on the crystal structure determinations and Professor M. C. R. Symons (University of Leicester) for assistance with e.s.r. measurements. Financial support from SERC is gratefully acknowledged.

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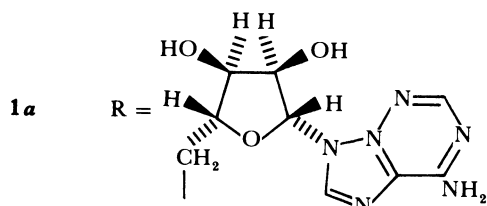
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APPENDIX



RCbl



- 1b** R = single electron
1c R = *R*-2,3-dihydroxypropyl
1d R = *S*-2,3-dihydroxypropyl
1e R = H
1f R = OH
1g R = HOCH₂CHOH(CH₂)₃
1h R = HOCH₂CHOH(CH₂)₄
1i R = HOCH₂CHOHCH₂CHMe
1j R = CH₂CH(CO₂R¹)C(=CH₂)CO₂R¹ (R¹ = tetrahydropyranyl)

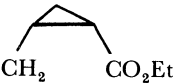
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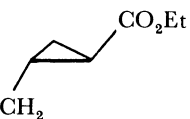
RUTH M. DIXON

2 $\text{RCo}(\text{dmgH})_2\text{py}$ (dmgH = monoanion of dimethylglyoxime; py = pyridine)

2a $\text{R} = \text{CH}(\text{CO}_2\text{Et})\text{CH}_2\text{CH}=\text{CH}_2$

2b $\text{R} = \text{CH}_2\text{CH}(\text{CO}_2\text{Et})\text{CH}=\text{CH}_2$

2c $\text{R} =$ 

2d $\text{R} =$ 

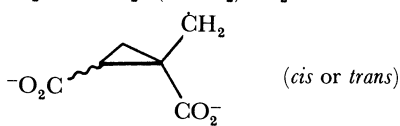
2e $t\text{-O}_2\text{CHMeCH}=\text{CHCO}_2\text{Et}$

2f $t\text{-CHMeCH}=\text{CHCO}_2\text{Et}$

3a-3d alkyl halides corresponding to R in compounds **2a-2d** (halide = Br in **3a**, I in **3b-3d**)

4a-4d alkyl radical corresponding to R in compounds **2a-2d**

4e $\text{HO}_2\text{C}\dot{\text{C}}\text{HCH}_2\text{C}(=\text{CH}_2)\text{CO}_2\text{H}$

4f  (cis or trans)

4g $\text{HO}_2\text{CCH}(\dot{\text{C}}\text{H}_2)\text{C}(=\text{CH}_2)\text{CO}_2\text{H}$